ACTA SOCIETATIS
BOTANICORUM POLONIAE
vol. XLI — nr 3
1972

Comparative investigations of the development of Fungi from the section of *Penicillium clavigerum*

III. Action of CO_2 on the morphology of coremia and dry weight of the mycelium

B. PISKORZ

Institute of Plant Physiology, Polish Academy of Sciences, Grodzka 53, Cracow, Poland

(Received: December 12, 1971.)

Abstract

The action of varying concentrations of CO_2 in light and in darkness on *P. claviforme* and *P. isariaeforme* was studied. In the former, the shape of coremia was influenced but dry weight was not. In *P. isariaeforme* increase of concentrations of CO_2 inhibits development of coremia, and in light, results in excess of dry weight. Experiments applying $C^{14}O_2$ revealed that both may assimilate carbon from the air.

INTRODUCTION

Investigations of the action of carbon dioxide on heterotrophic organisms have been made for several years. Already in 1918, Wherry and Ervin observed that the growth of the tuberculosis bacteria partly depends on the presence of CO_2. Rockwell and Highberger (1927) found that the growth and development of lower Fungi kept in an atmosphere devoid of carbon dioxide is commonly inhibited. Rippel and Bortles (1927) and Rippel and Heilman (1939) studied the influence of carbon dioxide on the germination and growth of mycelium of *Aspergillus niger*, and arrived at the conclusion that the presence of carbon dioxide is an important factor allowing normal development.

Lambert (1933) was the first to deal in a more detailed way with the action of an increased amount of carbon dioxide in the air on the growth and development of mushroom carpophores. Tschierpe (1959) and Tschierpe and Sinden (1964) showed that concentrations of CO_2 higher than 1.5 per cent have a stunting effect on the fruit body of *Agaricus bisporus*. A slight increase of the concentration of CO_2 reaching 0.3 per cent results in a clearly higher rate of growth of the stipe of the mushroom fruit body, while the growth of pileus is being inhibited. These phenomena are still more distinct at higher concentrations of CO_2. Cultures of mush-

rooms died at a concentration of 11 per cent. Rast and Bahofen (1965) showed that *Agaricus bisporus* is able to assimilate quite high amounts of CO_2. Wherli and Rast (1967) thought that carboxylation reactions explained the important influence of CO_2 on the growth and development of *Agaricus bisporus*; this was directly verified by Le Roux (1962, 1966).

It appeared that the Fungi from the *Penicillium clavigerum* section strongly react to the gas composition of the surrounding atmosphere. Both species from this section sensitive to light are also sensitive to the flow of air.

It was seen in *Penicillium claviforme* (Piskorz 1970) that an increasing rate of air flow resulted in a distinct shortening of coremia and their increasing density per unit urea of mycelium surface. With an increasing rate of flow, morphological effects of light diminish. At the highest rate applied, cultures of *P. claviforme* grown in light were but slightly different from those grown in darkness. Both light and aeration of cultures did not act on the amount of the dry mass of the mycelium formed.

Penicillium isariaeforme is still more sensitive to aeration. Cultures grown in Erlenmayer flasks tightly closed with cotton stoppers produce high elongation mycelia which do not join in coremia. Cultures grown in light form some typically developed coremia while the rate of flow increases. Beginning at the rate of 90 l per hour they become more elongate. In certain conditions, *P. isariaeforme* grown in light produces more dry weight of mycelium than in darkness, the excess being about 30 per cent. In cultures of this species, aeration appears to act strongly not only on the morphology, but also on the production of the dry weight, its excess obtained in light disappearing already at the lowest rate of flow applied. The dry weight of aerated cultures grown in light is equal to that of cultures grown in darkness.

These investigations allowed to assume that the factor cooperating with light and strongly acting on the morphology of coremia of the representatives of the *Penicillium clavigerum* section was some substance present in the air, most probably carbon dioxide.

Thus it seemed necessary to study the action of carbon dioxide on the production of dry weight and development of coremia in the *Penicillium clavigerum* section.

I. MATERIAL AND METHODS

I. 1. Material

The investigations concerned stocks of *P. claviforme* and *P. isariaeforme* obtained from Centraalbureau voor Schimmelcultures at Barn, Holland. Original cultures were grown on nutrient medium solidified by agar; its composition and preparation were described previously (Piskorz 1967).

I. 1. 2. Conditions of culture

Cultures have been grown in thermostats in darkness or light of an intensity of 900 lx. The optimum temperatures were 22°C and 25°C for *P. claviforme* and *P. isariaeforme* respectively.

I. 2. Apparature used to obtain flow of air with increased amount of CO_2

In the experiments on the action of aeration on the cultures of the representatives of the *P. clavigerum* section (Piskorz 1970) a prototype apparatus, namely a twelve canal gas-flow-meter was used. In the present experiments, this apparatus was modified (Fig. 1).

