EFFECT OF EXCESS CARBON DIOXIDE ON GROWING MUSHROOMS [1]

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INTRODUCTION

In mushroom culture the problem of ventilation has always been considered important, but so far as the writer is aware there has been no experimental work done on it. It is a common observation among growers employing underground caves that mushrooms have a tendency to "sulk" in certain pockets in the caves. It has also been known for many years that they have a tendency to grow abnormally long stems and small caps when confined in a limited atmosphere under a glass jar or similar container.

A consideration of these observations led the writer to make a series of experiments in which cultivated mushrooms (*Agaricus campestris* L.) of the white and cream varieties were grown in a modified atmosphere. Systematic observations were also made on the carbon dioxide content of commercial mushroom houses and caves under different conditions in order to determine the practical significance of the results of these experiments.

METHODS

The atmosphere was modified by slowly running carbon dioxide, nitrogen, and oxygen in different amounts and combinations into glass jars inverted over clumps of mushrooms growing on a standard mushroom bed. In some cases the gases were introduced into the compost beneath the clumps of mushrooms rather than into containers above the bed. The gases were obtained in standard commercial cylinders, and the flow was regulated by means of reducing valves, pinchcocks, and bubbling bottles. The arrangement of the apparatus is shown in figure 1. The only gases measured were carbon dioxide and oxygen. With the Orsat gas analysis apparatus used in these experiments the smallest portion of gas that it was feasible to determine was approximately 0.2 percent. Judging from the determination of replicate samples, the error inherent in the use of this apparatus is not more than 0.1 percent.

The effect of different treatments on the growth of the mushrooms was determined by weighing and making outline drawings of the four largest mushrooms in treated clumps and comparing these with similar data from untreated clumps having apparently an equal chance at the start.

It was necessary to exercise considerable care in selecting comparable clumps of mushrooms. Most of the experiments were made at temperatures approximating 15° C.

[1] Received for publication July 3, 1933; issued October 1933.

Except where otherwise specified, the atmosphere surrounding the clumps of untreated mushrooms, which were used as checks, contained less than 0.2 percent carbon dioxide and approximately 20.8 percent oxygen. In the interstices of a normal section of the compost under an actively growing clump of mushrooms there was approximately 20 percent oxygen, and the carbon dioxide content ranged from 0.3 to 0.4 percent.

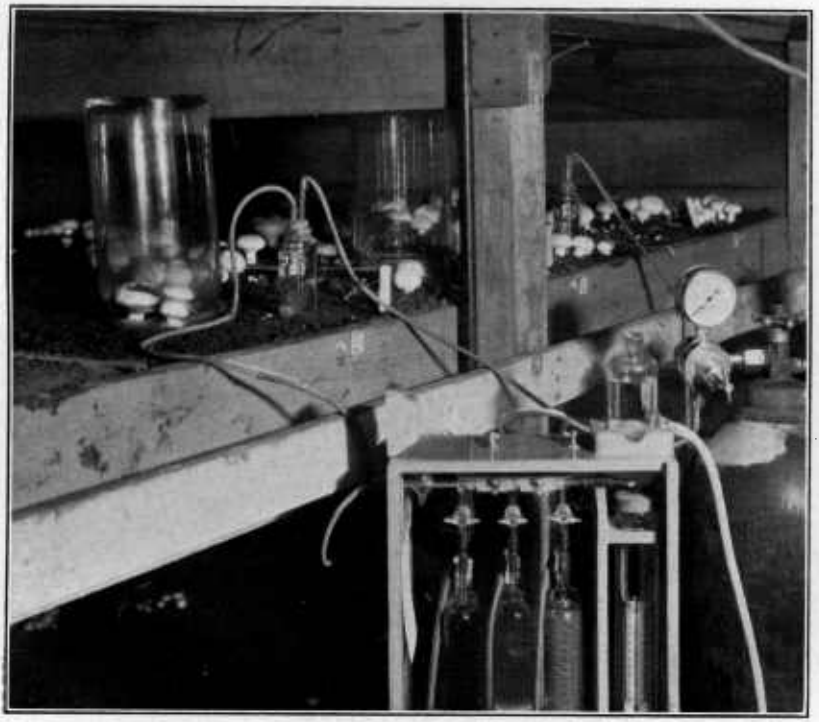

FIGURE 1.—Apparatus used for altering and testing the carbon dioxide and oxygen content of air surrounding growing mushrooms.

