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Sensitivity of *Fomes annosus* Fr. Cooke and *Schizophyllum commune* Fr. to air pollution with sulphur dioxide

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Abstract

The sensivity of mycelium to sulphur dioxide polluting the air depends on its physiological activity. As measure of activity growth was adopted, in the sense of the function of dry weight and diametre of the colony, intensity of respiration, amino acids and nitrogen content in dry mycelial mass. The influence of SO_2 on mycelium growth in dependence on its age and pH of substrate was also investigated.

INTRODUCTION

One of the most common, not only in Poland, and most dangerous in its effect, components of air pollution is sulphur dioxide. In industrial districts it is a specific and essential ecological factor exerting an influence on all the components of the forest as an ecosystem, including also fungi pathogenic to trees.

Disappearance of the following fungi from forests has been observed in industrial districts: *Microsphera alphitoides* Griff. et Maubl., *Rhitisma acerinum* (Pers.) Fr. (Köck, 1935), a number of species from the genera *Melampsora, Melampsoridium, Cronartium, Puccinia, Pucciniastrum* (Scheffer and Hedgcock, 1955; Heagle, 1969; Hibben and Stotzky, 1969) *Cronartium flaccidium* (Alb. et Sch.) Wint. (Linzon, 1958), *Lophodermium juniperinum* (Fr.) de Not. (Schönbeck, 1968). *Diplocarpon rosae* (Lib.) Wolf. and *Histericum pulicare* (Pers.) Rhem. are considered to be as sensitive to air pollution as are some lichens and epiphytic mosses so that they may serve as indices of its degree. The noxious influence of air pollution on mushrooms, mycorrhizal and air-borne fungi, those pathogenic to man and soil microfungi has also been demonstrated (Pachlewski, 1958; Hibben and Stotzky, 1961; Krutikow, 1969; Dubos, 1970).

Among the fungi activated by industrial emmission may be quoted: *Armillariella mellea* Vahl. Karst. (Novak et al., 1957, Jančařik, 1961; Kudela and Novakova, 1962; Darley and Middleton, 1966; Sierpiński, 1972; Grzywacz, 1973a), *Rhizosphaera kalkhoffii* Bubák (Chiba and Tanaka, 1968), *Lophodermium pinastri* (Schrad.) Chevall. (Costonis and Sinclair, 1967), *Hirschioporus abietinus* (Dicks. ex Fr.) Donk. (Novak et al., 1957, Jančařik, 1961; Grzywacz, 1973b), *Hirschioporus fusco-violaceus* (Ehrenb. ex Fr.) Donk. (Sierpiński, 1970, Grzywacz, 1973b) and *Schizophyllum* commune Fr., *Nectria cinnabarina* Fr., *Stereum pini* Fr. and other species of the genus *Stereum* devouring wood of deciduous tree species (Jančařik, 1961; Grzywacz, 1973b). In forests suffering from chronic air pollution the specific composition of the saprophytic fungal flora on the leaves changes (Manning, 1971). A number of studies, mainly experimental, concerning the influence of air pollution and fungal pathogens on crop plants in agriculture and gardening indicate that the interaction of fungi with air pollution is of synergic character (Yarwood and Middleton, 1954; Brisley et al., 1959; Schönbeck, 1960, Saunders, 1966; Manning et al., 1969, 1970, 1971).

The disappearance of some and activation of other pathogenic fungal species under the influence of changes in the forest ecosystem due to the phytotoxic components of air pollution will become a new problem in forest protection.

The reaction of pathogenic fungi to SO_2 in the air depends on many factors, mainly on the concentration, diurnal amplitude and seasonal variations of the concentration, on duration of exposure, composition of the vegetal cover and degree of damage to it caused by SO_2, the topography of the area, climatic conditions, kind of forest habitats and sensitivity of the given pathogens.

The purpose of the present study was to examine whether the sensitivity of the mycelium to SO_2 may be a significant factor decisive for the disappearance of the given species from the forests in industrial districts, and to establish the physiological conditionings of the sensitivity threshold to this phytotoxin.

