BOTANICA LITHUANICA 2003, **9(2)**: 171–177

GROWTH OF *STACHYBOTRYS CHARTARUM* STRAINS ON NATURAL AND ARTIFICIAL SUBSTRATES

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Abstract

Elansky S. N., Petrunina Ya. V., Likhachev A. N., 2003: Growth of *Stachybotrys chartarum* strains on natural and artificial substrates [*Stachybotrys chartarum* kamienų augimas ant natūralių ir sintetinių substratų]. – Botanica Lithuanica, **9(2)**: 171–177.

Growth of *Stachybotrys chartarum* (Ehrenb.) Hughes strains on plant and artificial materials in contact with water was analysed. After 84 days exposition at 25 °C material samples (35 × 240 mm) were divided into segments (60 mm length): A – the lower segment under the water level, B – directly over the water level, C and D – accordingly over B and C. Mycelium occupation of each segment was separately evaluated. Maximum fungal growth was on the segment B directly contacting with water, on segment A in most cases the lack of growth was observed. Fungal occupation of the segments C and D positively correlated with water capacity of the material. Mycelium occupation of the higher segments was quite weak, though the humidity was 100 %. The rate of growth on artificial materials was different for each tested *S. chartarum* strain. These differences were not observed when fungi grew on natural materials. The most suitable plant materials were stems and seeds of grain: oats, wheat, couch grass. The growth was weaker on other plant parts: maple leaves, barberry leaves and branches, rapeseeds, trefoil seeds, St.-Johns wort. In all cases the optimal temperature for growth was 25 °C.

Keywords: *Stachybotrys*, collonisation, cellulose, building materials, plant materials.

INTRODUCTION

Stachybotrys chartarum (Ehrenb.) Hughes is a saprotrophic fungus. In natural conditions it grows in soil, on cellulose rich materials such as plant remnants. Also it can grow on artificial materials (wallpaper, pasteboard, some building mixtures). *S. chartarum* is cosmopolite, widespread all over the Earth.

Majority of *S. chartarum* strains produce mycotoxins that can affect humans and animals. *S. chartarum* produces six types of macrocyclic tricothecenes: roridin E, satratoxins

F, G and H, trichoverrols, trichoverrins, verrucarol, and verrucarin J. These toxins occur in all parts of the fungus and in culture broth (NELSSON, 1999). In addition, the fungus produces immunosuppressors and possibly toxic volatile organic compounds (VOC). The combination of organic compounds depends upon the growth media. Four unique for *S. chartarum* VOCs, 1-butanol, 3-methil-2-butanol, 3-methil-1-butanol, and thujopsen, were detected on rice culture, and only 1-butanol was detected on gypsum board culture (GAO & MARTIN, 2002).

Abundant growth of the mold on water damaged cellulose-containing building materials (wallpaper, paper covering on gypsum wallboard, putty) presents the particular danger. In 1990s there was an outbreak of human illnesses even with lethal outcome in water-damaged homes in Cleveland, USA. It was determined that all these cases had arisen from the exposure to this fungus, which had grown profusely on the ground floors with moisture problems. Spores and volatile mycotoxins can be found in air-conditioning and ventilation systems of buildings and thus can induce the health disturbance in residents of homes and work premises. Casual relationship between human indisposition caused by indoor air quality problems and the level of *S. chartarum* contamination was repeatedly established (PAGE & TROUT, 2001, COOLEY et al., 1998).

Commonly used standards of tests for fungal resistance (GOST 9.048-89, 9.049-91, ISO 846) do not include the test for resistance to *S. chartarum*. Therefore, non-resistant materials are used and promote the fungal growth. Furthermore, the foregoing standards envisage the investigations under moist chamber conditions. But in real life conditions the presence of the liquid water in direct contact with the contaminated materials accelerates the growth of *S. chartarum* (LIKHACHEV & ELANSKY, 2001, 2002). In the present study our objectives were to analyse the resistance of some partly immersed in water natural and artificial materials to the growth of *S. chartarum* and to determine the optimal temperature for fungal growth.

MATERIALS AND METHODS

Six strains of *S. chartarum* from distant regions and different substrates were selected for this work. Strains 0 T 16.11-3 – Tomsk, air, 9 MPS – Moscow, paper, and 9 KP 1/2 – Karačaevo-Čerkessia, mountain soil were used in analysis of the growth on materials and in assay of the growth on different plant parts. Strains 9 NPO 1/1 – Nižnij Novgorod region, wallpaper; 9 MVC 1/2 – Moscow, air, and 9 KP 1/2 were used to determine the optimal temperature for fungal growth.

