ROLE OF CARBON DIOXIDE IN THE CONTROL OF FRUITING OF *SCHIZOPHYLLUM COMMUNE*

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ABSTRACT

NIEDERPRUEM, DONALD J. (Indiana University Medical Center, Indianapolis). Role of carbon dioxide in the control of fruiting of *Schizophyllum commune*. J. Bacteriol. **85**:1300–1308. 1963.—Compatible matings of *Schizophyllum commune* carried out in sealed chambers showed good vegetative growth and clamp-connection formation but fruiting was markedly inhibited. This inhibition was reversed by either aeration or the inclusion of alkali in the chambers. These phenomena occurred on a defined medium, with glucose as the primary carbon source, and were dependent on the level of carbohydrate employed. Gas mixtures of air-CO_2 (95:5) severely restricted the fruiting process when applied during mating or before the formation of fruit body primordia. It is proposed that respiratory CO_2 plays an important role in the regulation of form of *S. commune*.

Schizophyllum commune is a wood-rotting hymenomycete that exhibits an orderly sequence of morphological events during the sexual progression. Although a great deal of attention has been paid to the genetic bases of sexual incompatibility in this mushroom (Papazian, 1950, 1951; Raper and Miles, 1958; Raper, Baxter, and Middleton, 1958; Raper, Baxter, and Ellingboe, 1960; Parag and Raper, 1960; Parag, 1962), definitive biochemical studies of the developmental cycle are lacking. Raper and Krongelb (1958), in an extensive study of the genetic and environmental aspects of fruiting of *S. commune*, clearly demonstrated that the genetic background regulated both the occurrence of fruiting and the morphology of fruits. The biochemical mechanisms through which this genetic control is expressed remain to be extablished.

A possible approach to this problem came from the discovery of a volatile inhibitor of the fruiting process in *S. commune* (Niederpruem, 1962). This phenomenon appears to be of general significance to the regulation of form of *S. commune*, in that a variety of compatible matings as well as certain homokaryotic fruiting strains show this effect. Experiments concerning the nature and action of this inhibitor are the subject of the present report.

MATERIALS AND METHODS

S. commune Fr. was cultured and mated at 24 C (±2 C) in the light (fluorescent lamp, 175 ft-c ±50) on a solid medium. The strains of *S. commune* employed were obtained from P. Snider, Department of Botany, University of California, Berkeley. A detailed account of the life cycle and terminology peculiar to *S. commune* has appeared (Raper and Miles, 1958). In this study, the minimal medium contained (per liter of distilled water): D-glucose, 20 g; L-asparagine, 2.0 g; thiamine hydrochloride, 100 μg; KH_2PO_4, 0.46 g; K_2HPO_4, 1.0 g; $MgSO_4 \cdot 7H_2O$, 0.5 g; Noble's Agar, 20 g. A complex medium was also used, containing the same ingredients as the above, with the exception that peptone (2 g) was substituted for asparagine.

Matings leading to the production of fruit bodies were made by placing discs of mycelia of appropriate mating types a few millimeters apart on solid medium. Fruiting was scored after 7 days by macroscopic observation along lines defined by Raper and Krongelb (1958; i.e., the time at which the hymenial surface appears in developing fruits). In certain instances, longer time periods were necessary to evaluate fruiting. Crosses were performed in standard petri dishes incubated in the conventional manner and are designated in this work as "open." Sealed-chamber matings were obtained by sealing two standard petri dish bottoms together with time tape (Professional Tape Co., Riverside, Ill.). One side of the chamber contained the solid medium

and the appropriate mating. Aeration of the sealed crosses was achieved by removing the seal and incubating in the conventional manner.

The effects of various gas mixtures on morphogenesis were studied by incubating duplicate crosses contained in small plastic petri dishes (60 × 15 mm) in large desiccators (250 × 326 mm). Commercial gas mixtures (Matheson Co., Joliet, Ill.) were admitted after evacuation of the desiccators; this cycle was repeated three times.

