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Comparative investigations on the development of species from the *Penicillium clavigerum* section

II. Action of aeration on the morphology of the mycelium and coremia

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In a previous paper (P i s k o r z 1968) optimum growth conditions of three representatives of the section *Penicillium clavigerum* — *P. clavigerum, P. claviforme, P. isariaeforme* — were determined; optimum range of temperature, light intensity and time of exposure as well as optimum concentration of glucose in the nutrient solution were established. An elementary analysis of the mycelia developing in light and darkness was carried out for all species. Differences in dry weight and content of the essential elements in the mycelia of *P. isariaeforme* depending on culture conditions were determined. A temporary increase of dry weight of mycelia grown in light and the concomitant slight increase of carbon content were also found to occur in *P. claviforme.* As the amount of glucose taken up from the nutrient solution is almost the same in light and darkness, the observed differences are probably due either to changes in the respiration rate, or in its mechanism, or to changes connected with carboxylation processes occurring in certain conditions at higher light intensities. Preliminary tests seemed to indicate that the composition of the surrounding air exerted a marked action on the dry weight of the mycelia. Thus, the aim of the present paper was to examine the action of aeration of culture vessels on the dry weight of the mycelia and the morphology of fruit bodies in the above mentioned species of the section of *P. clavigerum.*

I. MATERIAL

Three species of moulds from the *Penicillium clavigerum* section, namely *P. clavigerum, P. claviforme, P. isariaeforme* obtained from the Centraalbureau voor Schimmelcultures in Baarn, were investigated. Stock cultures were kept on an agar nutrient solution, the composition of which was previously given (P i s k o r z 1967). The experiments were carried

out in Erlenmayer flasks containing 30 ml nutrient solution. Cultures were inoculated and allowed to germinate for 24 hours (*P. clavigerum* and *P. claviforme*) and 36 hours (*P. isariaeforme*) before the experiment started.

II. CULTURE CONDITIONS

II.1. Apparatus for aeration

A prototype apparatus called 12 channel airflowmeter was used for the investigations on the action of aeration on dry weight and morphology of the mycelia. The apparatus consisted of 3 main parts: a compressor, type Wan CF 1960, a set of 12 rheometers, and a culture arrangement. Air of normal composition (21% oxygen determined by Orsat's method, 0,034% carbon dioxide, determined chemically) compressed by the compressor, passed through a thick walled tube and through a pressure sta-

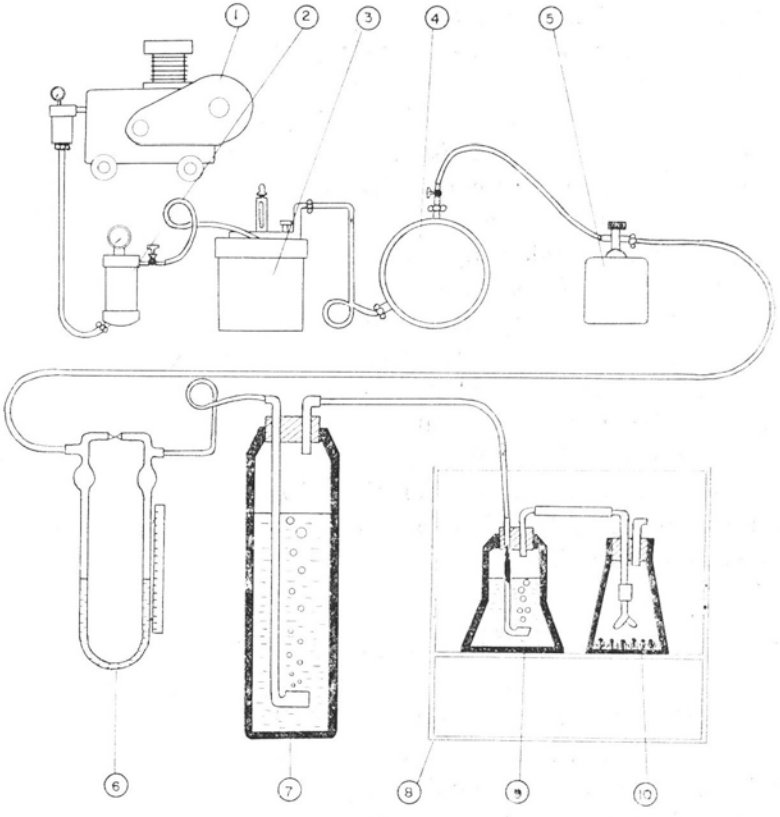

Fig. 1. Apparatus for measurements of the air flow rate (description in text).

bilizer (2) to an ultrathermostat (3), where it was warmed to 25°C. The warmed air passed through a compensation chamber (4) equipped with 12 taps connected with rubber tubes which directed the air to 12 small compensation chambers (5). Each of them could be individually regulated and set for a required rate of air flow. From these chambers the air passed to rheometers filled with a solution of eosin in water. Each rheometer was equipped with a set of capillaries of various diameters permitting to apply various rates of air flow. The air leaving the rheometer at an already established speed was directed to wash bottles filled with distilled water (7), where it was humidified and finally directed to culture flasks (8) placed in a light thermostat. A rotameter type TG — 300 connected with the flasks was used to measure the air flow rate. The highest speed obtained in the apparatus was 250 l/h. The scheme of this arrangement is presented in Fig. 1.

II.2. Culture flasks

Fungi were cultivated in 300 ml Erlenmayer flasks with tightly fitting glass stoppers, each with two glass tubes. Air was introduced into a flask by the longer one which ended in a double discharge tube slightly bent upward (Fig. 2) to improve air exchange in the flasks. The shorter

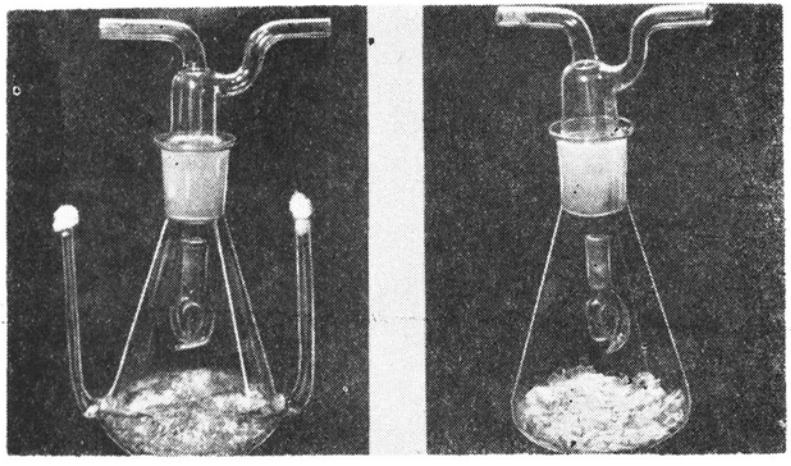

Fig. 2. Culture flasks.

tube was used to carry away the air. In order to check the efficiency of gaseous mixing in the flask smoke tests were made (T s c h i e r p e 1959). It appeared that 90 l/h is a satisfactory and uniform air flow rate. At the air flow rate 25 l/h and below the results were charged with an error due to incomplete mixing of air in the flask.

