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Effects of concentrated carbon dioxide on the fruiting of several cultivated basidiomycetes (II)

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Edible basidiomycetes *Flammulia velutipes* and *Pleurotus ostreatus* were cultivated in the usual manner on media based on sawdust and rice bran, and the cultures were exposed to slowly flowing CO_2-enriched air (550 (control), 3,000, 6,000, and 9,000 μl/l) for seven days at different stages of cultivation. When the cultures were exposed at the primordium stage (less than 10 mm in length), length and yield of fruit-bodies increased and pileus expansion was slightly inhibited in *F. velutipes*, while in *P. ostreatus* length increased, yield decreased, and pileus expansion was greatly inhibited. When the cultures with fruit-bodies larger than 10 mm were exposed, length and yield were insensitive and pileus expansion was greatly inhibited in *F. velutipes*, while in *P. ostreatus* length was insensitive, but pileus expansion was heavily damaged by trumpet-like deformation and yield decreased. The different action of CO_2 on the two species appeared to be due to the different anatomical structures of their fruit-bodies.

Key Words——carbon dioxide; *Flammulina*; *Pleurotus*; fruiting; mushroom cultivation.

Difficulties in cultivation of mushroom (*Agaricus bisporus* (J. Lange) Imbach) due to CO_2 accumulation in the air of mushroom houses (Lambert, 1933) has continued to attract biologists' interest (Plunkett, 1956; Niederpruem, 1963; Tschierpe and Sinden, 1964; Schwantes and Hagemann, 1965; Long, 1966; McLaughlin, 1970; Schwalb, 1971). Kinugawa et al. (1986) have reported the results of exposing some of the main Japanese cultivated edible mushrooms, *Flammulina velutipes* (Curt.: Fr.) Singer, *Hypsizigus marmoreus* (Peck) Bigelow (*Lyophyllum ulmarium* sensu auct. japon. p.p.), Pleurotus ostreatus (Jacq.: Fr.) Kummer, and *Pholiota nameko* (T. Ito) S. Ito & Imai, to two levels of CO_2 concentration (550 and 6,000 μl/l).

In this study, cultures of *F. velutipes* and *P. ostreatus* were exposed to air enriched with CO_2 at four levels of concentration (550, 3,000, 6,000, and 9,000 μl/l) at various stages of cultivation, and the effects on the activities of the fungi were compared.

Materials and Methods

Commercial stocks, T8 for *F. velutipes* and Hisamune for *P. ostreatus*, were used. All cultures were prepared by Chikuma Kasei Co. (hereafter, Chikuma). Media compositions, culture bottles, and sterilization temperatures are shown in Table 1. Cultivation schedules are outlined in Table 2. When cultures of *P. ostreatus* had passed through colonization (spawn-runs I and II, ca. 27 days at 20°C), spawn on the top of the medium was removed and the cultures were placed in cooler room (14°C) to induce fruiting, then fruit-bodies were harvested after growing. In the cultivation of *F. velutipes*, spawn-run II was omitted and cultures were subjected to low temperature of 3°C to suppress primordial growth and make their length uniform, then the primordia were allowed to grow at 6°C, surrounded with special paper to prevent outward bending of grown-up stipes. Harvest was made at the end of the experiment.

Cultures were separated into five groups which were exposed to CO_2-enriched air for 7 days at different stages of the cultivation schedule. The stage at which exposure began was as follows, and the corresponding stage used in the previous paper (Kinugawa et al., 1986) is given in parentheses.

Group A: middle of spawn-run I (B).
Group B: end of spawn-run I (*F. velutipes*) or II (*P. ostreatus*) (C)
Group C: when fruit-body primordia appeared (D-E).
Group D: when fruit-bodies attained ca. 10 mm in length (E-G).
Group E: time of suppression (*F. velutipes*) or the last period of cultivation (*P. ostreatus* (F-G).

Some features of fruiting were preliminarily measured at the start of exposure in groups D, and E (Table 3).

Exposure was carried out in the growth cabinet system (GCS) of the National Institute of Environment, Tsukuba, Japan as reported by Kinugawa et al. (1986).

Table 1. Media and sterilization.

Species	Composition of media		Volume of culture bottle	Sterilization temperature (time)
F. velutipes	Sawdust*	115 g	800 ml	98°C (3 h)
	Rice bran	93 g		
	Water content	63%		
P. ostreatus	Sawdust**	70 g	850 ml	106°C (2.5 h)
	Rice bran	60 g		
	Wheat bran	15 g		
	PA***	30 g		
	Water content	65%		

* *Cryptomeria japonica* D. Don.
** *Pinus* spp.
*** Dried pod-ale from whisky distillation.