RESULTS OF EXPERIMENTS

EXPERIMENTAL SERIES NO. 1

The first group of experiments indicates that mushroom growth is arrested by diluting the atmosphere surrounding the caps and the mycelium with carbon dioxide, but there is no evidence to indicate whether the stunting is due to high carbon dioxide concentration or to the dilution of oxygen caused by the addition of carbon dioxide, nor is it clear whether the stunting is due primarily to the modification of the atmosphere surrounding the sporophores above ground or the mycelium in the compost.

EXPERIMENT 1-A.—Carbon dioxide was run slowly into a jar over a clump of button mushrooms for 5 days. The concentration of carbon dioxide varied from 6 to 18 percent, and that of oxygen from 16 to 19 percent. In the atmosphere of the compost beneath this jar the carbon dioxide content was 4.6 percent and the oxygen content 20.4

percent. As a result of this treatment the growth of the button mushrooms was completely arrested, while similar clumps outside of the jar

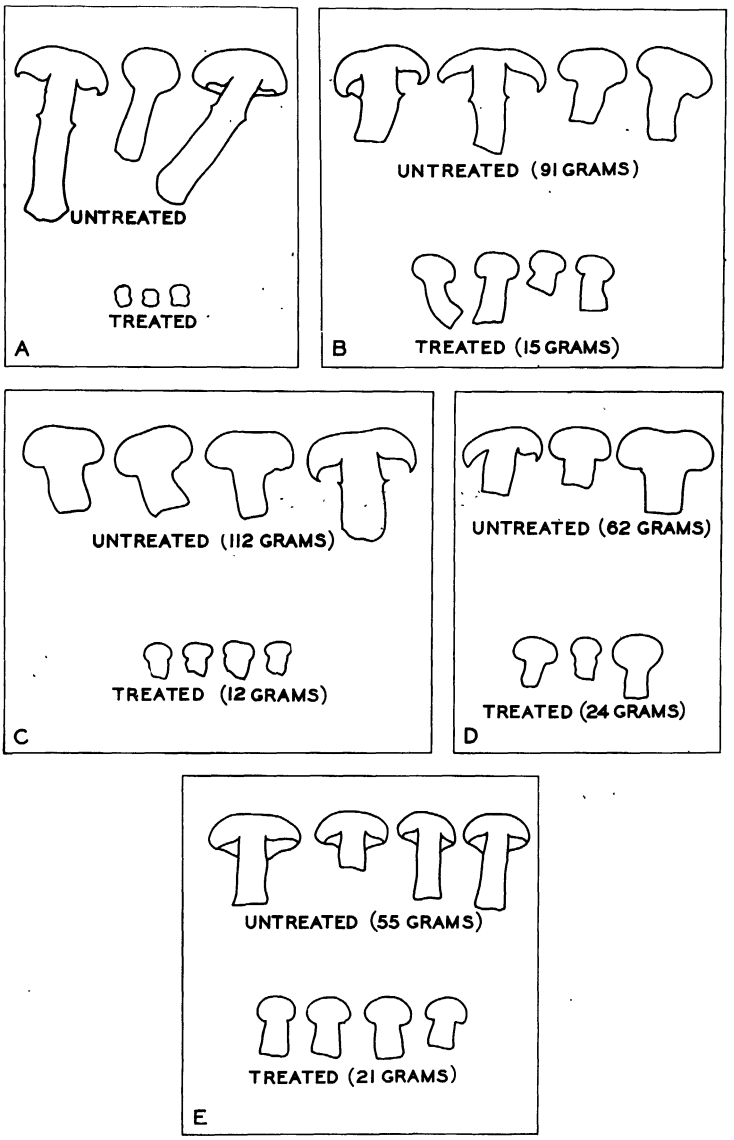

FIGURE 2.—Effect on the growth of mushroom sporophores of diluting the atmosphere with high concentrations of carbon dioxide. Treated mushrooms in group *A* were subject to 6 to 18 percent carbon dioxide for 5 days; group *B*, carbon dioxide 7.2 to 9.4 percent; *C*, carbon dioxide 8 to 9.6 percent; *D*, carbon dioxide 10.8 to 20 percent; *E*, carbon dioxide 15.6 to 22.4 percent. Untreated mushrooms in all had a comparatively equal start but were only subject to normal carbon dioxide in house, less than 0.1 percent.

