RESEARCH INTO THE INFLUENCE OF THE *IN VITRO* BIOLOGICAL PARAMETERS ON THE DEVELOPMENT OF *PHYTOPHTHORA INFESTANS* (MONT.) DE BARY FUNGUS COLONIES

Zsuzsanna NEMES*, Daniela POPA*, Anca BACIU*, Luiza MIKE*

*Potato Research and Development Station – Targu Secuiesc, Romania
zsuzsa20@yahoo.com

Abstract. In the period 2002-2003 under laboratory conditions it was determined the influence of biological parameters (temperature, relative air humidity, energetically and plastically resources and the composition of the cultivation environment) on the growth of the *Phytophthora infestans* fungus colonies.

The biological parameters determined *in vitro* for the Ph. infestans fungus showed that the fungus needs mesophyll conditions. The energetically and plastically resources are important to its growth, being indispensable to the colonies' evolution.

Keywords: Ph. infestans, biological parameters, development, colonies

INTRODUCTION

Ph. Infestans causes a well-known and extremely harmful disease, the late blight of potato. The *Ph. infestans* fungus grows well under laboratory conditions, on artificial media as well as on natural substrates (potato slices, fresh potato leaves).

In order that he can obtain well-grown *Ph. infestans* cultures, Stille carried on research to demonstrate that neither the zoospore nor the mycelia germination of the fungus's sporanges depends only on temperature but it is also influenced by the environmental humidity conditions [4].

The growth of the osmotic pressure in the liquid drop in which the inoculation had been done produced the same effect as the growth of the temperature, favoring the zoospore germination and not the mycelia one. The optimal pH for growth is between 5 and 7, but it prefers a more acidic environment [2]. Some researchers mention that the fungus procreates poorly in an alkaline environment, though the mycelium grows quite abundantly. The viability of a *Ph. infestans* culture kept under optimal conditions does not last longer than 2 months. At 20°C the cultures can be kept well only for 8-10 days [1].

There were effected researches about the influence of different culture medium concerning the development of *Ph. infestans* fungus [4].

Our experiments and observations were meant to relieve the optimal *in vitro* growth and development conditions of the *Ph. infestans* fungus colonies as well as the viability of these cultures.

MATERIALS AND METHODS

For the isolation of the pathogens from the potato leaves it was used the procedure described by Riker & Riker (1936) and Altman (1966): healthy tubers are washed, they are wiped with gauze, sterilized by tamponning with alcohol 95% (we used 70%), then they are cut aseptically into two. A leaf attacked by late potato blight is inserted between the two halves, the tuber is fixed with a rubber tape and it is put into a wet chamber, to 18°C. After 2-3 days the fungus has entered the tuber, which is then cut vertically on the surface affected by the parasite and in 4-5 days the fruit bodies of the fungus appear on the surface thus sectioned. The fruit bodies can be kept at 6°C for a week or more, or they can be transferred to the cultivation environment.

Another method used for the isolation of the pathogens was the method of direct isolation described by Ovidiu Constantinescu (1974). By this method a portion of the fungus mycelium is placed under binocular eyeglasses, and with a sharp inoculation loop, moistened in nutritive medium, one or more spores, conidia are detached, which are then inoculated on a cultivation environment. To avoid bacterial contamination there are bactericides added to the environment or it is acidified.

To isolate the pathogenic agent from the tubers an infected potato is chosen, it is washed on the outside and wiped well with clean gauze. A deep cut is made on the tuber, opposite to the blight lesion and then the tuber is split across the infected portion. The two potato halves obtained are placed into a Petri dish padded with blotting paper and they are kept at 18°C. After 4-5 days the *Phytophthora* mycelium appears on the potato's broken surface and then it is moved to the cultivating environment.

The cultivation environments used for the isolation of the fungus were: Czapek, CGA (with potato extract and glucose), Henniger and malt.

To determine the most adequate substrate on which it can be multiplied, the parasite was grown on the four substrates presented previously, preparated according to the standard recipies used in specialized laboratories. The media was divided into 8 cm diameter Petri dishes, the fungus or the infected tissue being singled on them. Three days, respectively seven days after the inoculation the diameters of the colonies were noted.

The biological parameters examined in the laboratory were:

- the influence of the temperature values on the growth of the *Ph. Infestans* fungus was realized by singling the fungus on media in 3.15 in diameter Petri dishes and then transferring the dishes to thermostats having temperatures of 6-34°C. The growth of the colonies' diameters was noted at intervals of 3 days, the observations lasted for 21 days.