Table 2. Cultivation schedules.

Species	Spawn-run I	Spawn run II	Spawn removal*	F. I.**	Supres-sion***	Growing Harvest
F. velutipes	18°C		+	13-15°C	3°C	6°C
	(20)			(7-9)	(14-15)	(12-13)
	⟩—A—|·····|B		+	|—C—|D—|E—|		
P. ostreaus	20°C	20°C	+			14°C
	(20)	(7)				(12)
	⟩—A—|—|B		+	|·····|·····|—C—D—E—|		

* Removal of spawn and surface layer of the medium to enhance fruiting.
** Fruiting induction by cooling of the cultures of *F. velutipes*.
*** Suppression of primordial growth to make the lengths of fruit-bodies uniform.
( ): Number of days. ···: Part of the process omitted. ⟩—: Inoculation. All groups were exposed to CO_2 for 7 days from the time indicated.

Table 3. Sizes of fruit-bodies at the onset of CO_2 exposure.

Species	Group	Fresh wt. /bottle (g)	Length (mm)	Diameter of pileus (mm)	Diameter of stipe (mm)	Number of fruit-bodies
F. velutipes	D	30.1±0.8*	8.1±0.7	1.3±0.02	—	—
	E	85.4±2.6	43.8±0.6	2.1±0.1	1.4±0.03	1,462± 87 (30 mm<)
P. ostreatus	D	33.2±2.7	28.0±0.8	2.9±0.1 ×2.5±0.1**	2.0±0.1 ×1.6±0.1**	1,016±132 (10 mm<)

* Average±S.E.
** Longer and shorter diameter.

GCS consists of a set of 4 cabinets, in each of which the CO_2 concentration (hereafter, CO_2 level) in the air, the rate of air flow, lighting (with white light fluorescent lamps), temperature, and relative humidity are automatically regulated (Aiga et al., 1978). All these conditions were carefully monitored throughout to avoid harmful shifts. Although light intensity and rate of air flow showed unavoidable positional differences in each cabinet due to disturbance by the shelves installed, the former was successfuly controlled to 13 to 72 lux and the latter to ca. 50-500 mm/sec on the top of culture bottles. Humidity and CO_2 levels of the air were set up before it was introduced. The exhaust air was not recirculated. The relative humidity and temperature in the cabinet were maintained at 90±2% and 13±0.5°C. CO_2 levels (μl/l) in the air of cabinets No. 1 to 4 were as below:

Growth cabinet	Level set (actual range), μl/l	
No. 1	550 (468- 684)	control
No. 2	3,000 (2,980-3,060)	
No. 3	6,000 (5,880-6,080)	
No. 4	9,000 (8,840-9,120)	

Cultures were transported from Chikuma to GCS in a cooled car. The numbers of cultures were 36 for groups A and B, and 16 for groups C, D, and E in each CO_2 level. At the end of exposure, half of the cultures of groups A and B were returned to Chikuma by cooled car and

Table 4. Rate of colony expansion in culture bottles (mm/day) at various CO_2 levels of room air.

Species	CO_2 levels (μl/l)			
	550	3,000	6,000	9,000
F. velutipes	3.5±0.1*	2.7±0.1	2.8±0.1	2.9±0.2
P. ostreatus	2.7±0.1	2.1±0.1	2.7±0.1	3.2±0.2

* Average±S.E.

replace on the regular cultivation schedule, and the yield of fruit-bodies was determined. Rate of colony expansion during exposure was determined in group A. For groups C, D, and E, yields, growth, and morphology of fruit-bodies were measured or observed during or at the end of exposure in GCS. The lengths of fruit-bodies were based on measurements between the bottom and the top of a bundle of fruit-bodies developed in a bottle in *F. velutipes*, and those of separate fruit-bodies in *P. ostreatus*.

Results

1. Rate of colony expansion In group A, the colony margin was observable during the spawn-run through the transparent bottle wall. Using 36 cultures, the distance from the top of the medium to the colony margin was measured every two days. A regression equation relating (y) to indicated data (x) gave the rate of colony expansion, which was calculated by the least squares method using the data obtained in the linear growth period of the colony. The results showed no significant difference in mycelial growth of *F. velutipes* and *P. ostreatus* between CO_2 levels (Table 4). These results agree with those of the previous report (Kinugawa et al., 1986). It is noted that CO_2 levels inside the bottles exceeded 5% (50,000 μl/l) irrespective of the levels set in the air of the cabinet.