The air of normal composition compressed in a WanCF 1960 compressor (9) passed by a pressure tube equipped with a manometer (8), a needle valve (7), and a rotameter (6), to the wider arm (5) of a copper tube. Carbon dioxide obtained from a cylinder (1) passed by a pressure tube also equipped with a manometer (2), a needle valve (3) and a rotameter, (4) to the narrower arm of (5) the same copper

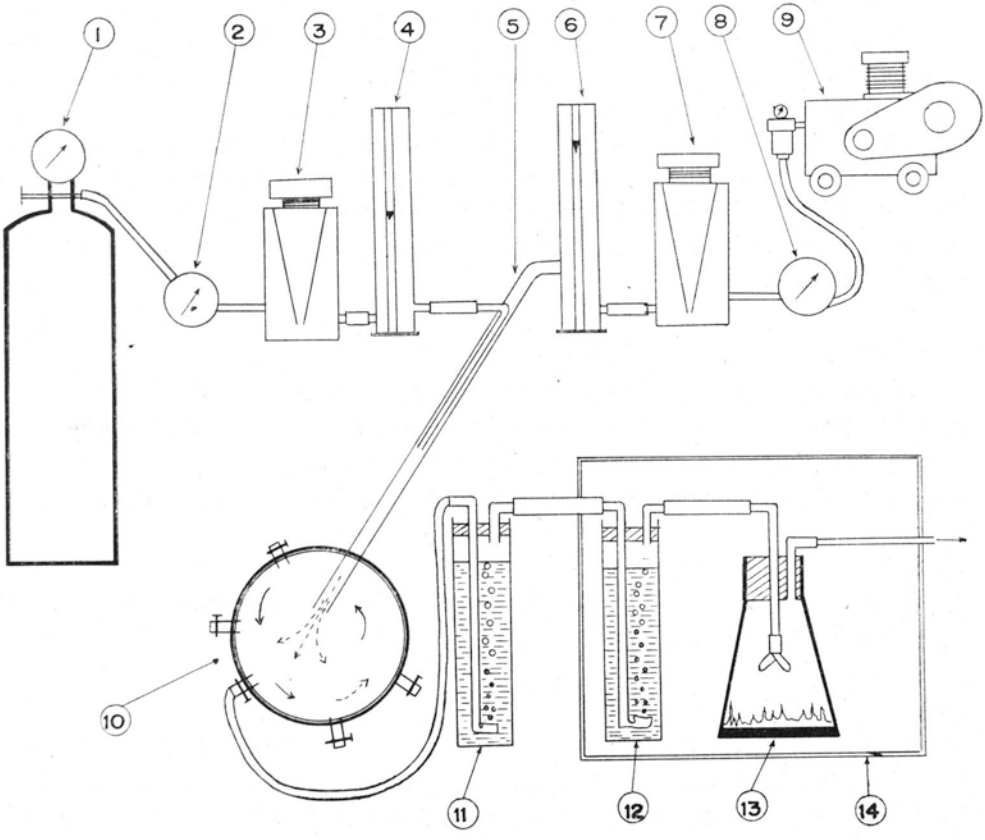

Fig. 1. Apparatus used for measuring air flow and amount of CO_2. For exlanation see the text.

tube. At the extremity of the copper tube, both gases, the air being at a pressure of 1.5 atm. and carbon dioxide at 5 atm., became mixed. The mixture subsequently flowed to a compensation chamber (10) with a set of taps, and then it was directed by rubber tubes to a set of wash bottles (11, 12) with water where it was humidified, to reach finally culture flasks (13), each provided with a rubber tube by which the gas mixture was diverted to a common chamber, and thence to the atmosphere.

The rate of flow of the gas mixture was measured by a TG-300 rotameter at the end of the system, namely at the exit from culture flasks.

I. 2. 2. Culture flasks

In the present series of experiments — in the same way as in that concerning aeration — were used Erlenmayer flasks of 300 ml capacity with tightly fitting polished glass stoppers.

I. 3. Measurements of concentration of carbon dioxide in the gas mixture

The percentage of carbon dioxide in the gas mixture contained in the system was established. The chemical method consisted in absorption of carbon dioxide from a sample by a solution of $Ba(OH)_2$, the rate of flow being known; the percentage of carbon dioxide was calculated taking into account the temperature and atmospheric pressure. This method was used mainly if the concentration of CO_2 was low. At higher concentrations, namely above 1 per cent, measurements were made by Orsat apparatus (Alekseiewskij et al. 1954). Measurements of per cent concentration of carbon dioxide were made separately for each culture flask prior to measurements. Five samples of gas mixture were taken and the mean of measurements calculated.

I. 4. Evaluation of results

After seven days of culture in the case of *P. claviforme* and twelve in that of *P. isariaeforme*, the nutrient medium was filtered out and morphological measurements of the shape and height of coremia were made. Mycelia were then dried and their dry weight measured. The results were presented in tables.

I. 5. Use of carbon dioxide with radioactive carbon

I. 5. 1. Preparation of chamber with atmosphere enriched in $C^{14}O_2$

The experiments were made in a tightly closed plexiglass chamber (Fig. 2), kept either in darkness or in a white light of 900 lx, depending on the effects studied. The chamber contained an atmosphere with 5 per cent of CO_2, obtained from $NaHCO_3$ and $NaHC^{14}O_3$ treated by an excess of 2 n H_2SO_4.

