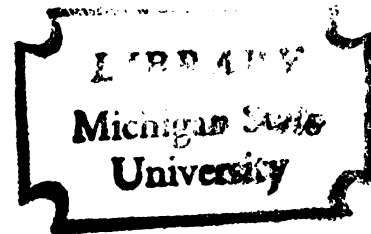


ECOLOGICAL AND PHYSIOLOGICAL STUDIES
OF THE CASING SOIL LAYER IN RELATION TO
FRUIT BODY DEVELOPMENT IN AGARICUS
BISPORUS (LANGE) SING

Thesis for the Degree of Ph. D.
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THESIS



This is to certify that the

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ABSTRACT

ECOLOGICAL AND PHYSIOLOGICAL STUDIES OF THE CASING SOIL LAYER IN RELATION TO FRUIT BODY DEVELOPMENT IN AGARICUS BISPORUS (LANGE) SING.

By

Cesar A. Escobar

An ecological study was undertaken to provide for a better understanding of the qualitative and quantitative changes in the fungal population in the casing soil throughout the mushroom growing period under different cultural practices. A diversity of genera of fungi were found and did not form discrete communities nor a pattern of associated distribution.

The severity of the disease caused by Mycogone perniciosa, Verticillium malthousei, Trichoderma lignorum and T. koningii on mushrooms was determined by inoculation of these organisms in autoclaved casing soil independently and in different combinations. When any combination in pairs of V. malthousei, M. perniciosa, T. lignorum and T. koningii were used, a significantly lower production of mushrooms was obtained than when each isolate was inoculated into the soil separately.

Mycogone perniciosa, Verticillium malthousei, T. lignorum and T. koningii were used in experiments to determine

the factors influencing their survival capacity in soil. In autoclaved soil a decrease of the survival capacity of M. perniciosa and V. malthousei was observed while an increase occurred with T. koningii and T. lignorum during a 30 day period. A marked decrease in survival capacity for these fungal species occurred in unautoclaved soil. Trichoderma koningii and T. lignorum increased in numbers when the autoclaved soil was on top of compost.

An investigation was made of the possibility of biological control of diseases caused by Mycogone perniciosa and Verticillium malthousei in combination with two saprophytic fungi, Penicillium corylophilum and P. asperosporum. A significant reduction or partial biological control of Verticillium and Mycogone diseases, as reflected by a significantly higher mushroom production and less severe expression of symptoms, was accomplished by Penicillium corylophilum.

Additional studies involved the effect of chemically treated wood for preservation on mushroom production. Neither the mushroom production nor the vigor of the mycelial growth of Agaricus bisporus appeared to be affected when grown in wooden trays treated with copper-8-quinolinolate.

Quantitative data on the carbon dioxide and ethylene evolution were taken above and under the casing soil throughout the growing period of mushrooms. Below the casing soil, the carbon dioxide remained nearly constant at 0.1% at the

different flow rates of air from 100 to 2500 ml air/min.

Above the casing there was a marked drop in carbon dioxide level at the time of primordial formation to less than

0.02% when the rate of air flow was at least 1000 ml air/min.

Higher yields of mushroom occurred under this rate of flow.

Ethylene seems not to play important role on the metabolism of mushrooms.



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By

Cesar A. Escobar Rez

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To my wife Lucia
and my three children: Carmen Lucia,
Julia Teresa, and Cesar Eugenio.



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INTRODUCTION

The mushroom industry in the United States has expanded and developed considerably during the last few years. The product, Agaricus bisporus (Lange) Sing., which provides richness and glamour to any food to which it is added, possesses a nutritional value that can be compared favorably with most fresh vegetables such as cauliflower (61). In 1968, mushroom production reached 181 million pounds in the United States and the total cost for the annual mushroom production reached about 61 million dollars (48). However, some factors such as diseases and unknown physiological influences exerted by the casing layer of the primordial formation and fruiting body development of the mushrooms, offer considerable limitation in production in this industry.

For a number of years attempts have been made to establish suitable methods for control of fungal, viral and bacterial diseases of mushroom in the casing soil. However, a number of diseases like "wet and dry bubbles" caused by Mycogone perniciosus Magnus and Verticillium dahliae Ware respectively, continue as problems.

Little or no information is available on the ecology of fungi in casing soil in mushroom houses. An ecological

study has been undertaken to provide for a better understanding of the qualitative and quantitative changes in the fungal population in the casing soil throughout the mushroom growing period under different cultural practices. This included the investigation of fungal contamination of the air in a mushroom house in relation to the microbial invasion of the casing soil. Verticillium malthousei, M. perniciosa, Trichoderma lignorum and T. koningii were used in experiments to determine the factors influencing their survival capacity in soil and to determine the severity of the disease when these organisms are inoculated in different combinations in the soil. This led to the investigation of the possibility of biological control of diseases caused by M. perniciosa and V. malthousei, in combination with some of the saprophytic fungi such as soil isolates of Penicillium, namely P. corylophilum and P. asperosporum. Additional studies involved the effect of chemically treated wood for preservation on mushroom production.

Quantitative studies were performed on the evolution of CO₂ and ethylene above and under the casing soil as well as on the influence of these gases, upon the primordial formation and sporophore development throughout the mushroom growing period.

LITERATURE REVIEW

In casing soil and in compost more than 20 different fungal species, in addition to bacterial and viral diseases (37, 2), affect mushroom production in the United States. Research has been done for a number of years in trying to establish suitable methods for control. However, many of the diseases like Mycogone perniciosa (wet bubbles disease) and Verticillium malthousei (dry bubbles disease) are still problems. Very little information is available on the ecology of fungi in casing soil in mushroom production in relation to the disease problem.

Cross and Jacobs (14) found that Verticillium malthousei is difficult to control as a mushroom pathogen because it produces abundant numbers of conidia which are well adapted for water (splashing droplets) and animal (insects and mites) dispersal. The sticky conidial surface enables the spores to cling tenaciously to materials on contact and to be carried for long distances. They also mentioned a possible means of dispersal by wind dissemination of dust or debris particles to which spores may have adhered. Furthermore, it was reported that V. malthousei has the capacity to infect the mushroom at all stages of development with equal facility. The same authors described Mycogone perniciosa

as a mushroom pathogen which is well adapted for wind dispersal. Dispersion by water and contact are also important but not to the same extent as for Verticillium. They also said that infection caused by Mycogone mainly occurs at the pin-head stage. The more mature fruiting bodies are attacked less frequently by this fungus.

Cross and Jacobs (13) found that conidia of V. malthousei remained viable for at least one year in moist soil and peat, and survived desiccation for up to seven months. In sterile soil the conidial germination rate was high and was followed by rapid mycelial growth and sporulation. In natural soil the germination rate was low and growth did not occur. Conidia of V. malthousei required an exogenous source of nutrients for germination. The required nutrients for conidial germination might be supplied from mushroom mycelium. They reported that conidia in soil are subjected to fungistasis, which they suggest is due to a microbial induced deficiency of essential nutrients (13).

