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Some results of systematical and ecological research on Agaricales, VIII.

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Abstract: Experimental investigations were carried out to study the effect of carbon dioxide on Agaricus macrosporoides. It can be stated that the mycelium growth, the fruit body formation and the development of this fungus is undisturbed even in a rather high concentration of CO_2. The dispersion in the fructification of the mycelium thalli was also examined.

The CO_2 concentration of the air and fruit body formation of Agaricus macrosporoides

On the basis of literature data and their own extensive research material, HINTIKKA & KORHONEN (1970) made the following statements: "Carbon dioxide seems to play a considerable regulatory role in the ecology of the higher basidiomycetes... However, the inhibition of growth seems to appear only at low oxygen tensions and evidently the inhibitory effect of carbon dioxide sets in earlier, at least for litterdecomposing species... When a fungus attacks wood, its hyphae must grow in vessels and small cavities where the ventilation is much more restricted than it is in the leaves of the litter layer and CO_2 may accumulate. The carbon dioxide tolerance of a fungus probably reflects its ability to grow in such atmospheres, which consequently have a selective effect of carbon dioxide on the fungal growth has been known about for 40 years... One of the factors regarded as responsible for this effect is the heterotrophic assimilation of carbon dioxide... The formative effects of carbon dioxide on mycelium morphology have been also studied... The formation of basidiocarps of Schizophyllum commune - and of some other fungi (Agaricus bisporus, Flammulina velutipes, Pleurotus ostreatus) - is a process very sensitive to excessive amounts of carbon dioxide... Ecologically, it is easy to understand the advantages of the changes in mycelium morphology brought about by carbon dioxide."

Our experimental investigations were carried out with such an Agaricus species about which it turned out during the experiments that its fruit body formation is much less sensitive to excessive amount of carbon dioxide than that of the cultivated mushroom, Agaricus bisporus, at which a little as 0.1-0.18% CO_2 retards the formation of the fruit bodies and their number (TSCHIERPE & SINDEN 1964). For the cultivation of Agaricus macrosporoides in laboratory such conditions were created (BOHUS 1978) that it can be considered as more or less closed system. About two thirds of glass vessels were filled with culture medium and one third of the vessels was air-space. The glass vessels were covered with Petridishes, ventilation took place through paper wadding (see the photo). Fruit bodies were formed on the surface of culture medium as it has been regarded in several hundreds of cases. In the course of their growth they either lifted up the cover or if it was tight they developed even while closed, only they became bent, filling up almost the

whole space. CO_2 concentration of the vessel's atmosphere above the culture medium, densely interlaced with mycelium, was 1.64% at the starting time of the fruit body growth (measured with a "Frenyó" apparatus), that is about ten times the concentration which has already an inhibitory effect in the case of Agaricus bisporus. (I express my gratitude also in this place to Professor V. FRENYÓ for his kind arrangement for carrying out of the measurements). In some cases the fruit bodies formed at the bottom of the vessels where CO_2 accumulation was the highest, and they grew upwards, along the wall of the glass vessels.

After these observations, two problems were to be examined: (1) Interlacing and the appearance of fruit bodies in hermetically closed vessels, (2) What kind of changes can be expected in the growth and the morphology of mycelium during the effect of replacing the atmosphere with CO_2 above the culture medium.

Mycelium growth in hermetically closed vessels

Two days after inoculation (mixing the culture medium with inoculum), the cover was sealed by means of adhesive tape so that ventilation stopped for 29 days. During this period mycelium interlaced the culture medium in a normal way and the primordia - even in this condition - appeared after such a long time as was taken in the case of ventilation through the wadding filter. After 29 days the adhesive tape was removed. The yield appearing in the subsequent period was of the usual quantity: up to the 83rd day it amounted to 109% in comparison with the dry weight of the culture medium. Number of replications: 2, in a similar way to the preceding one. The production was 100% and 104% respectively. Thus, CO_2 accumulation did not cause inhibition and did not bring about observable changes in the morphology of mycelium. The development of mycelium, densely interlacing the culture medium, and the fact that the synthetization of large quantity, nutritive in necessary for the growth of the rather large fruit bodies the while, seem to indicate that partly even a heterotrophic CO_2 utilization does occur.