For investigations of the growth under different temperatures, spores of tested strains were placed on Petri dishes with beer-wort agar (200 g wort, 800 ml distilled water, 14 g agar; sterilisation during 30 min, 1 atm.). Plates were incubated at 5, 10, 15, 20, 25, 30 °C for 20 days. The diameter of colonies was measured every 3 days.

For analysis of the growth on plant parts several substrates were used: stems of *Avena sativa, Triticum aestivum, Elytrigia repens*, stems and leaves of *Sonchus arvense, Hypericum perforatum, Acer campestre* leaves, leaves and fragments of branches of *Berberis vulgaris* and seeds of *Brassica napus*, *Linum usitatissimum, Trifolium pratense*, *Triticum aestivum*, *Avena sativa*. The plant parts were dried out, sterilised in autoclave, and regularly placed on the plate bottoms. Materials were inoculated by 10 ml of spores suspension (concentration 50000 spores/ml). All specimens were partly immersed in water. Plates were incubated at 25 °C for 14 days. The level of growth and intensity of spores formation were evaluated vi-

sually and observed with microscope (at 60× magnification). The contamination level was tabulated according to the following scales: 1 – fungal growth was observed on less than 30 % of the substrate, 2 – 30–60 %, 3 – 50–80 %, 4 – 80–100 % of substrate were occupied. The intensity of spores formation: 0 – no spore formation, 1– only single conidiophores were observed, 2 – spores were formed at less than 50 % of the substrate, 3 – profuse spore formation was observed over 50–100 % of the substrate.

The following samples were used in analysis of the *Stachybotrys chartarum* growth on the artificial materials:

1 – Gypsum plaster with paper covering
2 – Vinyl wallpaper with textile fiber base
3 – Vinyl wallpaper on paper base
4 – Washable vinyl wallpaper on paper base
5 – Washable raised vinyl wallpaper on paper base
6 – Paper wallpaper
7 – Partly washable paper wallpaper

Previously, the water absorption capacity (WAC) of the samples was detected. Specimens (1.5 × 1 cm) were dried out in desiccator at 70 °C for 6 hours and weighted. Thus, it was the weight of an absolutely dry sample (M dry). Then the samples were placed in plates with water for 1 day and weighted again. It was the weight of absolutely moist sample (M moist). The total water content formulation is:

$$(M_{moist} – M_{dry}) \times 100/M_{moist}$$

For the growth analysis the cultures of the selected isolates were prepared. Fungal spores were placed on the slanting beer-wort agar. Tubes were incubated in the dark, at 25 °C, for 10 days. Then the spore suspension was prepared (50000 spores/ml). The samples (35 × 240 mm) were sterilised in an autoclave. Spore suspension was sprayed on the samples. Inoculated samples were placed in 1 litre cylinders with 100 ml sterilised distilled water. The cylinders were plugged with a sealing stopper and incubated at 25 °C, for 84 days. Once a week the cylinders were opened for ventilation, and once in 14 days sterilised distilled water was added up to the initial level. For the experiment six samples of each type were used: 2 repetitions for every of 3 isolates used for inoculation. For the evaluation, the samples were divided into segments (60 mm): A – segment below the water level, B – directly above the water level, following C and D segments. The external and internal sides of each segment were observed visually and with microscope (at magnification 60×). Analysis was carried out according to the evaluating scale of fungal resistantce (GOST 9.048-89):

0 – conidium germintation is not found by microscoping,
1 – germinated conidia and poor mycelium are detected with microscope,
2 – mycelium is well detected with microscope, some sporulating mycelium may be found,
3 – mycelium and sporulation are hardly detected visually, but distinctly visible by microscope,
4 – fungal growth is well detected visually, 25 % of substrate is occupied,
5 – fungal growth is well detected visually, more than 25 % of substrate is occupied.

RESULTS AND DISCUSSION

Analysis of fungal growth under different temperature conditions

Maximum growth of all isolates was observed at 25 °C (Fig. 1). But the relative growth rate of each isolate was different: strain 9KP 2/1 grew slower than strains 9 MVC 1/2 and 9 NPO 1/1 (Fig.1).

Analysis of the *Stachyobotrys chartarum* growth on natural and artificial materials

In natural conditions *S. chartarum* occupies different substrates. The fungus was isolated from bean roots (Li et al, 2002), straw fed to animals (Bilaj & Pidoplichko,1970), straw-filled mattresses (Nelsson, 1999,) soil, wood debris, etc. The results of the analysis of fungal growth on natural substrates are given in Table 1. The most favourable were stems and seeds of gramineous (oats, wheat, couch grass). *S. chartarum* grows slower on other plant remnants. Three strains were tested (see Materials and Methods), but no obvious differences in growth and sporulation rates were found. Thus only one average value is tabled (Table 1).

