RESEARCH ON THE INFLUENCE OF TEMPERATURE, LIGHT AND CULTURE MEDIA ON GROWTH AND DEVELOPMENT OF *Pyrenophora teres* FUNGUS (IN VITRO)

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Abstract

Pyrenophora teres is the causal agent of net blotch fungus disease on barley, one of the most common, destructive foliar diseases in barley pathology. Abiotic factors play an important role for fungal development. Under laboratory conditions it was monitored the abiotic factors influence (temperature, light and culture media) on the *Pyrenophora teres* fungi growth and sporulation. The pathogen have been isolated from barley leaves of Dana variety and incubated at 22°C. Actual research monitored the biological thresholds on the growth and sporulation of *Pyrenophora teres* fungus. Regarding the temperature influence on the colonies development, the observations revealed that the pathogen growth begins at 6°C. The optimal pathogen development threshold is between 20 to 26°C temperatures, the fungus developing colonies measuring 90 mm after 9 days observations. Over 28°C temperature, the growth rate of the fungal colonies decreased, and at 34°C the fungus ceased sporulation. The light influence on the *Pyrenophora teres* fungi development showed that the colonies have been well developing under continuous light conditions, as well under 16/8 alternating light conditions, the colonies` diameter measuring 85 mm after 9 days observations. Under the continuous lack of light conditions (dark), the colony developed slowly, reaching a diameter of 65 mm after 15 days incubation. Under continuous light conditions, *Pyrenophora teres* had the fastest development rate, after 3 days of incubation the colony diameter reached 41 mm and respectively 87 mm, after 9 days of observations. *Pyrenophora teres* fungus had the optimal growth & development on the natural type culture media compared with artificial ones.

Key words: barley, fungus, abiotic factors

Pyrenophora teres is one of the most devastating pathogens from an economic point of view that cause diseases in barley (Weiland J.J*., et al,* 1999), being present wherever this plant is grown. Production losses are between 10% and 40% in years with favorable conditions for the pathogen (Shipton W.A. *et al,* 1973).

The pathogen produces on the leaves, from the leaf base to the flag leaf, initially yellow spots, which can reach up to 20-25 mm long and 1-3 mm wide. Gradually amid the yellow background of the spot, brown striations streaked longitudinally and transversely as a network, striations that are multiplying and the stain turns brown.

Often the spots are surrounded by a yellow border, and strongly affected leaves dry (Cristea S., 2005). On the surface of the specific stains, the fungus develop characteristic fructifications (Gheorghies C., Cristea S., 2001). Monitoring blotch attack requires a correct diagnosis of the disease, knowledge of epidemiology, prophylaxis and therapy of disease. Establishing the biological parameters of the phytopathogenic fungi in the laboratory can provide data on their requirements in terms of culture - "in vivo" (Cristea S., Oprea M., 1996).

MATERIAL AND METHODS

Research into the biology of the phytopatogenic fungus *Pyrenophora teres* were performed in laboratory conditions.

The fungus was isolated from the leaves of the barley Dana variety naturally infected and raised on PDA medium (agar-potato-glucose), method Ulster (Hulea A., 1969; Constantinescu O., 1974; Raicu C., Baciu D., 1978).

Experiences watched the temperature influence, the culture media and light, on growth and sporulation of the pathogen.

The substrates used were: Czapek Dox agar (synthetic environment), PDA (semi-synthetic environment) and natural environments with cornmeal-agar and oats-agar.

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The influence of light was studied by exposing cultures seeded with inoculum at different light: continuous light, continuous darkness and light/alternative dark for the versions 8h/16h, 12h/12h and 24h/24h.

The influence of temperature on growth and sporulation of the fungus was performed thermostatic cultures at temperatures between 2-40^{0}C. Observations were made at intervals of 3, 6, 9 and 15 days.

To determine thresholds for the development of the fungus, notes on the diameter of the colonies and notes regarding fructifications abundance were taken.

RESULTS AND DISCUSSIONS

Regarding the exposure to light (*figure 1*), the fungus *Pyrenophora teres* behaved differently. The Colonies developed very well in continuous light. The first measurements of the colony diameter (after 3 days) found the most developing at continuous light exposure, which is 36 mm. The growth rate was maintained throughout the period of examination of the culture, colony reaching a diameter of 90 mm with an abundant fructification, very good.