Working with M. perniciosa Fletcher and Ganney (21) found that inoculation of the soil soon after casing gave the highest incidence of disease, the amount decreasing steadily with subsequent inoculations. Furthermore, it seems that the time of symptoms caused by this pathogen appears to vary with the concentration of the inoculum. There appears to be very little growth of this pathogen in the casing soil

and it seems that infection develops in close proximity of the inoculum to the fruiting body. No evidence was found of parasitism of the mushroom mycelium by M. pernicios. They suggested that water might be a dispersal agent of the conidia.

Chlamydospores of Mycogone possess a thick-wall which make them capable of surviving in a dormant state for a long period of time. The conidia of V. malthousei can survive for at least 10 months in soil (14). Primary inoculum for both M. pernicios or V. malthousei could be brought in by flies, humans, wind, soil or debris from neighboring farms or soil for casing (14).

Quantitative data concerning microorganisms are subject to considerable variation due to several factors such as type of soil, seasonal distributions, other ecological conditions and isolation techniques employed for this purpose (24, 50).

The estimates of the number of fungi using the soil dilution and plate count method, refers just to the number of viable cells and mycelial fragments in the sample capable of growing on the medium employed in the test (34). It was pointed out that attempts to obtain exact estimation of the quantity of fungus flora by a plate technique fails to give a complete picture (28). On the other hand, Witkamp's (68) experiences reveal that the interpretable results of this method are valid, even though it enumerates only organisms that are nutritionally and competitively able to develop on the plates. The soil dilution plate is known to be strongly

biased in favor of heavily sporulating fungi, but if colonies are well spaced or about 20-25 on the plates in the final dilution, then competition is reduced (16). Garrett (24) says that some of the methods for isolation of microorganisms from soil are more selective than others.

In the study of casing soil several factors may limit the number of microorganisms. A series of these were mentioned: moisture content of soil, temperature, organic matter available and pH, as well as the survival capacity of the organisms (50, 28). A good casing soil should support a high population of fungi. This is the case for the mesic prairies in Wisconsin, while thin soil with low soil moisture and limited organic material was reported by Orput (50) to limit the number of soil fungi. He noted that *Aspergilli* were more common in prairie soil than *Fusaria*. Tresner (63) and Orput (50) found that *Aspergilli* were poorly represented in forest soil. The type and source of casing soil is important to consider.

Trenser (63) and Latter (42) found a variation in the organisms present in different types of soil and in different soil habitats during the winter and summer periods due to the available organic materials, amount of moisture and pH.

Treatment of soil with one of several chemicals including methyl bromide, promoted the development of dominant populations of Trichoderma, reaching about 100% of the

recolonizing fungal flora. Trichoderma viride and T. koningii were included among the dominant species (47).

Bliss (7) reported that a high population of Trichoderma (ascribed to be T. viride) followed after a fumigation of soil with carbon disulphide. Trichoderma viride established itself as the dominant fungus, at least for 6 months, in a soil treated with diluted formalin (1:500 to 1:1000). In sterile soil, the first colonizing fungi were fast-growing Phycomycetes and T. viride (16).

Methyl bromide has been reported relatively ineffective against Verticillium spp. (23). However, Hayes (29) recommends the use of methyl bromide as a substitute for steam as an after-crop sterilant. He failed to kill V. malthousei when fumigation was run at a temperature less than 65°F. He recommended the maintenance of minimum air temperature of 67°F during fumigation.

Trichoderma spp. and Verticillium spp. are important in mushroom diseases and are commonly isolated from casing soil. The above are among the reasons why the members of these genera are still problems.

Antagonism has been reported between pathogens and their vascular plant hosts (19, 35) as well as microorganisms and root parasites (9). Biological substances as gliotoxin and viridin, both produced by T. viride, have been reported to be antagonistic to the fungi (8, 10). Trichoderma viride is commonly found in casing soil.

Burnett (11) describes the "fungal antagonistic reaction" as a complex problem, in which several factors are involved such as competition for nutrients and sometimes competition for space, parasitic attack by other fungi and antibiosis. Ko and Lockwood (38) point out that soil fungistasis is due to the unavailability of nutrients required for spore germination, or the loss of them from the fungal spores.

It is believed that several pathogens could be effectively controlled by some natural enemy whose identity would depend on its need for rapid reproduction capacity, good soil colonizing characteristics, inhibitory or competitive effect against other fungal microorganisms, inexpensive culture production, and harmless effect to the crop. Myrothecium sp. was shown to restrict the spread or completely stop damping-off due to Rhizoctonia solanii on pepper seedlings. Some information is given on the promising controlled colonization technique which protects treated soil from introduced disease organisms (19).

Kneebone (36) describes Peziza ostrocoderma as a fast casing soil infesting fungus which, in severe cases, causes retardation on the mushroom breaks and yields are diminished somewhat perhaps due to the effect of excreted toxins into the soil. It spreads mainly by wind.

Cooper-8-quinolinolate has been widely used as a fungicidal agent to protect materials such as timber and wooden

fruit boxes. The life of chip baskets in continuous use to collect fruits was prolonged from 10 days to 10 weeks by treatment with this fungicide (32). Cunilate, a soluble form of copper-8-quinolinolate, has been recommended as an anti-microbial agent for control of accumulated microorganisms in dairy plants, on walls, ceiling and floors. This nontoxic material has been accepted for regular use, by some regulatory agencies of the government (57).

Microorganisms may or may not be associated with fruit body formation. Gerlin (25) reported that formation of fruiting bodies of the cultivated mushrooms is caused by certain bacteria. These organisms live in the casing soil. If they are sufficiently numerous they stop the growth of the mycelium. The formation of fruiting bodies is connected with this growth stoppage. Lockard and Kneebone (44) were able to induce production of mushrooms on sterilized substrates.

According to Lintzel as mentioned by Singer (61), 72 to 83% of the total nitrogen content of Agaricus bisporus is present in the form of digestible protein. "When used as the only source of dietary protein, 43 to 62 grams of mushroom protein or 100-200 gr dry weight of mushroom tissue per day, was required to maintain nutritional balance in a normal subject weighting 70 kgs." He concludes that mushrooms are relatively high in digestible protein among vegetable foods. The nutritional value, calories and vitamins of the mushrooms may be comparable with such vegetables as

cauliflower, besides adding flavor to other foods. To illustrate the importance of mushrooms, in 1968, the production of mushrooms in the United States totaled 181 million pounds (according to the Crop Reporting Board, statistical reporting service, U. S. Dept. of Agriculture). Pennsylvania State produced 113 million out of the above figure (more than 62% of the nation's production). Total cost for the entire production was \$61,750,000 or 34.1¢ per pound (48).