- the relative air humidity on the colony formation was realized in Petri dishes in which the fungus was

singled and which were kept without a lid, in exsicators with temperatures of 50-95%

- the energetically and plastically resources of the cultivating environment were metabolized differently by the fungus. The carbon and the nitrogen resources were replaced successively and it was followed the growth of the mycelium

RESULTS AND DISCUSSIONS

Results related to the influence of temperature on the growth of the Ph. Infestans fungus colonies

The growth of the *Ph. Infestans* fungus on CGA environment was strongly influenced by the temperature values. As table 1 show, the fungus colonies start growing at + 6°C, forming a white and quite loose mycelium. As the temperature increases, the colonies' diameters grow, the mycelium becomes densely tissue, with a felt like aspect. The optimal values for the growth of the fungus were those of 16 – 24°C. Above 30°C the fungus developed infirm colonies, and at 34°C the colonies stopped growing.

Table 1. The influence of the temperature on the growth of *Ph. infestans* (Mont) de Bary colonies

T°C	6	8	10	12	14	16	18	20	22	24	26	28	30	32	34
φ mm	4	9	15	22	40	72	75	75	75	70	45	30	10	2	0

DL 0.1% = 4.84
DL 1% = 2.28
DL 5% = 1.42

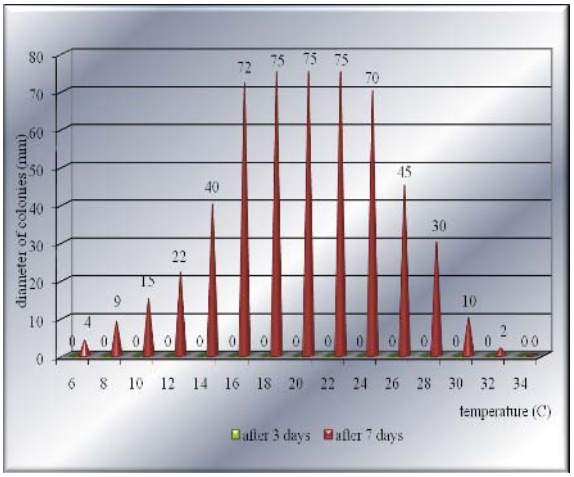

Figure 1. The influence of the temperature on the growth of *Ph. infestans* (Mont) de Bary colonies

Results related to the influence of the relative air humidity on the growth on the *Ph. infestans* fungus colonies

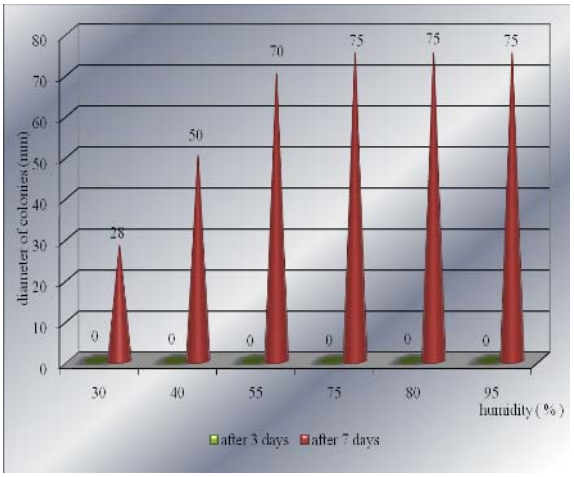

Figure 2. The influence of the humidity on the growth of *Ph. infestans* (Mont) de Bary colonies

According to the experiments carried on *in vitro* the growth of the *Ph. infestans* fungus colonies was influenced by values of the relative air humidity. As chart 2 shows, after 15 days of observation the colonies started developing at values of 30%, the mycelium being loose, and the colony reached 28mm. As the values of the relative air humidity increased, the colonies presented a good vegetative mass, the mycelium becoming denser, with a felt like aspect.

Results related to the influence of the energetically and plastically resources on the growth of the *Ph. infestans* fungus colonies

According to the experiments carried on *in vitro* the carbon element was indispensable to the growth of the fungus colonies. This element can be assimilated in a different way by certain resources, depending on the carbon's chemical bonds in the given molecule. The favorite carbon resources of the *Ph. infestans* are the glucose and the sucrose (the colonies' diameter was of 2.9527 in – 3.0709 in). The growth was poorer in the case of the rhamnose, xylose and arabinose (the colonies' diameter was of 0.3937 in). The lack of the carbon resource stopped the growth of the colonies completely **(Table 2)**.