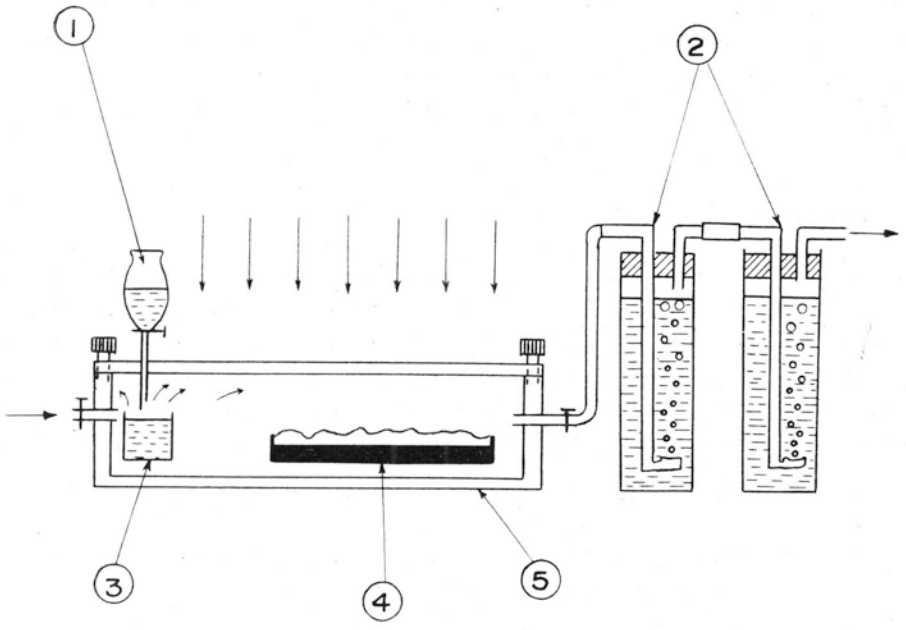

Fig. 2. Set used in experiments applying C^{14}: chamber — 5, Petri dish and mycelium — 4, vessel with acid solution sodium carbonate — 3, funnel used for adding H_2SO_4 — 1, rinsing bowls with 20% KOH—2.

I. 5. 2. Preparation of mould extracts with C^{14}

Following two hours of incubation in the chamber with CO_2 containing C^{14} the mycelia were quickly taken out and measured. The extracts were made using a modified Wherli and Rast (1967) method. After measurement of fresh weight 1 g of the mycelia was treated with 4 ml of boiling 80% ethyl alcohol, homogenized for 5 minutes, and then filtered in a funnel with a porous G5 plate. The filtrate was collected, and the residue once more treated with 80% ethanol and homogenized. Following each filtering, the funnel was washed with some absolute alcohol. The filtrates obtained by both filtrations were mixed, the residue was collected from the funnel and hydrolized in a reversible cooler successively for 2.5 hours with 2.5 ml of 0.7 n HCl and for 24 hours with 6 n HCl (Fraction III). The acidity of the mixture of filtrates from the first and second filtration was raised to pH 3. The dried filtrates containing Fractions I and II were dissolved in boiling water using 5 ml for 1 g of mycelium, mixed with diatom earth, and filtered in a G5 filter, washing four times using 5 ml of warm water. The filtrate was collected; it contained mainly organic acids, amino-acids, and simple sugars this was Fraction I. The residue in the filter, not soluble in water, was washed four times with absolute ethyl alcohol, using 20 ml for 1 g of mycelium. The successive filtrates were Fraction II, composed mainly of lipids, and Fraction III, which was a hydrolizate of the

residue of previous extractions insoluble in alcohol and consisting mainly of proteins and products of hydrolitic distintegration of polysaccharides. The filtrates were dried, and then, prior to measurements of radioactivity, dissolved using for 1 g of Fractions I, II and III respectively 1.25 ml of water, 1.5 ml of toluene, and 1.5 ml of water.

I. 5. 3. Measurements of radioactivity

All measurements of radioactivity of mould extracts were made in a scintillator Packard 2002, Tri-Carb Liquid Scintillation Spectrometers. The composition of the scintillating solution was the following: 75 g naphtalene, 5 g PPO, 0.15 g POPOP, 500 ml dioxan (scintillator with dioxan according to Davles and Hall). The general radioactivity of the total fraction was established on the results obtained, and the radioactivity of particular fractions referred to 1 g of fresh mass was calculated.

II. Action of various concentrations of CO_2 in the flowing air on the morphology of coremia, dry weight of mycelium and sporulation

Cultures of *P. claviforme* following a 24 hours preliminary incubation were placed for six days in a dark or light thermostat. The flowing air contained the following amounts of CO_2: 0.07, 0.15, 1.5, 3.0, and 6.0 per cent. The results of the experiments are presented in Table 1 and Fig. 3.

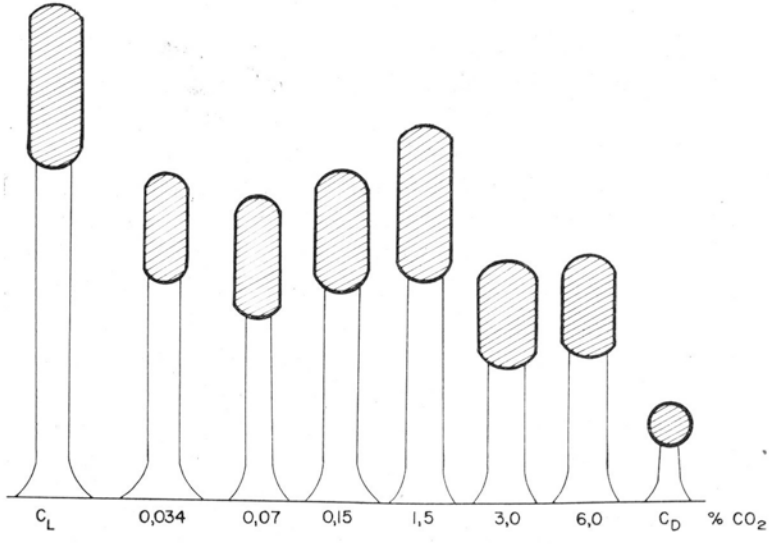

Fig. 3. Shape of coremia of *Penicillium claviforme* grown at various concentrations of CO_2, schematized; L_c — control culture grown in light without air flow, D_c — control culture grown in darkness without air flow.