The sporophores of the cultivated mushroom are sensitive to an excess of carbon dioxide in the surrounding air. For fruit body initiation the carbon dioxide content of the air in or directly above the casing soil must be less than 0.5%. If the rooms are not ventilated after casing, the fruit body formation does not initiate in the casing soil; instead the mycelium grows through the casing and spreads on the surface (64).

Numerous authors have studied the effects of carbon dioxide concentration on the development of fruit bodies in many types of mushrooms as well as the effect on the growth of the mycelium (64, 65, 41, 49, 51, 45, 56, 64, 46). Tschierpe and Sinden (65) found that levels of 0.10-0.15% carbon dioxide retards fruiting body formation and 1%-2% carbon dioxide caused a reduction in the number of sporophores produced. The concentrations of 0.2% carbon dioxide and 20.8% oxygen were found to be acceptable conditions by Lambert (41) for sporophore growth, while 5% or more caused abnormal growth.

Niederpruem (49) and Plunkett (51) found an arresting effect of carbon dioxide on the formation of fruiting bodies of Schizophyllum commune and Collybia velutipes respectively.

A carbon dioxide requirement has been demonstrated as necessary for growth of hyphal strands of Agaricus bisporus into sterilized or unsterilized casing, and the carbon dioxide range favoring sporophore initiation was found to be from 340 to 1,000 ppm (0.034% to 0.1%) by Long and Jacobs (45).

In compost carbon dioxide may accumulate up to 18-20% with no effect on yield, as shown by Rasmussen (56). At times a high carbon dioxide concentration may stimulate crop yields. Tschierpe (64) reported that the carbon dioxide content of the air in the compost decreased during the development of the sporophores under commercial and laboratory conditions. Concentrations of carbon dioxide less than 0.3% were seldom measured.

Tschierpe (64) postulated a theory concerning the functions of the casing soil and the cause of fructification of the mushroom. He stated "the object of the casing soil is to create a carbon dioxide gradient from the air in the compost to the air above it, and fructification takes place in the region of this carbon dioxide partial pressure gradient." However, Long and Jacobs (45) indicated that mushroom initiation is not dependent on a carbon dioxide gradient in the casing layer.

Mercier and MacQueen (46) reported that the rate of carbon dioxide evolution for mushrooms (commercial varieties) was about 200 grams of CO_2 per gram of mushroom in one hour (this amount is equivalent to about $10.2 \text{ ml } \text{CO}_2/100 \text{ g-hour}$) at room temperature (an open or closed system was not specified). An infra-red carbon dioxide analyser was used.

Wright et al. (69) gave the heat evolution of cultivated mushrooms stored at 50°F (10°C) as 22,000 BTU/ton-24 hour. This value in terms of respiration rate is equivalent to about $5.1 \text{ ml}/\text{CO}_2/100 \text{ g-hour}$. Besides that, Gill (26) reported that the rate of carbon dioxide evolution, during the first 24 hours, for mushroom stored at 10°C , was about $5.7 \text{ ml}/\text{CO}_2/100 \text{ g-hour}$. Both are in agreement. The RQ was not appreciably altered (average 0.85) by temperature in the range 5° to 15°C .

The production and role of ethylene in Agaricus bisporus, and its interaction with carbon dioxide still remain unknown. Ilag and Curtis (30) found that of 228 species of fungi tested for detectable amounts of ethylene production, approximately 26.5% produced ethylene. The concentration of ethylene varied from 0.18 to over 500 ppm. Fungi producing less than 0.16 ppm of ethylene were classified as nonproducers. They concluded that ethylene is a common metabolic product of fungi. Lockard and Kneebone (44) in analyzing collected gases produced by mycelium of Agaricus

bisporus found that at least five substances, ethylene, acetaldehyde, acetone, ethylacetate and ethanol, in addition to carbon dioxide, were produced. Tschierpe and J. W. Sinden (65) studied the sequences of production of acetone, ethanol, acetaldehyde and ethylacetate by the mycelium of Agaricus bisporus under oxygen concentrations of about 1.5% in which anaerobiosis is stimulated. They concluded that the above metabolic substances are not produced by either the vegetative mycelium or by the fruiting bodies under aerobic conditions.

Ethylene is well known to involve certain processes in vascular plants and microorganisms (52, 66, 22). Many of these processes may be inhibited by carbon dioxide (52).

Résumé of the general method of commercial mushroom culture. Mostly, mushrooms are grown on compost consisting of straw-bedded horse manure or hay and ground corn cobs supplemented with brewers' grain. Gypsum is added as a stabilizer, preventing adverse changes in pH from developing during and after composting phases (61). The composting process consists of two phases.

Phase I: The outdoor composting, in which the compost is held in long piles for 8 days. During this phase the pile is turned and watered at two day intervals. The temperature of the compost in this phase is in the range of 160-170°F. The N content varies from 1.5 to 2.0% of the dry weight and is present in the form of ammonia and amines. At this stage oxidation is limited, but carbohydrates lose "water" by

caramelization, resulting in a rapid darkening of the straw, hay and corn cobs (59).

Phase II: Phase I is followed by the indoor decomposition process which serves as a final conditioning and pasteurization to free the compost of organisms which might be pathogenic or antagonistic to mushrooms by interfering with their normal growth (60). During phase II, the temperature is raised to about 143°F and held there. The importance of this phase is to promote the active development of some thermophilic microorganisms which rapidly convert the ammonia and amine nitrogen into microbial protein that is readily used as a nutrient by the mushrooms. Phase II is accomplished in 5-7 days (59). The cooling off should be gradual (no greater than 12°F per day) and when the temperature has decreased to less than 100°F, and before it has reached 80°F, the spawning should be done.

Spawning is planting the mushroom mycelium or spawn in the compost. Spawn is composed of sterilized wheat or rye grains thoroughly impregnated with the mushroom mycelium. The spawn needs to grow in the compost for 2-3 weeks at an optimum temperature of 73°F. Moisture and ventilation should be kept at optimum levels and as uniform as possible during this time.

Casing, the next step, consists of placing a 1" layer of soil on the surface of the compost. Then the compost temperature is permitted to decrease slowly until it reaches

about 60°F. Good ventilation and sufficient water are important factors in mushroom production. Production of mushrooms will begin about 10-15 days after casing.

Harvesting, the last step, must be done when fruiting bodies offer the best characteristics for marketing.