RESULTS

Morphogenesis of S. commune. The mating of compatible strains (699 $A^{41}B^{41}$ × 845 $A^{51}B^{51}$) of *S. commune* leads to the production of sporulating fruits within 5 to 7 days; some of the gross morphogenetic changes that lead up to this event are shown in Fig. 1. The compatible homokaryotic mycelia first undergo vegetative growth and hyphal anastomoses, which allow nuclear exchange to occur (Fig. 1, 3 days). The conjugate nuclear division of the dikaryon is indicated by the presence of clamp connections, which provide the investigator with a microscopic indication that the dikaryon has been established. The formation of fruit body primordia (Fig. 1, 5 days) follows the vegetative growth of the dikaryon; the primordia develop pits and eventually en-

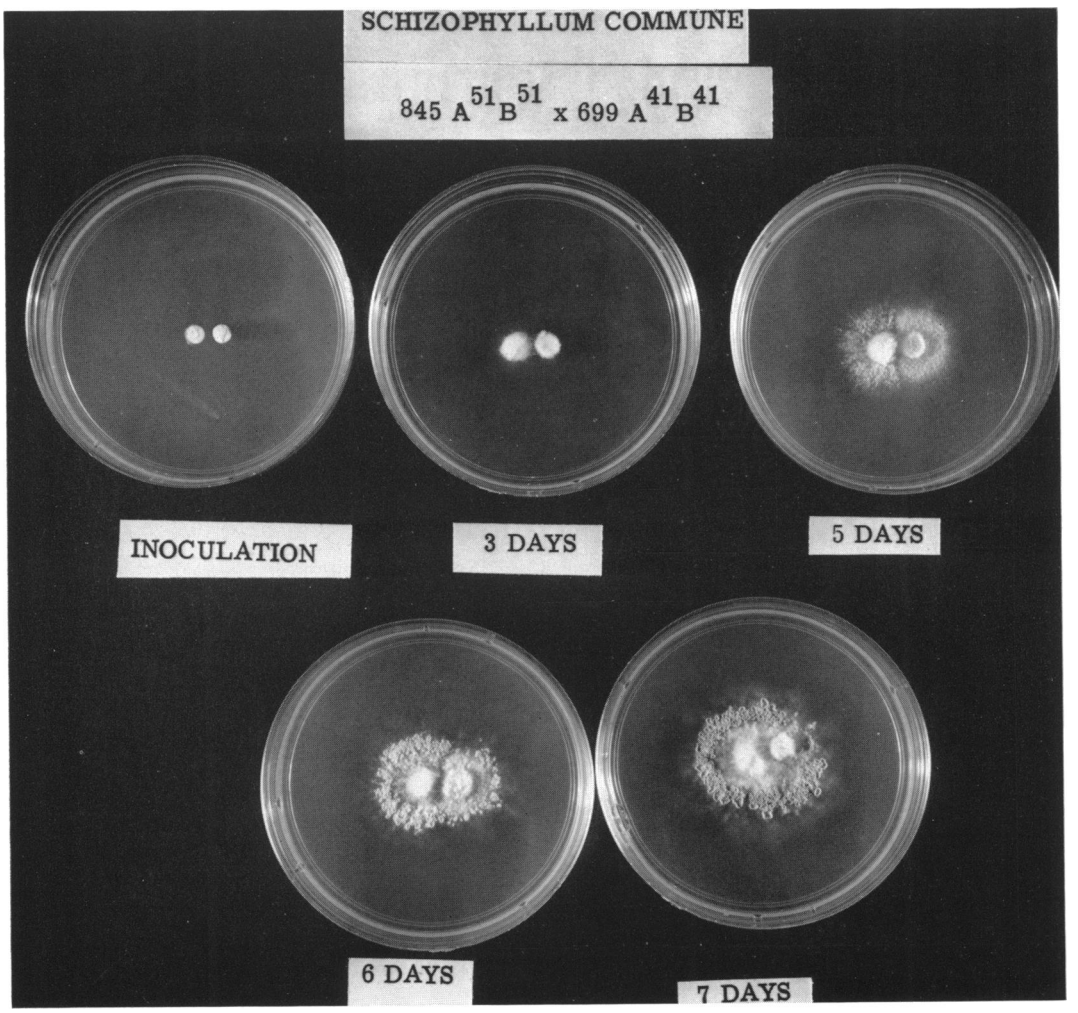

FIG. 1. *Stages of morphogenesis during a compatible mating of Schizophyllum commune.*

large to form the fruit bodies. The sporulating fruits (Fig. 1, 7 days) finally shed basidiospores, which can germinate under the appropriate conditions to produce septate, homokaryotic mycelia, thus completing the developmental cycle.

