GROWTH OF *MACROPHOMINA PHASEOLINA* ISOLATES DEPEND ON DIFFERENT TEMPERATURE

Csöndes Izabella, Kadlicskó Sándor, Gáborjányi Richard *

* Plant Protection Institute, Pannon University, Georgikon Faculty of Agriculture, Keszthely

Abstract

The charcoal root disease caused by Macrophomina phaseolina (Tassi) Goidanich may cause considerable damages in a hot as well as in dry seasons. The effect of temperature was investigated on the growing patterns of 35 Macrophomina phaseolina isolates, collected from different districts of Hungary. The fungal cultures in 90 mm Petri dishes held on 10, 15, 20, 25, 30, 35 and 40 °C were measured in four replications. For the isolates the most favourite temperature regime was 25 to 35 °C. At these temperatures mycelia and microsclerotial diameter of all isolate colonies reached 90 mm on the 5th day. Mycelia growth of this pathogen was very low at 10, 15 and 40 °C, and they did not form microsclerotia. The cultures were well grown at 20 °C, their colony size on the 3rd day were 14 times larger than at 10 and 15 °C. Even the extreme temperatures (10 and 40 °C) were not lethal, cultures started to grow when were kept to 25 °C.

Key words: Macrophomina phaseolina, temperature, fungal cultures, mycelia

INTRODUCTION

The economic importance of charcoal rot disease caused by *Macrophomina phaseolina* (Tassi) Goidanich [*Rhizoctonia bataticola* (Taubenhaus) E.J. Butler] is still considerable. This polyphagous pathogen infects more than 300 plant species. In Hungary it causes serious damage – especially in dry hot seasons – on sunflower, maize, legumes, paprika and many other plants (Békési, 1970, Varga et al., 1997). The damage influenced mainly by the season, location, water and nutrient supply. The disease can be diagnosed on the basis of the symptoms: ash grey spots on the stems and small, black microsclerotia developed in the pith and root tissues. The effect of temperature on the growth of *Macrophomina* has been investigated earlier by Das (1988), who found that the optimal temperature of for mycelia growth and microsclerotia development was 30 °C in India.

The aim of our study was to investigate the effect of temperature on growing pattern of 35 *Macrophomina phaseolina* isolates from different Hungarian locations.

MATERIAL AND METHOD

The experiments have been made under the same conditions for all *Macrophomina phaseolina* isolates, except the different temperatures, where

the growing pattern of the fungal colonies were measured. Initial test-material was collected from 32 different sunflower fields in September, 2005. From one site (Cserkeszőlő), two samples we collected, one from cultivated sunflower and the other one from a volunteer sunflower. From three sites (Bóly, Iregszemcse, Keszthely) samples were collected from soybeans, too. Scrapings from the infected plant debris were taken to potato dextrose agar (PDA) medium, at 25 °C. Pure cultures were made by threefold passage. PDA medium were poured into 9 cm diameter sterilized Petri dishes. Five mm diameter agar discs with over-wintering propagules were taken from the well growing cultures kept on 25 °C and transferred to agar media. Following the inoculation Petri dishes were taken in darkness into thermostats adjusted to 10, 15, 20, 25, 30, 35 and 40 °C. The effect of temperature on growing patterns of *Macrophomina* was tested in four replications. Colony diameters were measured after 3, 5 and 6 days, respectively following the inoculations. Statistical analysis was made by Microsoft Excel program. Because of the large extent of the samples this work shows only representative results.

RESULTS

It is typical for this pathogen, that first the mycelia start to growth and then follows the formation of microsclerotia. The heat demand and heat tolerance of isolates showed a wide range of differences.

At 10 °C no mycelia growth was observed on the 3rd day, therefore no data are present in the tables. Even on the 5th day some of the isolates showed a small mycelia growth. The spread of this pathogen at 10 °C was very slow, the highest daily growth rate was only 0,71 mm/day in the case of the soybean isolate (Iregszemcse).

At the 3rd day at 15 °C only two isolates started to grow, one from sunflower (Tordas) and the other one from soybean (Iregszemcse). In the average of four replication the growing rate of Tordas isolate was only 1,0 mm/day, and that of Iregszemcse was 4 mm (value of SD 5% was 0,33). The 5th day slow growing rates were observed in isolates of nine isolates (Bize, Dunaföldvár, Hódmezővásárhely, Kaposvár-Toponár, Keszthely, Lepsény, Nyíregyháza, Szederkény and Székkutas) from sunflower, and one (Keszthely) from soybean. On the 6th day seven other isolates started their mycelia growth. At 15 °C the Iregszemcse isolate showed the highest average daily mycelia growth rate (2,04 mm).

Mycelial growth was very low at 10 and 15 °C, without microsclerotia formation.