2. Yield, length, and water content of fruit-bodies The yields in groups A and B of both species measured at Chikuma showed no significant differences between CO_2 levels, suggesting that exposure to CO_2-enriched air up to 9,000 μl/l for 7 days in the incubation period before fruiting does not affect the final yield of fruit-bodies (ca. 140 g fr.wt./bottle in *F. velutipes* and ca. 63 g fr.wt/bottle in *P. ostreatus*).

Groups D and E of *F. velutipes* gave higher yields than group C due to their longer cultivation periods (Table 2). With increase in CO_2 level, yield and length of fruit-bodies increased in group C (Figs. 1, 2-1, 13-1), while yields in group E, where high CO_2 levels were applied during the last 7 days of cultivation, did not respond to the different CO_2 levels. In group D, where exposure began later than in group C, length of fruit-bodies was promoted by higher CO_2 levels (day 6, Fig. 2-2), but the difference virtually disappeared one day after exposure ended (day 8). Fruit-bodies at an earlier stage, including primordia, seem to be more sensitive to ambient high CO_2 level than those at a later stage, presenting more yield and length at harvest.

In contrast, cultures of groups C and D of *P. ostreatus* gave lower yields than controls at higher CO_2 levels (Fig. 3). However, length increased at higher CO_2 levels in group C (Fig. 4) and was virtually independent of CO_2 level in group D (Fig. 5). The increase in length of fruit-bodies in group C does not mean an increase in the number of larger fruit-bodies, but an increase in the number of the fruit-bodies of moderate length. The number of lager fruit-bodies was reduced (Figs. 8, 13-2). No difference was found in final water content between fruit-bodies grown at different CO_2 levels. Fruit-bodies of this fungus seemed to be more sensitive to higher CO_2 levels throughout their development than those of *F. velutipes*.

3. Numbers of fruit bodies of different sizes CO_2 level of air affected the number or fruit-bodies of different sizes at the time of harvest. In *F. velutipes*, as shown in Figs. 6-1 and 6-2 the number and yield of lager fruit-bodies (larger than 30 mm in length) of group C increased with increase in CO_2 level, except for a sudden drop in number at 9,000 μl/l. In group D, no significant differences in number of yield were noted between CO_2 levels, except for the greater yield observed in the control. As for smaller fruit-bodies (less than 30 mm in length) no significant difference in yield or number were noticed between CO_2 levels in groups C and D (Figs. 7-1, 7-2). It seems that the growth of younger fruit-bodies, which comprise physiologically active cells, was promoted even by a moderate increase of ambient CO_2 level to 9,000 μl/l.

Fruit-bodies of *P. ostreatus* in group C were also divided into larger and smaller fruit-bodies. The larger fruit-bodies were more than 30 mm in length and their fused basal parts were longitudinally separated from each other. The smaller fruit-bodies were less than 30 mm in length and their fused basal parts were left intact. Both were measured separately by yield. As shown in Figs. 8 and 9 for groups C and D, yields of the larger fruit-bodies decreased with increase in CO_2 level, while those of the smaller fruit-bodies increased.

4. Morphogenesis In groups A and B of *F. velutipes*, exposure to CO_2 at any level had no effect on the area of the culture surface from which primordia arose. In group C, where the exposure started from earliest phase of primordial development, primordia tended to occur over wider areas of the culture surface and to grow faster (Fig. 2-1), while pileus enlargement tended to be inhibited (Fig. 10). Exposure during the period after fruit-bodies had reached over 10 mm in length (group D) showed a slight tendency for the length of fruit-bodies to be longer with higher CO_2 levels (Fig. 2-2), while pileus expansion was

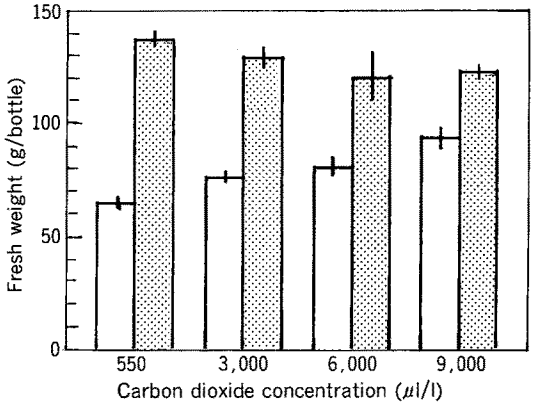

Fig. 1. Yield of fruit-bodies of *F. velutipes* in groups C and D. □, group C; □, group D. Vertical bar indicates S.E.