Table 1

Action of carbon dioxide on the dry weight of the mycelia and morphology of coremia *in Penicillium claviforme*

Concentration of CO_2 %		Dry weight in g	Number of coremia per cm²	Height of coremia in mm	Length of foot in mm	Breadth of foot in mm		Length of head in mm	Breadth of head in mm
						top	base		
Control	L	0.602 ±0.003	12±2	9.38	6.09 ±0.03	0.66 ±0.02	1.50 ±0.06	3.29 ±0.04	0.95 ±0.06
Control	D	0.616 ±0.009	64±5	1.69	1.17 ±0.08	0.33 ±0.02	0.90 ±0.05	0.52 ±0.03	0.50 ±0.03
0 034	L	0.562 ±0.016	24±4	5.84	3.90 ±0.02	0 54 ±0.04	1.47 ±0.08	1.94 ±0.02	0.76 ±0.01
	D	0.737 ±0.009	58±6	1.96	1.31 ±0.05	0.49 ±0.05	0.95 ±0.08	0.65 ±0.06	0.60 ±0.06
0.07	L	0.681 ±0.020	19±4	5.38	3.20 ±0.07	0.48 ±0.07	1.49 ±0.04	2.18 ±0.06	0.85 ±0.04
	D	0.619 ±0.019	63±5	2.29	1.50 ±0.08	0.42 ±0.05	0.90 ±0 06	0.79 ±0.04	0.83 ±0.04
0.15	L	0.633 ±0.016	18±7	6.18	3.86 ±0.04	0.57 ±0.04	1.20 ±0.05	2.32 ±0.06	1.01 ±0.07
	D	0.649 ±0.025	63±7	2.31	1.26 ±0.05	0.46 ±0.07	0.81 ±0.03	1.05 ±0.04	0.91 ±0.05
1.5	L	0.605 ±0.023	17±6	6.85	3.99 ±0.0	0.59 ±0.07	0 96 ±0.07	2.86 ±0.06	0.96 ±0.04
	D	0.566 ±0.018	64±5	2.57	1.48 ±0.05	0.36 ±0.07	0.87 ±0.05	1.09 ±0.03	0.92 ±0.03
3.0	L	0.639 ±0.012	28±4	4.30	2.38 ±0.03	0.52 ±0.06	1.16 ±0.08	1.92 ±0.04	1.20 ±0.05
	D	0.659 ±0.019	abundant	1.54	1.10 ±0.06	0.28 ±0.08	0.85 ±0.05	0.44 ±0.04	0.48 ±0.04
6.0	L	0.743 ±0.016	27±5	4.59	2.61 ±0.05	0.47 ±0.09	1.01 ±0.06	1.98 ±0.05	1.03 ±0.05
	D	0.511 ±0.010	—	1.53	1.11 ±0.04	0.30 ±).05	0 91 0.07	0.42 ±0.05	0.45 ±0.06

L — cultures in light.
D — cultures in darkness.

It may be seen in Table 1 that an increase of CO_2 in the air from 0.07 to 3.0% does not influence the amount of the dry mass produced either in light or in darkness. A slight excess of dry mass occurs in cultures grown in light at the highest amounts of carbon dioxide applied, i.e. 6.0%.

On the other hand, increased concentrations of CO_2 have a strong morphogenetic effect, both on the shape and the number of coremia. In cultures grown in light, the amounts of CO_2 increasing from 0.07 to 1.5% resulted in an increase of the

height of coremia of 1 mm in relation to the control cultures, which were grown in light at a constant flow of air with normal amount of CO_2. Further increase of the amount of CO_2, from 1.5 to 6.0%, resulted in a shortening of the coremia. The conidial heads are also sensitive to the changing CO_2 concentrations. At low concentrations, between 0.07 and 1.5%, the head is cylindrical. Increased concentrations result in the shapes of heads approaching spherical, and thus becoming similar to those obtained in cultures grown in darkness (Fig. 3).

The cultures of the second species studied, namely *P. isariaeforme*, were incubated for 36 hours, and then treated with flowing air for twelve days, applying the same concentrations of CO_2. Cultures were grown both in light and in darkness. The results of the experiments are shown in Table 2 and Fig. 4. As previously demonstrated, in *P. isariaeforme* some excess dry weight is produced in cultures grown in light. This excess disappears in aerated cultures.