MATERIAL AND METHODS

The mycelia were fumigated in an air-tight chamber with 6 compartments into which ventilators were introduced in order to ensure a uniform SO_2 concentration. Appropriate concentrations were obtained by acting with sodium pyrosulphate aliquots and hydrochloric acid. The influence of SO_2 on the mycelium was investigated at concentrations of 0.01, 0.1, 1, 10, 100 and 1000 mg/m^3, with time of exposure 30 min, 1, 6, 12, 24 h and 3, 5, 10, 14 and 20 days. Of the more than ten fungal

species tested, pure cultures were chosen of the following: *Schizophyllum commune* Fr. as relatively resistant and rather common in avenues and in forests of the Upper Silesian Industrial District (Grzywacz, 1971, 1973 b) and *Fomes annosus* Fr. Cooke as sensitive to SO_2 and disappearing from forest areas with polluted air (Grzywacz, 1973 a, 1973 b). The fungi were cultured on malt-agar medium, and in investigations on the influence of SO_2 on the reaction of the medium, amino acids and total nitrogen content in the mycelium, as medium for the culture brewer's wort from barley malt was used. The cultures were kept under natural light at room temperature and 80 per cent relative air moisture. After closing of the compartments and switching on of the ventilators, the moisture inside was 96 per cent. Each variant of the experiments was replicated 10 times (10 mycelium colonies).

Respiration intensity measurements were performed on the mycelium in the closed system of a gas CO_2 analyzer of Infralyt II type in unchanging laboratory conditions (25.0°C, air flow velocity 40 l./h) in a dark chamber.

Amino acids were analyzed in an automatic, Carlo Erba type 3 A 27 amino acid analyzer according to the method described by Spackman, Stein and Moore in 1958. The extracts for analysis were prepared from powdered 10-day mycelium (6 days after fumigation) dried at 80°C (Whitaker, 1971).

RESULTS AND DISCUSSION

1. Influence of various SO_2 concentrations and time of exposure on the growth of test fungal species

The investigations demonstrated that the SO_2 influence on fungi depends on the concentration and the time of exposure to it, as has been reported earlier (Couey and Uota, 1961, Nelson and Backer 1962, Couey, 1965). It was found that fumigation (in the time period tested) with a 0.01 mg/m^3 SO_2 concentration did not produce significant differences in the growth of the mycelium, and in a 0.1 mg/m^3 concentration slightly stimulated growth. This was hardly noticeable when fumigation was short and statistically nonsignificant, but distinct when the treatment lasted 5 or more days. SO_2 in 10, 100 and 1000 mg/m^3 concentrations inhibited growth in the species tested in various degrees. *Schizophyllum commune* still showed growth 20 days after fumigation at a 1000 mg/m^3 concentration, whereas the mycelium of *Fomes annosus* exposed to the same ceased growing after 14 days.

A single 30 min fumigation at a 0.01 or 0.1 mg/m^3 concentration did not produce any difference in mycelium growth, but with 1 mg/m^3 of SO_2 the difference was visible. When an equal concentration was used

for fumigation, the growth was reduced proportionally to the time of exposure in the chamber (Fig. 1).

Sulphur dioxide stimulated linear growth of the hyphae when the concentration was low and the time short. This phenomenon is noticeable

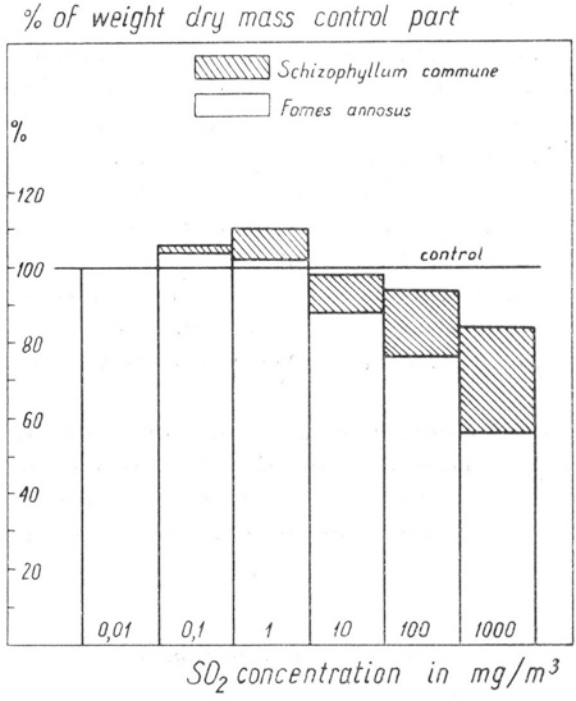

Fig. 1. Influence of various SO_2 concentrations on dry weight of mycelium, expressed as per cent of weight of control mycelium (exposure to fumigation — 10 days)

in what is called escape of mycelium visualized in the graphic comparison of the colony diametre and the dry weight of the mycelium (Fig. 2).

