THE INFLUENCE OF TEMPERATURE ON INFECTION OF PHYTOPATOGENIC FUNGUS *BOTRYOTINIA FUCKELIANA* (DE BARY) WHETZEL

VLIV TEPLOTY NA INFEKČNOST FYTOPATOGENNÍ HOUBY *BOTRYOTINIA FUCKELIANA* (DE BARY) WHETZEL

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ABSTRACT

B. fuckeliana (de Bary ex de Bary) Whetzel is plant parasite, which reproduction is with conidia, sclerotia, but it exists as microconidia and mycelial characters too. Detrimental factors of this patogen (conidies production and germination) are optimal temperature and high relative huminidity (RH).

Optimal temperature for growing and sporulation is from 20 to 22°C. 95-98% RH is necessary for germination too. At this temperature patogen make the best sporulation and the biggest infection. The high summer temperature of summer months last years enabled the incidence of new warm climate diseases, pests and weeds which has not been found during last years in the Czech republic.

Our aim was to study the temperature response of fungi and their ability to adapt to changing temperature. *B. fuckeliana* (de Bary) Whetzel was used as a model organism. During our *in vitro* experiment was watching differences among single strains. Great differences show for possibility of very fast selection in population and organism responce to adapt for changing temperatures.

Keywords: moulds, temperature adaptation

ABSTRAKT

Botryotinia fuckeliana (de Bary ex de Bary) Whetzel je rostlinný parazit, rozmnožující se především konidiemi, sklerociemi, ale tvoří také mikrokonidie a infekční mycelium. Limitujícími faktory pro šíření a infekci rostlin (tj. tvorba a klíčení spór) jsou optimální teplota a vysoká relativní vzdušná vlhkost.

Teplotní optimum pro růst a sporulaci patogena je v rozmezí od 20-22°C. Podmínkou je také 95-98% vzdušná vlhkost, která napomáhá klíčení spór. Při této teplotě houba nejlépe fruktifikuje a dochází k nejintenzivnější infekci. V posledních letech vlivem zvyšujících se

teplot zejména během letních měsíců dochází k novému výskytu patogenních chorob, škůdců a plevelů, které se na území České republiky předtím nevyskytovaly.

Cílem studia je sledování schopnosti teplotní adaptace fytopatogenních hub při různých teplotách. Jako modelový organismus byla použita fytopatogenní houba *B. fuckeliana*. Během pokusu *in vitro* byly sledovány rozdíly mezi jednotlivými kmeny. Velké rozdíly poukazují na možnost velmi rychlé selekce v rámci populace a na schopnost organizmu přizpůsobit se změně teplot.

Klíčová slova: plísně, teplotní adaptace

INTRODUCTION

Botrytis cinerea Pers. ex Fries represents the highly variable conidial form of a series of distrincts *Botrytotinia* species related to *B. fuckeliana* (de Bary) Whetzel (syn. *Sclerotinia fuckeliana* (de Bary) Fuckel).

B. fuckeliana is found on various plant scraps and on live plants, were makes deseases or plant rots. It raises tomatoes rots and rot of vine (*Vitis vinifera*). We can find it on strawberry fruits but also on stock vegetables (cucumbers, celeriacs, onions).

The variability was described by Hansen to the existence of mycelial and a conidial basic type and used as an example of the „ dual phenomenon“. Greater possibilities for variation exist in respect of conidium size, sclerotium formation, mycelial characters and formation of microconidia. The high genetic variabilty is prescribed to the presence up to 20 different nuclei in mycelial cells. Under the situation of the exogenous selection effect, e.g. selection pressure of fungicide, the nucleus containing the gen of resistance to the fungicide takes a control in the cell.

The high summer temperature of summer months last years enabled the incidence of new warm climate diseases, pests and weeds which has not been found here at all during previous time. On the contrary low precipitation decreased the incidence and losts caused by harmfull organismus prefering high humidity especially Oomycota, e.g. *Phytophthora infestans*.

Aim was to study the temperature response of fungi and their ability to adapt to changing temperature. *B. fuckeliana* (de Bary) Whetzel was used as a model organism. During our *in vitro* experiment was watching differences among single strains. Great differences show for possibility of very fast selection in population and organism responce to adapt for changing temperatures.