MATERIALS AND METHODS

Soil sampling. For ecological studies of the fungal population in the casing soil, periodic samples were collected from commercial mushroom houses located in Lapeer and Utica, Michigan. Forty grams of casing soil samples taken with a sterile spatula were collected at random from mushroom growing beds throughout several growing periods. The soil samples were placed into sterile plastic bags and transported immediately to the laboratory. Soil samples were collected from four different locations inside the mushroom houses included the front and back parts at the upper and lower levels.

Soil isolation methods. Each soil sample was sifted through a 10-mesh sieve and the moisture content was calculated by drying the samples over night at 100°C. The pH of each soil sample was obtained by means of a Coleman Metrion meter. Twenty five grams of each sample were placed in a graduated cylinder and filled to a volume of 250 ml with distilled water. This suspension was stirred and poured into a 1000 ml Erlenmeyer flask, then shaken on a mechanical shaker for 30 minutes. Ten ml of this suspension were used to make dilutions. One ml of each desired dilution

was transferred aseptically into each of five Petri dishes and fifteen ml of culture medium were added to each dish. The dishes were swirled to disperse the diluted soil in the medium before solidification. The plates were incubated at 24°C for 5-6 days. For calculation of the number of fungi, bacteria or actinomycetes present, the average number of colonies per dish was multiplied by the dilution factor.

Soil dilutions ranging from 1×10^1 to 1×10^5 were used in order to establish a suitable dilution for isolation of fungi from the casing soil. The most uniform distribution and convenient number of colonies was obtained at dilutions of 1×10^3 and 1×10^4 .

Two different culture media were tried for isolation of fungi from casing soil. Peptone dextrose agar with rose bengal and streptomycin, and potato glucose agar with novobiocine, as recommended by Johnson (33) and Pramer and Schmidt (53), were used for isolation of fungi from some soil samples. Both culture media showed good selectivity for fungal growth. However, fungal species such as Mycogone sp., Verticillium sp., Nigrospora sp., and some genera of Mucorales failed to grow on the former culture medium. In addition, morphology of the fungus colony on the medium with rose bengal was modified in many instances. On the basis of the above results, potato glucose agar with novobiocine* was used as standard culture medium for isolation of fungi from the casing soil samples.

*Furnished by The Upjohn Co.

Identification of the fungi. Single fungal colonies were transferred from the Petri dishes for further study. The Riddell slide culture method (58) was used when necessary for identifying fungal isolates. Standardized cultural methods adopted from Raper and Thom (54), Kulik (40), Raper and Fennell (55) and Toussoun and Nelson (62), were used for identification of species of Penicillium, Aspergillus and Fusarium, respectively. Species of Trichoderma were identified utilizing references by Mughogho (47), Bisby (6), Webster (67), and Gilman (27). For identification of species of Mucorales and most isolates of Deuteromycetes, several additional keys and references were used (4,5,18,20,27,31). For Chaetomium, a monograph by Ames (1) was used.

Method for cultivation of mushrooms in the experimental house. Autoclaved wooden boxes, 13"x13"x8", each with about 1200 g (dry weight basis) of commercially pasteurized compost were transported from a commercial mushroom house to the experimental mushroom house. Prior to filling, each box was covered by a sterile plastic bag after autoclaving. The plastic case was partially removed at the filling time inside of the mushroom house, and each box was fully covered by the plastic during transportation. The temperature of the compost at the time of filling of the boxes was 100°F. The material was placed inside of the experimental mushroom house which is a walk-in incubator air-conditioned to provide

suitable environment for mushroom culture or, in some instances, inside of a walk-in growth chamber. Both places were chemically treated with 10% Clorox before using. About 55 g of fresh spawn, white commercial strain 310, were aseptically mixed with the compost. The spawn was allowed to develop at 73°F for at least two weeks. The procedures already described were followed throughout the growing period.

Nitrogen analysis. Samples of the compost were analyzed for nitrogen content. The Kjeldahl procedure was used (15). The nitrogen content for different samples ranged between 1.89-1.95%.

Casing soil. A silt-loam soil was used as standard casing for all the experiments. The pH of the soil was corrected to neutral by adding pulverized limestone. Soil was autoclaved for six hours. The same type of soil, without autoclaving, was used as control where indicated.

Preparation of the soil inoculum. In some experiments, soil inoculation was required. The fungal isolates were grown on slants of the most suitable standard culture medium for 8 days. Five ml of sterilized distilled water was added to each tube and the fungal growth was scraped with a sterile needle. The product of several tubes was collected in a centrifuge tube. Conidial suspensions were washed three times with sterile distilled water by centrifugation

at 3500 rpm for 5 minutes. Different concentrations of conidial suspensions were prepared, utilizing a hemocytometer to determine the number of conidia.

Soil inoculation method. Two methods of soil inoculation were tested. In one trial, conidial suspensions were poured onto the soil immediately after casing. The other trial consisted of mixing the conidial suspension with the soil followed immediately by casing. More satisfactory results were obtained with the former method.

Recovery of fungi from casing soil. Soil samples taken at random with a sterile spatula were collected immediately after soil inoculation and after 2, 4, 8, 16 and 30 days. Samples from the same treatments were mixed and by use of the plate dilution technique, the number of fungal colonies was estimated.

Methods for study of contamination of the air and the casing soil. Fungal contamination of the air in a commercial mushroom house was studied by periodic exposure of Petri dishes containing potato glucose agar and novobiocine at different locations inside the house throughout the growing period.

Chemically sterilized plastic boxes containing a 1" thick autoclaved soil layer were distributed in different places inside the mushroom house on the beds, in order to study the invasion which takes place in the casing soil.

Soil samples from the casing layer were periodically collected at random from different locations of the house, and at the same time samples were collected from the soil in plastic boxes. In this study colonies of fungi, bacteria and actinomycetes were counted and the number recorded based on the number of propagules per g of dry soil from all the soil samples. Water agar and Thornton's standardized medium plus PCNB (17,43) were used for isolation of actinomycetes and bacteria respectively. For bacteria, incubation was for 15 days at 32-35°C. Fungi and actinomycetes were incubated at 24°C. In this experiment, only the fungi were classified to species.

Methyl bromide was used to study the effectiveness of soil treatment on control of fungi. Soil for casing was treated with two levels of methyl bromide: 4 pounds/100 sq. ft./1 ft. depth, designated as H (high), and 2 pounds/100 sq. ft./1 ft. depth designated as L (low). The treatment period of the soil was two days beneath a gas-proof cover. After several weeks storage, soil was aerated for 5 days before use.

Preparation of jars for growth chambers for gas studies.