II.3. Rate of air flow

The following speeds of air flow were applied: 3, 5, 10, 25, 90, 250 l/h. As already mentioned above, the speed of air flow was measured at the outlet of the culture flask by means of a rotameter (type TG — 300) and was regulated by the dimensions of the outlet of a small compensation chamber and the length of the capillary in the rheometer.

The speed of the air flow changed slightly during twenty four hours (about 3 l/h) but only at high flow speeds; therefore it was regulated once a day according to the measurements read on the rheometer.

II.4. Changes in the water vapour content in the flowing air; evaporation of the liquid nutrient solution

Preliminary experiments were performed to establish the changes of water vapour content and air temperature resulting from the aeration of cultures. A plexiglass chamber was mounted in the thermostat instead of the flasks. The chamber was connected to a thermograph (type TZ—8MZM Kraków). It appeared that, excepted temporary disturbances at switching on and off the apparature, both the temperature and air humidity remained at the same level for a long time (24 hours). An intensive evaporation of the nutrient solution might be a side effect of the air flow through the culture flasks. The measurements showed that the flasks containing nutrient solution lose about 0,2% water during a week when not exposed to aeration. Inoculated flasks lose 1,7—2,0% water whereas inoculated aerated (25 l/h) flasks lose about 1,8% water per week if one set of the wash bottles is placed outside and the other inside the thermostat. Above 50 l/h the water loss due to evaporation increases significantly; therefore at high flow speeds modified culture flasks with two side tubes through which the water loss could be replaced were used; in this way concentration of the nutrient solution was stabilized.

II.5. Air sterilization

No additional treatment was applied to sterilize the air flowing through the apparatus. The wash bottles used for humidifying the air were equipped with densely porous glass plates performing a double role: 1° they turned the flowing air into a stream of small bubbles permiting to saturate uniformly the air with water vapour, 2° they retained all the microorganisms present in the air. From time to time these plates were cleaned in chromic acid mixture. This way of air sterilization proved to be quite satisfactory, as no bacterial or fungal infections were observed even in control flasks containing a not sterile nutrient solution.

II.6. Withdrawing carbon dioxide from air

In order to obtain air devoid of CO_2, necessary in the present experiments, wash bottles with 20% KOH were placed before the wash bottles filled with water. Their length and capacity were chosen so that the path of the flowing air was sufficiently long to assure the complete absorption of carbon dioxide even for the highest speeds of the air flow (90 l/h). The efficiency of absorption was checked by means of an URAS and in a set of wash bottles filled with barium hydroxide placed the end of the aeration system. Calculations revealed that it was sufficient to change daily the KOH solution in the wash bottles.

II.7. Evaluation of results

After a growth period lasting for 7 day for *P. clavigerum* and *P. claviforme* and 12 days for *P. isariaeforme*, cultures were described and photographed and the dry weights determined. More exact measurements of coremia were performed for *P. claviforme*.

III. ACTION OF THE RATE OF AIR FLOW ON DRY WEIGHT, MORPHOLOGY OF COREMIA AND SPORULATION

After a preliminary period of incubation the moulds were cultivated in constant air flow during the whole period of each species (7 days — *P. clavigerum* and *P. claviforme* and 12 days — *P. isariaeforme*). The effect of three rates of air flow (25, 90, 250 l/h) on the growth and development in darkness of the species under study was examined. Only *P. isariaeforme* was cultivated in light at lower rates of flow. The results of this series of experiments are presented in tables 1, 2, 3 and illustrated by photographs.

Table 1

Action of air flow rate on the dry weights of the mycelia in *P. clavigerum*

Rate of flow in l/h	Dry weight in g
Control L	0.439 ± 0.007
Control D	0.443 ± 0.005
25 L	0.447 ± 0.027
90 L	0.470 ± 0.018
90 D	0.537 ± 0.017
250 L	0.368 ± 0.017

L — cultures in light
D — cultures in darkness

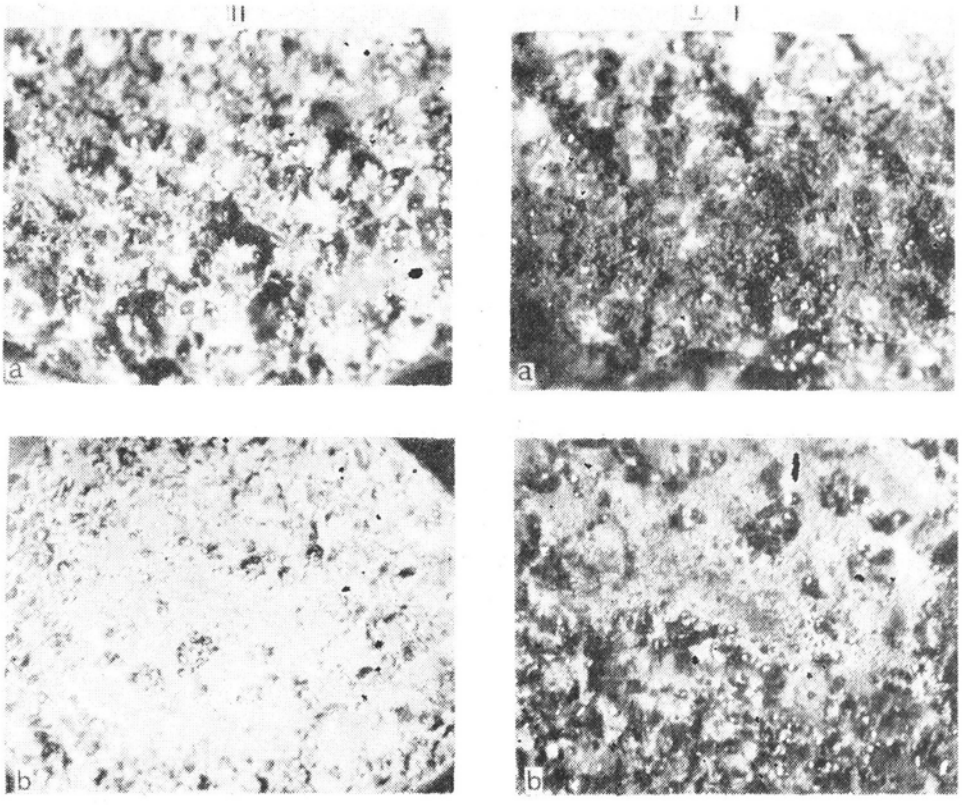

Fig. 3. Action of the rate of air flow on the shape of coremia of *P. clavigerum*; I — light cultures, II — dark cultures, *a* — control cultures, *b* — cultures aerated at 90 l/h.

The growth of *P. clavigerum* (Table 1 and Fig. 3) is almost identical in aerated conditions and in controls without aeration.

The highest speed of air flow, i.e. 250 l/h, causes a slight decrease of dry weight; aerated cultures and those growing in darkness manifest a small excess of dry weight. These changes, however, are not associated with any morphological changes of the mycelium. Sporulation proceeds in a normal way: in aerated cultures, however, it starts 1—2 days earlier than in controls.