grew to maturity. Figure 2, *A*, shows the comparative sizes of treated and untreated mushrooms at the end of 5 days.

EXPERIMENT 1-B.—Carbon dioxide was run into a jar over button mushrooms more slowly than in experiment 1-A. The concentrations of carbon dioxide in the atmosphere under the jar ranged from 7.2 to 9.4 percent; the range of the oxygen concentration was 19.0 to 20.2 percent. Growth was arrested, as shown in figure 2, *B*.

EXPERIMENT 1-C.—Carbon dioxide was run into a jar over button mushrooms, as in experiment 1-B. The carbon dioxide concentration of the atmosphere ranged from 8.0 to 9.6 percent, and the oxygen value ranged from 19.0 to 20.4 percent. The carbon dioxide content of the atmosphere in the compost beneath the clump of mushrooms ranged from 3.8 to 4.0 percent, while the oxygen remained at 20.2 percent. The results of this treatment are shown in figure 2, *C*.

EXPERIMENT 1-D.—A comparatively rapid flow of carbon dioxide was run into a jar over a clump of mushrooms. The carbon dioxide concentration in the jar ranged from 10.8 to 20.0 percent, while the oxygen was so diluted that it ranged from 16.8 to 18.4 percent. The atmosphere of the compost beneath the jar contained 6.0 to 6.6 percent carbon dioxide and 19.0 to 19.6 percent oxygen. The effect of this treatment on the growth of the mushrooms under the jar is shown in figure 2, *D*.

EXPERIMENT 1-E.—A comparatively rapid flow of carbon dioxide was passed into the jar. The carbon dioxide content of the atmosphere in the jar was 15.6 to 22.4 percent, the weighted average being 18.4 percent. The oxygen content ranged from 15.0 to 17.6 percent and the weighted average was 16.6 percent. The effect of this treatment on the growth of the mushrooms is shown in figure 2, *E*.

EXPERIMENTAL SERIES NO. 2

The second group of experiments shows that moderate dilution of the atmosphere with nitrogen has little effect on the growth of the mushrooms, whereas dilution with carbon dioxide even in the presence of an excess of oxygen causes a distinct stunting of the mushrooms. It would seem from these results that the increase in the concentration of carbon dioxide rather than the dilution of oxygen is the important factor.

EXPERIMENT 2-A.—Nitrogen was run into the jar to dilute the oxygen. The oxygen content ranged from 7.8 to 20.0 percent, and the carbon dioxide concentration ranged from 0.3 to 0.7 percent. The average oxygen concentration for the entire experiment was 15.7 percent. The nitrogen content was not determined. The growth under these conditions compared favorably with that under jars arranged as checks with similar concentrations of carbon dioxide, 0.4 to 0.8 percent, but with very slight dilutions of oxygen, 20.1 percent. This is shown in figure 3, *A*.

EXPERIMENT 2-B.—This experiment was essentially a duplicate of experiment 2-A. The concentration of carbon dioxide ranged from 0.2 to 1.0 percent, while the oxygen content ranged from 10.4 to 19.6 percent, averaging 16.3 percent. The effect of this treatment on growth is shown in figure 3, *B*.

EXPERIMENT 2-C.—In this experiment both carbon dioxide and oxygen were run into the jar in order to obtain a high concentration

of carbon dioxide in the presence of an excess of oxygen. The oxygen concentration ranged from 25.8 to 26.4 percent, while the carbon dioxide concentration ranged from 7.6 to 9.2 percent. The effect of the treatment is illustrated in figure 3, *C*.