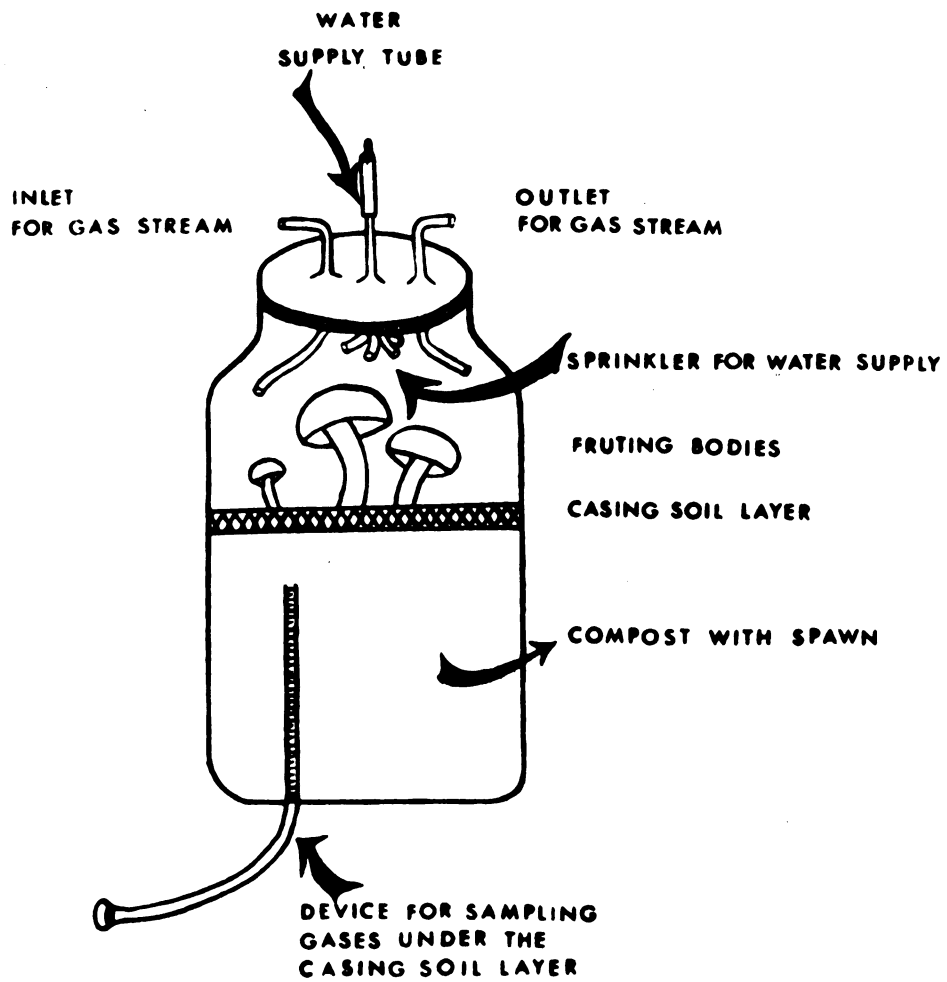
One gallon jars with metal lids, were provided with three short pieces of copper tubing as inlet and outlet for gas stream and a supply of water. These were used as growth chambers. One perforation of 2 mm. in diameter was made at

the bottom of each jar for collection of gas samples from the compost. A diagram of the growth chamber is shown in Fig. 1.

Two thirds of the volume of each jar was filled with commercially pasteurized compost (250 g. dry weight). Twenty five grams of fresh spawn, white commercial strain 310, were aseptically mixed with the compost, the jar mouth was covered with a 2 x 15 cm. Petri dish cover, and the mycelium was allowed to grow for 13-16 days at 75°F. When complete coverage of the compost by the growing mycelium was accomplished, an autoclaved casing soil layer of about 3/4" thick was placed on top. A silt-loamy soil was used for this purpose and it was buffered with pulverized limestone to pH 7, and adjusted to approximately 90% of its water capacity. After this step, desired flow rates of air were given continuously and the temperature of the mushroom house was lowered 2°F every day to 60°F. This last temperature was maintained throughout the growing period. For those experiments in which gas samples were collected throughout the mycelial growing period on different substrates, the different flow rates of air were provided immediately after placing the materials inside the jars.

By means of two capillary flowmeters designed by Claypool and Keefer (12), a continuous flow, of a desired amount of air ranging from 50-2,500 ml/minute, was passed through the growth chambers. In some experiments, gas

Figure 1.--Growth chamber device used for gas studies.



samples were collected from growth chambers with all outlets closed (closed system). A general view of the growth chambers and flowmeters are shown in Fig. 2.

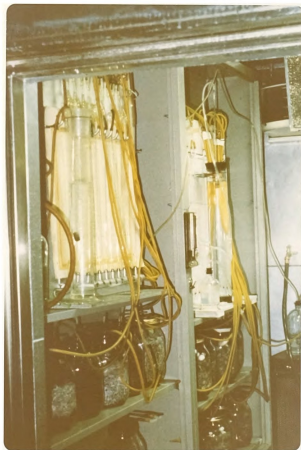
A short piece of rubber tubing with a glass rod at the end was attached to the outlet of the growth chamber one minute before the gas sample was collected. Before collecting the 7 ml sample from each growth chamber, the gas was withdrawn by a syringe and reinjected three times in order to obtain an homogeneous mixture. After about 30 seconds an equilibrium occurred inside the syringe, and the rubber tubing was removed. Immediately a gentle pressure was exerted on the syringe's plunger, the syringe was partially submerged in water, and the needle was replaced by a serum cap. The syringes were left partially submerged in water until gas analysis.

The carbon dioxide and oxygen were analyzed by means of a thermal conductivity gas chromatography unit. A flame ionization gas chromatography unit was used for ethylene analysis. One ml of the gas sample was injected into the machine. Measurements of carbon dioxide, oxygen and ethylene were made daily or every other day.

These experiments were normally conducted in a walk-in incubator to provide suitable environment for mushroom culture. The jars used in these experiments were autoclaved at 121°C for 30 minutes at 15 pounds pressure. All the necessary components and attachments of the flowmeters were



Figure 2.--The growth chambers and the flowmeters used for gas flow experiments.



sterilized with sodium hypochlorite (Clorox) in order to obtain a better accuracy on the ethylene measurements. The air flow was filtered, prior to humidification through sterile cotton. Additional sterile water was supplied to the casing soil, when required, through the attached sprinkler by means of a 20 ml sterile syringe.

The soil used was autoclaved at 121°C for 6 hours at 15 pounds pressure. Other materials, such as compost or rye grain, were autoclaved in smaller portions only once for two hours if sterile conditions were required. All the results were confirmed by repetition of the experiments.