Aeration exerts a marked influence upon the development and growth of the cultures of *P. claviforme*, as shown by the results of experiments presented in Table 2 and Figure 4. Air flow does not modify the dry weight when this organism is cultivated in light; in darkness however aeration leads to a considerable increase of dry weight; the cause of this effect is as yet unknown. Both in light and dark cultures the shape and the number of coremia formed per surface unit area of the mycelium depend, to

Table 2

Action of the rate of air flow on dry weight of the mycelium and shape of coremia in *P. claviforme*

Rate of flow in l/h	Dry weight in g	Number of coremia per cm²	Height of coremia in mm	Length of foot in mm	Breadth of foot in mm top	Breadth of foot in mm base	Length of head in mm	Breadth of head in mm
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
25 L	0.585 ± 0.013	11 ± 3	9.20	5.40 ± 0.02	0.53 ± 0.04	1.39 ± 0.07	3.80 ± 0.04	0.66 ± 0.04
90 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
90 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 ± 0.03
250 L	0.583 ± 0.030	49 ± 7	3.02	1.86 ± 0.04	0.61 ± 0.03	1.40 ± 0.09	1.16 ± 0.04	1.12 ± 0.05

L — cultures in light
D — cultures in darkness

Table 3

Action of the rate of air flow on dry weight of the mycelium of *P. isariaeforme*

Rate of flow in l/h	Dry weight in g
Control L	0.406 ± 0.008
Control D	0.306 ± 0.010
3 L	0.283 ± 0.017
5 L	0.321 ± 0.018
10 L	0.296 ± 0.013
25 L	0.234 ± 0.006
90 L	0.292 ± 0.009
90 D	0.324 ± 0.010
250 L	0.260 ± 0.014

L — cultures in light
D — cultures in darkness

a great extent, on the rate of air flow (Fig. 4 and 5). The number of coremia per unit area increases with the rate of air flow; and at 250 l/h it attains 49/cm², thus approaching the value of 64/cm² obtained in this species in dark cultures. Aeration of cultures grown in permanent dark-

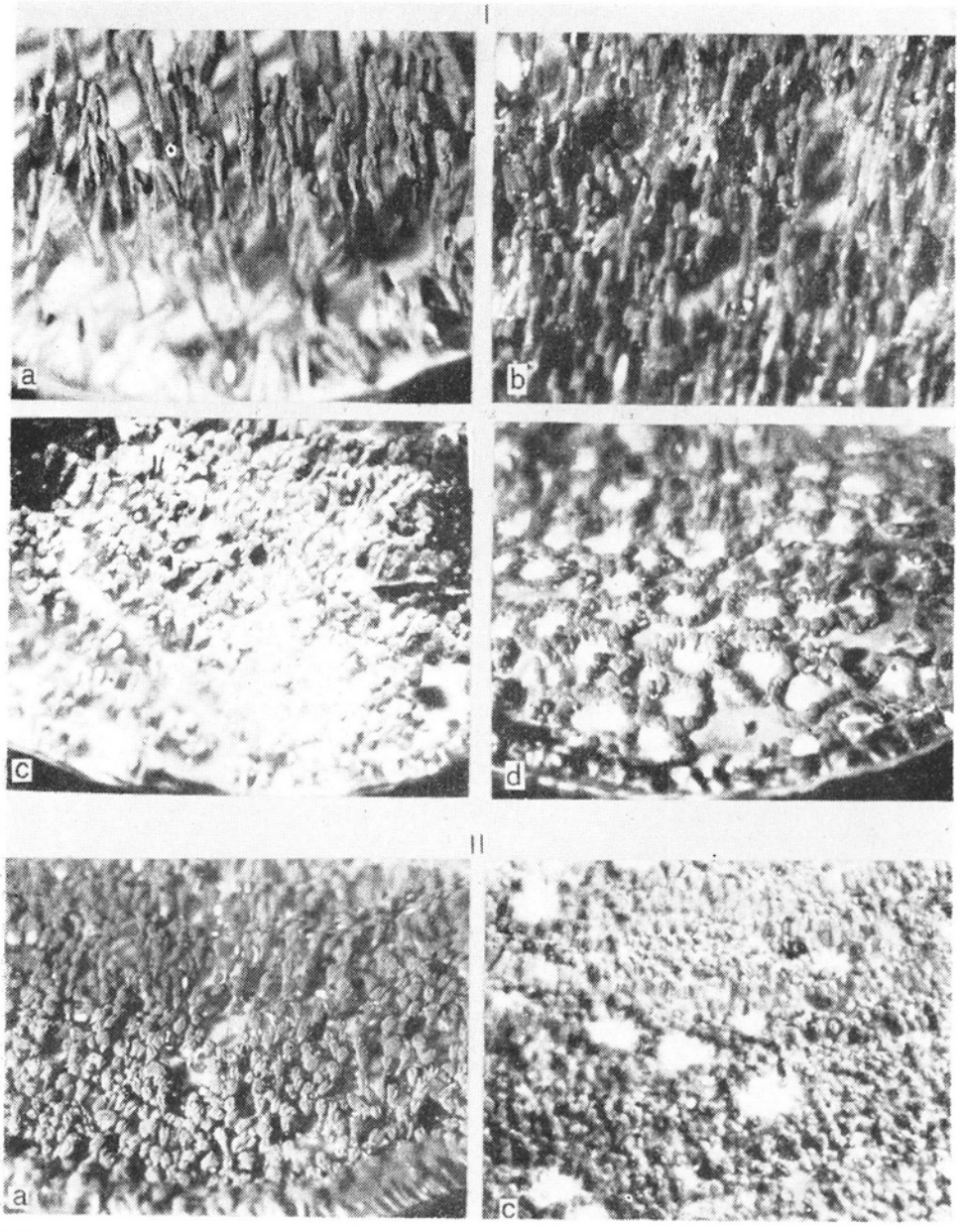

Fig. 4. Action of the rate of air flow on the shape of coremia of *P. claviforme;* I — light cultures, II — dark cultures, *a* — control cultures, *b* — cultures aerated 25 l/h, *c* — cultures aerated 90 l/h, *d* — cultures aerated 250 l/h.

ness does not modify the number of coremia and only slightly changes their shape.

Cultures grown in light, on the other hand, even at relatively low flow rates (25 l/h) show a great morphogenetic effect consisting in the