EXPERIMENT 2-D.—This experiment was performed in an attempt to duplicate experiment 2-C. Carbon dioxide concentration ranged from 16.0 to 22.6 percent and oxygen from 26.4 to 26.6 percent. The effect on growth and weight are shown in figure 3, *D*.

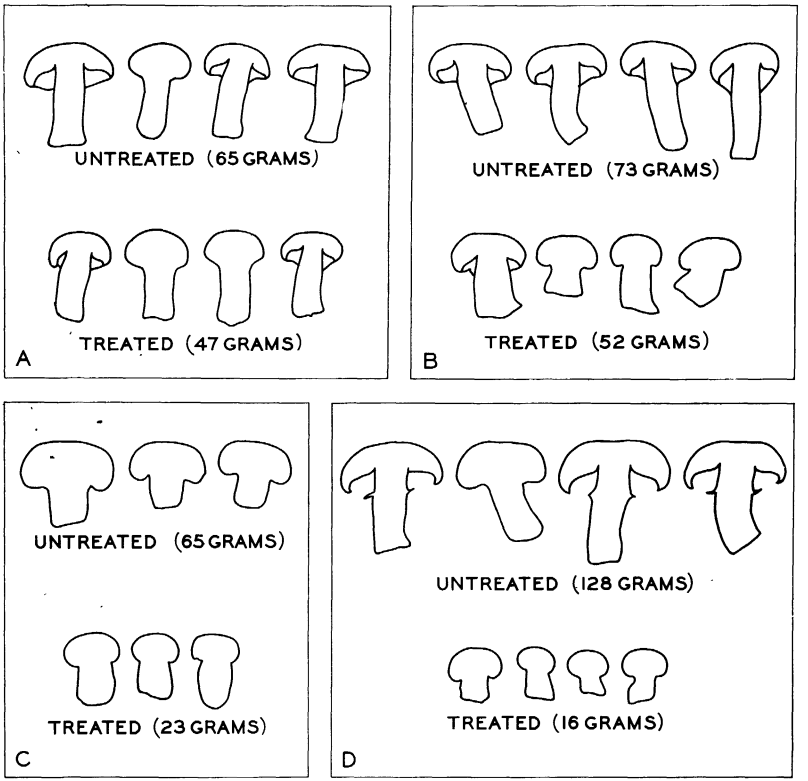

FIGURE 3.—Group *A* and group *B* show the effect of diluting the atmosphere with nitrogen. Groups *C* and *D* show the effect of high concentrations of carbon dioxide together with high concentrations of oxygen. Treated mushrooms in group *A* grew in an atmosphere with oxygen concentrations 7.8 to 20.0 percent and carbon dioxide 0.3 to 0.7 percent; group *B*, oxygen 10.4 to 19.6 percent, and carbon dioxide 0.2 to 1.0 percent; group *C*, oxygen 25.8 to 26.4 percent, carbon dioxide 7.6 to 9.2 percent; group *D*, oxygen 26.4 to 26.6 percent, carbon dioxide 16.0 to 22.6 percent. Untreated mushrooms grew in normal carbon dioxide and oxygen content of house.

EXPERIMENTAL SERIES NO. 3

The third series of experiments indicates that the stunting effect of an excess of carbon dioxide is due principally to its effect on the caps rather than on the mycelium in the compost.

EXPERIMENT 3-A.—Carbon dioxide was run slowly into the compost under a clump of button mushrooms growing in the open air on the bed. The carbon dioxide content reached 1.0 percent, but there was

no appreciable effect on the growth of the mushrooms, as shown in figure 4, *A*.

EXPERIMENT 3-B.—Carbon dioxide was run into the bed more rapidly than in experiment 3-A, and a higher carbon dioxide content resulted, 6.4 to 9.0 percent; yet there was no appreciable effect on the growth of the mushrooms. (See fig. 4, *B*.)