RESULTS

Ecological study of the fungal population in the casing soil of a commercial mushroom house. During a 3 month period in the winter and in the summer of 1969, samples of the casing soil were periodically collected from a large commercial mushroom house located in Lapeer, Michigan in order to study the qualitative and quantitative changes in the fungal population throughout several cropping periods. Each house has approximately 13,500 sq. ft. and contains a shelf bed system. There are 4 1/2 to 5 breaks per fill, depending on schedules, disease and insect problems. Zineb (zinc ethylene bis dithiocarbamate) 15% dust and Malathion [S-(1,2, bis (ethoxycarbonyl) ethyl) 0,0-dimethylphosphorodithioate] 4% dust plus pyrethrins are the chemicals most commonly used in this mushroom plant. Each application of zineb on the casing soil was 3/4 lb./4,000 sq. ft. Up to 11 applications of zineb were put on the casing soil during this 6 months study.

The results are based on collection of samples from the casing soil in different houses, using the same type and source of soil, at different stages of the cropping period. The fungi came from the casing soil samples collected from the front and back and from the upper and lower part of each



of these houses. The relative numbers of fungal micro-organisms, per g of dry soil, present in the casing soil from four different mushroom houses during two different seasons, winter and summer, are showed in Table 1 and Fig. 3. The highest number of fungi was in the untreated casing soil during the summer period. In the same period, samples were taken 18 days after casing with 4 applications of zineb and continued up through 47 days with 11 applications of zineb. In these samples the colony remained lower than in the untreated soil. In the winter the colony count was lower in the untreated casing soil than in summer, and much lower after the casing soil was treated with zineb.

The genera of fungal isolates from the casing soil, and their relative frequency in each of the four locations in each of the commercial houses are indicated in Table 2. The number of genera and species which appeared in the untreated casing soil was similar both in the summer and in the winter. There was some variation between the lower front and lower back of the mushroom house. The front lower casing soil samples had fewer genera.

There was a marked difference in the number of genera present after the various applications of zineb, especially in the winter. Species of Aspergillus were strongly reduced after the fungicide treatment on day 18, while several species of Penicillium continued to appear throughout the sampling period. Species of Trichoderma remained abundant during the



TABLE 1.--Numbers of fungi present in the casing soil of 4 different mushroom houses during winter and summer seasons and their relationship to the number of applications of zineb after casing and mushroom production.

House	Days after casing	Applications of zineb	Fungal colonies/ g dry soil (1x10 ³)	Production lbs./ sq. ft.	Season
1	2	-	22.2	2.0	W I N T E R
2	18	4	9.1	1.5	
3	34	8	5.9	2.6	
4	47	11	7.5	2.3	
1	1	-	54.1	2.0	S U M M E R
2	6	1	35.8	2.2	
3	30	8	24.7	2.3	
4	40	10	35	2.3	

TABLE 2.--Genera of fungi isolated from casing soil in any one of 4 mushroom houses during winter and summer seasons, and their occurrence in each of the four locations inside the houses.

GENERA OF FUNGI	WINTER				SUMMER			
	DAYS AFTER CASING				DAYS AFTER CASING			
	2	18	34	47	1	6	30	40
(0)*	(0)*	(4)	(8)	(11)	(0)*	(1)	(8)	(10)
F F B B F F B B F F B B F F B B F F B B	F F B B F F B B F F B B F F B B F F B B	F F B B F F B B F F B B F F B B F F B B	F F B B F F B B F F B B F F B B F F B B	F F B B F F B B F F B B F F B B F F B B	F F B B F F B B F F B B F F B B F F B B	F F B B F F B B F F B B F F B B F F B B	F F B B F F B B F F B B F F B B F F B B	F F B B F F B B F F B B F F B B F F B B
L L L L L L L L L L L L L L L L L L L L	L L L L L L L L L L L L L L L L L L L L	L L L L L L L L L L L L L L L L L L L L	L L L L L L L L L L L L L L L L L L L L	L L L L L L L L L L L L L L L L L L L L	L L L L L L L L L L L L L L L L L L L L	L L L L L L L L L L L L L L L L L L L L	L L L L L L L L L L L L L L L L L L L L	L L L L L L L L L L L L L L L L L L L L
<i>Aspergillus</i> spp.	X X	X			X X X X	X		X X
<i>A. niger</i> Tieghem	X				X			X X
<i>A. fumigatus</i> Fresenius	X	X						
<i>A. clavatus</i> Desm.					X X			
<i>Alternaria</i> sp.	X	X			X X		X	
<i>A. tenuis</i> Nees.					X X			
<i>Absidia spinosa</i> Lendner					X X			X X
<i>Botrytis</i> sp.	X X		X		X			
<i>Chaetomium globosum</i> Kunze			X	X				X
<i>Cephalosporium</i> sp.	X X	X			X			
<i>Cladosporium</i> sp.	X				X X		X X	X
<i>Charopsis</i> spp.	X							
<i>Fusarium</i> sp.	X X	X			X X	X		
<i>F. oxysporum</i> Schl.					X X			
<i>Gilmaniera</i> sp.	X				X X			
<i>Geotrichum</i> sp.	X	X			X			
<i>Glyocladium roseum</i> (Link) Thom.	X X				X			
<i>Humicola brevis</i> (Gilm. & Abbot) Gilman					X X			
<i>Myrothecium</i> sp.	X		X		X			
<i>Mucor</i> spp.		X			X X X	X	X X	X X
<i>M. racemosus</i> Fresenius					X X			X X
<i>Mycogone perniciosa</i> Magnus	X	X			X X	X		X X
<i>Mycogone nigra</i> (Morgan) Jensen	X	X		X	X X			
<i>Nigrospora sphaerica</i> (Saccardo) Mason	X	X	X		X			X
<i>Oedocephalum</i> sp.	X X							

* () Number zineb applications

F = front

B = Back

L = lower part

U = upper part of the mushroom houses



entire 47 days. Chaetomium globosum and Mycogone perniciosa, reoccurred in the samples on day 47, after being absent while Verticillium albo-atrum was present again in the samples on day 34.

The fungi isolated from the casing soil in the summer followed a similar pattern for species of Aspergillus, Penicillium and Trichoderma. A few of the other genera were more prevalent after the application of zineb to the casing soil in the summer period. Alternaria spp. commonly found in air and soil in the summer was markedly reduced in the casing soil by the use of zineb.

Fungal contamination of the air in a mushroom house and microbial invasion of the casing soil. During a complete cropping period beginning in March 1970, colony counts of microorganisms were made from samples of casing soil and sterile soil in plastic trays placed on the mushroom beds. These were collected every 14 days from a commercial mushroom house located in Utica, Michigan. In addition, fungi were isolated in culture media exposed to the air at different places inside the house for 15 minutes during the time of the soil samples collection.

The casing soil analyzed in this study was treated with chloropicrin (a commercial product) at a rate of one quart per 6 cubic yards of soil, stored for about one year under a plastic covering. The number of colonies of fungi, bacteria and actinomycetes per g of dry soil, obtained from the



casing soil and from the sterile soil in plastic trays throughout a mushroom growing period is presented in Table 3.