Reversible inhibition of fruiting. When compatible matings were carried out in sealed petri dishes, the mycelium showed good vegetative growth and clamp-connection formation but fruiting did not take place. When the same crosses were made in "open" petri dishes, fruiting did occur. This suggested that some volatile material influences the development, and attempts were made to trap this substance. When small wells containing KOH, soda lime, or Ascarite were included in the sealed petri dishes, the inhibition was released and fruiting occurred. A number of other materials were placed in the wells, including H_2SO_4, acidic $KMnO_4$, activated charcoal, paraffin oil, water, basic fuchsin, lead acetate, and mercuric chloride, but none of these was effective in eliminating the inhibition. These experiments suggested that a volatile inhibitor of fruiting accumulated under conditions of limited aeration and that this material was removed by alkali.

TABLE 1. *Effects of culture conditions on fruiting of Schizophyllum commune*

Cross	Fruiting		
	Open chamber	Sealed chamber	
	− Alkali	− Alkali	+ Alkali
Compatible matings			
845 $A^{51}B^{51}$ × 699 $A^{41}B^{41}$	+	−	+
12 $A^{3}B^{3}$ × 43 $A^{41}B^{2}$	+	−	+
667 $A^{2}B^{2}$ × 693 $A^{35}B^{35}$	+	−	+
12 $A^{3}B^{3}$ × 693 $A^{35}B^{35}$	+	−	+
666 $A^{4}B^{2}$ × 12 $A^{3}B^{3}$	+	−	+
693 $A^{35}B^{35}$ × 845 $A^{51}B^{51}$	+	−	+
70 $A^{2}B^{1}$ × 699 $A^{41}B^{41}$	+	−	+
33 $A^{9}B^{9}$ × 38 $A^{10}B^{10}$	−	−	−
Common A matings			
667 $A^{2}B^{2}$ × 70 $A^{2}B^{1}$	−	−	−
43 $A^{41}B^{2}$ × 699 $A^{41}B^{41}$	−	−	−
Common B matings			
667 $A^{2}B^{2}$ × 43 $A^{41}B^{2}$	−	−	−
666 $A^{4}B^{2}$ × 667 $A^{2}B^{2}$	−	−	−

A number of compatible matings of *S. commune* showed arrested fruiting when they were carried out in sealed chambers; in every case, the inhibition was reversed by alkali, and the data are summarized in Table 1. Clamp connections were observed in all cases. In many instances, fruiting was greater in sealed chambers containing alkali than in "open" culture vessels (see Fig. 4). This suggested that matings performed in the conventional manner may also be limited to some extent.

Compatible matings of *S. commune* occurred when the A and B incompatibility factors were heterozygous in the two strains; these crosses led to sporulating fruits. If matings were performed between strains having identical A factors but different B factors, a "flat" reaction occurred which was characterized by the lack of aerial mycelia together with gnarled, knobby hyphae. Similarly, crosses between strains having different A but identical B factors led to a characteristic "barrage" phenomenon. Fruiting was absent in both cases. The possibility therefore existed that this lack of fruiting may be due to an unusually high sensitivity of these reactions to the volatile inhibitor. However, common A- and B-crosses failed to fruit in sealed chambers containing alkali, even though they exhibited the morphological attributes associated with these reactions (see Table 1). In addition, the cross (33 $A^{9}B^{9}$ × 38 $A^{10}B^{10}$) involving poor fruiting strains of *S. commune* failed to fruit in a sealed chamber containing alkali, although clamp connections were abundant.

Arrested fruiting in sealed chambers and alkali-reversal of this effect are not peculiar to the sexual cycle of *S. commune*. A haploid (homokaryotic) fruiting strain 1082 (A^{41} sup. B^{41}-mutated) also showed these characteristics. In addition, recent nutritional studies on the development of *S. commune* (Niederpruem and Hobbs, 1963) indicated that nutritionally induced fruiting in the haploid strain 699 $A^{41}B^{41}$ was inhibited in a sealed chamber and reversed by the inclusion of alkali. These experiments indicate the general importance of this phenomenon in the regulation of the development of *S. commune*.