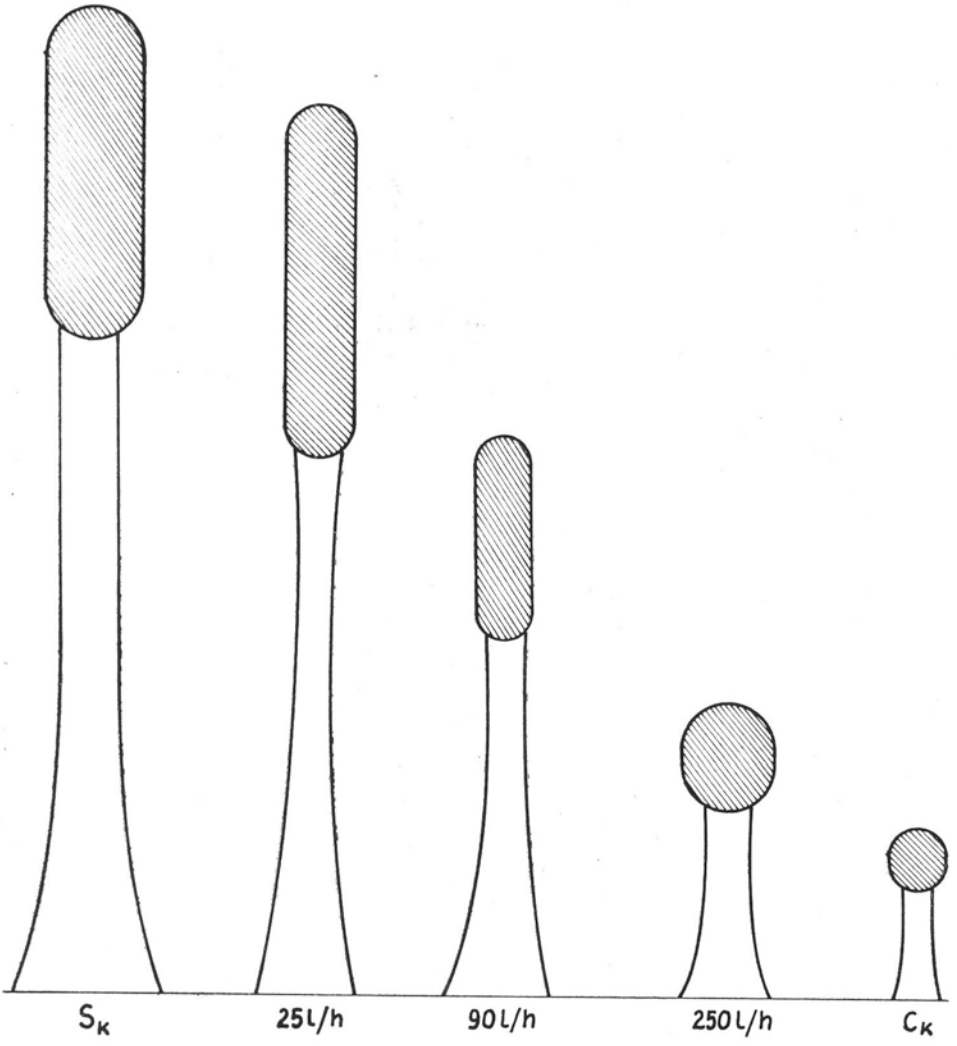

Fig. 5. Shape of coremia of *P. claviforme* as dependent on the rate of air flow.

modification of the apical part of the coremium — the coremial head. With the increase of speed of the air flow the coremial head becomes more and more spherical approaching the shape obtained in darkness. The height of coremia also decreases. The highest flow rate induces changes making coremia from light cultures similar to those from cultures grown in darkness. The coremial heads are almost round and their heights are equal to those of the heads from dark aerated cultures.

In Table 3, Figures 6 and 7, are given the results concerning the action of aeration on the growth and development of *P. isariaeforme*. In this species, excess dry weight of the mycelium (P i s k o r z 1967 and 1968) was established in light cultures. In the hitherto applied conditions the

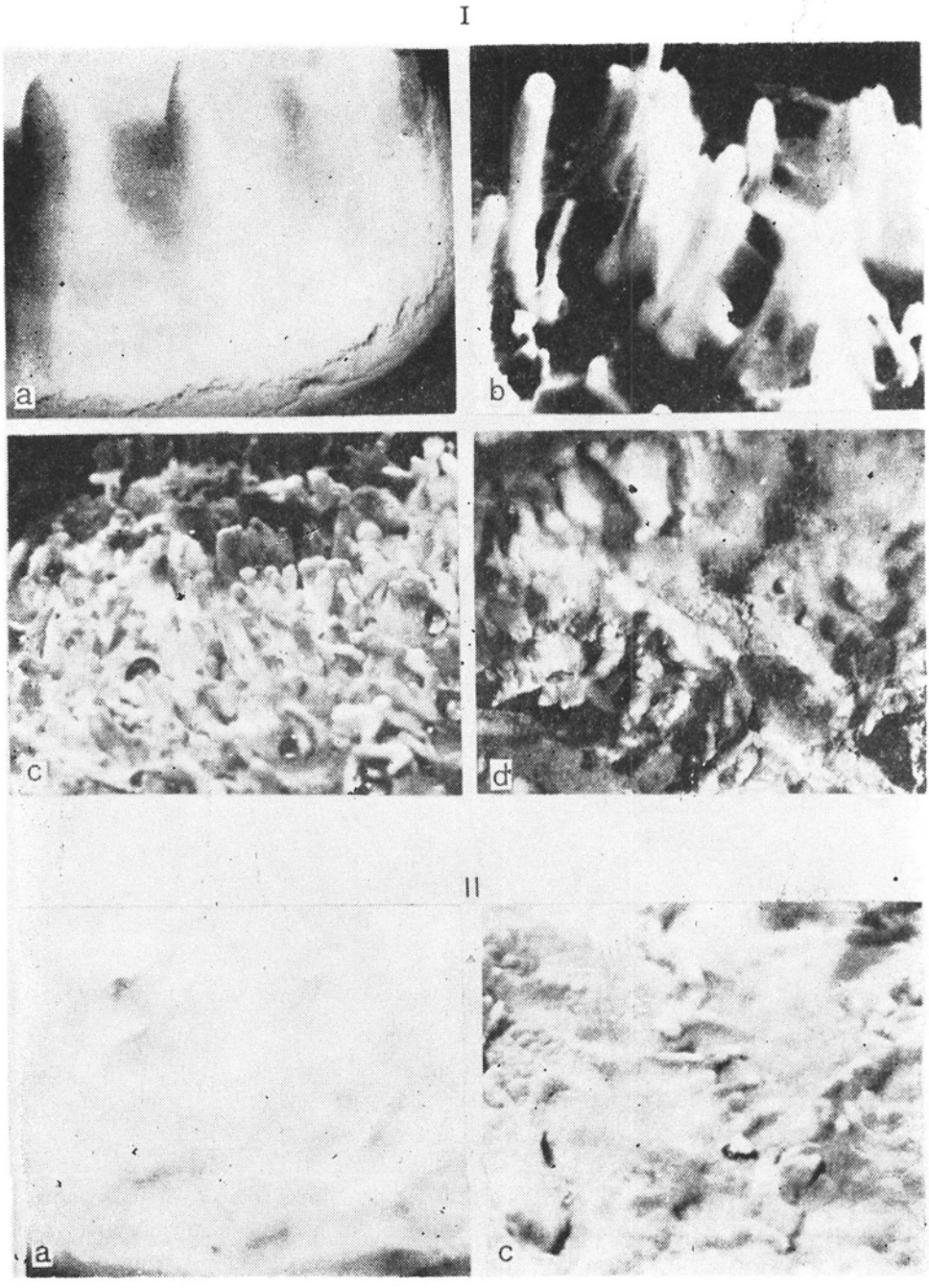

Fig. 6. Action of the rate of air flow on the shape of coremia of *P. isariaeforme*; I — light cultures, II — dark cultures, *a* — control cultures, *b* — cultures aerated 25 l/h, *c* — cultures aerated 90 l/h, *d* — cultures aerated 250 l/h.

excess attained 30% of dry weight observed in dark cultures. It follows from the present investigations that even the lowest rate of air flow (about 3 l/h) suffices to make away the excess dry weight occurring in not aerated light cultures, so that no differences in dry weight between light and dark cultures are observed.