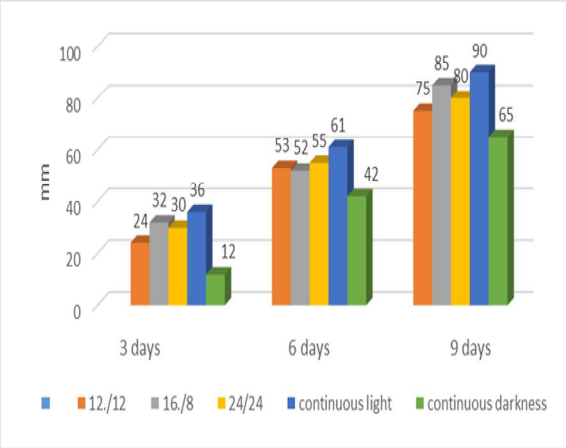

Figure 1 **The diameter of the colonies at alternative exposure**

The fungus incubated at 22^{0}C and exposed to different intervals of alternating light, developed in 6 days a diameter range of 53 mm for the 12/12 interval, 52 mm at 16/8, respectively 55 mm at 24/24. At 9 days, the growth speed was constant, the values recorded in alternating light-dark periods were 75 mm, 85 mm and 80 mm, with a good fructification.

In conditions of continuous darkness, the colony developed slowly, the diameter is 12 mm after 3 days and at the end of the incubation period it measured diameter was 65 mm, the colony presenting a very poor fructification.

Regarding the influence of the culture substrate, the data in *table 1* shows that the fungus *Pyrenophora teres* fungus was favored by the natural culture media (cornmeal and oats) where it formed a very good vegetative mass, abundance and sporulated very well on a semisynthetic PDA culture medium the fungus presented a good vegetative mass and a good sporulation.

The weakest development of the fungus colony formed by *Pyrenophora teres* was on a Czapek-Dox Agar synthetic medium, where it formed a weaker vegetative mass with a weaker sporulation than the other culture media.

Table 1

The influence of the different culture media on the development of *Pyrenophora teres* fungus

Culture medium 22^{0}C	Colony diameter(mm)/days			
	3	6	9	fungus behaviour
synthetic culture media: Czapek-Dox Agar	32	49	75	Mv + Sp+
semi-synthetic culture media: PDA	34	55	80	Mv++, sp++
natural culture media: cornmeal+agar	37	57	85	Mv+++ Sp+++
natural culture media: oatmeal+agar	38	59	85	Mv+++ Sp+++

With regard to temperature influence on the development of the fungus colonies, the data presented in *table 2* shows that the minimum threshold of its development may be considered 6^{0}C, where the colony diameter was 9 mm in 6 days.

The optimum threshold of the development for the fungus ranged between 20-26^{0}C, where in 15 days the fungus colonies formed 90 mm, with an abundant vegetative mass and very good sporulation, fungus resembling felt, brown colour, with brown reverse (*figure 2*).

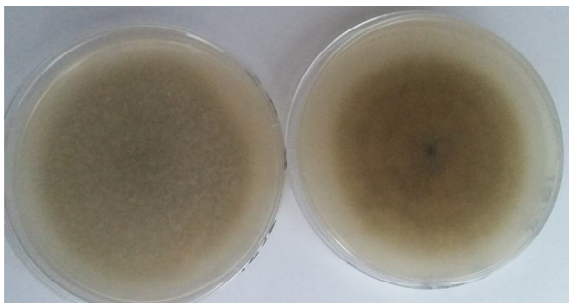

Figure 2 **Aspects of the *Pyrenophora teres* fungus colony**

Table 2

The development of *Pyrenophora teres* fungus at different incubations temperatures