The aim of our *in vitro* trials was to compare:

- the growth of several isolates at the same temperatures,
- the responce of individual isolates on different temperatures,
- to reveal, if there is any adaptation to low or to high temperatures after prolonged cultivation on corresponding temperatures.

MATERIAL AND METHODS

B. fuckeliana was isolated from red cabbage *(Brassica oleracea* L. var.*capitata* L.*)*, carrot *(Daucus carota* L.*)* and from grapes *(Vitis vinifera)*. Single strains from red cabbage and carrot heve their origin in the Czech republic, grapes were from Italy. Isolates were indicated by No.:

- 2, 3, 5 isolated from red cabbage,
- 7 isolated from carrot,
- 10 isolated from grapes.

Strains cultivation was on dextrose agar at 5, 8, 22, and 27°C. Petri´s plates were inoculated by transfering the piece mycelia in the midle. Radial growth of the colony was measured (diameter of the colony). Mycelial growth was measured during a days (first observation) and after covering whole plates, the pieces of mycelium from the first trial were transfered to next Petri´s plates (second observation). Diameter of the Petri´s plate was 8 cm. The second observation was only at 5, 8 and 22°C.

RESULTS

Growth of isolates can be compared as:

- colony diameter after x days,
- the number of days needed to reach certain diameter of colony.

We will compare the number of days. Because the differences between isolates are increasing with the time, the comparison should begin from the day, when the first isolates reaches the edge of the Petri´s plate. In this case 8 cm diameter was taken as reference.

From the first observation we see that at 5°C isolates No.3 and 7 reached the end of the plate 19 days after inoculation, the others, No.2, 5 and 10 were slowest. They covered whole plate after 23 days. Between the slowest and the fastest isolates was difference 4 days (Fig. 1). At 8°C the fastest were isolates No. 2, 3, 5 and 7. Covering time was 15 days. The last one, No. 10 was the slowest, time for covering was 19 days. Difference between them was 4 days too (Fig. 2). At 22°C first covering was with isolates No. 3 and 7. Time was 8 days. Isolates No. 2, 5 and 10 finished growing after 11 days. The difference between the slowest and the fastest is 3 days (Fig. 3). And at 27°C the fastest one was isolate No. 10 with covering 15 days after inoculation. Isolates 3, 5 and 7 growed 19 days and Isolate No. 2 23 days. The difference between No. 10 and No. 2 is 7 days (Fig. 4).

In second observation we can see that at 5°C the first one was isolate No. 3. Covering time was 13 days. Slower growing had isolates No. 7 and 10. Their time was 15 days. The last one in growing were isolates No. 2 and 5. They covered whole plate after 17 days. The difference between the fastest and the slowest was 4 days (Fig. 5). At 8°C the diameter of the colony was reached as first with isolates No. 3 and 7 with covering plate after 10 days. Isolate No. 10 was slower. Covering time was 15 days. The slowest one was isolates No. 2 and 5 with time 17

days. The difference between No. 3, 7 and 2, 5 was 7 days (Fig. 6). In the end at 22°C the fastest was isolate No. 3. Growing time was 6 days. Isolate No. 7 covered whole Petri´s plate after 8 days and the last isolates with No. 2, 5 and 10 covered plate after 10 days. The difference between No. 3 and No. 2, 5 and 10 was 4 days (Fig. 7).

DISCUSSION

Despite of the low number of isolates in the trial, substancial differences between them were found. Isolates do not respond to changing temperatures consistently. No one of the isolates has its growth shifted to low or to high temperatures. From both observations isolates No.3 and No. 7 are fast groving at the temperatures. On the other hand isolates No. 2 and No 5 was slow groving. Very interesting is isolate No. 10. During the first observation belongs to the slow growing (except 27°C). In second observation is in the middle. This means, that thanks to origin in Italy, this strain has temperature optimum higher than others strains. We can see, that middle position means ability to adapt changing temperatures. Thanks to existence of differences between single strains occures to fast selection and to responce to adapt for changing temperatures too. The difference in the growth rate of isolates was higher than the difference
in their responce to different temperatures.