In technogenic environment *S. chartarum* can occupy cellulose rich artificial materials: paper, pasteboard, wallpaper, some building mixtures, plywood and wood elements, etc. (Nelsson, 1999). Analysis of capacity of *S. chartarum* strains from distant geographical regions to occupy artificial materials considering their WAC and capillary power of suction of the material was performed (Table 2).

The fungal growth on segments of samples was different. The B segment (placed directly above the water-level) was maximally occupied; the next C and D segments were less occupied. The A segment (below the water level) was very little occupied.

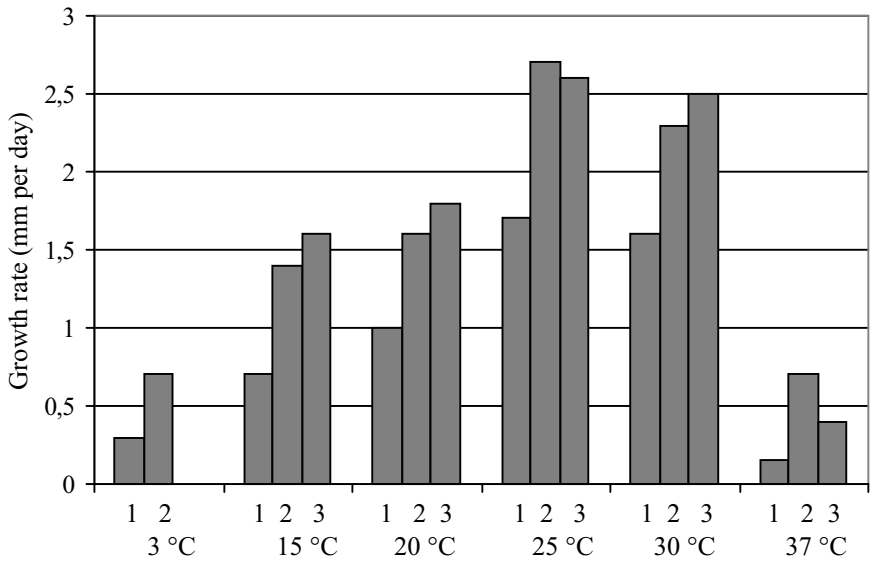

Fig. 1. Growth (increase of the colony diameter) of *S. chartarum* strains under different temperatures. Strains: 1 – 9 NPO 1/1; 2 – 9 KP 1/2; 3 – 9 MVC 1/2

Table 1.

Mycelial growth and sporulation of *S. chartarum* on plant parts and seeds

Substratum	Mycelium growth, scores	Sporulation intensity, scores
Dried plant parts		
Oats, stems	4	3
Wheat, stems	4	3
Couch grass, stems	4	3
Sow-thistle, stems and leaves	3	2
St.-John's wort, stems and leaves	2	0
Maple, leaves	1	1
Barberry, branch fragments and leaves	3	2
Seeds		
Rape	1	1
Trefoil	1	1
Wheat	4	3
Oats	4	3

Table 2.

Fungal growth rating on material segments after 84 days of exposition

Abbreviations. Tested material segments (A – segment below the water-level, B – directly above the water-level, C and D – upstream segments). E – external, I – internal sides of the sample. Data a/b/c/ means: a – contamination with 9 KP1/2 strain, b – with 0 T 16.11-3, c – 9 MPS (average value for two repetitions)

No. of sample[1]	Total water content (%)	Tested segments of materials							
		A		B		C		D	
		E	I	E	I	E	I	E	I
1	51,4	5^4/5/5	5/5/5	5/5/5	5/5/5	5/5/5	5/5/5	5/5/5	5/5/5
2	10,28	1/1/2	1/2/1	4/4/4	4/4/4	0/0/0	0/1/0	0/0/0	0/0/0
3	65,6	1/1/4	1/1/3	2/2/4	3/3/4	3/3/3	4/4/4	3/3/3	3/3/5
4	35,6	0/0/2	3/3/2	2/0/2	3/4/5	2/0/0	4/4/4	1/0/0	2/2/1
5	69,9	0/-/3	4/-/4	5/-/4	4/-/5	5/-/3	5/-/5	4/-/3	4/-/4
6	64,8	3/1/-	3/3/-	4/3/-	4/4/-	5/1/-	4/3/-	4/0/-	3/2/-
7	54,8	5/3/-	3/4/-	5/5/-	4/4/-	3/4/-	0/4/-	3/5/-	0/4/-

[1] Number of sample – according to the numbers in Materials and Methods.