On the 3rd day at 20 °C the Kecskemét isolate had the highest growing rate (38,75 mm in diameter). Most of the isolates showed a full (90 mm

diameter) mycelium size 5th day after inoculation. It means a growth rate of 15 mm/day. First microsclerotia formation was observed at the 5th day at 20 °C. It is typical, that even at the 6th day the microsclerotia did not reach the side of the Petri dishes. The sunflower isolate from Lepsény showed the largest diameter (85,25 mm), with a daily growth rate of 14,21 mm.

On the 3rd day Szigetvár sunflower isolate showed the largest mycelia colony diameter (85,25 mm) at 25 °C, while the largest microsclerotial diameter (65,25 mm) was observed by the sunflower isolate of Sármellék.

Mycelia colony diameter of the isolates of Balatonújlak, Bóly, Kecskemét and Lepsény from sunflower at 30 °C reached the maximal diameter on the 3rd day. The microsclerotial diameter of the isolate from Balatonújlak showed just one mm smaller value. The Iregszemcse isolate from soybean showed the slowest growth both at 20 and 25 °C.

The 3rd day at 35 °C colonies of thirteen isolates from sunflower reached the side of the Petri dishes. At this temperature, as well as at 30 °C, the Tordas isolate showed the slowest growing rate (70,25 mm diameter).

At 25, 30 and 35 °C mycelia colony diameters have reached the sides of Petri dishes (90 mm) in the 5th and 6th days, except the soybean isolate of Iregszemcse. This value on the 5th day at 25 °C was 45,75 mm, at 30 °C was 47,25 mm, and at 35 °C was 41,75 mm. On the 6th day at 25 °C the diameter of the colonies was 54,25 mm, at 30 °C was 56,50 mm, and at 35 °C was 46,0 mm, respectively, that means, that this isolate significantly differed from the other isolates.

At 25, 30 and 35 °C the values of microsclerotial diameters all isolates still reached a value of 90 mm at the 5th and 6th days, except the soybean isolates of Iregszemcse. This value on the 5th day at 25 °C was 31,25 mm; at 30 °C was 33,50 mm; at 35 °C was 32,0 mm. On the 6th day at 25 °C was 42,00 mm; at 30 °C was 47,25 mm; at 35 °C was 35,0 mm. This isolate significantly differed from the other ones.

Ten isolates showed no mycelia growth at 40 °C on the 3rd day. Isolates of Röjtökmuzsaj and Tordas from sunflower and the isolate of Iregszemcse from soybean did not start growth neither on the 5th nor on 6th day. A wide growth interval could be observed on the 6th day. The largest diameter had the isolate of Kéthely (68,75 mm), and lowest daily growth rate was observed by the isolate of Lepsény (0,71 mm/day).

It is interesting to note, that while the isolate of Iregszemcse from soybean had the best growing at 10 and 15 °C, it could not grow larger as 60 mm at 20, 25, 30 and 35 °C, at a temperature range which all other isolates reached 90 mm mycelia diameter. Also its microsclerotial diameter was about 50 % of the others even on the 6th day.

The Figure 1 shows the average colony diameters of 35 isolates measured at different temperatures, on the 3rd, 5th and 6th day.

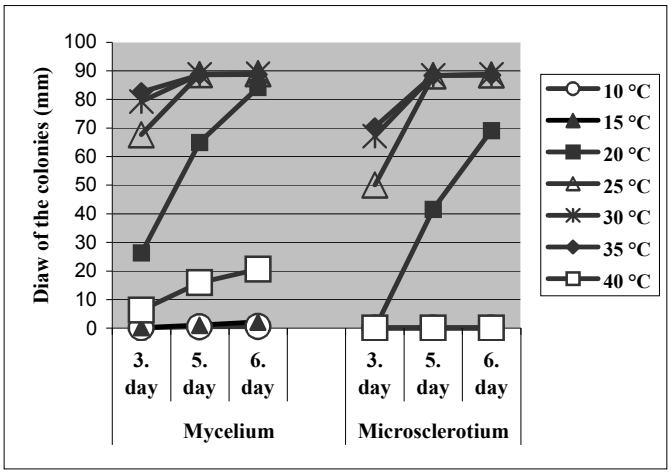

Figure 1. Mycelial (A) and microsclerotial (B) colony diameters depending on the temperature and incubation time

The most favourable temperature regime for the development of the isolates were between 25 to 35 °C. At 20 °C the isolates were growing relatively well. However the low temperatures as 10 and the high 40 °C was not lethal for the isolates: giving back them to 25 °C, they started to grow.

DISCUSSION

Effect of temperature on the growth of 35 *Macrophomina* isolates was studied. Their growth at 10 and 15 °C was very slow, the average daily growing rate was only 0,71 and 2,04 mm/day, respectively. *Macrophomina phaseolina* did not developed microsclerotia at the too low and at too high temperatures. However, at 20 °C on the 5th day they did. The most favourable temperature regime was between 25 and 35 °C for all the isolates. On the 5th day at favourable temperatures all the isolates grown well. Microsclerotial colonies reached the 90 mm diameter, except for one isolate.

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