Schizophyllum commune growing in an SO_2-polluted atmosphere aged sooner and produced fruitbodies much earlier. After 10 days neither fruitbodies nor their primordia were found in the control group (20 slides), and in the fumigated group with 1 mg/m^3 25 per cent of the colonies had fruitbodies and in the group fumigated with 100 mg/m^3 60 per cent. It results from the experiments that SO_2 in low concentration stimulates the growth of vegetative mycelium, and in high concentration (10 and 100 mg/m^3) fructification processes, while the amount of vegetative mycelium is considerably reduced.

Sulphur dioxide caused changes in the morphological structure of the mycelium colonies. Mycelium fumigated with 10 mg/m^3 and higher concentrations, after 14 days of this treatment showed a dense compact

arrangement of hyphae, and lost its fluffy apperance. After long-lasting exposure to SO_2, brown necrotic regular spots appeared on the mycelium surface. This phenomenon was observed in both species examined.

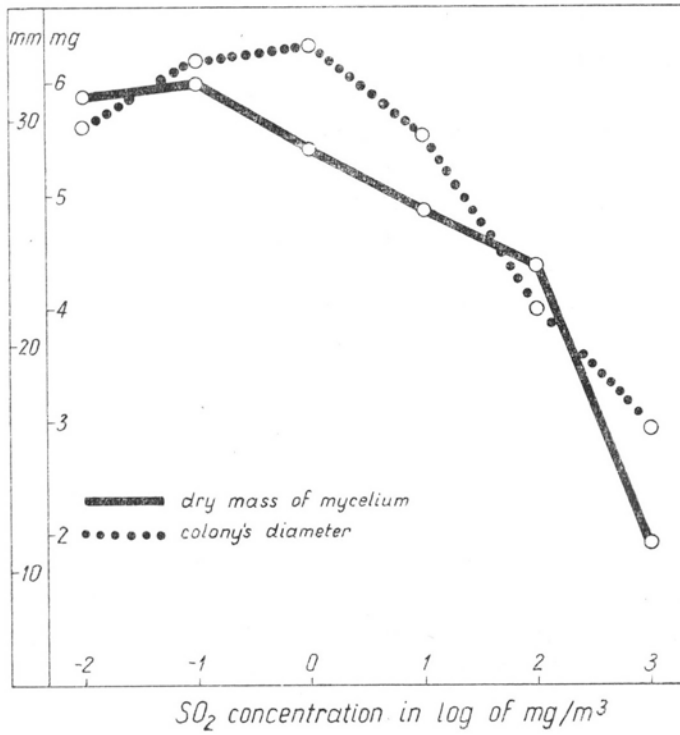

Fig. 2. Influence of 3-day fumigation with SO_2 on dry weight and diametre of *Fomes annosus* mycelium colony

Thus, SO_2 in concentrations of 0.1, 0.5 and 1.0 mg/m^3 (Godzik and Piskornik, 1969, 1970) noxious to most green plants only caused growth inhibition in the tested fungi and for *S. commune* was a stimulator of growth and fructificaton. Death of mycelium occurred only at high concentrations (100 and 1000 mg/m^3) not found in nature. It should be stressed here that the results concerned only a relatively short time period (20 days).

2. Influence of SO_2 on mycelium growth in dependence on its age

The mycelium was most sensitive to SO_2 at the moment of most intensive growth. At this period the intensity of respiration of the mycelium is very high, and this causes an increased inhalation of air together with the sulphur dioxide polluting it into the hyphae. The relatively greater amount of SO_2 absorbed, as compared to the cell mass in the mycelium growing most intensively, enhances the toxic effect

of SO_2. The youngest and oldest mycelia, in which the intensity of metabolic processes is relatively low, showed much lower sensitivity to SO_2. The age of the mycelium is understood as time from its inoculation into the medium to the end of the experiment. After the end of short-lasting fumigation growth was somewhat inhibited for a time, but this was followed by accelerated growth and even regeneration of the mycelium (renewed growth after its complete inhibition). In this experiment, on the 12-14th day of mycelium growth (after the culmination of daily increment of the mycelial mass in the control), a new increase of daily growth increments was observed in the fumigated group.