P. isariaeforme grown in tightly stopped flasks by means of cotton-wool plugs forms a very high aerial mycelium; in optimum light conditions coremia do not develop in a typical way. If aeration of such cultu-

Fig. 7. Shape of coremia of *P. isariaeforme* as dependent on the rate of air flow.

res is poor few coremia are formed but if the flow rate is increased to 25 l/h almost the whole mycelial surface is covered with coremia. Some of them form on their tops a delicate brush of loose mycelium hyphae. Typical coremia are formed at the flow rate of 90 l/h. Their height, however, decreases distinctly. At the rate 250 l/h the formed coremia are typical, they are however very low. Moulds cultivated in darkness at air flow rate 90 l/h form few coremial primordia and even normally developed but low coremia. Thus in this species air flow through culture flasks may replace the action of light in initiating coremial primordia, but is unable to induce elongation of these primordia. Mycelia of *P. isariaeforme* grown in not aerated flasks sporulate very weakly, whereas even the lowest applied rate of air flow already suffices to stimulate sporulation. At the rate 25 l/h the whole surface of the mycelium and coremia sporulate.

IV. ACTION OF THE RATE OF AIR FLOW DEVOID OF CARBON DIOXIDE ON THE DRY WEIGHT OF THE MYCELIUM AND MORPHOLOGY OF COREMIA

The further part of the present paper deals with the results of investigations on the effect of the flow of air devoid of carbon dioxide. Experiments were carried out only for *P. claviforme* and *P. isariaeforme* in light and dark cultures. Two rates of air flow were applied: 25 and 90 l/h. The experimental results are presented in Table 4 and 5, and Figure 8.

Dry weight of cultures of *P. claviforme* aerated with air deprived of CO_2 does not differ from that observed in control cultures i.e. cultures not aerated or aerated with air containing the usual amount of carbon

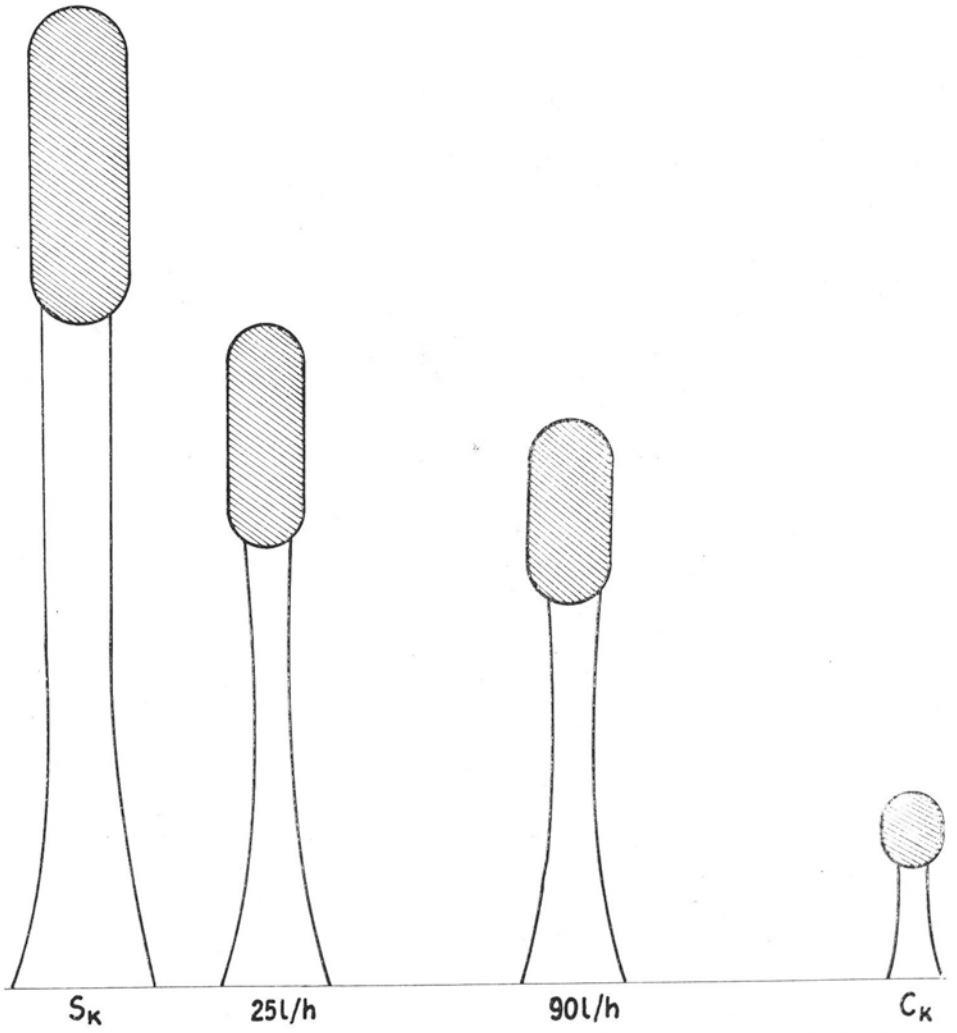

Fig. 8. Shape of coremia of *P. claviforme* as dependent on the rate of air flow — air devoid of CO_2.

dioxide. Withdrawal of carbon dioxide from air does not modify the number of developing coremia; it causes, however, pronounced morphological effects (Fig. 8, Table 4). In cultures aerated at the rate 25 l/h the coremial foot is considerably shortened, and at this rate of flow the head is already almost spherical. Increase of the flow rate to 90 l/h leads to a still greater shortening of the coremial foot and of the head. Therefore, air devoid of CO_2 has a similar effect as the flow of normal air with atmospheric content of carbon dioxide, but morphological changes occur already at lower rates of flow.

In air without CO_2, *P. isariaeforme* produces the same amount of dry mycelial weight as in normal air passing over the cultures. Also in this

Table 4

Action of the rate of air flow devoid of CO_2 on dry weight of the mycelium and shape of coremia in *P. claviforme*

Rate of flow in l/h	Dry weight in g	Number of coremia per cm^2	Height of coremia in mm	Length of foot in mm	Breadth of the foot in mm top	Breadth of the foot in mm base	Length of head in mm	Breadth of head in mm
Control L 25	0.585 ± 0.013	11 ± 3	9.30	5.40 ± 0.02	0.53 ± 0.04	1.39 ± 0.07	3.80 ± 0.04	0.66 ± 0.04
Control L 90	0.562 ± 0.016	24 ± 4	5.84	3.90 ± 0.02	0.54 ± 0.03	1.47 ± 0.08	1.94 ± 0.02	0.76 ± 0.01
25 — CO_2	0.512 ± 0.018	13 ± 3	7.33	4.90 ± 0.04	0.47 ± 0.05	0.99 ± 0.09	2.43 ± 0.03	0.86 ± 0.05
90 — CO_2	0.529 ± 0.006	24 ± 5	5.75	3.95 ± 0.03	0.49 ± 0.03	1.29 ± 0.07	1.80 ± 0.02	0.91 ± 0.03

L — 25 — cultures in light aerated 25 l/h
L — 90 — cultures in light aerated 90 l/h

Table 5

Action of the rate of flow of air devoid of CO_2 on the dry weight of the mycelium of *P. isariaeforme*

Rate of flow in l/h	Dry weight in g
Control L 25	0.234 ± 0.006
Control L 90	0.292 ± 0.009
L 25	0.270 ± 0.009
L 90	0.303 ± 0.012

case the trend of morphological changes, i.e. shortening of the coremia concomitant with the increase of the flow rate, persists; at lower rates, however, changes were observed similar to those shown by cultures aerated with normal air and at a higher speed.