Optimal temperature was 22°C. By the temperature 27°C was the growth of the fungus strongly inhigited (except isolate No. 10). The results are surprising, inhibition was supposed by the temperatures above 30°C.

Great differences between single strains in field conditions showes for occurrence as trials, which growth is in lower temperatures as trials, which prefere warmer climate. So that, during changing temperatures will dominate either one or second.

With regard to get warmer climate is very important to find out if temperature adaptation exists.

The wether conditions of last years were very different from the average of previous 30 years. Temperature of summer months was often on the level of thirty years average. We can see it from the Tab. I, where are compared the average monthly temperatures at Žatec region (the warmest place of Czech republic) from 1999 to 2003 with a) the 30 year average (1961 – 1990) of Žatec and b) with the data of stations from southern European regions.

Tab. 1: Comparison of average temperatures

Station – average 1961-1990	Elevation (m)	Latitude (°, ´)	March (°C)	April (°C)	May (°C)	June (°C)	July (°C)	August (°C)
Bělehrad	132	44°44´	7, 1	12, 2	17, 3	20, 1	21, 6	21, 2
Zagreb	156	45°44´	7, 1	11, 7	16, 0	19, 3	21, 2	20, 4
Pécz	202	46°00´	5, 6	10, 7	15, 6	18, 7	20, 5	20, 1
Ljubljana	298	46°04´	5, 6	9, 6	14, 4	17, 5	19, 8	19, 3
Žatec	250	50°20´	3, 6	8, 5	13, 4	16, 7	18, 0	17, 4
Žatec 1999	250	50°20´	5, 4	9, 5	15, 0	16, 7	20, 7	17, 1
Žatec 2000	250	50°20´	5, 4	11, 1	15, 5	18, 3	17, 1	19, 3
Žatec 2001	250	50°20´	4, 3	8, 8	16, 3	16, 6	20, 2	20, 7
Žatec 2002	250	50°20´	6, 1	9, 6	16, 9	19, 6	20, 6	21, 2
Žatec 2003	250	50°20´	6, 3	9, 3	16, 6	21, 6	20, 2	22, 2

The data presented that at Žatec region the average monthly temperatures during most of the summer months were remarkably higher than the 30 – year average.

In practice it means, that growth of the fungus and plant infection at high temperatures is not prevented by low moisture connected with high temperature under our conditions as supposed up to know, by the temperature itself.

If hot summer months will be continue is very probable, that *B. fuckeliana* should have tendentions to step back.