Characteristics of the volatile inhibitor. When concentrated H_2SO_4 was added to samples of alkali that had been incubated in the presence of crosses performed in sealed chambers, a gas was

evolved. On addition of 10% $BaCl_2$ (w/v) to the alkali, a copious precipitate formed. These results suggested that respiratory CO_2 was being trapped by the alkali in the sealed chambers. The inhibition of fruiting and its reversal by alkali were observed on minimal medium, complex medium, and on a minimal medium containing ammonium sulfate as the primary nitrogen source. The latter implicated glucose as a possible source of the volatile material. Moreover, the level of glucose supplied in the basal minimal medium also appeared to be of some importance. For example, fruiting was observed in sealed chambers if the mating was performed on a minimal medium containing a low level of glucose (0.5%), whereas similar crosses performed at higher glucose levels (2%, 3%) showed arrested fruiting. Control matings performed on these media in sealed chambers containing alkali fruited in every case. All these data are consistent with the hypothesis that a volatile inhibitor of fruiting, possibly respiratory CO_2, accumulates during crosses performed under conditions of limited aeration.

It appeared of interest to determine the level of alkali required to reverse the inhibition of fruiting in sealed chambers. These studies are presented in Fig. 2. The end point for reversal appears between 0.1 and 0.05 N KOH.

Effect of carbon dioxide on fruiting. The determination of the amount of alkali required to reverse the inhibition of fruiting in sealed chambers allowed a calculation of the theoretical level of carbon dioxide which may have accumulated under these conditions. From a knowledge of the chamber volume, normality and volume of the KOH, and stoichiometry of the reaction

$$2\ KOH + CO_2 = K_2CO_3 + H_2O$$

calculations of the levels of CO_2 that could

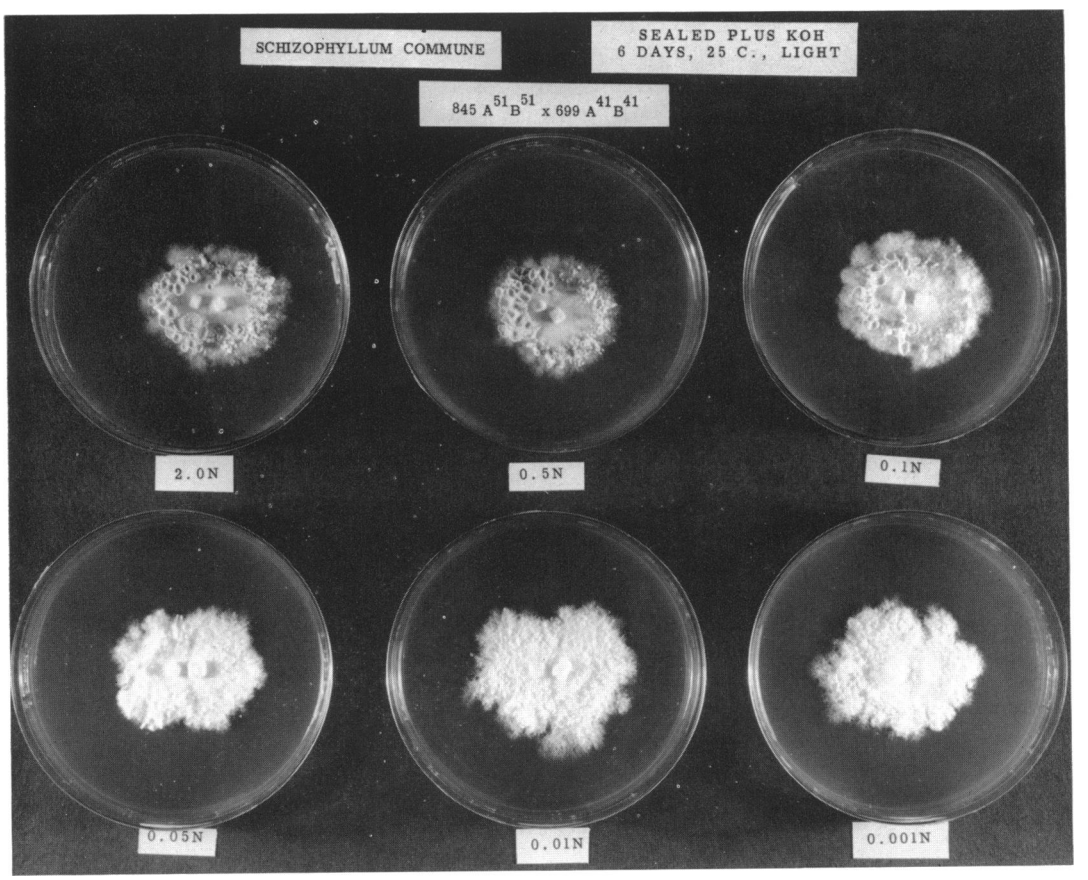

FIG. 2. *Effect of alkali concentration on fruiting of Schizophyllum commune in sealed chambers.*