Table 2

Action of carbon dioxide on the morphology and dry weight of the mycelia in *Penicillium isariaeforme*

Concentration of CO_2		Dry weight in g	Morphology
Control	L	0.406±0.008	High mycelium S +++
Control	D	0.306±0.010	Low mycelium S ++++
0.034	L	0.292±0.009	Coremia S ++++++
	D	0.324±0.010	Coremial primordia S ++++
0.07	L	0.320±0.018	Coremia S ++++++
	D	—	— —
0.15	L	0 310±0.015	Scarce coremia S ++++
	D	0.276±0.017	Absence of coremial primordia S +++
1.5	L	0.330±0.010	High mycelium S +++
	D	0.314±0.020	Low mycelium S +++
3.0	L	0.458±0.019	High mycelium S +++
	D	0.310±0.015	Low mycelium S +++
6.0	L	0.433±0.015	High mycelium S +++
	D	0.276±0.018	Low mycelium S +++

L – cultures in light.
D – cultures in darkness.
S – sporulation.

In a similar way, the amount of dry weight of the mycelium of *P. isariaeforme* remains constant in cultures grown in light at concentrations of CO_2 from 0.07 to 1.5 per cent, and is equal to that produced by control cultures. An increase of concentration to 3.0 per cent results in an increase of dry weight from 330 to 458 mg, but an increase of concentration to 6.0 per cent does not result in a further increase of dry weight. In cultures grown in darkness at a constant flow of air concentrations of CO_2 exceeding the normal have no effect on the dry weight produced in the whole range of concentrations applied in relation to control cultures.

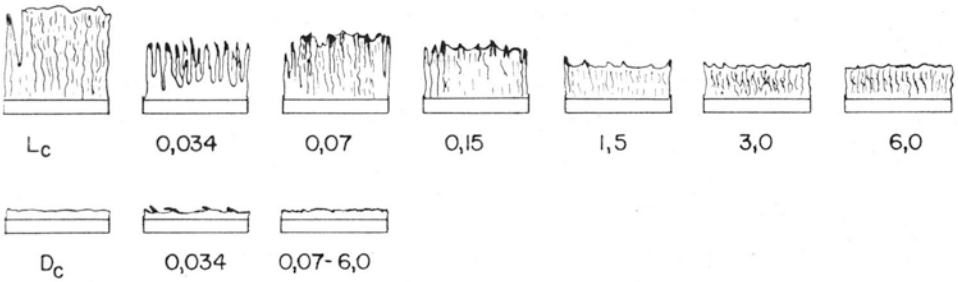

Fig. 4. Schematized shape of coremia and mycelium of *Penicillium isariaeforme* as dependent on the concentration of CO_2. Above cultures grown in light, L_c — control culture grown in light without air flow. Below: cultures grown in darkness, D_c — control culture grown in darknest without air flow.

On the other hand, increased concentrations of CO_2 in the flowing air exert clear morphological effects. Even a concentration of 0.07 per cent, which is only twice as much as in the normal air, results in the coremia becoming scarcer and mostly fused one with another. Further increase of concentration of CO_2, above 1.5 per cent, results in all coremia being fused together and an elongation mycelium consisting of parallel hyphae. Concentrations of 3 and 6 per cent result in a complete inhibition of formation of coremia. The aerial mycelium is shorter than in control cultures and consists of rather chaotically disposed hyphae. In cultures grown in darkness and aerated by a flow of air containing CO_2 at various concentrations from 0.07 to 6.0 per cent formation of coremial primordia was not seen. In all the cases dealt with a low aerial mycelium was observed, as usual in darkness.

Sporulation does not seem to depend on the concentration of CO_2. In both present species, it was not disturbed at higher concentrations of CO_2, and the spores produced could develop normally.

III. Uptake of gaseous CO_2 by the mycelia of *P. claviforme* and *P. isariaeforme*

Previous work (Piskorz 1968, 1970) allowed to suppose that both in *P. claviforme* and *P. isariaeforme* the uptake of atmospheric CO_2 may occur. It seemed therefore advisable to make some preliminary investigations using C^{14}.

A concentration of 5 per cent CO_2 was chosen in experiments made both in light and in darkness. The cultures dealt with were 3.5 days old in the case of *P. claviforme* and 4 days old in the case of *P. isariaeforme*. Cultures were grown either in light or in darkness on Petri dishes 10 cm in diameter, and then placed for two hours in the plexiglass chambers previously described. The mycelia were then weighed and the material extracted in the way described above. In Table 3 are indicated the products of metabolism marked by C^{14} found in the mycelia of *P. claviforme*. The data obtained suggest an ability to take up carbon dioxide both in light and darkness from the atmosphere. The highest radioactivity occurs in Fraction I com-

posed mainly of organic acids, and the lowest radioactivity, in Fraction II composed mainly of lipids. The general radioactivity, and also that calculated for 1 g of fresh mass of particular fractions, seem to be slightly higher in cultures grown in light than in those grown in darkness.