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APPENDIX

First observation

Figure 1: Mycelial growth of single isolates at temperature 5 °C

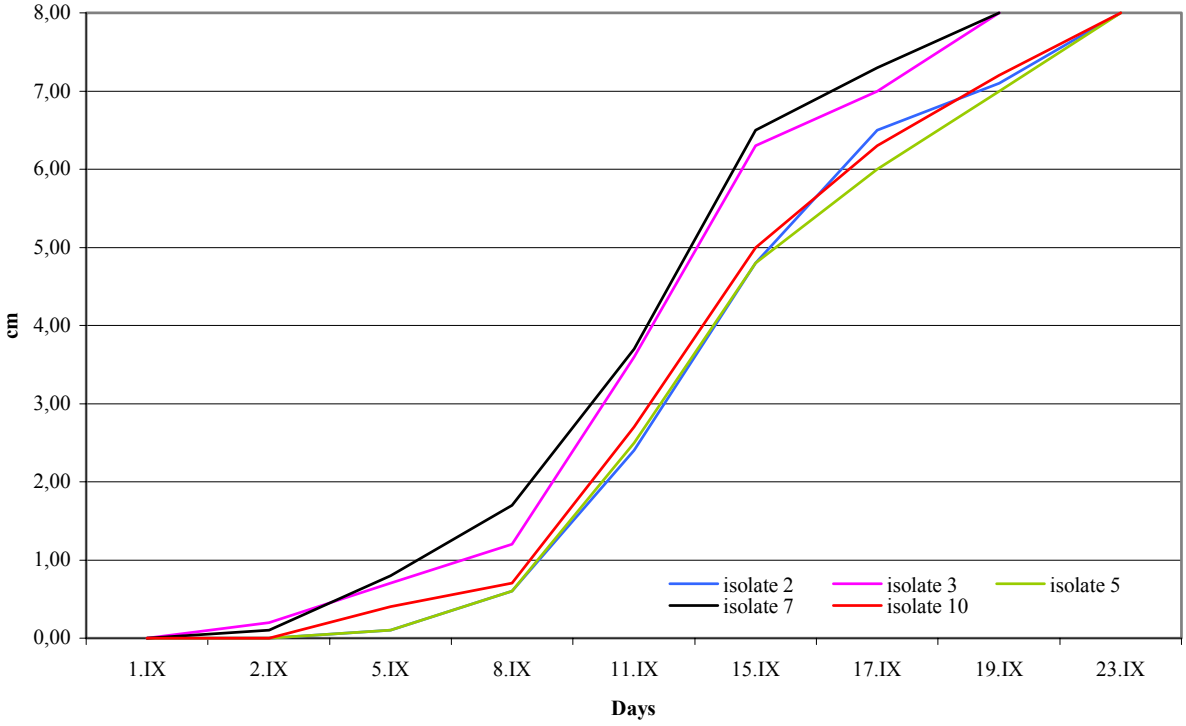


Figure 2: Mycelial growth of single isolates at temperature 8 °C

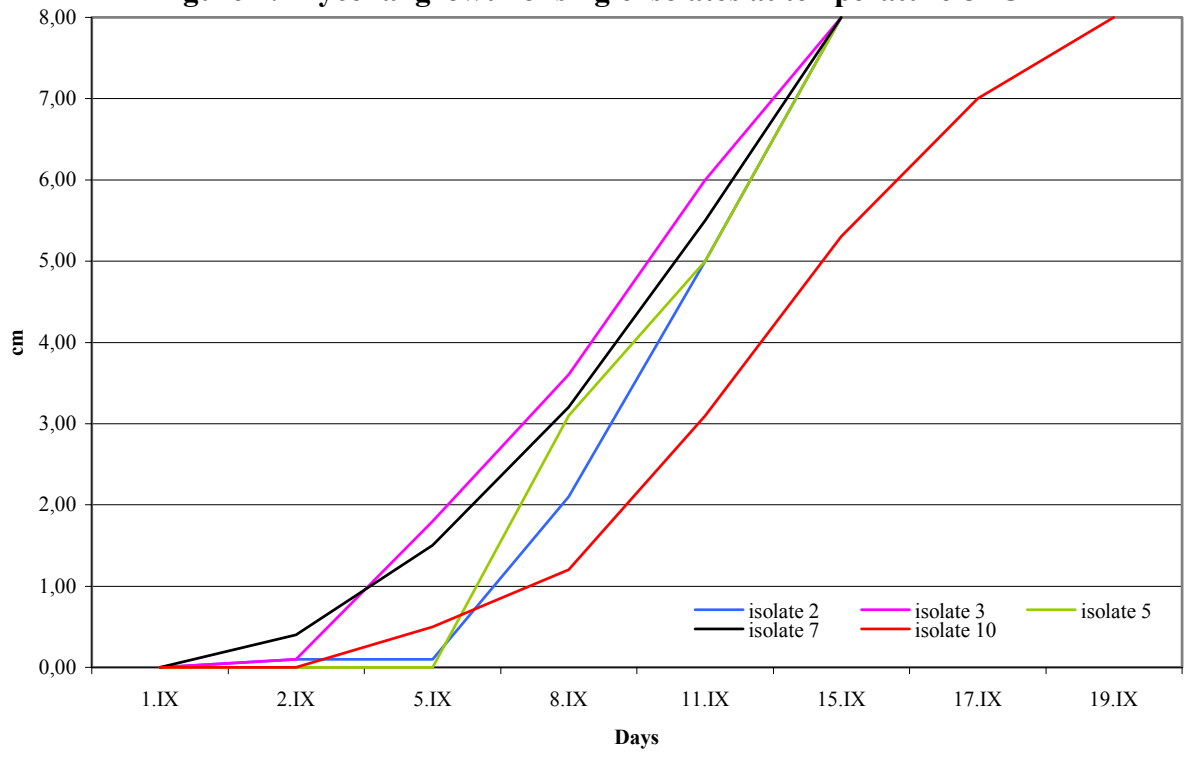