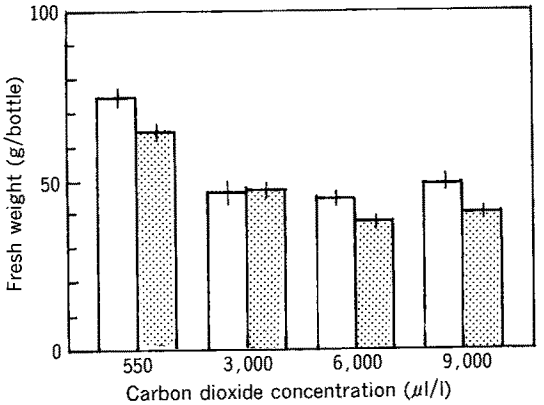

Fig. 3. Yield of fruit-bodies of *P. ostreatus* in groups C and D. For legend, see Fig. 1.

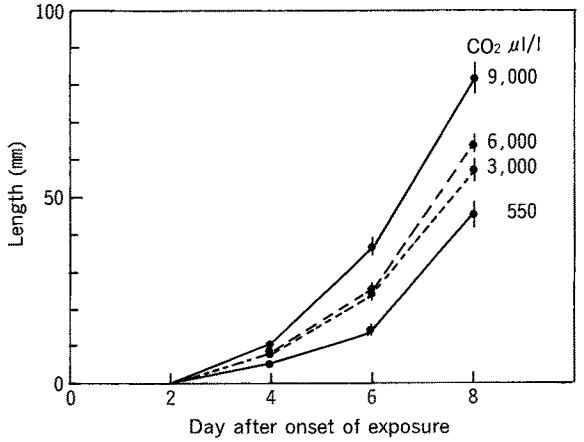

Fig. 2-1. Growth of fruit-bodies of *F. velutipes* in group C. Vertical bar indicates S.E.

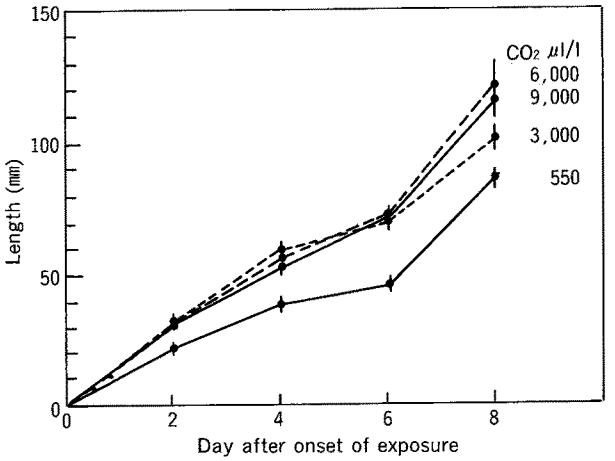

Fig. 4. Growth of fruit-bodies of *P. ostreatus* in group C. Vertical bar indicates S.E.

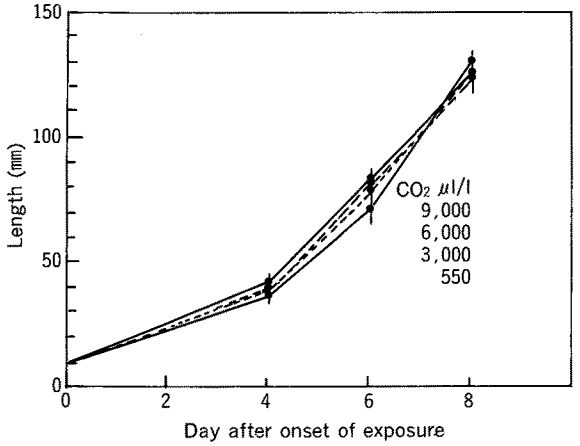

Fig. 2-2. Growth of fruit-bodies of *F. velutipes* in group D. Vertical bar indicates S.E.

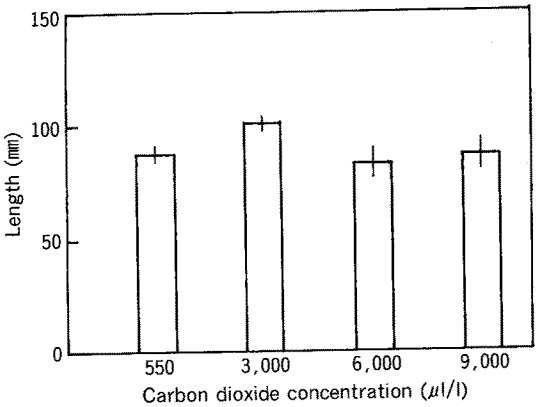

Fig. 5. Length of fruit-bodies of *P. ostreatus* in group D. For legend, see Fig. 1.