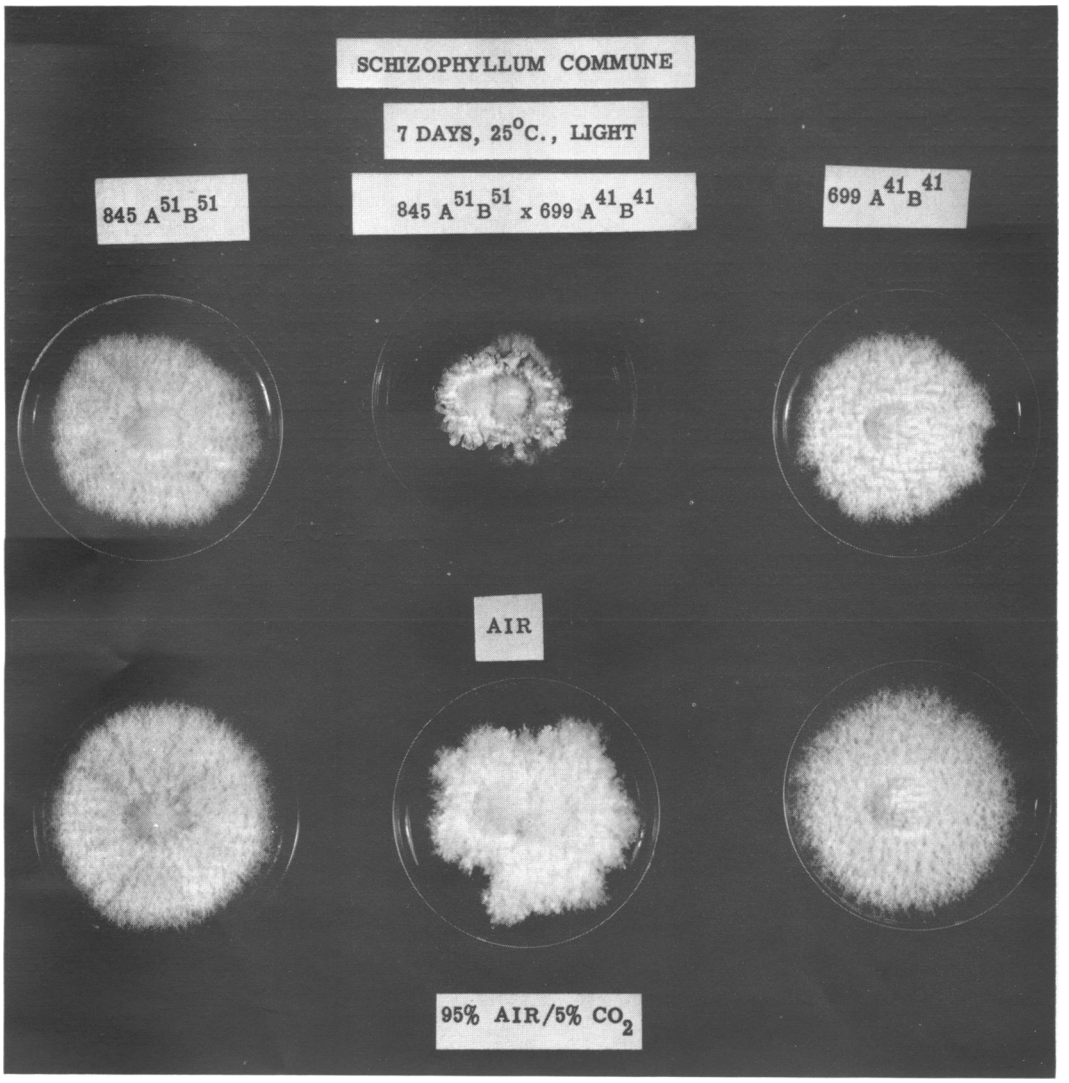

FIG. 3. *Effect of CO_2 on fruiting of Schizophyllum commune.*

accumulate under these conditions gave values between 4 and 8% CO_2. The effects of a gas mixture, containing 95% air and 5% CO_2, on the vegetative growth and fruiting of *S. commune* are shown in Fig. 3. Good vegetative growth and clamp-connection formation (as evidenced by microscopic examination) took place, but fruiting was markedly inhibited. Control crosses performed in a 95% air-5% N_2 atmosphere fruited in every case. These data support further the idea that respiratory CO_2 arrests fruiting of *S. commune*.