Such differences arised, probably, from the difficulties in air exchange. It cannot be excluded that spores were washed away during the experiment. Positive correlation between fungal growth and water capacity of the material was marked. On samples with low WAC (2) the profuse fungal growth was observed near air–water boundary level; in samples with higher water capacity (4) all interior of the B segment was occupied with fungal mycelium; in samples with the highest water capacity (1, 3, 5, 6, 7) all above-water segments were occupied. The most profuse fungal growth was on the plaster (1) – non-waterproof material. In samples of vinyl wallpaper with paper base the paper interior was most occupied, though the plastic cover was almost unoccupied. In this case plastic cover was smooth and thick enough. In relief vinyl wallpaper samples (5), where thick and thin plastic parts were alternated and formed hillocks, this effect was less defined: mycelium grown through thin plastic was very evident, but where the cover was thick it did not come out. The paper wallpaper samples (6, 7) were equally occupied from interior and exterior sides. It was also determined that the rate of growth on artificial materials was different for each *S. chartarum* strain. Strain 9 KP 1/2 grew slowly throughout the above-water segments of sample 7, though the 0 T 16.11-3 grew more rapidly.

Our experiments reveal that in analysis of the material resistance to *S. chartarum* the direct contact of tested materials with aqueous phase in moist chamber is necessary for both artificial and plant materials. Maximum sporulation and mycelium growth were observed near boundary between air and water. Mycelium occupation of samples correlated with water capacity and chemical characteristics of the material. Thick plastic cover of the water absorbing vinyl wallpaper prevents mycelium from coming out to the external side, but the fungus profusely grows on the internal side. Such *S. chartarum* growth on the internal wallpaper side was mentioned by other researchers (Cooley et al., 1998). Samples, which were tested in moist chamber and got mark "2" (according to GOST 9.048-89), in direct contact with aqueous phase were occupied with fungus by 70–100 %. In real-life conditions of flooding this material could be hardly damaged with mold. The growth analysis of isolates sampled from different regions and different substrates shows some differences in fungal occupation of the artificial materials. These differences were not found while fungi grew on plant parts. It is probably explained by the adaptation of fermentative apparatus to the growth on plant debris during evolution. Each strain has its own rate of adaptation to the artificial substratum.

S. chartarum is one of those fungi, which induce human indisposition. It is necessary to put this fungus into the list of the test cultures for the fungal resistance analysis. It is also important to carry out investigations on fungal resistance of materials to *S. chartarum* and to select strains for the fungal resistance tests.

ACKNOWLEDGEMENTS

We thank Yu. Chikin and M. Sogonov for the help in collecting isolates and V. Apryshko for the assistance in strain analysis. This work was supported by a grant of the Russian Foundation for Basic Research, № 00-04-48750.

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STACHYBOTRYS CHARTARUM KAMIENŲ AUGIMAS ANT NATŪRALIŲ IR SINTETINIŲ SUBSTRATŲ

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Santrauka

Buvo tyrinėjamas grybo *Stachybotrys chartarum* (Ehrenb.) Hughes augimas ant augalų dalių (vienaskilčių ir dviskilčių augalų lapų, stiebų ir sėklų) ir dirbtinių medžiagų (gipskartonio ir popierinių bei vinilu dengtų tapetų), kontaktuojančių su vandeniu. Po 84 dienų substratų inkubacijos 25 °C temperatūroje buvo įvertintas grybo augimas skirtingose substrato dalyse. Nustatyta, kad micelis intensyviausiai augo ant substrato, tiesiogiai kontaktuojančio su vandeniu (B), ir dažniausiai visai neaugo zonoje, esančioje po vandeniu (A). Tarp grybo augimo ant C ir D dalių, esančių virš vandens linijos ir aukščiau B zonos, ir medžiagos adsorbcinių savybių nustatyta tiesinė teigiama priklausomybė. Įvairūs *S. chartarum* kamienai ant tų pačių dirbtinių medžiagų augo skirtingai, o ant augalinių substratų – vienodai. Grybas geriausiai vystėsi 25 °C temperatūroje.

Received: September 26, 2002
Accepted: March 7, 2003

Gautas: 2002 m. rugsėjo 26 d.
Priimtas: 2003 m. kovo 7 d.