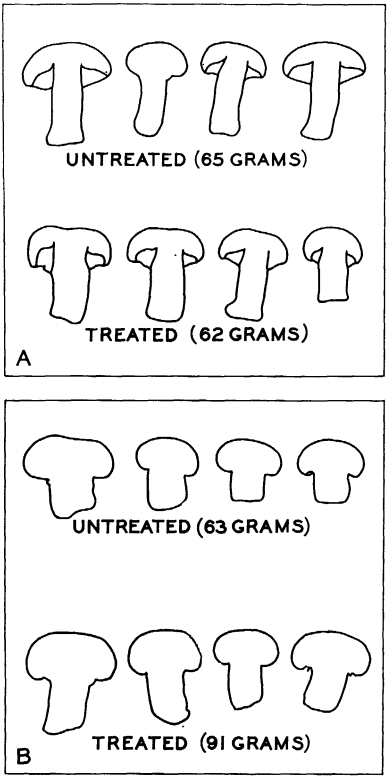

FIGURE 4.—Effect of high concentrations of carbon dioxide in the interstices of the compost under growing mushrooms. Under treated mushrooms in group *A*, carbon dioxide 1.0 percent; under treated group *B*, carbon dioxide 6.4 to 9.0 percent; untreated in both groups, carbon dioxide 0.3 to 0.4 percent. In all instances the sporophores grew in normal air.

EXPERIMENT 4

In an attempt to revive the mushrooms stunted by high concentrations of carbon dioxide in experiments 1-B and 1-C, several stunted mushrooms were left on the bed after the jars were removed. Growth was not resumed, and it was evident that the young mushrooms had been killed by the exposure to excess carbon dioxide.

EXPERIMENTAL SERIES NO. 5

It would seem from the fifth series that there is a slight tendency for the stems of mushrooms to elongate at a concentration of carbon dioxide of about 1 percent. At 1.8 percent this tendency is quite marked.

EXPERIMENT 5-A.—The concentration of carbon dioxide ranged from 0.4 to 0.8 percent and averaged 0.5 percent. The effect on the growth of mushrooms is shown in figure 5, *A*.

EXPERIMENT 5-B.—The carbon dioxide concentration ranged from 0.2 to 1.0 percent and averaged 0.6 percent. The results are shown in figure 5, *B*.

EXPERIMENT 5-C.—The carbon dioxide concentration ranged from 2.2 to 2.6 percent and averaged 2.3 percent. The results are shown in figure 5, *C*.

EXPERIMENT 5-D.—The carbon dioxide concentration was 0.9 percent. Results are shown in figure 5, *D*.

EXPERIMENT 5-E.—The carbon dioxide concentration was 0.6 percent. The results are shown in figure 5, *E*.

EXPERIMENT 5-F.—(Replication of 5-E.) The carbon dioxide concentration was 0.6 percent; a comparison of the treated and untreated mushrooms is shown in figure 5, *F*.

EXPERIMENT 5-G.—This experiment was similar to the others in this series, except that it was made on a floor bed in a cave at St. Paul,