Site of action of carbon dioxide. Experiments were designed to gain some information on the timing of the CO_2 effect during the sexual cycle of *S. commune*. Previous data indicated that development is arrested before the formation of fruit body primordia.

Since light is required for normal fruiting of *S. commune* (Raper and Krongelb, 1958), it was of interest to determine whether the CO_2 effect was manifested during crosses incubated in the absence of light. Control matings are compared to crosses incubated in sealed chambers (±KOH) in the presence and absence of light in Fig. 4 and 5. Although normal fruiting occurred in

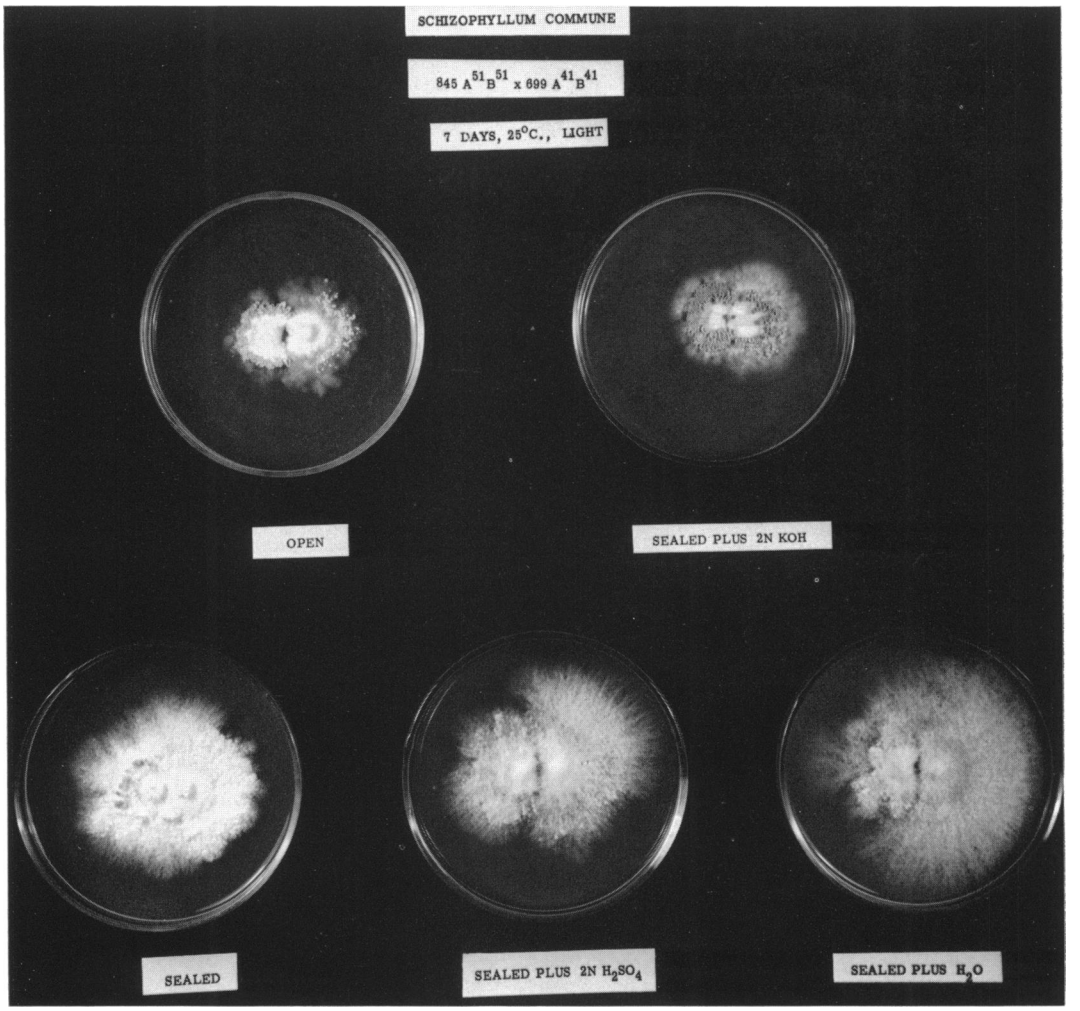

FIG. 4. *Effect of incubation conditions on development of Schizophyllum commune in the light.*