V. RELATION BETWEEN THE TIME OF EXPOSURE TO AIR FLOW AND DEVELOPMENT

In the previous parts of the present study air flow was applied continuously during the whole time of growth; it appeared that this modification of normal growth conditions greatly influenced the growth and development of *P. claviforme* and *P. isariaeforme*. Thus it became necessary to establish whether the effect is caused by continuous aeration, or if there exists a certain phase of sensitivity to aeration. In order to de-

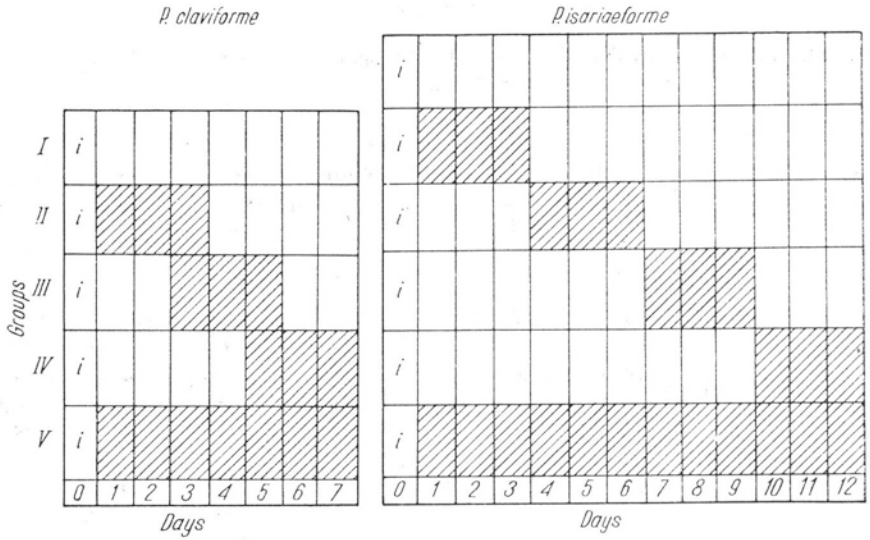

Fig. 9. Scheme of the time of applying the air flow: i — period of preliminary incubation, hatched area — period of application of air flow, free area — period when air flow was not applied.

cide this question flasks with nutrient solutions were prepared and distributed in the required number, to several groups (Scheme Fig. 9). In the case of *P. claviforme,* the first group of cultures was not aerated at all, the second one was aerated between the 1 and 3 day of growth (always at the rate 90 l/h), the third one between the 3 and 5, and the fourth one between the 5 and 7 day. After aeration, cultures were taken out from the apparatus, and allowed to grow in the same conditions excepting aeration till the end of the experiment (7 days). The last group was aerated continuously. Cultures of *P. isariaeforme* were treated in the same way; the periods of aeration, however, were different: the first group was aerated from the 1 till the 3 day, the second from 4 to 6, the third from the 7 to 9 and the fourth was aerated from the 10 till 12. All cultures were grown in light and the flow rate of air devoid of carbon dioxide was 90 l/h. The results obtained for *P. claviforme* are presented in Table 6 and Fig. 10. In very early growth stages of *P. claviforme* (from the 1 till the 3 day) aeration induces an increase of the number of coremia per surface unit area. This number is similar to that obtained for dark not aerated cultures, and identical with that obtained for continuously aerated cultures. Interruption of air flow causes a normal elongation of coremia. Thus the shape of coremia is identical to that observed in parallel not aerated cultures growing in light, but their number is such as if they were kept in darkness for the whole time. Compared with not aerated cultures, the coremia are slightly shorter, but also narrower; presumably this is due to the fact that the same amount of nutrient substances is used in normal

Table 6

Action of the length of time of exposure to aeration with air devoid of CO_2 on dry weight of the mycelium and shape of coremia of *P. claviforme* (light intensity 900 lx)

Time of flow 90 l/h	Dry weight in g	Number of coremia per cm²	Height of coremia in mm	Length of foot in mm	Breadth of the foot in mm top	Breadth of the foot in mm base	Length of head in mm	Breadth of head in mm
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
Flow between 1—3 day	0.759 ± 0.014	24 ± 5	8.49	4.87 ± 0.06	0.56 ± 0.04	1.01 ± 0.05	3.62 ± 0.04	0.75 ± 0.08
Flow between 3—5 day	0.758 ± 0.010	7 ± 3 cor. low	6.34	3.99 ± 0.04	0.82 ± 0.05	1.20 ± 0.09	2.35 ± 0.03	1.39 ± 0.05
		5 ± 1 cor. h	9.55	6.22 ± 0.03	0.61 ± 0.07	1.29 ± 0.09	3.33 ± 0.04	0.64 ± 0.04
Flow between 5—7 day	0.690 ± 0.011	7 ± 3	8.63	5.90 ± 0.02	0.97 ± 0.04	1.48 ± 0.07	2.75 ± 0.04	1.33 ± 0.05
Continuous flow L	0.562 ± 0.015	24 ± 4	5.84	3.90 ± 0.02	0.54 ± 0.04	1.47 ± 0.08	1.94 ± 0.02	0.76 ± 0.01

Cor. low — coremia low
Cor. h — coremia high

Table 7

Action of the length of time of exposure to aeration with air devoid of CO_2 on dry weight of the mycelium of *P. isariaeforme*

Time of aeration 90 l/h	Dry weight in g
Control L	0.406 ± 0.008
Continuous flow L	0.292 ± 0.009
Control D	0.306 ± 0.010
Flow between 1—3 day	0.280 ± 0.014
Flow between 4—6 day	0.340 ± 0.009
Flow between 7—9 day	0.415 ± 0.010
Flow between 10—12 day	0.400 ± 0.012

conditions in light cultures for the formation of 12 (per cm²) coremia and for the formation of 24 coremia in the present case.

In order to obtain morphological effects similar to those observed in continuous aeration, air flow should be applied between the 3 and 5 day of growth. The height and shape of coremia are then very similar to those obtained in continuous air flow. The coremial foot is shorter than in not aerated cultures, but the shape of coremia does not change. The number