Mycelium growth in CO_2 atmosphere

(1) Inoculum was placed on to the surface of the culture medium. It took 20 days for the mycelium to cover the surface of the culture medium and it intruded into the medium 4 cm deep. Then the normal atmosphere of the culture vessels was substitued for CO_2 and ventilation was stopped in the vessel for 20 days. Under the effect of these conditions mycelium ceased to intrude deeper into the culture medium. (During this time, the mycelium reached the lower level of the culture medium in the control.) After 20 days the normal air condition was replaced. After this, mycelium still stagnated for 1-2 days, then it interlaced also the lower part of the culture medium.

(2) Inoculum was mixed with the culture medium. Interlacing reaches such an extent at 25°C during 10 days that the cultures can be put to a temperature at around 17°C, for the inducement of fruit body production. Three experiments were carried out in a way, that after 10 days' interlacing period the normal atmosphere of the vessels was substituted for CO_2 and then the cultures were kept without ventilation. In the first case of this serie of experiments - it seems - the substitution of the atmosphere for CO_2 could not be successful enough, and after 10 days one fruit body started to grow, earlier than in the case of the control (seemingly the remaining small quantity of oxygen made it possible that CO_2 inhibition could not yet prevail). Then CO_2 was removed. In the medium treated with CO_2 the fruit body production was 122%, during 90 days, from the beginning of inoculation in the CO_2 treated cultures, while in the control it was 109%. In the second experiment the procedure was the same as in the first one. During the 30 days while the cultures were kept in CO_2 atmosphere, no change could be visible. After CO_2 removal, the life activity of the fungus continued. The production was 56% during 90 days in the case of CO_2 treatment, while in the control it was 103%. A production of 56% is equivalent to the value as usual for 60 days, thus CO_2 treatment, lasting for 30 days, had no harmful effect on the cultures, it only caused a stagnation. Apparently, during the third experiment - the procedure was identical with that of the

two former ones - the substitution of normal atmosphere for CO_2 was successful, because during the CO_2 treatment, lasting for 30 days, caused some changes in the cells of the mycelium. After CO_2 removal, a thick stroma-like mycelium layer developed spreading up to glass-cover, and no fruit body grew either.

Another experiment, closing also with interesting results, was carried out in the same topic. It could be noticed in the earlier examinations that if part of the surface of the cultures was covered with a plastic sponge full of water, the fruit bodies appeared only on the uncovered surface, and not on the plastic sponge in spite of the fact that mycelium did spread over the surface as well. This phenomenon was similar to that when part of the surface of the cultures was covered with water. In the present experiment, the surface of the cultures was fully covered with a plastic sponge full of water, with the exception of the area of 2 cm dia which had been left uncovered as a hole. The situation became unfavourable for the fruit bodies on the surface covered with a sponge, nevertheless, thus numerically 6 (see the photo) did not appear at the hole but at the bottom of the culture vessel, on the lower side of the culture medium, where the carbon dioxide was accumulated. This area can be considered as completely closed at the beginning of fruit body growth since the mycelium interlaces the culture medium to such an extent that the water, poured on the upper surface, remains on the surface without changing in its quantity for weeks, it cannot get downwards. Naturally in the course of fruit body growth, the interlaced culture medium, separated, as a result of a decrease in its volume, from the wall of the vessel.

Discussion

It could be stated that the mycelium growth, the fruit body formation and the growth of fruit bodies of Agaricus macrosporoides were undisturbed even at a rather high CO_2 concentration. At the time of the appearance of the fruit body a CO_2 concentration of 1.65% was measured, but carbon dioxide does not effect as inhibitor for fructification even at a higher concentration, fruit bodies can appear and grow in a space without ventilation, moreover, at the bottom of the vessels in hermetically closed situation. Compared the observations on Agaricus macrosporoides with for example that of Pleurotus ostreatus, it can be stated, that the CO_2 tolerance of mycelium naturally extends to a higher percentage at Pleurotus ostreatus, since it can interlace the hard wood where CO_2 can accumulate in a considerable amount. There is, however, an essential difference in the CO_2 tolerance of the fruit bodies. In cultivation of Pleurotus ostreatus, owing to the CO_2 sensitivy of the fruit bodies, the keeping of the CO_2 content of the atmosphere at a low level causes a problem; if we cannot succeed in this, the quantity of the production is not satisfactory. This is well indicated by the investigations of SCHÁNĚL, KOZLÍK & JABLONSKY (1974) according to which Pleurotus ostreatus in an atmosphere without ventilation, when the diffusion of gases was arrested, did not produce fruit bodies and at CO_2 concentration of 0.012% half the quantity of fruit bodies grew than that produced at 0.0068% CO_2 concentration. As against this, the fruit body production of Agaricus macrosporoides is much less influenced by CO_2 concentration, this species is more resistive to CO_2.