the light with control crosses or with crosses in sealed chambers containing alkali, matings performed under similar conditions in the dark proceeded only to the stage of fruit body primordia. Those crosses performed in sealed chambers, in the dark or light, showed clamp connections, but the formation of primordia was arrested. These experiments indicated that the CO_2 effect was also manifested in the dark and suggested that the action of CO_2 was to inhibit the formation of fruit body primordia. The latter is further supported by the finding that a gas mixture of 95% air and 5% CO_2 inhibited fruiting severely when introduced at the time of inoculation or before the formation of fruit body primordia, but not thereafter.

Discussion

It has long been known that conditions of limited aeration alter the developmental processes of certain fungi (*see* Hawker, 1950). Although CO_2 has been suggested as the active principle responsible for the inhibition of sporangial formation of *Choanephora cucurbitarum* (Barnett and Lilly, 1955) and for the abnormal growth and development of mushrooms (Lambert, 1933), other factors may be involved (Mader, 1943; Stoller, 1952). The experiments of Plunkett

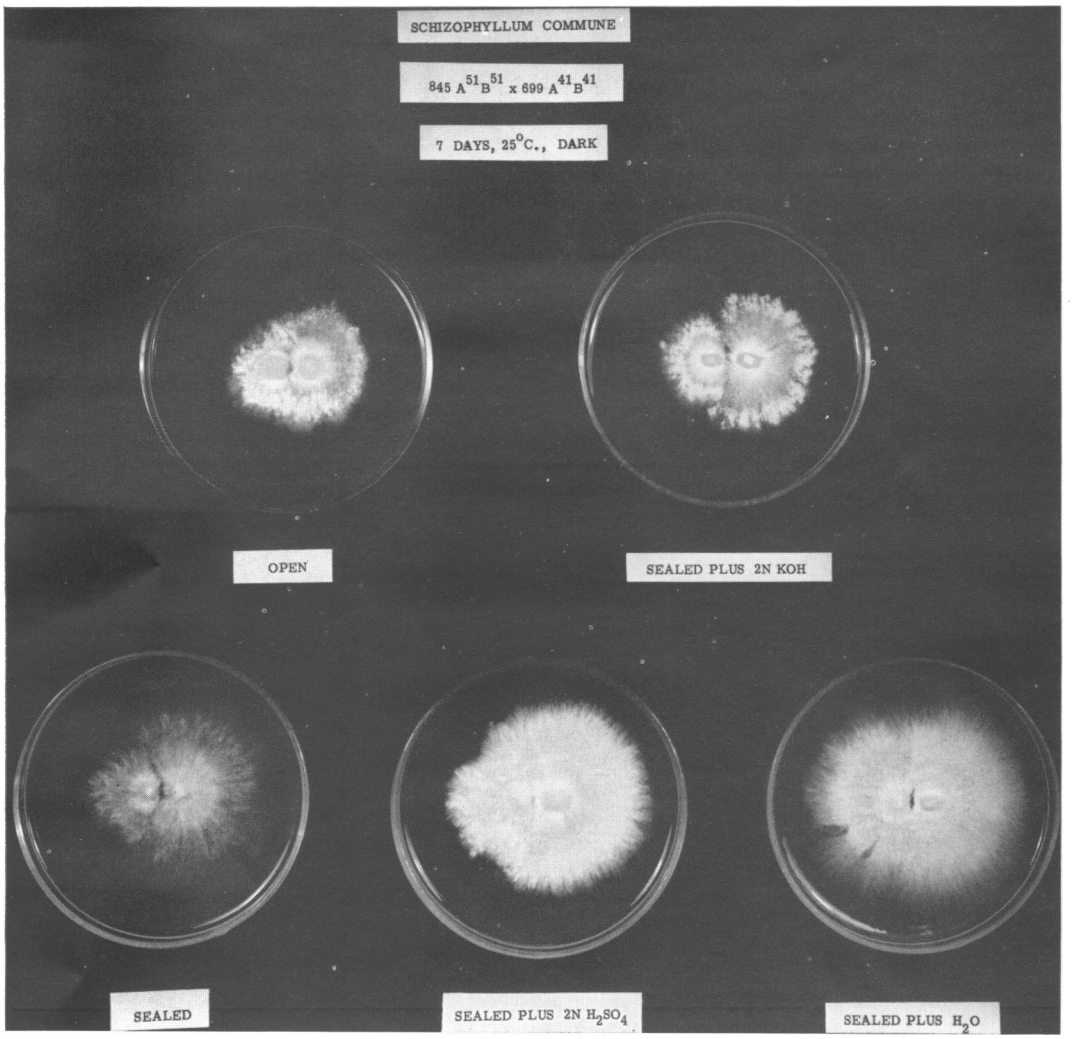

FIG. 5. *Effect of incubation conditions on development of Schizophyllum commune in the dark.*

(1956) indicated that arrested fruiting can be reversed by the inclusion of alkali in the case of *Collybia velutipes*, but not with *Polyporus brumalis*. In addition, gas mixtures of air-CO_2 failed to affect fruit body development of *P. brumalis*. Although abnormal fruiting has been reported for a dikaryon of *S. commune* when placed under conditions of restricted aeration for 4 weeks (Raper and Krongelb, 1958), no data were offered to support the view that respiratory CO_2 was involved. It was important in the present investigation, therefore, to establish the nature of the volatile inhibitor of fruiting of *S. commune*.

Several lines of evidence presented in this paper indicate that respiratory CO_2 plays an important role in the morphogenesis of *S. commune*. Arrested fruiting in sealed chambers is reversed by the inclusion of KOH, soda lime, or Ascarite, all of which are known to combine chemically with CO_2. Acid treatment of this material liberated a gaseous product, while addition of $BaCl_2$ gave a copious precipitate, presumably $BaCO_3$.

Nutritional studies indicated that the volatile inhibitor was produced during a compatible mating on a minimal medium containing glucose as the primary carbon source. In addition, decreasing the glucose level of the medium allowed