Table 3

Absorption of carbon dioxide by mycelia of *Penicillium claviforme*

Material	Fresh weight in g	Radioactivity per 10 minutes				
		Fraction I	Fraction II	Fraction III	Total per 1 g	%
Mycelium 3.5	exp. 1	1.2×10^6	2.4×10^4	7.8×10^5	4.4×10^5	134.2
days old	4.5	2.6×10^5/1g	5.4×10^3/1g	1.7×10^5/1g		
grown in	exp. 2	1.7×10^6	1.6×10^4	11.5×10^5	5.9×10^5	
light	4.9	3.5×10^5/1g	3.4×10^3/1g	2.4×10^5/1g		
Mycelum 3.5	exp. 1	1.2×10^6	4.2×10^4	5.8×10^5	3.2×10^5	100.0
days old	5.4	2.1×10^5/1g	7.3×10^3/1g	1.1×10^5/1g		
grown in	exp. 2	1.4×10^6	4.9×10^4	7.7×10^5	4.4×10^5	
darkness	5.0	2.8×10^5/1g	9.9×10^3/1g	1.6×10^5/1g		

In Table 4 are presented measurements of radioactivity in metabolic products in the mycelia of *P. isariaeforme*. This species is also able to take up gaseous CO_2 both in light and darkness. The radioactivity of the fraction of organic acids is the highest, that of lipids, the lowest. In all fractions, radioactivity is higher if cultures were grown in light. The greatest differences occur in Fraction I, composed mainly of organic acids. The results obtained allow to suppose that in *P. isariaeforme* carbon dioxide uptake is more intense in light.

Table 4

Absorption of carbon dioxide by mycelia of *Penicillium isariaeforme*

Material	Fresh weight in g	Radioactivity per 10 minutes				
		Fraction I	Fraction II	Fraction III	Total per 1 g	%
Mycelium 4	exp. 1	4.7×10^6	1.9×10^5	1.2×10^6	1.6×10^6	151.2
days old	3.8	12.0×10^5/1g	5.2×10^4/1g	3.2×10^5/1g		
grown in	exp. 2	3.1×10^6	1.2×10^5	1.2×10^6	1.3×10^6	
light	3.3	9.4×10^5/1g	6.6×10^4/1g	3.7×10^5/1g		
Mycelium 4	exp. 1	1.7×10^6	1.3×10^5	1.2×10^6	0.85×10^6	100.0
days old	3.5	4.8×10^5/1g	3.5×10^4/1g	3.4×10^5/1g		
grown in	exp. 2	2.2×10^6	1.3×10^5	2.1×10^6	1.0×10^6	
darkness	4.1	5.3×10^5/1g	3.2×10^4/1g	5.1×10^5/1g		

IV CONCLUSIONS

It appeared that in the development of the representatives of the *Penicillium clavigerum* section, namely *P. claviforme* and *P. isariaeforme*, there occur four phases characterized by different reactions to the composition of air and intensity of light. These are the following:

1. Formation of basal mycelium,
2. Formation of coremial primordia,
3. Elongation of coremia,
4. Sporulation.

Formation of the basal mycelium lasts three days in *P. claviforme* and five days in *P. isariaeforme*. At this time, no morphological differences were seen in the cells of mycelium developing in light and those in darkness. The rate of growth of mycelium as established by Ryan's method (Ryan 1943; Piskorz 1967a) and the rate of increase of dry weight in light and in darkness are identical.

In the second phase, in the already well developed basal mycelium there appear coremial primordia. Microscope analysis did not reveal any differences in the morphology of the cells initiating the primordia. However, both cytological and morphological differences may appear if more detailed studies were made. The initiation of coremia seems to depend on three factors, namely light, temperature, and composition of air surrounding the mycelium.

In *P. claviforme*, numerous coremial primordia are formed in darkness. The number of primordia per unit area diminishes following illumination by light of 100 to 2000 lx intensity (Piskorz 1967), i.e. in the range of intensities causing very pronounced elongation. Formation of coremial primordia clearly depends on temperature (Piskorz 1967 b), being most intense at about 20°C. The flow of air has an inhibiting effect: with the increasing rate of flow, the number of primordia approaches that obtained in darkness (Piskorz 1970). Increased amount of carbon dioxide in the flowing air inhibited the formation of coremia, but throughout the wide range of concentrations applied the number of coremia remained practically constant.

In *P. isariaeforme*, formation of coremial primordia also depends on the conditions of culture. If grown on Petri dishes with access of air, numerous primordia are formed in light, and none appear in darkness. In Erlenmayer flasks tightly closed with cotton stoppers, no primordia are formed either in darkness or in light. Adequate aeration of cultures causes formation of coremial primordia both in light and in darkness. Formation of coremia on Petri dishes in light permits to suppose that in these conditions there occur in the mycelium processes changing substantially the composition of air and causing effects similar to those of aeration. Addition of carbon dioxide to the flowing air, beginning with a concentration of 0.07 per cent, results in diminished densities of coremial primordia.

It seems therefore that the initiation of coremia is a process, or rather a set of processes, essentially not depending on the action of light. Initiation begins only if the composition of air is adequate, namely if the concentration of carbon

dioxide or of "factor x" in the surrounding atmosphere is sufficiently diminished. Analogous results were obtained by Tschierpe (1959) and Bartnicki-Garcia (1964), who showed that carbon dioxide concentration of 0.1% is optimum for the formation of fructifications of mushrooms.

It follows that critical concentrations of carbon dioxide allowing initiation of coremia differ from species to species.

It should be now considered, why the concentration of carbon dioxide increases in light both in *P. claviforme* and *P. isariaeforme*. If it be assumed that carbon dioxide really determines initiation of coremia, it would follow that light may result in increasing amounts of carbon dioxide in culture vessels only in one way, namely by making the respiration more intense. The action of light on respiration has not been hitherto studied in the *Penicillium clavigerum* section. However, some data concerning *Aspergillus giganteus* mut. *alba* were established (Zurzycka and Pasiut 1970).