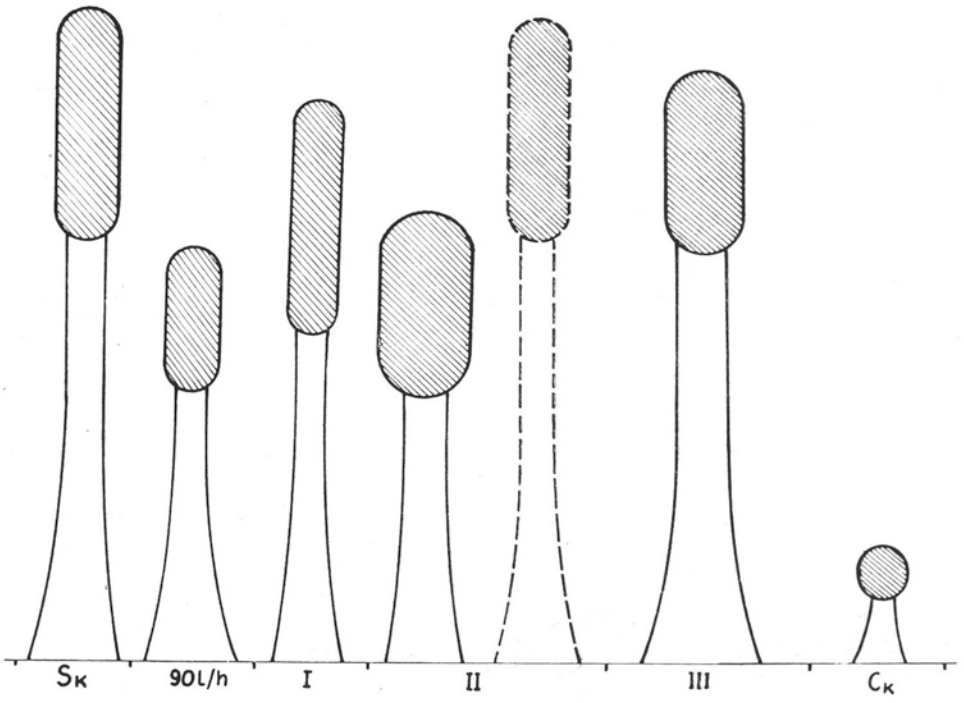

Fig. 10. Shape of coremia of *P. claviforme* as dependent on the time the air flow was applied (90 l/h, air devoid of CO_2).

of coremia remains unchanged, and is thus a characteristic of not aerated cultures grown in light. Aeration applied in this time inhibits the growth of newly formed coremial primordia so that it is only after ceasing aeration that their further growth is resumed. Their height is identical with that of coremia observed in not aerated cultures but, on the other hand, they are much weaker. Inhibition of growth of coremial primordia seems to cause a decrease of the number of coremia when aeration was applied between the 5 and 7 day of growth; let us recall that the experiments were finished on the 7 day and the coremial primordia had no more possibility of development. Thus it appears that it is not necessary to apply continuous aeration. Aeration during the initial days of culture determines the number of coremia per surface unit, and aeration between the 3 and the 5 day produces morphological effects. *Penicillium isariaeforme* cultures aerated between the 1 and 4 day demonstrate a slight but distinct tendency to form coremia (Table 7, Fig. 11), but there is no excess dry weight characteristic of not aerated cultures. Aeration between the 4 and 6 day of culture induces formation of numerous and typical coremia and their strong elongation. Already a slight increase of dry weight occurs. Aeration in the following growth period (7 — 9 day) leads to a further excess of dry weight, which is identical with that in not aerated controls.

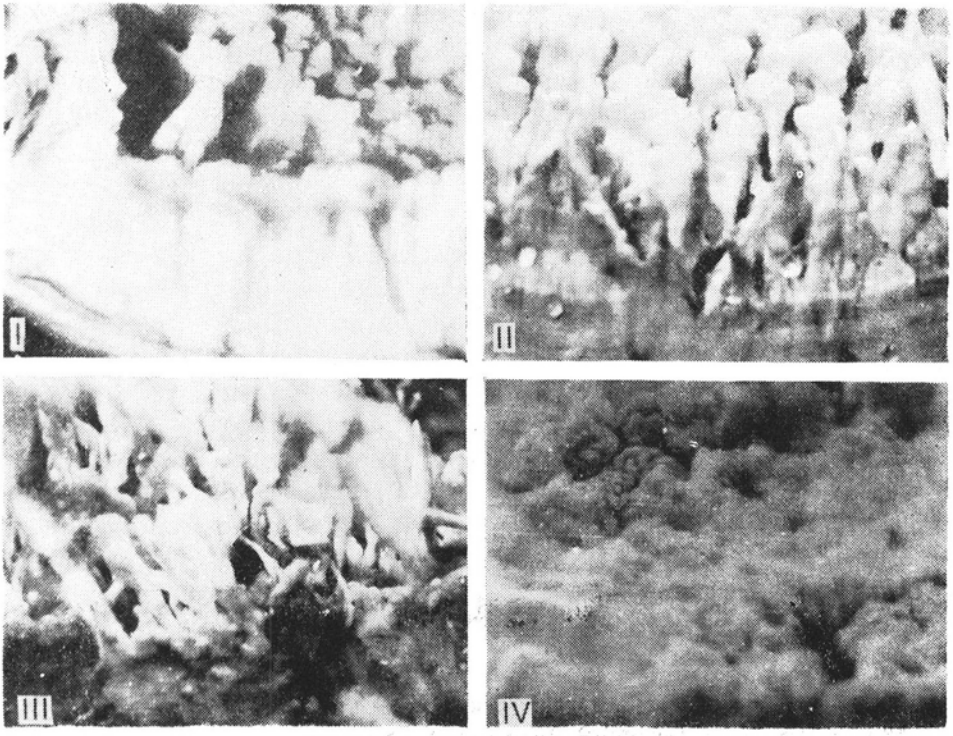

Fig. 11. Action of time of flow of air devoid of CO_2 on the formation of coremia in *P. isariaeforme* (90 l/h).

In the last stage of development, aerated and not aerated cultures behave essentially in the same way. In order to obtain the maximum effect of aeration on dry weight of the mycelia, elongation, and formation of coremia, cultures should be aerated between the 4 and 9 day of growth.

CONCLUSIONS

Since a long time it has been known that the composition of air and the presence of volatile metabolites secreted by mycelia influence strongly the morphology and physiology of many microorganisms, and especially of fungi.

Lambert (1933) established that an increase of CO_2 concentration to 1—5% in the surrounding air causes a deformation of fruit bodies of mushrooms characterized by a considerable elogation of the stipe of fruit bodies and reduction of the pileus. This was confirmed by Tschierpe (1959) who found that an increase of CO_2 concentration in the air to 0,36% leads to a notable acceleration of stipe growth and a concomitant inhibition of development of the pileus. These phenomena become more pronounced in still higher concentrations of carbon dioxide. Concentrations

of CO_2 higher than 1,5% evidently inhibit sporulation, and concentrations exceeding 11% are lethal. Further investigations revealed (Tschierpe and Sinden 1965) that the number of fruit bodies formed in an environment containing CO_2 is smaller. Beginning with 0,15% CO_2 the number of fruit bodies diminishes with increasing CO_2 concentration and at 1—2% CO_2 their number is below 1% of the value obtained in controls kept in the air of normal CO_2 content. Le Roux (1966) found in fruit bodies of mushrooms treated for 30 minutes with C^{14} ($C^{14}O_2$) a great amount of labelled free amino acids and organic acids. Similar results were obtained by Rast (1961) for homogenized fruit bodies of *Psalliota campestris*. The influence of carbon dioxide on morphogenesis of *Collybia velutipes* and *Polyporus brunnalis* was also examined by Plunkett (1956). The former species reacts to the removal of carbon dioxide by forming abnormal fruit bodies. In tightly closed culture flasks long stems with residual heads are formed. *Polyporus brunnalis* did not react to changes in the concentration of carbon dioxide, but was sensitive to the content of water vapour in the air. Niederpruem (1963) established that the fructification process of *Schizophyllum commune* is inhibited when allowed to grow in closed flasks; this inhibition, however, is reversible by means of aeration or absorption of carbon dioxide. He believes that carbon dioxide emitted in respiration acted morphologically, as a decrease of glucose in the nutrient solution had a similar effect as aeration of culture chambers. It may be added that Tschierpe and Sinden (1964) kept mushrooms in flasks closed with cotton wool plugs and found that during growth there occurred important changes in the composition of the atmosphere. After 24 days oxygen content was reduced to the level of 5%, and carbon dioxide content increased to 16%. Thus the lethal concentration of CO_2 is possibly higher than it was previously supposed by these authors.