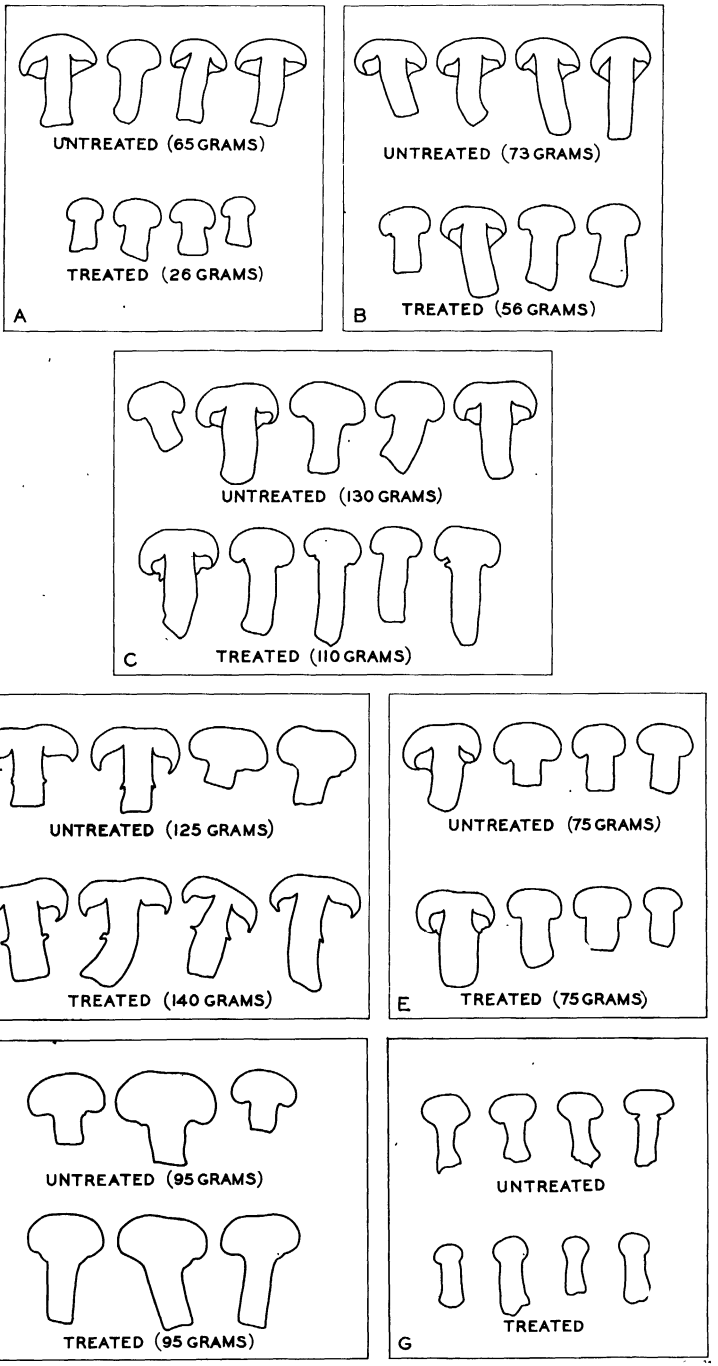

FIGURE 5.—Effect on the growth of mushrooms of small increases in concentration of carbon dioxide in the air. Treated sporophores in group *A* were subject to carbon dioxide average 0.5 percent; group *B*, carbon dioxide 0.6 percent; group *C*, carbon dioxide 2.3 percent; group *D*, carbon dioxide 0.9 percent; group *E*, carbon dioxide 0.6 percent; group *F*, carbon dioxide 0.6 percent; group *G*, carbon dioxide 1.8 percent untreated in all cases exposed to less than 0.1 percent carbon dioxide.

Minn., in 1931. The carbon dioxide accumulation under the jar reached 1.8 percent on the second day. The results are shown in figure 5, *G*.

EXPERIMENTAL SERIES NO. 6

In the sixth series a large excess of oxygen was run into jars containing clumps of mushrooms. There seemed to be a slight tendency for the mushrooms to be firmer and heavier in atmospheres containing a large excess of oxygen.