The phase of formation of coremial primordia is immediately followed by the phase of elongation of coremia. This is connected with the appearance of parts of mycelium capable of intense divisions, called elongation mycelium. This capability persists in *P. claviforme* for two or three days, and in *P. isariaeforme*, almost till the completion of the development. Elongation of coremia — a process much less dependent on temperature than initiation of coremial primordia (Piskorz 1967) — seems to be determined both by the action of light and by the composition of the air surrounding the mycelium. Both species revealed the same dependence on the intensity of light, though elongation coefficients were different.

Optimum illumination intensities lie in the range of 30 to 2000 lx. At higher intensities, in a similar way as in *Aspergillus giganteus* mut. *alba* (Zurzycka 1963), elongation becomes gradually inhibited. Elongation of coremia never occurs in darkness. It occurs in light only at adequate carbon dioxide concentrations. Experiments on aeration and applying air flow with increased amount of carbon dioxide revealed that the carbon dioxide concentration is very much higher than that necessary for the initiation of coremial primordia. In order to obtain the optimum course of elongation, careful matching of both factors, light intensity and carbon dioxide concentration, is necessary. Even slight deviations from optimum conditions result in disturbance of elongation and of dry weight production. In this respect, *P. isariaeforme* is much more sensitive. In this species, there occurs a phenomenon seldom seen in Fungi, namely a distinct increase of dry weight in light. The increase of the amount of carbon in the mycelia grown in light is not connected with the higher rate of glucose uptake (Piskorz 1968). It may be explained either by a change in the respiratory mechanism or conceived as connected with the uptake of carbon dioxide from the air, similar to that found by Cantino et al. (1958) in *Blastocladiella emersonii*. Experiments using C^{14} partly confirmed these suggestions, but the subject should be investigated further.

Sporulation of the above species also depends, as well known (Ingold and Nawaz 1967; Cochrane 1958; Hawker 1950), on the composition of air. A factor

causing earlier appearance of sporulation and its greater intensity is an adequate access of oxygen. In *P. clavigerum*, *P. claviforme* and *P. isariaeforme*, sporulation does not depend either on illumination or on carbon dioxide concentration.

The above results allow to believe that factors determining morphogenesis of *P. claviforme* and *P. isariaeforme* are light and carbon dioxide. Experiments applying different rates of air flow, concentrations of carbon dioxide, and illumination intensities, allowed to establish a method of an almost arbitrary control of the shape of these organisms.

The present study closes a series of experiments on the physiological effects of light and carbon dioxide on the moulds from the *Penicillium clavigerum* section. The results obtained allow to believe that the physiological changes observed may be explained by carboxylation processes.

SUMMARY OF RESULTS

The present investigations concerned the action of various concentrations of carbon dioxide on the morphology of coremia and dry mass of the mycelium.

In *P. claviforme*, increasing concentration of carbon dioxide results in morphological changes of the coremia. The effect of low concentrations in the range of 0.07 to 1.5 per cent is an increase of the mean height of coremia in cultures grown in light. Higher concentrations, between 1.5 and 6.0 per cent, result in diminishing height of coremia, whose shape becomes different. Cultures grown in darkness seem to be less sensitive to the increased concentrations of carbon dioxide. Increased concentrations of carbon dioxide result in diminished densities of coremia. Dry weight of the mycelium is essentially independent of the concentration of carbon dioxide.

In *P. isariaeforme*, the effects of the increased concentration of carbon dioxide in the air surrounding the mycelium are important. An increase of concentration to 0.07 per cent already results in a clearly diminished number of coremia. Further increase from 1.5 to 6.0 per cent causes a complete inhibition of formation of coremial primordia, and a high aeration mycelium appears. At low concentrations, the dry weight produced remains constant; however, excess dry weight appears in cultures grown in light at concentrations from 3.0 to 6.0 per cent.

The results of the experiments applying $C^{14}O_2$ suggested that the present moulds are able to assimilate fairly high amounts of carbon dioxide in cultures grown both in light and in darkness. It seemed that in *P. isariaeforme* assimilation in light was more intense.

Radioactivity of the organic acids fractions was the highest, that of lipid fraction the lowest, and that of protein fraction, intermediate.

The author wishes to express her deep gratitude to Docent A. Koj for enabling her to carry out experiments and to Mgr A. Dubin for the help in experiments.

REFERENCES

Aleksiejewskij R., Golc A., Musakin A., 1954, Analiza ilościowa, PWN, Warszawa.

Bartnicki-Garcia S., 1964, Carbon dioxide-dependent morphogenesis in *Arthrobotrys conoides*, Nature 204.

Cantino E. C., Horenstein E. A., 1956, The stimulatory effect of light upon growth and CO_2 fixation in *Blastocaldiella*. I. The SKJ Cycle, Mycol. 48: 777–799.

