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RESEARCH ON THE BIOLOGICAL FEATURES OF *ALTERNARIA BRASSICAE* PATHOGEN ISOLATED ON RAPE

E. RADU, STELICA CRISTEA, C.R. ZALĂ

University of Agronomic Sciences and Veterinary Medicine of Bucharest

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Abstract

Alternaria brassicae fungus is manifesting on every plant organ in every stage of plant development.

The abiotic factors play an important role during the fungus 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 occur.

Alternaria brassicae fungus is passed on from one year to another through the seed which comes from sick plants. Under laboratory condition, the phytopathogenic fungus Alternaria brassicae was isolated on potato-glucose agar. The sick biological material of rape, formed from leaves and stems with specific disease symptoms, was put in a wet room where the mycelium formation was observed after 3 days. The conidions germination is possible at 4°C, and 16 hours are necessary.

Alternaria brassicae fungus colonies developed extremely well in the presence of light. As relative atmospheric humidity value grows higher, the colony development is very good, and the vegetative mass is extremely dense, thick, grey in colour. Temperature, relative atmospheric humidity and light are important factors in the Alternaria brassicae fungus evolution.

INTRODUCTION

Alternaria brassicae fungus produces the black stain of rape leaves. The disease is manifesting on every plant organ in every stage of plant development.

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

Therefore, a certain biological study is necessary. The study concerns the research of the *Alternaria brasicae* pathogen fungus 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 know the moment when the first infections occur. Also, the study is aimed at analysing the evolution of the infection in the field, depending on the climatic conditions during the year.

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

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 the plants left over after harvesting.

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

MATERIAL AND METHODS

In laboratory conditions, phytopathogenic fungus *Alternaria brassicae* was isolated on potato-glucose agar. The sick biological material of rape, which was formed from leaves and stems with specific disease symptoms was put in a wet room, where, the mycelium formation was observed after 3 days. Placed on CGA culture medium again, in Petri recipients, colonies were formed; after 6 days, they purified the *Alternaria brassicae* species.

The abiotic factor was established in laboratory conditions. They influenced the *Alternaria brassicae* fungus development, after Tuite's method, 1968.

RESULTS AND DISCUSSION

The influence of temperature upon *Alternaria brassicae* fungus colonies development. On CGA culture medium, the *Alternaria brasicae* fungus formed light-grey colonies, with silky aspect, and dark-grey on the back. After 8 days, it formed pluri-septate vertical and horizontal conidions, of brown colour. The conidions had the following dimensions: 67.9-96.9 x 16.8-19.2 μm. *Alternaria brassicae* fungus was placed on CGA culture medium again, in Petri recipients of 8 cm in diameter, and then each of them was placed in thermostates at temperatures of 2-40°C. At a 3 days interval, the constant colonies diameter growth was registered. Also, fructifications growth was registered. The observations lasted for 15 days.

The colonies growth and fructification of *Alternaria brassicae* fungus are influenced by the thermal values. As we can see (Table 1), the minimum temperature for the colony formation was 2°C; they occurred under the form of a lax mycelium, of light-grey colour, with a grey back. Fructifications were absent. The aspect was the same at higher temperatures as well, such as 4°C and 6°C. The 8°C temperature determined a better colony development, so that the mycelium was compact, with silky aspect, of grey colour, with alight-grey back. The conidions presence was registered. They were rare on the mycelium's surface. At

12°C and 14°C, colonies presented the same characteristics, and at 16°C colonies formed a good vegetative mass.

Table 1

Influence of temperature upon *Alternaria brassicae* fungus development

t°C/days	2	4	6	8	10	12	14	Observations after 14 days	
	Colonies diameter (mm)								
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	

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

Over 36°C, colony 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 were not even formed any longer.

The influence of temperature upon the *Alternaria brassicae* fungus conidions germination (Figure 1). In order to analyse the influence of temperature upon the conidions germination, in Petri recipients and water-agar environment, the fungus conidions were arranged; they were placed in thermostates at temperatures between 2°C and 44°C, and kept for 24 hours in these conditions. At a 2 hours interval, germination was examined in 100 conidions for each variant. The first figure

shows that conidions germination is possible at 4°C, as 16 hours are necessary the minimum threshold (value).

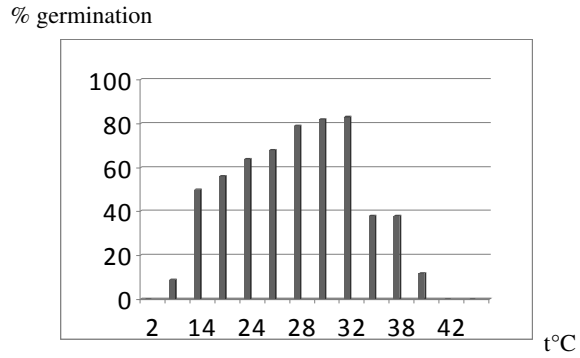

Fig. 1. *Alternaria brassicae* conidions germination depending on temperature

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

The maximum temperature was 40°C and letal temperature was identified at 42°C when conidions did not germinate even when the Petri recipient was further set to an optimum temperature of 32°C.

The influence of atmospheric relative humidity upon the *Alternaria brassicae* fungus colony development (Table 2). Different humidity values were created in exicators, from 15% up to 100%, using superconcentrated solutions of some salts. The Petri recipient with CGA environment, in which the fungus was placed again, was introduced in exicators, and kept for 21 days, without a Petri top. The atmospheric relative humidity represents an important factor in the fungus evolution. Table 2 shows that, at values of 15%, the colonies were not formed. At an atmospheric humidity of over 36.8% the formed mycelium was lax and conidions did not form. At values of 66-72%, the 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 conidions was noted on the colony surface. As the relative atmospheric humidity grows higher, the colony development is very good, and the vegetative mass is extremely dense, think, grey in colour. Also, fructifications are abundant.

The influence of light upon the *Alternaria brasicae* fungus development (Table 3). Light recorded a different action on the *Alternaria brassicae* fungus colony 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 observations were made after 15 days, when fungus growth and fructification was noted.

Table 2

Influence of relative atmospheric humidity upon *Alternaria brassicae* fungus colony development

Relative atmospheric humidity (RH%)	Colony 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 = fungus did not fructify
Fr ± =very weak fructification
Fr + = weak fructification
Fr.++ = good fructification
Fr+++ = abundent fructification

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

Table 3

Influence of light upon *Alternaria barsicae* fungus development

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

CONCLUSIONS

1. Temperature, relative atmospheric humidity and light are important factors in the *Alternaria brassicae* fungus evolution.
2. Concerning temperature, the minimum temperature for the colonies to be formed was 2°C, optimum necessary for the colonies to develop is 28°C and 36°C, and the maximum threshold (value) can be considered at 42°C when colonies formed have a weak-looking aspect, and fructifications do not form any longer. The optimum temperature for the conidions to germinate is between 28-32°C, when 79-83% of conidions germinated, while the lethal temperature was identified at 42°C.
3. Related to humidity, it was noted that on relative values of 15% colonies did not form, and as the relative atmospheric humidity values grow (superior to 75.6% value), colony development is very good, the vegetative mass is dense, thick-looking, and fructifications are abundant.
4. On permanent and alternative light, the vegetative mass of formed colonies was rich, the mycelium was silky, and sporulation was abundant.

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