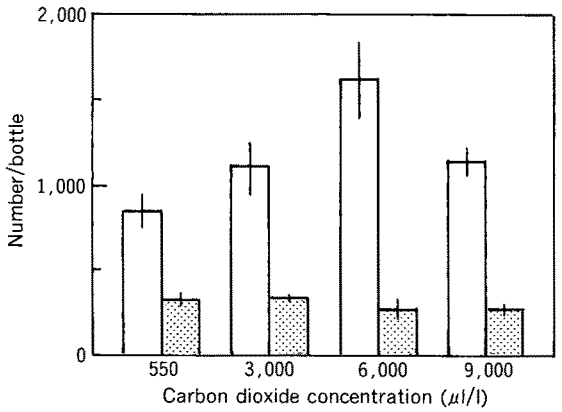

Fig. 6-1. Number of larger fruit-bodies of *F. velutipes* in groups C and D. For legend, see Fig. 1.

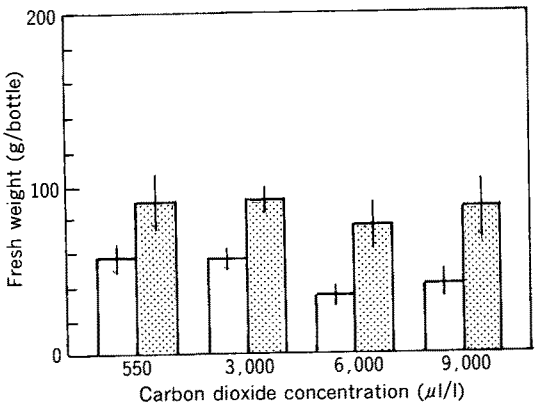

Fig. 7-2. Yield of smaller fruit-bodies of *F. velutipes* in groups C and D. For legend, see Fig. 1.

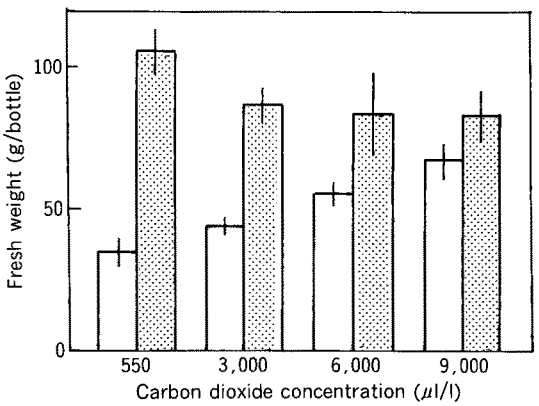

Fig. 6-2. Yield of larger fruit-bodies of *F. velutipes* in groups C and D. For legend, see Fig. 1.

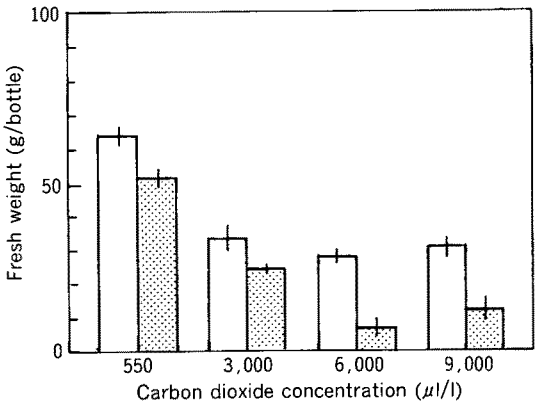

Fig. 8. Yield of larger fruit-bodies of *P. ostreatus* in groups C and D. For legend, see Fig. 1.

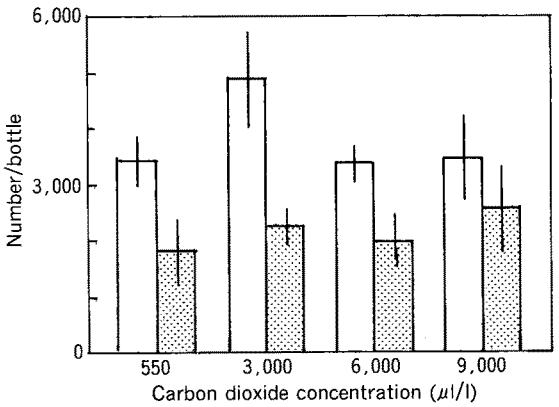

Fig. 7-1. Number of smaller fruit-bodies of *F. velutipes* in groups C and D. For legend, see Fig. 1.