Cochrane V. W., 1958, Physiology of Fungi, Wiley and Sons Inc. N-J.
Hawker I. E., 1950, Physiology of Fungi, Un v. Lond. Press, London.
Ingold C. T., Nawaz M., 1967, Carbon dioxide and fruiting in *Sphaerobolus*, Ann. of Bot. 31 (122): 352–357.
Lambert E., 1933, Effect of excess carbon dioxide on growing *Mushrooms*, J. of Agricult. Res. 47: 599–608.
Piskorz B., 1967, Investigations on the formation of coremia. I. Action of light on the formation of coremia in *Penicillium isariaeforme*, Acta Soc. Bot. Pol. 36 (1): 123–131.
Piskorz B., 1967, Investigations on the action of light on the growth and development of *Penicillium claviforme* Bainier, Acta Soc. Bot. Pol. 36 (4): 677–698.
Piskorz B., 1968, Comparative investigations on the development of Fungi from the *Penicillium clavigerum* section, Acta Biol. Cracov. 11: 159–178.
Piskorz B., 1970, Comparative investigations on the development of species from the *Penicillium clavigerum* section. II. Action of aeration on the morphology of the mycelium and coremia, Acta Soc. Bot. Pol. 39 (4): 711–732.
Rast D., Bachofen R., 1965, CO_2 Fixirung in *Agaricus bisporus*, Verh. Schweiz. Nat. Forsch. Ges. 125–129.
Rippel A., Bortles H., 1927, Vorläufige Versuche über die allgemeine Bedeutung der Kohlensäure für die Pflanzenzelle (Vers. an *Aspergillus niger*), Biochem. Z. 184: 237–244.
Rippel A., Heilmann W., 1930, Quantitative Untersuchungen über die Wirkung von Kohlensäure auf Heterotrophen, Arch. Mikrobiol. 1: 119–136.
Rockwell G. E., Highberger J. H. 1927, The necessity of carbon dioxide for the growth of Bacteria, Yeast and Molds. J. Inf. Dis. 40: 438–446.
Ryan F. J., Beadle W., Tatum E. L., 1943, The tube method of measuring the growth rate of *Neurospora*, Am. J. Bot. 20: 784–799.
Le Roux P., 1962, Metabolisme des acides organiques dans les carpophores d'*Agaricus campestris*, Mushroom Sci. 5: 525–539.
Le Roux P., 1966, Quelques aspects du metabolisme respiratoire du carpophore d'*Agaricus campestris* var. *bisporus* (Lge.), Thèses Fac. Science Univ. Paris.
Tschierpe H. J., 1959, Die Bedeutung des Kohlendioxyd für den Kulturchampignon, Gartenbauwissenschaft 24: 18–75.
Tschierpe H. J., Sinden J. W., 1964, Weitere Untersuchungen über die Bedeutung von Kohlendioxyd für die Fruktifikation des Kulturchampignons *Agaricus campestris* var. *bisporus* (L.) Lge., Arch. Mikrobiol. 49: 405–425.
Wherli M., Rast D., 1967, Stoffwechsel von $C^{14}O_2$ in Plektenchymsschnitten von *Agaricus bisporus*, Z. Pflanzenphysiol. 56: 305–324.
Wherry W., Ervin D. M., 1918, The necessity of carbon dioxide for growth of *B. tuberculosis*, J. Inf. Dis. 22: 194–197.
Zurzycka A., 1963, Studies on the photomorphogenesis in *Aspergillus giganteus mut. alba*. II, Acta Biol. Cracov. 66: 103–113.
Zurzycka A., Pasiut H., 1971, Study of endogenous respiration in the mycelium of *Aspergillus giganteus* mut. *alba*, Bull. Acadèmie Polonaise Série des sci. biol. Cl. II vol. XIX, 11.

Badania porównawcze nad rozwojem grzybów z sekcji Penicillium clavigerum

III. Wpływ CO_2 na morfologię koremiów i suchą masę grzybni

Streszczenie

Przedmiotem obecnych badań był wpływ różnych stężeń CO_2 na morfologię koremiów i suchą masę grzybni.

U *P. claviforme* ze wzrostem stężenia CO_2 zmienia się morfologia koremium. Niskie stężenie tego gazu w granicach od 0.07 do 1.5% powoduje zwiększenie się średniej wysokości koremiów w kulturach hodowanych na świetle. Podniesienie stężenia od 1.5 do 6.0% działa hamująco na wysokość koremiów, zmieniając także ich kształt. Mniej wrażliwe na zwiększoną zawartość CO_2 w przepływającym powietrzu wydają się być kultury hodowane w ciemności. Zwiększone stężenie CO_2 działa raczej hamująco na liczbę tworzących się koremiów. Sucha masa grzybni w zasadzie nie zależy od stężenia CO_2.

P. isariaeforme reaguje silnie morfotycznie na zwiększoną zawartość CO_2 w powietrzu otaczającym grzybnię. Już podniesienie stężenia do wartości 0.07% działa wyraźnie hamująco na liczbę tworzących się koremiów. Dalszy wzrost stężenia CO_2 od 1.5 fo 6.0% powoduje całkowite zahamowanie tworzenia zawiązków koremialnych, tak, że w tych warunkach obserwujemy wysoką grzybnię powietrzną. Sucha masa grzybni w niskich stężeniach nie zależy od zawartości CO_2. Nadwyżkę suchej masy obserwowano u kultur hodowanych na świetle i przy stężeniu CO_2 3.0–6.0%.

Wyniki doświadczeń sugerują, że grzyby te posiadają zdolność przyswajania dość znacznych ilości CO_2 tak w hodowlach na świetle, jak i w ciemności. Natomiast *P. isariaeforme* wydaje się posiadać większą zdolność wiązania tego związku w hodowlach prowadzonych na świetle. Najwyższą radioaktywność posiadała frakcja kwasów organicznych, niższą frakcja białkowa i najniższą frakcja lipidowa.