T/ ^{0}C	Colony diameter (mm)					after 15 days
	3	6	9	12	15	
0	0	0	0	0	0	0
2	0	0	0	0	0	0
4	0	0	0	0	0	0
6	0	9,0	28,0	34,0	37,0	Mv± 0
8	0	23,0	34,0	40,0	56,0	Mv+ sp±
10	22,0	32,0	38,0	40,0	58,0	Mv+ sp+
12	25,0	31,0	38,0	42,0	60,0	Mv+ sp+
14	25,0	34,0	39,0	46,0	62,0	Mv++ sp+
16	26,0	36,0	41,0	52,0	80,0	Mv++ sp+
18	32,0	44,0	56,0	69,0	85,0	Mv++ sp++
20	35,0	45,0	58,0	72,0	90,0	Mv+++ sp+++
22	37,0	48,0	68,0	79,0	90,0	Mv+++ sp+++
24	41,0	50,0	72,0	80,0	90,0	Mv+++ sp+++
26	41,0	54,0	77,0	82,0	90,0	Mv+++ sp+++
28	32,0	45,0	69,0	80,0	87,0	Mv+++ sp+++
30	22,0	31,0	39,0	45,0	70,0	Mv+ sp+
32	10,0	16,0	22,0	30,0	34,0	Mv± sp±
34	0	1,0	3,0	8,0	10,0	Mv± sp 0
36	0	0	0	0	0	0
38	0	0	0	0	0	0
40	0	0	0	0	0	0

mv± = very poor vegetative mass
mv+ = poor vegetative mass
mv ++ = good vegetative mass
mv +++=very good vegetative mass
0= fungus did not sporulated
Sp ± = very poor sporulation
Sp+ = poor sporulation
Sp ++ = good sporulation
Sp+++ = abundant sporulation

The growth rate was slower for the colony after 30^{0}C, after 34^{0}C the colony diameter greatly diminished and the fungus did not fructified anymore, and at temperatures above 36^{0}C it never developed. The mycelium growth was maximum at temperature values between 20 and 26^{0}C after 15 days of observation (*figure 3*).

CONCLUSIONS

Regarding the exposure of *Pyrenophora teres* fungus at different intervals of light and darkness a very good development of the colonies was observed at continuous lights and alternative 16/8. In conditions of continuous darkness the colony developed slowly.

The *Pyrenophora teres* fungus colony developed a very good vegetative mass on natural culture media (cornmeal and oats) and a good development on the semisynthetic PDA media.

The optimum temperature for the development of the *Pyrenophora teres* fungus in controlled conditions is between 20 to 26^{0}C, with a minimum threshold of 6^0 C and a maximum of 34^{0}C.

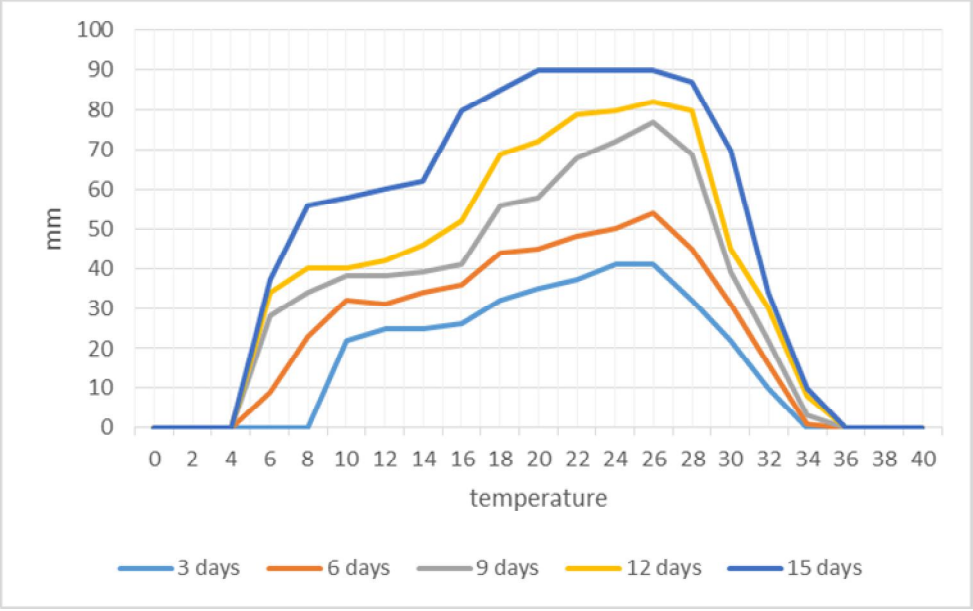

Figure 3 **The develoment of *Pyrenophora teres* fungus at different incubation temperatures**

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