Figure 3: Mycelial growth of single isolates at temperature 22 °C

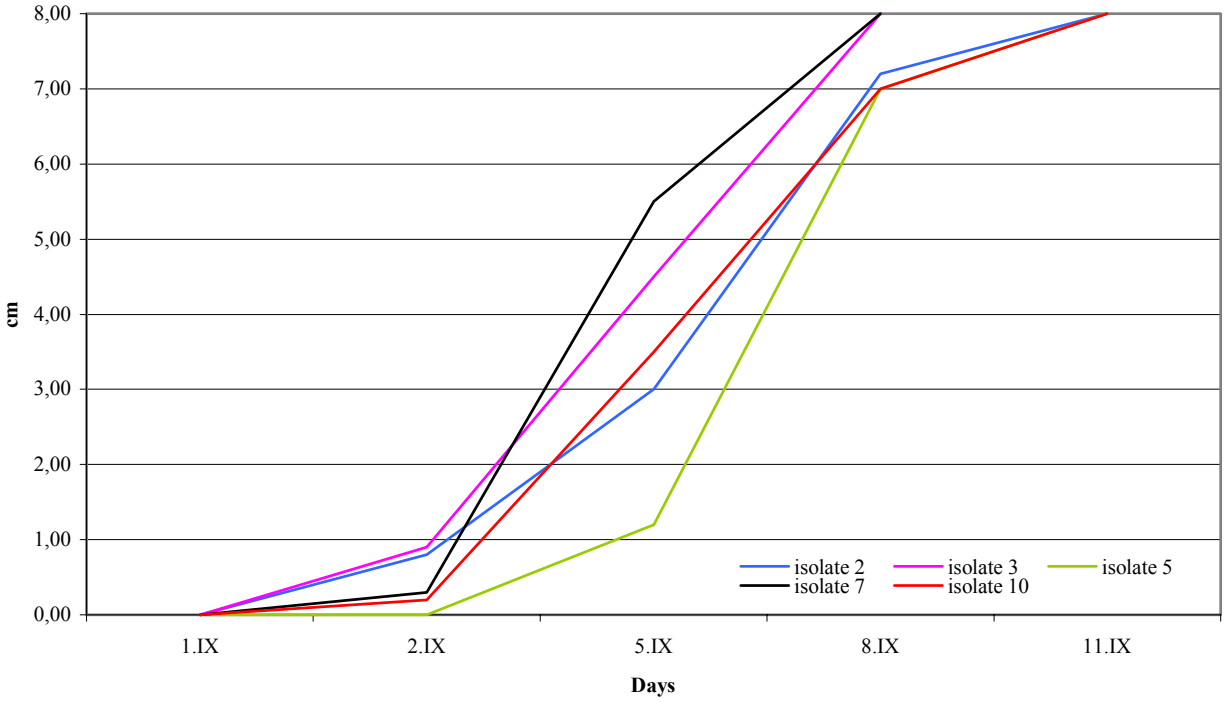
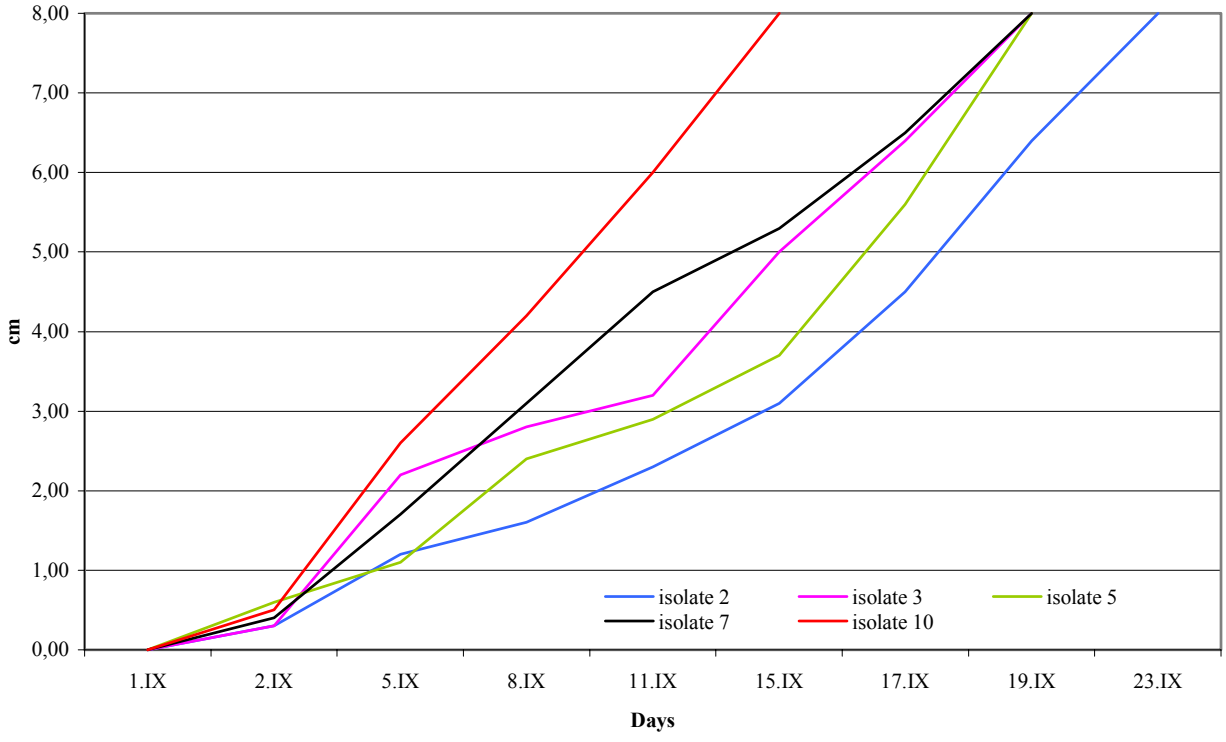