Table 2. The influence of the carbon resource on the growth of the *Ph. infestans* (Mont) de Bary fungus colonies

Sugars	Diameter of colonies (mm)
Monoses Fructose	69
Galactose	30
Glucose	75
Manose	65
Rhamnose	10
Arabinose	10
Lyxazone	10
Disaccharides and trisaccharides Lactose	50
Maltose	15
Sucrose	78
Polysaccharides Celluloses	30
Inulin	50
Lignin	48
Free carbon	0

DL 0.1% = 1.44
DL 1% = 0.92
DL 5% = 0.72

The growth of the *Ph. infestans* fungus was influenced by the nitrogen resource present in the substrate. From **table 3** it can be observed a good development of the colonies on media which had nitrogen in their composition in the form of anorganic salts: ammonium nitrate, potassium nitrate. The nitrate in the organic substances was well metabolized from: L – asparagine, DL – asparagine, L – norvaline, DL – norvaline, cysteine and glycine. In the presence of the DL – alanine the growth of the fungus was stopped. The lack of the nitrate in the substrate stopped the growth of the colonies completely.

Table 3. The influence of the nitrate resource on the growth of the *Ph. infestans* (Mont) de Bary fungus colonies

Nitrate resource	Diameter of colonies (mm)
Organic substance Potassium nitrate	74
Sodium nitrate	70
Ammonium nitrate	74
Ammonia sulphate	50
Organic substances DL - alanine	0
L - norvaline	75
DL - norvaline	75
Cysteine	75
L - Asparagines	74
DL - asparagines	70
Glycine	69
Nitrogen free	0

DL 0.1% = 1.08
DL 1% = 0.80
DL 5% = 0.56

Results related to the influence of some cultivating environments on the growth of the *Ph. infestans* (Mont) de Bary fungus colonies

The growth of the colonies was influenced by the composition of the cultivating environments (**Chart 3**). The most favourable to the growth of the colonies was the potato medium – the glucose – agar: after three days the diameter of the colony was of 0.87 in, and after seven days it reached 2.20 in. On the other three media there were formed colonies with a good vegetative mass and characteristic fruit bodies.

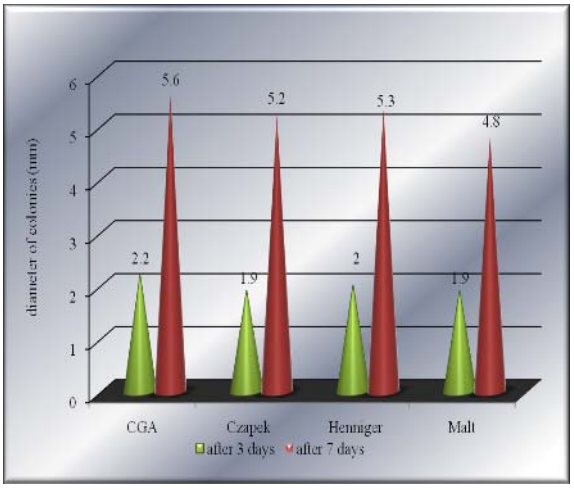

Figure 3. The influence of the culture medium on the growth of *Ph. infestans* (Mont) de Bary colonies

CONCLUSIONS

The biological parameters determined *in vitro* for the *Ph. infestans* fungus showed that the fungus needs mesophyll conditions

- the growth temperature of the colonies on cultivating environments is situated between large limits of 6 - 32°C with an optimum of 16 - 24°C;
- the relative air humidity influenced the growth of the colonies, the optimal needs being of 75 – 95%;
- the energetically and plastically resources are important to the growth of the fungus, being indispensable in the evolution of the colonies. The lack of the carbon resource stopped the growth of the colonies. The fungus metabolized the carbon in monoses (glucose, fructose, manose, galactose), disaccharides (sucrose, lactose) and polysaccharides (inulin, lignin, cellulose). The fungus metabolized the nitrate in the anorganic substances (potassium nitrate, ammonium nitrate, sodium nitrate) and organic substances (norvaline, asparagine and glycine).
- the favourite cultivating environments for the growth of the Ph. Infestans fungus are: CGA, Henninger, Czapek. On the malt medium there take place poorer growths.

REFERENCES

[1] Lacey, J., (1965). Infectivity of soils containing *Phytophthora infestans* spores. *Ann. Apl. Biol.* 56 (3), pp. 363-380.

[2] Perez, G., Medeum, C., (1965). Obtaining haploid potato plant of Solanum tuberosum varieties resistant to *Phytophthora infestans Amer. Potato Journal.* 42, pp. 296.

[3] Robert, G., Griffiths, Jane Dancer, Elisabeth, O'Neil, John, l., Harwood, (2003). Effect of culture conditions on the lipid composition of *Ph. infestans*. *New Phytologist Trust*.

[4] Still, B., (1965). Das Kaimver halten der Sporangien von Phytophthora infestans in Adhangigkeit von Temperatur und Hydratubedingungen, *Zeitshr, Pflnzenkrank*, 72 (4) Germania, pp. 193-200.