In the casing soil low numbers of fungal colonies on the first day increased by day 14, then leveled off on days 28, 42 and 55. In contrast the sterile soil with a zero count on day 1, continued to increase when checked every 14 days until the colony count was nearly twice that of the casing soil treated with chloropicrin.

The bacterial count in the casing soil continued to increase during the first 28 days and leveled off. In contrast the sterile soil with a zero count on day 1, had a rapid rise in the first 14 days then a gradual increase through day 55.

The actinomycetes showed a higher number of colonies on day one and a slower rate of increase in number of colonies than the other two groups. In the casing soil a small increase occurred in the first 14 days then leveled off. In the sterile soil with a zero colony count on day 1, an increase occurred up to day 14 and leveled off.

In making comparisons of the genera of fungi found in casing soil, sterilized soil and in the air of a commercial mushroom house, a number of similarities can be noted between genera isolated in the air and casing soil throughout the sampling period. Some of the important fungus pathogens of mushrooms such as Trichoderma koningii, Verticillium malthousei and Mycogone perniciosa were detected by the

TABLE 3.--Number of microorganisms found in casing soil treated with chloropicrin and microbial invasion of sterile soil in different locations in a commercial mushroom house.

Days after casing	NUMBER OF COLONIES /g. DRY SOIL											
	Fungi (10^3)				Bacteria (10^5)				Actinomycetes (10^5)			
	Casing F	Casing C	Soil B	Sterile F	Casing F	Casing C	Soil B	Sterile F	Casing F	Casing C	Soil B	Sterile F
1	11	15	16	0	0	0	0	0	28	29	41	0
14	39	23	46	19	23	23	23	21	43	48	32	19
28	43	21	41	21	38	58	58	38	36	54	28	13
42	41	27	34	41	39	72	72	50	46	70	63	20
55	45	31	41	77	69	101	101	61	41	68	36	15
F front	C center	B back of the mushroom house										

Each figure is a mean of four plates.

TABLE 4.--The most common genera of fungi found in the casing soil, in the soil of the plastic trays, and in the air of a commercial mushroom house over a 55 day period.*

Days after casing	Casing Soil	Sterile soil in boxes	Air
1	<u>Trichoderma</u> spp. <u>T. koningii</u> Oudemans <u>T. viride</u> Pers. <u>T. lignorum</u> (Tode) Harz <u>Penicillium</u> spp. <u>Zygorhynchus</u> sp. <u>Mucor</u> sp. <u>Aspergillus</u> spp. <u>Fusarium</u> spp. <u>Verticillium</u> spp.	<u>Trichoderma</u> sp. <u>T. koningii</u> Oudemans <u>T. viride</u> Pers. <u>Mucor</u> sp. <u>Rhizopus</u> sp. <u>Penicillium</u> spp.	<u>Trichoderma</u> spp. <u>T. lignorum</u> (Tode) Harz <u>T. viride</u> Pers. <u>T. koningii</u> Oudemans <u>D. dendroides</u> (Bulliard) Fries <u>Mucor</u> sp. <u>Rhizopus</u> sp. <u>Cladosporium</u> sp. <u>Gloeosporium</u> sp. <u>Penicillium</u> sp.
14	<u>Nigrospora</u> sp. <u>Humicola</u> sp. <u>Sporobolomyces</u> sp. <u>Alternaria</u> sp. <u>Botrytis</u> sp. <u>Geotrichum</u> sp.	<u>Trichoderma</u> sp. <u>T. koningii</u> Oudemans <u>T. viride</u> Pers. <u>Mucor</u> sp. <u>Rhizopus</u> sp. <u>Penicillium</u> spp.	<u>Geotrichum</u> sp. <u>Peziza ostromoderma</u> Korf. <u>Aspergillus</u> sp. <u>Cunninghamella</u> sp. <u>Rodotorula</u> sp.
28	<u>Peziza ostromoderma</u> Korf. <u>Dactylium dendroides</u> (Bulliard) Fries <u>V. malthousei</u> Ware	<u>V. malthousei</u> Ware <u>T. lignorum</u> (Tode) Harz	<u>Verticillium</u> sp. <u>V. malthousei</u> Ware <u>Monilia</u> sp.
42	<u>Mycogone perniciosa</u> Magnus	<u>D. dendroides</u>	<u>M. perniciosa</u> <u>Sporotrichum</u> sp.
55		<u>M. perniciosa</u>	<u>Chaetomium</u> sp. <u>Ch. perpulcrum</u> Ames <u>Cladosporium</u> sp.



exposed plate method as well as the soil sampling method. These fungi except for V. malthousei were usually detected in the sterilized soil samples after two weeks. Verticillium malthousei was found in all samples on day 28. One of the disease producing fungi, Dactylium dendroides, was isolated from the casing soil and sterile soil and early in the air samples. Severe infection of fruiting bodies by Verticillium malthousei was observed in several spots in the mushroom beds along with an abundant growth of the mycelium of Dactylium dendroides, which was present in scattered spots in the house as shown in Fig. 3a, b.

Effect of methyl bromide on the fungal population of casing soil under commercial conditions. Soil samples were collected at random from 3 trays treated with the high and the low concentration of methyl bromide. Samples were taken 10, 30, and 60 days after casing. The number of fungi obtained from these samples as well as from the soil piles before casing are presented in Table 5 and Fig. 5.

The number of fungal organisms from the treated soil on the pile before casing was 19×10^3 and 23×10^3 for high and low levels of the fungicide respectively. The main genera isolated from these soil samples were Mucor spp., Trichoderma spp. and Penicillium spp. Most of the other genera were absent. The number of fungal isolates from untreated soil before casing was higher, 28×10^3 . The main fungus species

Figure 3a.--Severe infection of fruiting bodies by Verticillium malthousei in the mushroom bed.

Figure 3b.--Abundant growth of mycelium of Dactylium dendroides in the mushroom bed.



TABLE 5.--Number of fungi recovered from casing soil treated with two levels of methyl bromide.

Days After Casing	Fungal colonies /g dry soil (10^3)		
	Methyl Bromide (lbs./100 sq. ft./1 ft. depth)		Control
	4	2	
10	25	40	27
30	27	42	37
60	32	43	40
Before casing	19	23	28

Each figure is a mean number of colonies from 5 plates per sample in 3 trays. Only one sample was collected from each of the two treated soil piles before casing.



isolated from this soil were Trichoderma spp., Mucor spp., Penicillium spp., Verticillium spp., Rhizopus spp., Fusarium spp., Chaetomium spp., Gliocladium spp., Alternaria spp., Aspergillus spp., Geotrichum spp., and Rodotorula spp.