Figure 4: Mycelial growth of single isolates at temperature 27 °C



Second observation

Figure 5: Mycelial growth of single isolates at temperature 5 °C

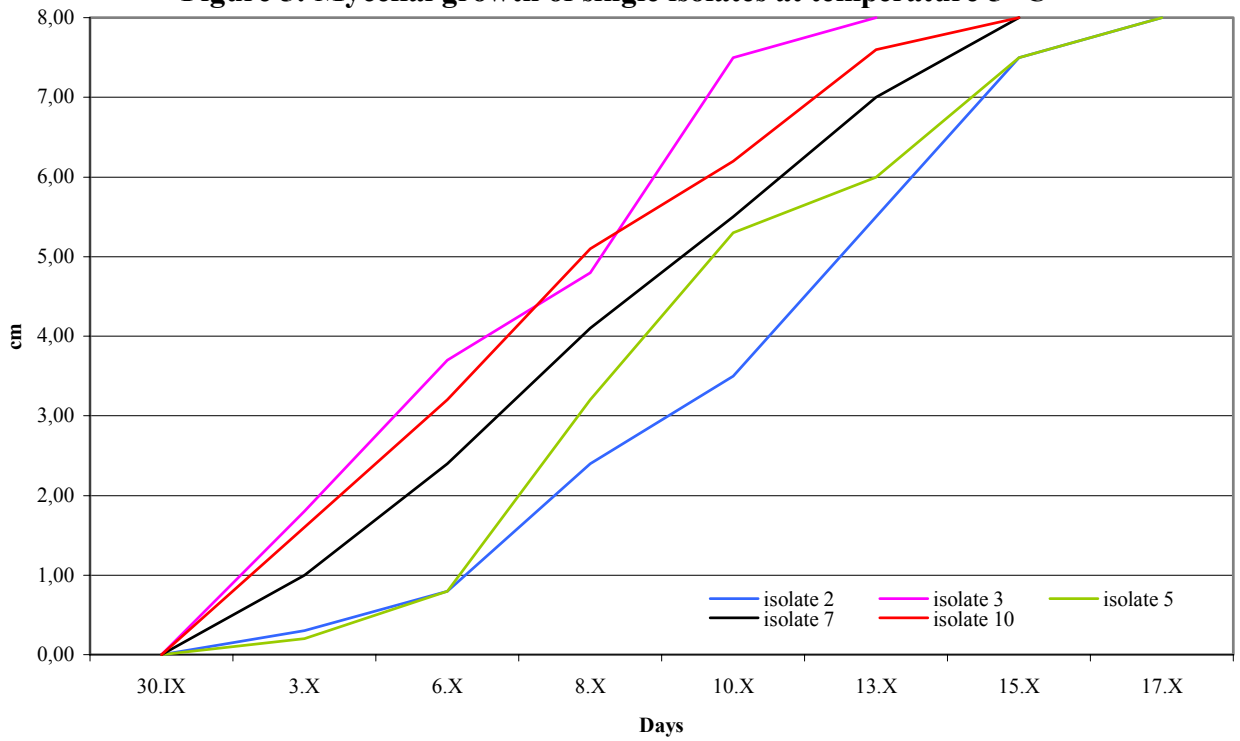


Figure 6: Mycelial growth of single isolates at temperature 8 °C

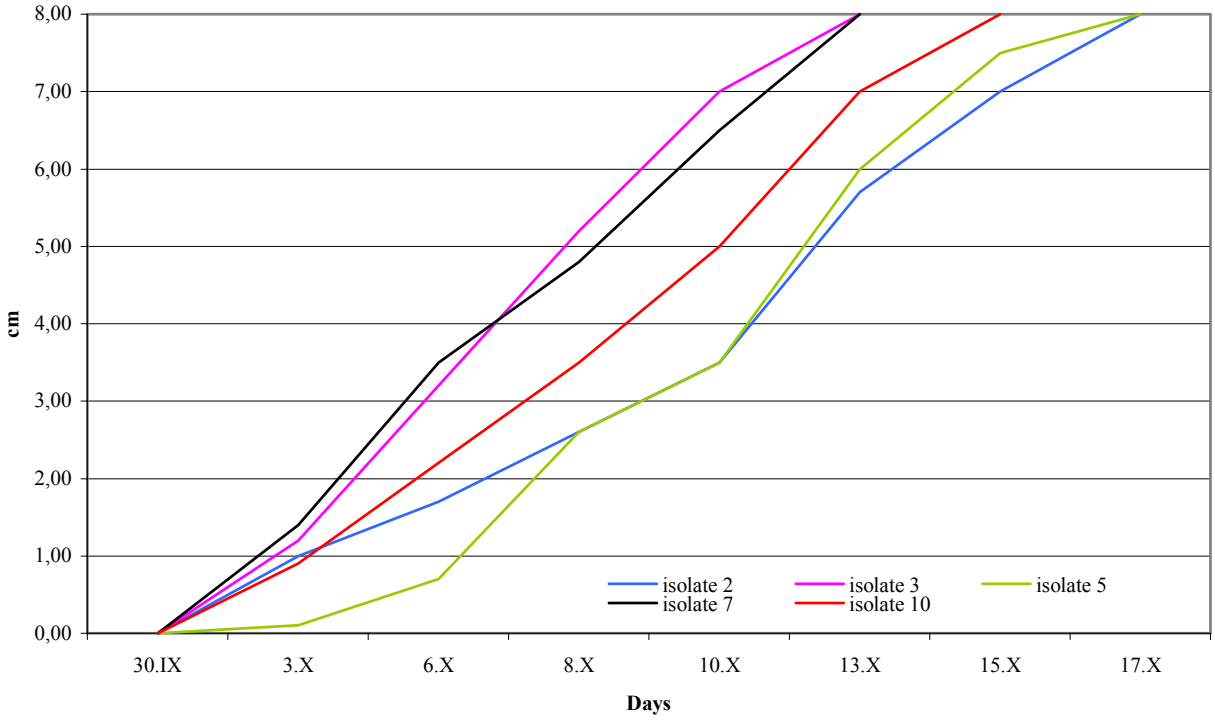


Figure 7: Mycelial growth of single isolates at temperature 22 °C