Ecologically, the advantage of the CO_2 tolerance of this fungus is understandable. Its habitat is in prairie environments where in the more or less dense soil the CO_2 content can increase considerably. In heathy soils values as high as even 1.5% were found (ROMELL 1928). In the air layer above the soil surface, the CO_2 concentration is, of course, considerably lower than that. Nevertheless, the fruit body production of Agaricus macrosporoides seems to be accustomed to the higher CO_2 concentration. This fact is supported by the experimental observation, that the fructification of the fungus takes place in the soil when the water content of the soil decrease simultaneously with a favourable air temperature.

Examination about the dispersion of the fructification of mycelium thalli

Record production of soil inhabiting macrofungi can be observed under especially favourable weather conditions. On the basis of our experiments, described below, it can be supposed that a part of mycelium thalli of an area belonging to the identical species and congested reserve food,

do not fructificate at all, or some of them take part in fructification if the favourable conditions last.

In this respect we succeeded in carrying out experimental observations with a prairie-species, Agaricus macrosporoides. The thoroughly mixed culture medium was divided into a number of culture vessels and sterilized in a special autoclave (the circumstances are homogenous). Inoculation was carried out from one inoculum (spawn) onto the surface of the culture medium; in some cases, 5-8% of the small sized spawn were mixed with the culture medium. In this last case a great number of mycelium thalli were produced during the period of interlace - thalli were started from several points of the medium and posses different growth intensity - the intensity differences become eliminated and the culture medium was fully interlaced. The parallel cultures were kept in a thermostate at 25°C during the period of interlace, then the cultures were placed in a thermostat at 16-18°C during the fructification period. The fruit bodies appeared in 21% of the cultures approximately at the same time; the appearance of the fruit bodies took place in some cases at other time, sometimes it takes long time. Rarely it also was observed that one of the parallel cultures did not produce fruit body at all (Table 1). On the basis of these observations, it can be supposed, that the dispersion of fructification in time is considerable, and it is possible that this is characteristic for other species, too.

A further observation: IMREH (1969) carried out observations for decades in connection with fruit body formation of another prairie species, Agaricus maskae, about on 260 mycelium thalli. He found, that some 10 days after a substantial rainfall there occurred many fruit bodies but certain fairy-rings - next to the fructifying mycelium thalli - did not fructificate at all, or rarely produced fruit bodies. This observation was made possible by the fact that, by discolouring of grasses the boundary of the mycelium thalli is well visible even if the fungus does not produce any fruit body in the given case. Since the soil in these examined pastures is rather homogeneous, it has no mosaic pattern, so it is impossible that the differencies, observed in fructification, could be caused by soil factor. The observation related to Agaricus maskae, seems to be a reliable one.

Table 1. Harvesting time of the first fruit bodies

Date and method of inoculation		Number of days from the beginning of production period[x] in parallel culture		
		1st	2nd	3rd
1970				
6.IV.	A[xx]	10	25	31
11.V.	A	22	22	38
10.VIII.	A	3	17	
17.IX.	A	6	16	37
14.XI.	A	16	18	18
1971				
4.I.	A	18	21	31
16.II.	A	18	31	
24.III.	A	26	30	
1972				
4.II.	A	15	16	
11.II.	A	15	17	
31.III.	A	21	22	
28.VI.	A	34	38	
18.XII.	A	14	26	
1973				
22.I.	A	37	50	
7.IV.	A	15	17	
9.V.	A	15	15	15
31.V.	A	17.	31	34
4.VI.	A	34	44	46
26.VI.	A	14	14	15
13.VII.	A	27	29	32
11.X.	A	20	34	
24.X.	A	19	19	30
1974				
17.I.	A	14	28	
22.I.	A	16	38	
23.I.	A	15	36	
25.I.	A	23	38	
31.I.	A	24	27	35
14.II.	A	19	22	