Change in the sensitivity of the mycelium to SO_2 depending on age is not a specific phenomenon for the action of this phytotoxin. Similar dependences were observed in treatment of the mycelium with other toxic substances, particularly with fungicides (Damaschke and Backer, 1965).

3. Influence on respiration of fungi

Fumigation of mycelium for several days with sulphur dioxide causes changes in the respiration intensity. Concentrations of 0.1, 1, 10 and 100 SO_2/m^3 stimulated respiration in *S. commune* as compared to that in nonfumigated mycelium.. Only a concentration as high as 1000 mg/m^3 produced a fall in respiration intensity. On the other hand, the mycelium of *F. annosus* showed a stimulation of respiration only at 0.1 mg/m^3 SO_2 concentration. Higher doses caused a fall of respiration intensity. When

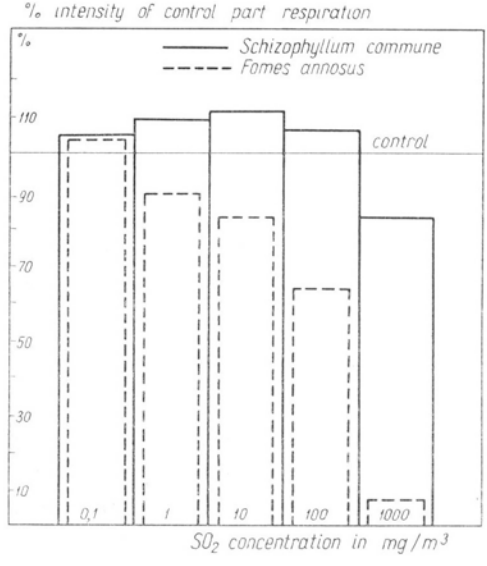

Fig. 3. Respiration intensity of mycelium exposed to SO_2 as compared to that of control mycelium

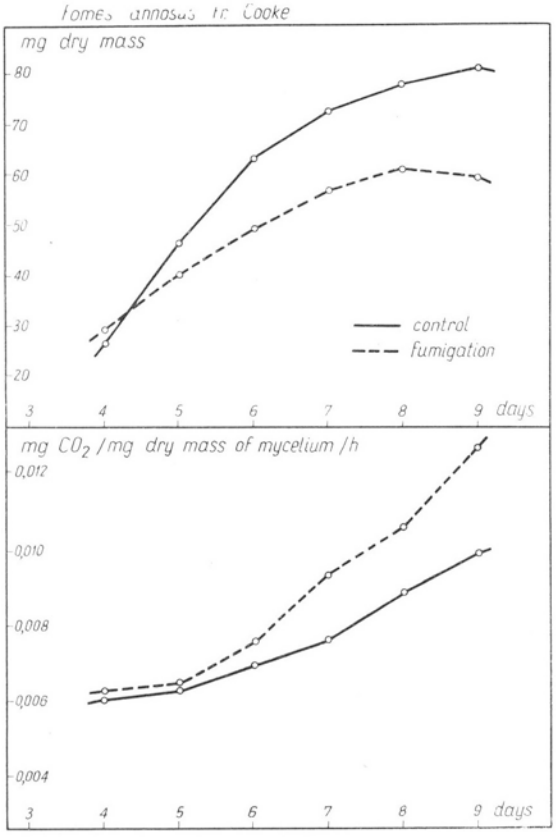

Fig. 4. Influence of SO_2 on dry weight and respiration intensity of mycelium depending on its age (1 mg/m^3)

1000 mg/m^3 was applied, respiration of *F. annosus* decreased to 7.8 per cent of that of the control mycelium (Fig. 3).