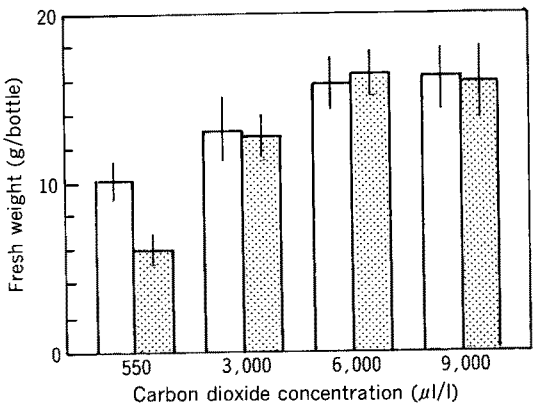

Fig. 9. Yield of smaller fruit-bodies of *P. ostreatus* in groups C and D. For legend, see Fig. 1.

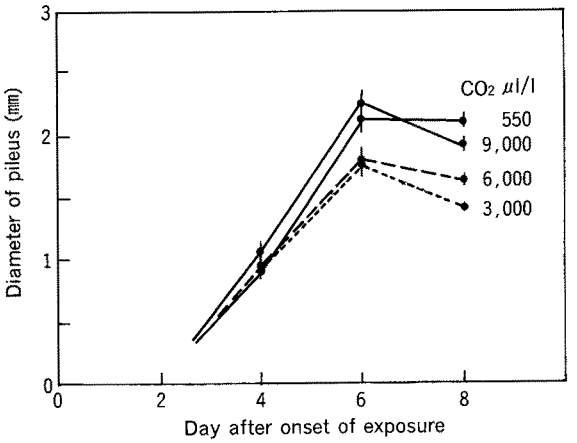

Fig. 10. Pileus expansion of *F. velutipes* in group C. Vertical bar indicates S.E.

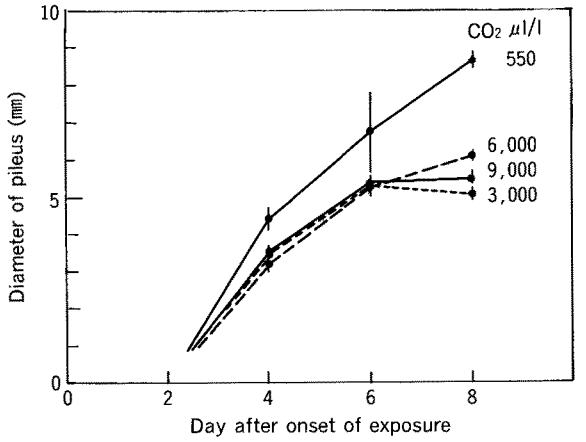

Fig. 11. Pileus expansion of *F. velutipes* in group D. Vertical bar indicates S.E.

retarded with levels higher than 3,000 μl/l (Fig. 11). Exposure of fruit-bodies which exceeded 20 to 30 mm in length (group E) showed no effect on length or pileus diameter, but the pileus tended to turn pale yellow and open early. Thus, the morphological effect of higher CO_2 level was highest when fruit-bodies were exposured in the formative stages, and varied with different parts of the fruit-body. In particular, it was noted in *F. velutipes* that promotion of stipe elongation by exposure was highest in the primordial stage (shorter than 1 mm in length), and inhibition of pileus enlargement by exposure was highest in the final stage of fruit-body development.

Inhibition of pileus expansion by high CO_2 levels was conspicuous, in particular, in *P. ostreatus* (Figs. 12 and 13-2). It always caused a trumpet-shaped deformation of the pileus (Fig. 13-2), sometimes swelling of the stipe accompanied by sponge-like tissue, and infrequently malformed small pilei on pileus surfaces.