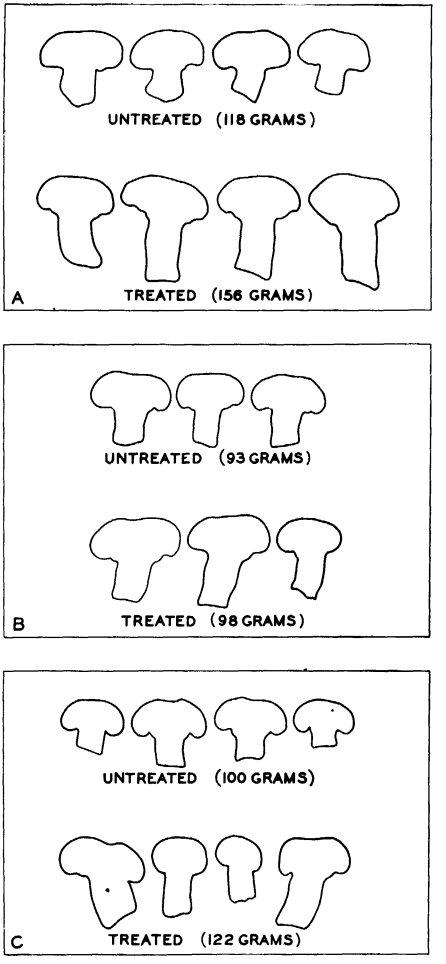

FIGURE 6.—Effect of an excess of oxygen on the growth of sporophores. In group *A* the treated mushrooms grew in an atmosphere containing approximately 37 percent oxygen and about 0.8 percent carbon dioxide; group *B*, oxygen 58 to 61 percent, carbon dioxide 0.4 percent; group *C*, oxygen 28 percent, carbon dioxide 0.6 percent.

EXPERIMENT 6-A.—The oxygen content under the jar ranged from 36.7 to 38.4 percent; the carbon dioxide ranged from 0.7 to 0.8 percent. A comparison of treated and untreated mushrooms is shown in figure 6, *A*.

EXPERIMENT 6-B.—The oxygen content under the jar varied from 58 to 61 percent; the carbon dioxide content was 0.4 percent. A comparison of the treated with the check mushrooms is shown in figure 6, *B*.

EXPERIMENT 6-C.—The oxygen content was 28 percent and the carbon dioxide 0.6 per cent. A comparison of treated with check mushrooms is shown in figure 6, *C*.

EXPERIMENT 7

An excess of oxygen was run into the compost under several clumps of mushrooms growing in the open air. There was no apparent stimulation from this treatment.

EXPERIMENT 8

Clumps of mushrooms were removed from the beds, and jars were inverted over these as well as over places in the bed containing tiny rudiments of mushrooms. After 5 days the carbon dioxide content under these jars averaged 0.2 percent. This is considerably lower than the concentration in series 5, indicating, as would be expected, that the sporophores liberate carbon dioxide freely during active growth.

OBSERVATIONS IN CAVES AND COMMERCIAL HOUSES

In the summer of 1931, samples of air were analyzed from different parts of three sandstone caves devoted to mushrooms culture at St. Paul, Minn. In one cave the beds were spawned but not yet in bearing. In this cave the carbon-dioxide content averaged only about 0.2 percent, in spite of the fact that the cave had been closed up tightly for several days. In the second cave there were actively growing mushrooms, but the door was left open at night for ventilation. The air in this cave had approximately the same carbon dioxide content, 0.2 percent. The third cave was dug somewhat closer to the outside wall and could not be kept cool during hot weather unless the doors were tightly closed. There were growing mushrooms in this cave, and after the door had been closed for a few days the carbon dioxide would reach a concentration of 1 percent. The mushrooms in this cave had a slight tendency to grow long stems with small caps as compared with those in the second cave.

In May 1932, air was analyzed from the mushroom houses of three commercial growers in Chester County, Pa. In all cases mushrooms were growing on the beds. After the doors had been closed for several hours the carbon dioxide content of the air at the ground level varied from 0.2 to 0.5 percent, and at the top of the house from less than 0.2 to 0.4 percent. Opening the doors overnight reduced the concentration to less than 0.1 percent.

DISCUSSION AND CONCLUSIONS

The foregoing experiments should perhaps be considered as field rather than as laboratory experiments. With a plant as variable as the cultivated mushroom, and with the comparatively crude methods used for maintaining and determining the composition of the atmosphere, it was not possible to establish precisely the limits of carbon dioxide and oxygen concentration tolerated by the mushroom. In spite of these limitations, however, the results of most of the experiments were clear-cut and justify definite conclusions.

When compared to the germinating spores and growing mycelium studied by Lopriore,[2] Brown,[3] Fellows,[4] and Neal and Wester,[5] the growing sporophores of cultivated mushrooms appear to be exceptionally sensitive to an excess of carbon dioxide in the air. Under the conditions of the experiments, concentrations of more than 5 percent carbon dioxide in the air arrested the growth of the sporophores and in some cases apparently injured them beyond recovery. At lower concentrations (2 percent, and perhaps even 1 percent) there was a tendency for the pileus to be retarded more than the stipe so that the mushrooms were out of proportion, having long stems and small caps.