By performing measurements on each of six successive days of fumigation of *F. annosus* mycelium with 1 mg/m^3 SO_2 it was demonstrated that stimulation of respiration due to the action of this substance increases with the age of the mycelium. Enhanced respiration intensity points to the occurrence of detoxification processes in the mycelium. In the end stages of the experiment the dry weight of the mycelium decreased, as compared with the previous day, owing to the prevalence of dissimilation processes over those of assimilation (Fig. 4).

4. Influence on amino acids content in mycelium

Investigation of amino acids content in dry mycelial mass of fungal species submitted to fumigation with SO_2 showed a considerable fall of their content with increasing SO_2 concentration. This substance acts on

Table 1

Amino acids content in *Fomes annosus* Fr. Cooke mycelium subjected to fumigation with SO_2 of various concentrations

Amino acids	Amino acids content in 1 g dry mycelial mass, µmoles			
	control	SO_2 mg/m³		
		1	10	100
aspartic acid	154.00	119.42	59.14	56.00
threonine	69.57	47.65	20.61	23.54
serine	182.85	105.15	49.28	46.85
proline	72.06	46.02	26.75	20.99
glutamic acid	+	+	+	+
glycine	157.25	119.74	86.34	86.40
alanine	162.18	111.04	47.55	41.15
cystine	+	+	+	+
valine	102.53	65.02	36.03	32.13
methionine	32.51	26.37	24.83	17.86
isoleucine	95.36	82.82	39.36	31.62
leucine	113.34	62.34	35.97	35.90
tyrosine	+	+	+	+
phenylalanine	46.63	36.61	17.08	17.09
lysine	59.71	34.37	13.38	20.22
histidine	26.24	+	+	+
arginine	47.04	30.78	+	+
Total amino acids	1320.26	861.09	453.89	432.18
%	100.0	65.2	34.3	32.7

+ trace amounts were found

the organism as a whole, therefore it does not show any special influence on any specific amino acid. No amino acids were found to be unsusceptible. The greatest decrease as compared with control values was noted in histidine and arginine in both fungal species, and in *F. annosus* also in alanine and serine. Histidine was detected both in *F. annosus* and *S. commune* only in nonfumigated mycelium (Tables 1, 2). In that exposed to 1 mg/m³ SO_2 only trace amounts could be found. The control mycelium of *F. annosus* contained about two times less amino acids in 1 g of dry matter than the *S. commune* mycelium, thus, in this species the process of amino acids degradation was much more intensive.

5. Influence on total nitrogen content in mycelium

Exposure to sulphur dioxide caused changes in total nitrogen content in the mycelium mainly by reducing the amino acids content. The increase, and afterwards fall, in nitrogen content with increasing SO_2

Table 2

Amino acids content in *Schizophyllum commune* Fr. mycelium fumigated with various SO_2 concentrations

Amino acids	Amino acids content in 1 g dry mycelial mass, µmoles			
	control	SO_2 mg/m³		
		1	10	100
aspartic acid	40.25	38.72	37.20	31.84
threonine	30.75	30.34	29.47	14.24
serine	70.50	72.96	56.70	28.42
proline	30.88	32.51	29.63	18.08
glutamic acid	+	+	+	+
glycine	140.51	161.22	122.37	62.72
alanine	35.20	23.46	27.46	13.25
cystine	+	+	+	+
valine	22.08	20.90	16.42	7.90
methionine	33.82	22.92	19.90	12.03
isoleucine	52.78	46.78	45.95	31.94
leucine	60.03	64.00	45.06	26.43
tyrosine	+	+	+	+
phenylalanine	13.44	22.40	17.06	8.22
lysine	21.98	21.89	14.40	9.60
histidine	38.75	+	+	+
arginine	26.34	17.65	22.40	+
Total amino acids	617.27	575.75	484.02	264.67
%	100.0	93.2	78.4	42.8

+ trace amounts were found

Table 3

Percentual total nitrogen content in dry mycelial mass after fumigation with various SO_2 concentration

Concentration SO_2 mg/m³	*Schizophyllum commune*	*Fomes annosus*
control	1.71	4.44
0.1	1.72	4.47
1	1.82	4.56
10	1.63	4.20
100	1.57	4.04

concentration had a different and slower course than the changes in amino acids content (Table 3). Probably the composition of other nitrogen compounds did not change under the action of SO_2 as much as did proteins. This would probably refer to such a chemically relatively stable substance as chitin (linear acetylglucosamine polymer). The reduced

content of nitrogen compounds in the fumigated mycelium cells produced a relative increase of the contribution of substances composing the cell walls to 1 g of dry weight of the mycelium. Hence, the total nitrogen content in the fumigated mycelium did not probably change as much as did the amino acids content. The problem whether the cell walls thicken in mycelium exposed to fumigation, as a defence reaction of the fungus to conditions unfavourable to growth in the environment requires further elucidation.