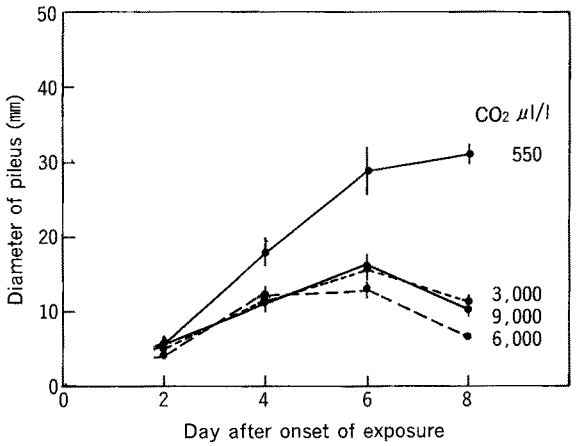

Fig. 12. Pileus expansion of *P. ostreatus* in group D. Vertical bar indicates S.E.

Discussion

It has been observed that basidiomycete mycelium responds to high CO_2 level in air in a wide range of activities, including promotion of mycelial growth, inhibition of fruit-body formation and pileus growth, and increase of fruit-body weight (Plunkett, 1956; Ingold and Nawarz, 1967; Niderpruem, 1963; Tschierpe and Sinden, 1964; Long, 1966; McLaughlin, 1970; Schwalb, 1971; Long and Jacobs, 1974; Zadražil, 1978; Hintikka, 1982; and Kinugawa et al., 1986). Long and Jacobs (1974) devised a culture chamber for *A. bisporus* whereby CO_2-enriched air flowed into the space of the chamber and was exhausted after passing through the colonized media, and showed that higher CO_2 levels stimulated hyphal growth of the fungus but inhibited basidiocarp formation. In the present experiment, a high CO_2 level of up to 9,000 μl/l in room air did not affect the rate of mycelial growth of *F. velutipes* and *P. ostreatus*, because the CO_2 concentration of the medium inside culture bottles exceeded 5%. However, fruiting and morphogenesis were affected by the CO_2 levels of room air, not by the very high concentration of CO_2 contained in the medium. Any effect on the mycelia by the excessive concentration of CO_2 in the medium did not extend to the primordia or fruit-bodies growing on the medium surface.

Plunkett (1956) reported with *Collybia velutipes* (Curt.: Fr.) Kummer, (F. velutipes) that normal pileus and stipe elongation were inhibited by concentrated CO_2 in air (up to 4.9%). Long (1966) showed that 4-day application of 3% CO_2 to *C. velutipes* severely inhibited pileus expansion (Figs. 10 and 11) and promoted stipe elongation (Figs. 2-1 and 2-2). In the present study with *F. velutipes*, higher levels of CO_2 inhibited pileus expansion and promoted stipe elongation, agreeing with Long's results. It appears that the stipe and pileus of *F. velutipes* may follow separate patterns of growth. Stipe growth is largely due to mycelial extension growth in the portion just below the pileus, and the pileus growth is due