(Continuation of Table 1)

Date and method of inoculation		Number of days from the beginning of production period[x] in		
		1st	2nd	3rd
		parallel culture		
1974				
1.III.	A	22	45	
7.III.	A	18	26	27
9.IV.	A	35	36	36
3.V.	A	26	37	
23.V.	A	23	26	32
14.VI.	A	4	7	
10.VII.	A	29	29	
22.VII.	A	24	26	
27.VII.	A	13	26	
27.VII.	A	37	43	
30.VII.	A	16	18	20
8.VIII.	A	10	17	32
18.VIII.	A	25	27	35
18.IX.	A	17	20	25
12.X.	A	14	15	17
25.X.	A	14	24	35
31.X.	A	30	47	
21.XI.	A	21	35	
21.XI.	A	40	42	
25.XI.	A	13	14	28
6.XII.	A	40	42	
18.XII.	A	31	31	
1975				
22.I.	A	56	58	
12.II.	A	22	24	
24.II.	A	21	21	
7.III.	A	21	22	
13.III.	A	34	34	
19.III.	A	19	22	
27.III.	A	24	31	32
8.IV.	A	17	20	
8.V.	B[xxx]	22	27	
24.V.	B	16	22	
30.V.	B	24	27	38
20.VI.	B	27	28	36
10.VII.	B	37	49	
21.VII.	B	29	33	
5.VIII.	B	26	26	
5.X.	B	28	29	
4.XI.	B	24	ø	
19.XI.	A	18	18	
9.XII.	B	21	25	
1976				
16.I.	A.	15	19	22
20.I.	B	24	32	
30.I.	A	28	30	

Date and method of inoculation		Number of days from the beginning of production period[x] in		
		1st	2nd	3rd
		parallel culture		
1976				
7.II.	B	28	28	
13.II.	B	20	20	
5.III.	B	22	ø	
17.III.	B	23	28	
18.III.	A	19	33	37
18.III.	A	19	19	29
24.III.	B	21	22	
8.IV.	B	26	27	
15.IV.	A	13	14	37
13.V.	B	20	28	
19.VIII.	A	19	19	
8.IX.	A	23	30	30
10.IX.	A	20	20	24
13.IX.	A	25	31	31
7.XII.	A	21	23	
16.XII.	A	21	21	
17.XII.	A	17	26	30
1977				
11.I.	A	26	31	
13.I.	A	24	24	
8.II.	B	18	22	
2.III.	B	19	26	33
29.III.	B	34	34	40
9.IV.	B	24	25	35
16.IV.	B	30	37	63
27.V.	B	21	28	
6.VII.	B	25	25	
22.VIII.	B	25	29	
13.X.	B	26	33	
2.XII.	B	29	32	
12.XII.	B	27	31	
13.XII.	B	21	25	
23.XII.	B	24	38	
1978				
23.I.	B	20	27	
31.I.	B	24	27	
4.II.	B	27	27	
10.II.	B	18	25	
17.III.	B	23	27	
15.IX.	B	28	35	
15.X.	B	25	25	
21.XI.	B	31	33	
1979				
4.V.	B	24	28	
11.X.	B	38	41	

(Continuation of Table 1)

Date and method of inoculation		Number of days from the beginning of production period[x] in		
		1st	2nd1	3rd
		parallel culture		
1980				
3.I.	B	37	40	
3.I.1	B	27	36	
5.I.	B	39	44	
8.I.	B	29	47	

x Production period begins when the cultures after interlacing were put to a temperature at around 17°C

xx A = Inoculation on the surface of the culture medium

xxx B = Inoculum (spawn) mixed with the culture medium

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Fig. 1. The appearance of the fruit bodies at the bottom of the culture vessel