

RESEARCHES REGARDING THE *ALTERNARIA BRASSICAE* FUNGUS BIOLOGY

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Abstract: The *Alternaria brassicae* fungus manifests on every plant organ and in every stage of development of the plant. The abiotic factors have an important role during the fungus's development and pathogenicity. The study must be done in order to establish its needs regarding certain abiotic factors such as: temperature, humidity, atmospheric pressure and light. The study is necessary in order to get to know the moment when the first infections appear. By knowing these elements, a prognosis can be made, and also a warning for the recommended treatment, in order to stop the disease. The *Alternaria brassicae* fungus is passed on from one year to another, through the seed which comes from sick plants. In terms of laboratory conditions, on crop environment potato-glucose agar, phytopathogenic fungus *Alternaria brassicae* was isolated on potato-glucose agar on oilseed turnip. The sick biological material, which was formed from leaves, and stems, which had specific disease symptoms, was put in a wet room, where, after 3 days, the mycelium formation was observed. On CGA environment, the *Alternaria brassicae* fungus formed light-grey colonies, with silky aspect, and

dark-grey on the back. The optimum temperature which is necessary in order for the conidia to develop is between 28°C and 36°C degrees. The maximum value of temperature can be considered to be at 42°C. The formed colonies have a weak aspect, and fructifications weren't even formed anymore. The conidia's germination is possible at 4°C, 16 hours being necessary. The optimum temperature can be considered the 28-32°C temperature interval, when 79-83% of conidia germinated. The maximum temperature was discovered to be 40°C and lethal temperature was identified as being 42°C, when conidia did not germinate, not even when the Petri recipient was further set to an optimum temperature of 32°C. The *Alternaria brassicae* fungus' colonies developed extremely well in the presence of light. As the relative atmospheric humidity value grows higher, the colonies' development is very good, and the vegetative mass is extremely dense, thick, of grey colour. Temperature, relative atmospheric humidity and light are important factors in the *Alternaria brassicae* fungus evolution. dense, thick, of grey colour.

Key words: fungus, biology, conidia

INTRODUCTION

The *Alternaria brassicae* fungus produces the black stain of turnip leaves. The disease manifests on every plant organ and in every stage of development of the plant.

The abiotic factors have an important role during the fungus's development and pathogenicity. The fungus produces diseases on crop plants, these being very sensitive during their whole vegetation period.

For this reason, a certain biological study is needed. The study is about studying the *Alternaria brassicae* pathogen fungus's biology. The study must be done in order to establish its needs regarding certain abiotic factors such as: temperature, humidity, atmospheric pressure and light. The study is necessary in order to get to know the moment when the first infections appear. Also, the study wishes to look at the infections's evolution on the field, depending on the climatic weather conditions during the year.

By knowing these elements, a prognosis can be made, and also a warning for the recommended treatment, in order to stop the disease.

The *Alternaria brassicae* fungus is passed on from one year to another, through the seed which comes from sick plants. And it can also be passed on through the remains of plants, which are left over after harvesting.

The primary infections are made through conidions from the vegetal remains, which are left on the field, or through sick see. The plants' newly formed organs are infected the whole time during the whole year. They are conditioned by : weather, pH value and applied fertility. The fungus's nucleum invades the tissues. It migrates 3-10mm from the stain's border, going forward through the veins.

MATERIAL AND METHODS

In terms of laboratory conditions, on crop environment potato-glucose agar, phytopathogenic fungus *Alternaria brassicae* was isolated on potato-glucose agar on oilseed tunip. The sick biological material, which was formed from leaves, and stems, which had specific disease symptoms, was put in a wet room, where, after 3 days, the mycelium formation was observed. Put again on CGA environment, in Petri recipients, colonies were formed, which, after 6 days, purified the *Alternaria brassicae* species.

In laboratory conditions the abiotic factor were established. They influence the *Alternaria brassicae* fungus's development, after Tuite's method, 1968.

RESULTS AND DISCUSSION

The tempreature's influence upon the *Alternaria brassicae* fungus's colonies development:

On CGA environment, the *Alternaria brasicae* fungus formed light- grey colonies, with silky aspect, and dark-grey on the back. After 8 days, it formed pluriseptate vertical and horizontal conidions, of brown colour. The conidions had the following dimensions: 67,9 – 96,9 x 16,8-19,2µm. The *Alternaria brassicae* fungus was put again on CGA environment, Petri recipient,s with an 8 cm diameter, and then they were put (each of them) in termostates with temperatures 2 - 40°C. At a 3 days interval the constant colonies's diameter growth was registered. Also, the fructifications's growth was registered. The obervations lasted 15 days.