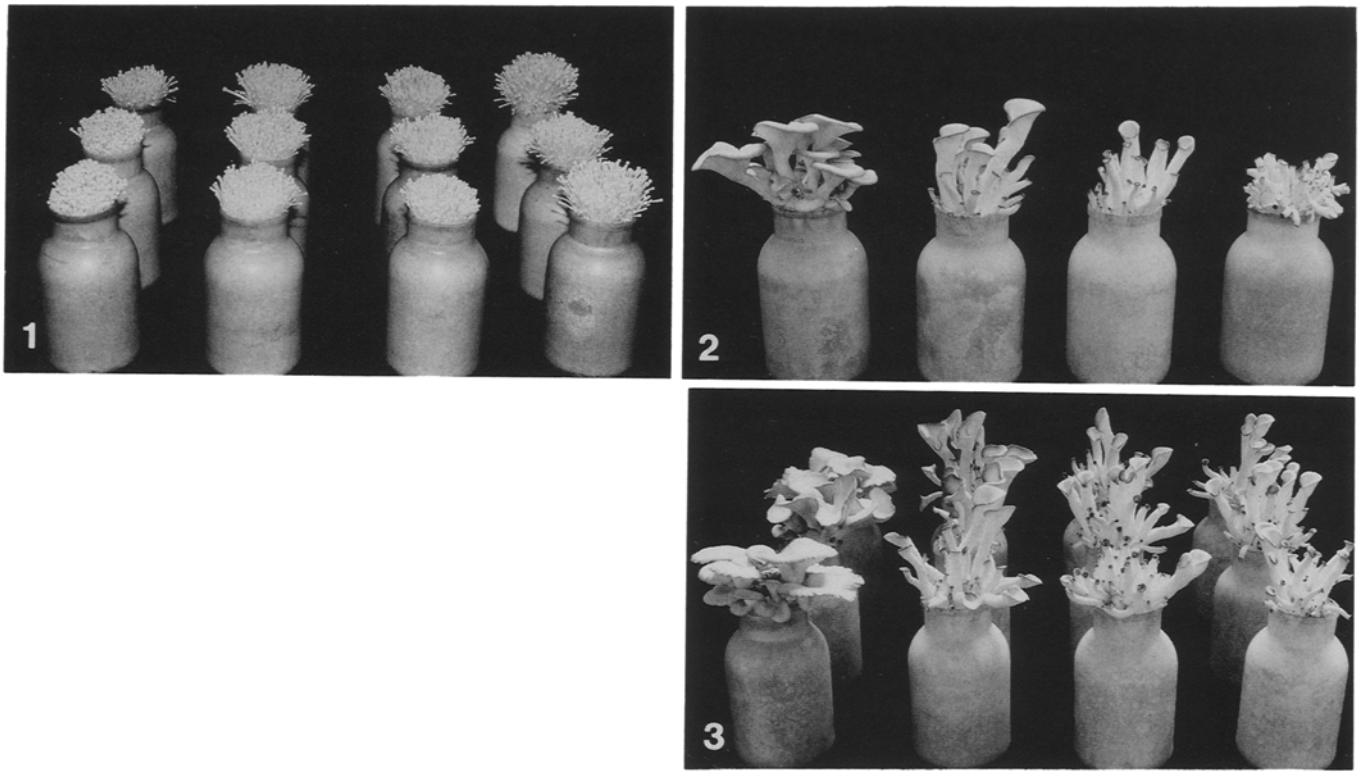

Fig. 13. Morphogenetic effect of CO_2 levels on fruit-bodies, Left to right: 550, 3,000, 6,000, and 9,000 μl/l CO_2. 1, *F. velutipes* in group C. 2, *P. ostreatus* in group D.

to mycelial extension growth and concurrent propagation by branches. It is probable that a moderate rise of ambient CO_2 level tends to promote extension growth of mycelium while inhibition branchig or propagation in the fungi used. In the case of *P. ostreatus*, therefore, a high CO_2 level in air necessarily resulted in reduction of the yield of fruit-body, since most of the fruit-body is the pileus itself, the enlargement of which is arrested by inhibition of mycelial propagation in this environment. Thus, the strong inhibition of marginal widening of the pileus with lesser inhibition of its radial growth seems to have caused the trumpet-like deformation of the pileus, with the margin standing upright around the top. The measured length of the fruit-body of this form is susceptible to overestimation.

Using *Schizophyllum commune* Fr.: Fr. Niederpruem (1963) reported that CO_2 inhibits fruiting, and Schwlb (1971) reported that CO_2 inhibits further growth of fruit-bodies when applied before the primordia open but not thereafter. Considering that fruit-bodies of *S. commune* are almost totally lacking in stipes, these results can be understood in connection with the inhibition of pileus expansion by CO_2.

Sietsma et al. (1977) suggested that CO_2-enriched air must indirectly inhibit pileus growth of *S. commune* by diminishing R-glucan content in wall composition of previously formed mycelium. This may explain the inhibition of pileus growth, but it does not explain the promotion of stipe elongation that was observed in *F. velutipes*. In *P. ostreatus*, high CO_2 levels in air were estimated to decrease stipe length. In Fig. 4, the longer fruit-bodies at 3,000 μl/l and higher levels probably resulted from the increase of length due to the upright trumpet-like form of the pileus. The occurrence of similar lengths of fruit-bodies at higher CO_2 levels was also interesting (Fig. 4). This could be the result of compensation of negative and positive effects of CO_2 on the lengths of fruit-bodies (Fig. 13-2). Prevention of stipe elongation was reported by McLaughlin (1970) in *Boletus rubinellus* Peck (*Chalciporus rubinellus* (Peck) Singer) in non-ventilated culture. This could be an indirect effect through inhibition of normal pileus formation by CO_2 accumulation. Hagimoto and Konishi (1959, 1960) and Hagimoto (1964) showed that an agent migrated to the upper portion of the stipe from the lamellae in *A. bisporus* (*A. brunnescens* Peck) and supported stipe elongation. Gruen (1969) also showed that stipe elongation of *F. velutipes* depended on the lamellae and not on the cap trama. In the present experiment, as shown in Fig.13-2 some amount of lamellae still remained on fruit-bodies of both species even under the highest level of CO_2 in air (9,000 μ/l). It is likely that the remaining amount of lamellae is capable to support, though in lesser degree, stipe elongation and radial development of the pileus (but not marginal enlargement).

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