Flow of air with various amounts of CO_2 affects also the development of *Blastocladiella emersonii* (Cantino and Goldstein 1967). These authors applied an air flow with various amounts of CO_2, from 0,03 to 27%. In low CO_2 concentrations, elongation of *Blastocladiella* cells was observed, their volume did not increase, however; 12%CO_2 was found to be the optimum concentration for this organism and causes uniform increase in the length and volume of the cells. In cultures exposed to light the optimum concentration dropped to 6,7%. As to the influence of carbon dioxide on morphogenesis, and especially photomorphogenesis of fungi, a possible role of volatile metabolites produced by these organisms cannot be neglected. In anaerobic conditions the mushroom produces several volatile organic compounds e.g. ethyl acetone, acetic aldehyde and acetone (Lockard and Kneebone 1962, Lockard 1967). Tschierpe and Sinden (1965) also found that in anaerobic conditions the mushroom produces small amounts of acetone (about 7 μg per

1 liter of air), but principially ethanol. No detailed information, however, is available concerning the action of these compounds on initiation and development of fruit bodies either in mushrooms or other fungi.

It may be supposed on the present evidence that species from the *Penicillium clavigerum* section react strongly to gas conditions in the air surrounding the culture. Both photosensitive species are also sensitive to the air flow. Under the influence of the increasing rate of air flow a considerable shortening of the height of the fruit bodies and an increase of their number per mycelial area unit were observed in *P. claviforme*. The higher the flow rate, the weaker the light effect; in the highest applied flow rates cultures did not differ significantly from dark cultures. Neither light nor air flow had any effect on dry weight produced by the given species. In *P. isariaeforme* the influence of air flow is not limited to a shortening of the height of coremia. In flasks closed tightly with cotton plugs we observe an elongation of aerial mycelium which fails to coalesce into coremia. At very low flow rates, already, scarce and nontypical coremia appear (with a brush of uncoalesced hyphae on top).

With the increase of the rate of flow coremia become typical. In rates higher than 25 l/h the typically developed coremia begin to shorten. Even the lowest of the applied flow rates causes already a decrease of the excess dry weight of the mycelium in *P. isariaeforme*. From the results of our experiments it may be concluded that a compound present in the surrounding air, presumably carbon dioxide — is a factor cooperating with light and influencing the number of fruit bodies, their shape and height. The evidence hitherto available does not allow, however, to exlude definitely a morphogenetic influence exerted by the volatile metabolites of the mycelium, which will be called below „factor x". The following pattern of the influence of light and composition of air might be suggested:

Light	+ high concentration of CO_2 (or of factor x)	⟶	1. strong elongation of coremia 2. increase of dry weight 3. decrease of ability to initiate coremia
Light	+ low concentration of CO_2 (or factor x)	⟶	1. inhibition of elongation 2. no increase of dry weight 3. high production of coremia
Darkness	+ low concentration of CO_2 (or of factor x)	⟶	1. high production of coremia

Experiments concerning the time of application of air flow (which gives the same effects as a decrease of CO_2 concentration in culture flasks) showed that there exists a certain period in the life of fungi and certain light conditions and air composition in which the above described processes proceed at optimum intensity. Changes in these conditions induce immediate disturbances in optimum morphogenesis, shifting the balance to one of its integrant processes.

SUMMARY

1) Previous investigations showed that cultures of *Penicillium isariaeforme* grown in light produce ca. 30 per cent more dry weight than those grown in darkness.

2) Elementary analysis of the mycelia of the species from the section of *P. clavigerum* revealed a certain excess of carbon in the mycelium of *P. isariaeforme* grown in light and also a double increase of the content of mineral salts.

3) Aeration of cultures of representatives of the section of *P. clavigerum* led to important morphological changes in *P. claviforme* and *P. isariaeforme*. In *P. claviforme* the form and number of coremia per cm^2 of surface of the mycelium vary depending on the rate of air flow. An analogous phenomenon occurs in *P. isariaeforme*, particularly concerning the formation of coremia, their number and height. In this species, grown in light and in aerated environments, there is no excess of dry weight.

4) Acceleration of morphological effects due to aeration may be obtained by flow of atmospheric air devoid of CO_2.

5) The cultures of the investigated moulds are most sensitive to aeration in early stages of development, namely *P. claviforme* from the 1 to 3 and *P. isariaeforme* from the 4 to 9 day of growth. At this time, it is possible to regulate morphogenesis by aeration.

6) Sporulation was accelerated in aerated cultures.

7) The results obtained allowed to present a hypothesis concerning the regulation of morphogenesis by external factors, particularly light and composition of air surrounding the mycelium.

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Badania porównawcze nad rozwojem grzybów z sekcji Penicillium clavigerum
II. Wpływ przewietrzania na morfologię grzybni i koremiów

Streszczenie

1. W badaniach poprzednich stwierdzono, że *Penicillium isariaeforme* tworzy więcej suchej masy w hodowlach na świetle w porównaniu do kultur pochodzących z ciemności o około 30%.

2. Analiza elementarna grzybni gatunków z sekcji *P. clavigerum* wykazała pewną nadwyżkę zawartości węgla w grzybni *P. isariaeforme* z hodowli pochodzących ze światła i także dwukrotne zwiększenie zawartości soli mineralnych.

3. Przewietrzanie kultur przedstawicieli sekcji *P. clavigerum* wykazało, że *P. claviforme* i *P. isariaeforme* reagują silnymi zmianami morfologicznymi na ten czynnik. *Penicillium claviforme*; u tego gatunku kształt i liczba koremiów na cm^2 powierzchni grzybni zmienia się w zależności od szybkości przepływu powietrza. Bardzo silnymi zmianami morfologicznymi reaguje na przepływ *P. isariaeforme;* tworzenie koremiów ich liczba i wysokość. Gatunek ten hodowany na świetle w kulturach przewietrzanych nie wykazuje nadwyżki suchej masy.

4. Przyspieszenie efektów morfologicznych pod wpływem przewietrzania można uzyskać przez zastosowanie przepływu powietrza pozbawionego CO_2 atmosferycznego.

5. Największą wrażliwością na przewietrzanie charakteryzują się kultury omawianych gatunków we wczesnych stadiach rozwoju; *P. claviforme* pomiędzy 1—3 dniem rozwoju a *P. isariaeforme* pomiędzy 4—9 dniem wzrostu. Przewietrzając kultury w tych stadiach rozwoju można regulować przebieg morfogenezy.

6. W kulturach przewietrzanych obserwowano przyspieszenie procesu zarodnikowania.

7. Na podstawie otrzymanych wyników postawiono hipotezę dotyczącą regulacji morfogenezy przez czynniki zewnętrzne szczególnie światło i skład powietrza otaczającego grzybnię.

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