The colonies's growth and fructification of *Alternaria brassicae* fungus are influenced by the termic values. As we can see in table 1, the minimum temperature for the colonies' formation was of 2°C, them being presented under the form of a lax mycelium, of light-grey colour, with its back grey. Fructifications were absent. The aspect was maintained the same also on higher temperatures, such as 4°C and 6°C. The 8°C temperature determines a better colonies' development, so that the mycelium is compact, with silky aspect, of grey colour, with the back light-grey. The conidions's presence was registered. They are rare on the mycelium's surface. On 12°C and 14°C, colonies presented the saame characteristics, and on 16°C colonies formed a good vegetative mass.

The optimum temperature which is necessary in order for the conidions to develop is between 28°C and 36°C degrees, when 50mm colonies' diameter was registered, with silky aspect, dense, of grey colour, with the light-grey back side. Fructification was very good, and the colonies' number was high (table 2).

Over 36°C the colonies's development was weaker, and also the number of formed conidions was smaller.

The maximum value of temperature can be considered to be at 42°C. The formed colonies have a weak aspect, and fructifications weren't even formed anymore.

The temperature's influence upon the *Alternaria brassicae* fungus's conidions germination: In order to analyse the temperature's influence upon the conidions's germination, in Petri recipients, with water-agar environment, the fungus's conidions were arranjed, and

then the following thing was made: their placement in thermostates at temperatures between 2°C and 44°C, and kept like that 24 hours. At a 2 hours interval, the germinations was examined, at 100 conditions, for each variant. From the first figure we can see that the conditions's germination is possible at 4°C, 16 hours being necessary – they represent the minimum threshold (value).

The optimum temperature can be considered the de 28-32°C temperature interval, when 79-83% of conditions germinated.

The maximum temperature was discovered to be 40°C and letal temperature was identified as being 42°C, when conditions did not germinate, not even when the Petri recipient was further set to an optimum temperature of 32°C.

The atmospherical relative humidity's influence upon the *Alternaria brassicae* fungus' colonies' development: In exicators, different humidity values were created, from 15% up to 100%, using superconcentrated solutions of some salts. The Petri recipient with CGA environment, in which the fungus was put again, were introduced in exicators, and kept there for 21 days, without the Petri's top. The atmospherical relative humidity represents an important factor in the fungus's evolution. From table 2 we can see that at values of 15%, the colonies were not formed. At atmospheric humidity of over 36,8% the formed mycelium was lax and conditions did not form. At values of 66 -72% formed colonies had a thick-dark aspect, of grey colour, and fructifications did not form. From values of over 75,6% the formation of developed conditions was noted, on the colonies' surface. As the relative atmospheric humidity value grows higher, the colonies' development is very good, and the vegetative mass is extremely dense, think, of grey colour. And fructifications are abundant.

The light's influence upon the *Alternaria brassicae* fungus's development: Light acted differently on the *Alternaria brassicae* fungus colonies's development, as a result of the crop's constant light exposure, continuous darkness and also light/darkness alternation 8/16 or 12/12. The final observation were made after 15 days, when the fungus's growth and fructification was noted.

The *Alternaria brassicae* fungus' colonies developed extremely well in the presence of light, as it can be observed in table 3. On permanent or alternative light, the colonies' vegetative mass was rich, it had a silky mycelium, of grey colour, and sporulation was abundant. Permanent darkness, throughout the whole experiment duration led to a very weak vegetative mass, and conditions very rarely appeared on the mycelium's surface.

CONCLUSIONS

Temperature, relative atmospheric humidity and light are important factors in the *Alternaria brassicae* fungus evolution.

Concerning temperature, the minimum temperature for the colonies to be formed was of 2°C, optimum necessary for the colonies to develop is 28°C and 36°C, and the maximum threshold (value) can be considered to be at 42°C, when colonies formed have a weak-looking aspect, and fructifications do not form anymore. The optimum temperature for the conditions to germinate is between 28-32°C, when 79-83% of conditions germinated, while the letal temperature was identified as being of 42°C.

Related to humidity, it was noted the fact that on relative values of 15% colonies did not form, and as the relative atmospheric humidity values grow, (superior to 75,6% value) the colonies' development is very good, and the vegetative mass is dense, thick-looking, and fructifications are abundant.

On permanent and alternative light, the vegetative mass of formed colonies was rich, the mycelium was silky, and sporulation was abundant.