fruiting to occur under conditions of limited aeration. Finally, CO_2 arrested fruiting severely when applied at the time of mating or before the formation of fruit body primordia, but not thereafter.

While the nature of the environmental control of morphogenesis of good fruiting strains of *S. commune* has been elucidated, the present studies offer no clue as to the mechanism responsible for arrested fruiting of crosses between poor fruiting strains of this mushroom. In addition, common A- and common B-crosses also failed to fruit under conditions of CO_2 deprivation. The failure to induce fruiting in these forms lends added significance to the importance of genetic background in regulating the development of *S. commune.*

Although the biochemical basis of arrested fruiting of *S. commune* by CO_2 remains to be determined, some insight may be gained from studies dealing with developmental phenomena in other fungi. Recent studies by Bartnicki-Garcia and Nickerson (1962*a*, *b*, *c*) indicated that the induction of yeastlike development in *Mucor rouxii* by CO_2 is accompanied by an increased level of protein and mannose in the yeast walls. This presumably occurs via an assimilation of CO_2 (Bartnicki-Garcia and Nickerson, 1962*d*).

The aquatic phycomycete, *Blastocladiella emersonii*, is also markedly affected by CO_2 (*see* Cantino, 1961). In this organism, the normal developmental sequence leading to a thin-walled colorless plant is drastically altered in the presence of bicarbonate, with the result that a thick-walled, pitted, resistant sporangial plant is formed (Cantino, 1951). Subsequent biochemical studies (*see* Cantino and Turian, 1959) indicated that bicarbonate triggers a shift from the normal tricarboxylic acid cycle reactions to a triphosphopyridine nucleotide-specific reductive carboxylation of α-ketoglutarate to isocitrate. Apparently, this metabolic shift leads to the formation of a resistant sporangium. These studies may provide a starting point from which to approach the mechanism of action of CO_2 on fruiting of *S. commune.* The recent work of Long (1961, 1962) dealing with the effects of inhibitors, light, and CO_2 on sporocarp development of *C. velutipes* appears to support this contention. Biochemical studies dealing with the role of CO_2 in the regulation of form of *S. commune* are now in progress.

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