6. Influence on reaction of medium

Sulphur dioxide changes the reaction of the medium by acidifying it and by changing the reaction in the mycelium cells. This leads to disturbances of the metabolic processes. The following processes affect the pH of the medium: absorption of cations and anions in the growth

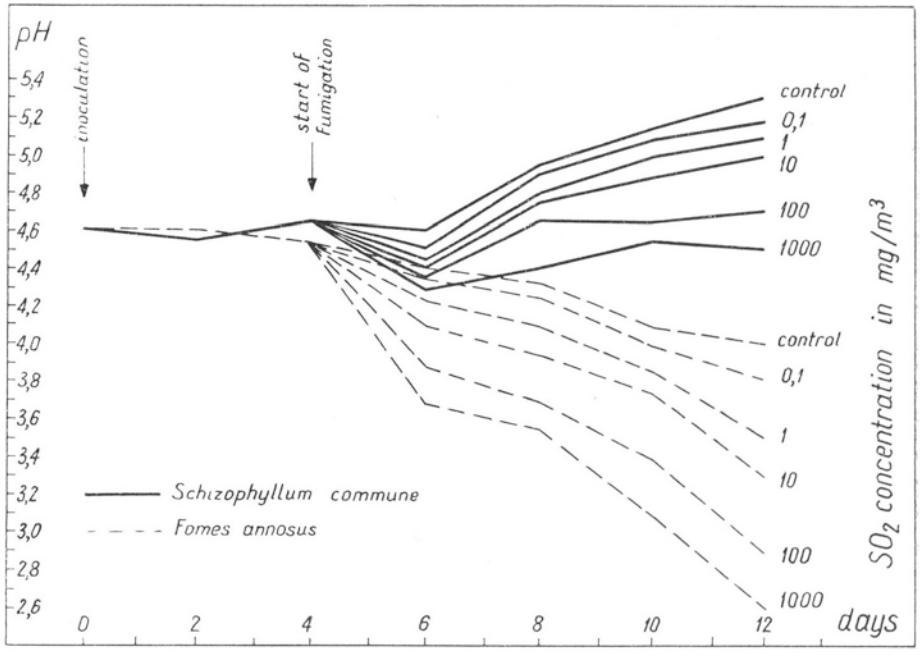

Fig. 5. Changes in pH of medium under the influence of fumigation in cultures of the species tested

process of the fungi, formation of acids from neutral metabolites and of bases, mainly from amino acids and proteins. Changes in pH are resultants of all these processes.

Most important are the pH changes due to disturbance of metabolic processes after fumigation, since changes of pH in medium without mycelium are only slight (when SO_2 particles fall on the medium surface

freely). Five days after exposure to SO_2 in a concentration of 100 mg/m^3 the medium changed only by 0.2 pH and at lower concentrations still less.

F. annosus mycelium acidified the medium in normal conditions. This process is markedly accelerated when the mycelium grows in a SO_2-polluted atmosphere. As early as after 2 days of fumigation (6th day of mycelium growth) the medium in Petri dishes exposed to 100 mg/m^3 showed pH 3.8, whereas the pH of the control was 4.3. After further exposure the difference became wider. *Fomes annosus* exhibits but slight buffer properties, and is uncapable to counteract the acidification of the medium by SO_2 (Fig. 5).

Schizophyllum commune reacts differently to changes in pH of the medium due to SO_2. It has buffer qualities and can maintain pH almost unchanged even when exposed to high concentration of the gas. Non-fumigated mycelium raised the pH as it grew to the optimal value of 6.0. Exposure of the mycelium to concentrations as high as 100 and 1000 mg/m^3 SO_2 did not after 9 days lower the acidity below the initial pH of the medium.