It is of considerable interest that growing mushrooms are more sensitive to high concentrations of carbon dioxide surrounding the

[2] LOPRIORE, G. UEBER DIE EINWIRKUNG DER KOHLENSÄURE AUF DAS PROTOPLASMA DER LEBENDEN PFLANZENZELLE. Jahrb. Wiss. Bot. 23: [531]-636, illus. 1895.

[3] BROWN, W. ON THE GERMINATION AND GROWTH OF FUNGI AT VARIOUS TEMPERATURES AND IN VARIOUS CONCENTRATIONS OF OXYGEN AND OF CARBON DIOXIDE. Ann. Bot. [London] 36: [257]-283, illus. 1922.

[4] FELLOWS, H. THE INFLUENCE OF OXYGEN AND CARBON DIOXIDE ON THE GROWTH OF OPHIOBOLUS GRAMINIS IN PURE CULTURE. Jour. Agr. Research 37: 349-355, illus. 1928.

[5] NEAL, D. C., and WESTER, R. E. EFFECTS OF ANAEROBIC CONDITIONS ON THE GROWTH OF THE COTTON-ROOT-ROT FUNGUS PHYMATOTRICHUM OMNIVORUM. Phytopathology 22: 917-920, illus. 1932.

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sporophores above ground than to similar concentrations in the interstices of the compost occupied by the mycelium. A possible explanation for this is that the comparatively loosely woven structure of the sporophore allows a direct diffusion of gases into the air from most of the hyphae, whereas the mycelium in the compost is buried deeply in the organic material through which it is growing and is therefore only partly in contact with the atmosphere of the compost. It may also be of some significance that when the hyphae of the sporophores are growing and respiring actively the mycelium in the compost has already finished most of its growth and is chiefly active in absorbing and translocating nutrients. In any case there is no justification for assuming that the evidence presented applies to young actively growing mycelium, or to sporophores in storage.

Analyses of gas in commercial houses and caves indicate that in practice harmful concentrations of carbon dioxide occur when the caves or houses have been closed up tightly for 24 hours or more. Theoretically the excess of carbon dioxide may be removed by air currents, by diffusion, or by absorption. Harmful concentrations of carbon dioxide can be avoided during the winter by opening the doors or ventilators for a few hours each day. In the case of summer crops grown in water-refrigerated houses where the air is recirculated to minimize the introduction of warm outside air it would seem advisable to make the water in the cooling sprays alkaline in order to absorb the accumulating carbon dioxide, and also to open the ventilators at the top of the house to allow for the escape of carbon dioxide by diffusion.

The evidence that a moderate dilution of oxygen is not detrimental to the growth of *Agaricus* is in agreement with the work of others, notably that of Porodko [6] and Brown,[7] which indicates that aerobic microorganisms usually are more tolerant of moderate reductions in oxygen pressure than of excesses of carbon dioxide.

SUMMARY

By running carbon dioxide under bell jars inverted over growing mushrooms (*Agaricus campestris* L.) it was found that an accumulation of 5 percent or more of carbon dioxide in the air caused abnormal growth, stunting, and even death of the mushrooms. Further experiments indicate that this effect was due to the presence of carbon dioxide and not to the dilution of oxygen. It was also demonstrated that the harmful effect was due principally to the carbon dioxide surrounding the sporophores rather than to that in the interstices of the compost in the bed. Approximately 1 percent of carbon dioxide was the lowest concentration that was noticeably injurious. This concentration was not encountered in commercial mushroom houses or caves except where they had been closed up tightly for 24 hours or more. An abnormally high concentration of oxygen seemed to produce more compact and heavier mushrooms. It is evident that mushroom houses should be ventilated as much as possible without interfering with temperature and humidity control or causing excessive evaporation from the beds by cross drafts.

[6] PORODKO, T. STUDIEN ÜBER DEN EINFLUSS DER SAUERSTOFFSPANNUNG AUF PFLANZLICHE MIKROORGANISMEN. Jahrb. Wiss. Bot. 41: 1-64. 1904.

[7] BROWN, W. (See footnote 3.)