BIBLIOGRAPHY

1. CONSTANTINESCU O., 1974 – Metode și tehnici în micologie. Editura Ceres, București
2. GEORGHIEȘ C., STELICA CRISTEA, 2001 – Fitopatologie-vol. Editura Ceres, București
3. STELICA CRISTEA, C.R. ZA, MALI-SANDALĂ MANOLE, FLORICA-FULVIA VLAD, 2005 – Fitopatologie generală-lucrări practice, AMD-USAMV București

Table 1

Temperature's influence upon the *Alternaria brassicae* fungus's development

t°C/days	Colonies diameter(mm)							Observations after 14 days	
	2	4	6	8	10	12	14		
2	0	0	0	0	2	4	10	Mv±	Fr 0
4	0	0	1	8	11	13	14	Mv +	Fr 0
6	0	0	1	8	11	13	14	Mv±	Fr 0
8	0	0	2	8	12	16	15	Mv+	Fr ±
10	0	1	4	13	15	27	24	Mv +	Fr +
12	0	4	13	17	31	33	30	Mv +	Fr+
14	2	7	12	20	30	30	33	Mv ++	Fr +
16	2	9	12	20	24	32	38	Mv ++	Fr +
18	2	9	14	20	25	36	38	Mv ++	Fr +
20	2	10	15	23	27	37	40	Mv ++	Fr +
22	2	12	17	27	30	37	40	Mv++	Fr +
24	2	12	18	27	30	38	40	Mv ++	Fr +
26	2	14	22	28	35	38	40	Mv ++	Fr +
28	3	17	30	40	70	70	70	Mv+++	Fr +++
30	3	19	35	45	70	70	70	Mv +++	Fr +++
32	3	22	38	45	70	70	70	Mv +++	Fr +++
34	3	25	40	45	70	70	70	Mv +++	Fr +++
36	2	30	40	45	56	59	62	Mv +++	Fr +++
38	2	20	30	30	38	40	40	Mv ++	Fr +
40	0	10	15	16	18	18	18	Mv +	Fr +
42	0	0	0	0	0	0	0	Stops growth	

% germination

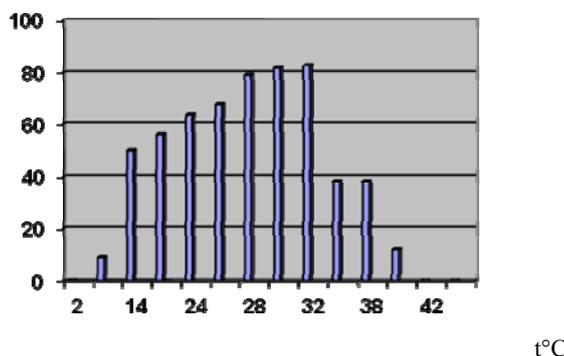


Fig. 1- *Alternaria brassicae* conidions' germination depending on temperature

Table 2

The relative atmospheric humidity's influence upon the *Alternaria brassicae* fungus colonies' development

Relative atmospherical humidity RH%	Colonies' diameter after 12 days	Observations
15	0	Colonies are not formed
36,8	20	Weak growth
43	32	Mv± Fr.0
56	37	Mv± Fr.0
66	70	Mv ++: Fr 0
72	70	Mv ++: Fr 0
75,6	70	Mv ++: Fr +
78.6	70	Mv+++ Fr++
82,9	70	Mv+++ Fr+++
88.5	70	Mv+++ Fr+++
90	70	Mv+++ Fr+++
92,7	70	Mv+++ Fr+++
96.1	70	Mv+++ Fr+++
98,5	70	Mv+++ Fr+++
99	70	Mv+++ Fr+++

Legend:

- mv± = very weak vegetative mass
- mv.+ = weak vegetative mass
- mv ++ = good vegetative mass
- mv +++ = very good vegetative mass
- 0= the fungus did not fructify
- Fr ± =very weak fructification
- Fr + = weak fructification
- Fr.++ = good fructification
- Fr+++ = abundant fructification

Table 3

Light's influence upon ciupericii *Alternaria barsicae* fungus's development

Light	Colonies' development
Light 24 hours	Rich vegetative mass, thick-looking mycelium, of grey colour, rich sporulation
Light/Darkness alternation 12/12 hours	Rich vegetative mass, thick-looking mycelium, of grey colour, rich sporulation
Light/Darkness alternation 8/16 hours	Rich vegetative mass, thick-looking mycelium, of grey colour, rich sporulation
Permanent darkness	Very weak vegetative mass, weak fructifications