The considerably higher respiration intensity depends on the content of protein compounds in the mycelial cells of *F. annosus*, as compared to that of *Schizophyllum commune* mycelium, causes a more active SO_2 adsorption from the polluted air, and this in turn produces intensive disturbances in metabolic processes responsible for changes in the pH of the medium.

CONCLUSIONS

1. The reduction in growth of the mycelium fumigated with SO_2 is proportional to the concentration of the gas and the time of exposure to it in the fumigation chamber.

2. The mycelium is most sensitive at the moment of its most intensive growth.

3. Low SO_2 concentrations stimulate linear growth of the hyphae, whereas high concentrations inhibit it and cause quicker ageing of the mycelium with necrotic colour changes.

4. Sulphur dioxide produces morphological changes in the mycelial colony and disturbs fructification processes.

5. Mycelium growing in an atmosphere polluted with SO_2 shows changes in respiration intensity. Low concentrations stimulate and high ones inhibt this process. The extent of the respiratory disturbances due to SO_2 changes with the age of the mycelium.

6. Under the influence of fumigation with SO_2 the amino acids content changes in the mycelium, and these changes become wider with higher

concentrations. The greatest decrease was noted in histidine, and arginine, and also in alanine and serine content.

7. The total nitrogen content in dry mycelial mass is decreased by exposure to SO_2.

8. Fumigation with SO_2 influences the changes in medium pH. The species tested differ in buffer qualities which counteract the acidification of the medium by SO_2.

9. The sensitivity of the tested fungal species to SO_2 depends on their physiological activity. The higher the protein content in the hyphal cells and the higher the respiration intensity, the more sensitive is the species to SO_2.

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Wrażliwość Fomes annosus Fr. Cooke i Schizophyllum commune Fr. na dwutlenek siarki zanieczyszczający powietrze

Streszczenie

Zanik pewnych i aktywizacja innych gatunków grzybów patogenicznych drzew pod wpływem zmian w ekosystemach leśnych spowodowanych fitotoksycznymi składnikami zanieczyszczeń powietrza stanowi nowy problem ochrony lasu. Reakcje grzybów na SO_2, główny składnik zanieczyszczeń, zależą od wielu czynników, między innymi od ich wrażliwości.

W pracy badano wpływ SO_2 na wzrost grzybni *Fomes annosus* i *Schizophyllum commune* w warunkach laboratoryjnych, stosując różne koncentracje i czas działania. Stwierdzono, że zmniejszony wzrost grzybni fumigowanej SO_2 jest odwrotnie proporcjonalny od koncentracji i czasu ekspozycji w kamerze. Najbardziej wrażliwa jest grzybnia w momencie najintensywniejszego wzrostu. SO_2 powoduje zmiany morfologiczne kolonii grzybni, zaburzenia w procesach fruktyfikacji, szybsze starzenie się i powstawanie nekrotycznych przebarwień. Niskie stężenia stymulują liniowy wzrost strzępek grzybni. Bardzo wysokie stężenia nie występujące już w przyrodzie (100 i 1000 mg/m^3) nie spowodowały jeszcze całkowitego zabicia grzybni po 20-dniowej fumigacji, dotyczy to względnie odpornego gatunku, jakim jest *S. commune.*

Niskie stężenia symulują, wysokie inhibują procesy oddychania. Wielkość zmian intensywności oddychania pod wpływem SO_2 zmienia się wraz z wiekiem grzybni. Dwutlenek siarki powoduje spadek zawartości aminokwasów w grzybni, pogłębiający się w miarę wzrostu jego stężenia. Największemu ubytkowi ulegają: histidyna, arginina, alanina i seryna. Ulega obniżeniu zawartość azotu ogólnego w suchej masie grzybni. Badane gatunki grzybów posiadają różne zdolności buforowe, pozwalające na przeciwdziałanie wzrostowi kwasowości pożywki pod wpływem fumigacji.

Badane gatunki wykazały duże różnice we wrażliwości na SO_2, wypływa to z ich aktywności fizjologicznej. Fakt ten pozwala wytłumaczyć zanik *Fomes annasus* oraz aktywizację *Schizophyllum commune* w lasach okręgów przemysłowych.

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