The number of fungi increased to almost double in number after 10 days of casing when a low concentration of the fungicide was used, but after 30 and 60 days the colony count showed only a slight increase. At the high level of the fungicide, an increase from 19×10^3 to 25×10^3 in the number of fungal microorganisms was noted after 10 days of casing; the number of colonies increased up to 32×10^3 on the last day of sampling.

The most common species isolated from treated soil were Trichoderma viride, T. lignorum, T. koningii, Penicillium spp., Verticillium spp., Zygorhynchus spp., Mucor spp., and Rhizopus spp. Some colonies of Mycogone perniciosa were found at the end of the cropping period.

The number of fungal colonies isolated from the untreated soil before and 10 days after casing was very similar. A significant increase in the number of colonies was found after 30 and 60 days of casing. Fungal species of Trichoderma spp. and genera of the Mucorales prevailed under this condition during the 60 days after casing. No fungi pathogenic to mushrooms were isolated.



Pathogenicity of five fungal isolates on mushrooms.

Pathogenicity of five fungal isolates, Trichoderma lignorum, T. koningii, T. viride, Mycogone perniciosa and Verticillium malthousei was tested. A volume of 100 ml of conidial suspensions at a concentration of 6×10^6 /ml for the Trichoderma species, 4×10^6 /ml for V. malthousei, and 4×10^6 /ml for M. perniciosa (including conidia and chlamydospores) was used for infestation of the sterile soil, 12x12x1", in each of 4 trays for each treatment immediately after casing. A total of 8 trays were cased, 4 with autoclaved soil and 4 with unautoclaved soil, and a volume of 100 ml of sterile distilled water was poured onto each of the 8 trays.

After casing and inoculation of the casing soil with the fungi, the compost temperature was gradually lowered to 62°F. This temperature was maintained for the next 10 days to provide more suitable conditions for mushroom infection by Verticillium malthousei and Mycogone perniciosa; then it was maintained at 60°F.

The number of fruiting bodies showing symptoms of the disease and mushroom production during the first 30 days after casing were the two parameters used to measure the deleterious effect of the fungal isolates tested. One hundred per cent, 75%, 50%, and 25% of fruiting bodies showing symptoms of disease were used to express the severity of the diseases.

When the casing soil was inoculated with Mycogone perniciosa all the mushrooms that developed showed typical symptoms of "wet bubbles disease". The fruiting bodies of A. bisporus were covered with a dense white cottony mass of mycelium of the pathogen. In the more severe cases of the disease, a distorted or spherical mass of fruiting bodies was observed, covered by a dense velvety white mycelial growth. An abundant amber exudation was noticed as shown in Fig. 4a. An external deformation of the lamellae was noticed in some developed fruiting bodies as shown in Fig. 4b. In spite of the severe infection caused by this pathogen, a large number of fruiting bodies developed throughout the 30 days of the growing period and an average total weight of 239 g was obtained as a yield with these infected carpophores (Table 6). Fruiting bodies in trays in casing soil inoculated with Verticillium malthousei were not infected as severely as in the case of M. perniciosa. Changes in the color of the pileus were usually observed as symptoms of diseased sporophores and tiny, irregular brown spots appeared. Sometimes brown blotches were formed by the extension of the diameter and coalescence of primary necrotic spots. A sequence of the increase in severity of this disease, dry bubbles, and appearance of the old brown sunken lesions on the pileus are showed in Fig. 5a and 5b. It was estimated that 25% of the fruiting bodies did not show any symptoms of infection and an average production of 189 g was obtained throughout the 30 days of the cropping period (Table 6).

Figure 4a.--Fruiting bodies infected by Mycogone perniciosa showing typical symptoms of "wet bubbles disease". The distorted mass of mushrooms is covered by a dense velvety white mycelial growth of the pathogen. An abundant amber exudation is typical of the disease.

Figure 4b.--Agaricus bisporus basidiocarp with deformation of lamellae caused by Mycogone perniciosa.



TABLE 6.--Presence of disease symptoms and yield of mushrooms when pathogenicity of 5 fungal isolates was tested.

TREATMENT	Yield of mushrooms (g)			Fruit bodies with symptoms %
	Days 10-18	after casing 24-30	Total	
<u>Trichoderma lignorum</u>	79	120	199	25
<u>Trichoderma koningii</u>	68	97	165	75
<u>Trichoderma viride</u>	136	132	268	
<u>Mycogone perniciosa</u>	125	114	239	100
<u>Verticillium malthousei</u>	108	81	189	75
Unautoclaved soil	136	182	318	
Autoclaved soil	148	190	338	

Each figure is the average production of 4 trays.

Figure 5a.--A group of mushrooms showing the increase in severity of the symptoms of "dry bubbles disease" caused by Verticillium malthousei.

Figure 5b.--Detail of old brown sunken lesions of the pileus of Agaricus bisporus infected by Verticillium malthousei.





In the case of fruiting bodies grown in trays in casing soil inoculated with Trichoderma koningii, about 75% showed brownish color, burned-like appearance and poor development. The average production of mushrooms obtained from this treatment was 165 g (Table 6).

When casing soil was inoculated with Trichoderma lignorum 25% of the fruiting bodies showed a brownish color and poor development. The rest of the carpophores were normal but only an average production of 199 g was obtained.

Apparently all the developed fruiting bodies in the trays inoculated with Trichoderma viride were normal and an average production of 268 g was obtained. On several occasions, a grayish or green mycelial growth was observed on the surface of the casing soil inoculated with species of Trichoderma.

Carpophores developed in trays with autoclaved and unautoclaved soil were normal and an average production of 338 g and 318 g was obtained respectively (Table 6).

Survival capacity in casing soil of some mushroom fungal pathogens. For the study of the behavior and survival capacity of M. perniciosus, V. malthousei, T. lignorum, and T. koningii, 50 ml of the standard conidial suspensions were placed in casing soil in sterile plastic trays, 25x17x9 cms. A layer of silt-loam soil at a pH of 7, 1" thick, was used alone or on top of a 2" layer of pasteurized compost when indicated. Four trays were used for each treatment as follows:

1. Unautoclaved soil.
2. Unautoclaved soil on top of the compost.
3. Autoclaved soil.
4. Autoclaved soil on top of the compost.

Soil samples were collected at 0, 2, 4, 8, 16, and 30 days after the soil was inoculated with the conidial suspensions. The plastic trays were watered at the same frequency as required in a regular mushroom growing period.

The number of fungal colonies recovered from each of the above treatments during a period of 30 days is shown in Table 7.

In the autoclaved soil there was a decrease in the number of colonies of M. perniciosa and V. malthousei while T. koningii and T. lignorum showed an increase. A somewhat similar situation occurred for the autoclaved soil on top of the compost except for T. koningii and T. lignorum which showed a more marked increase in the number of colonies during the 30 day period, while V. malthousei remained about constant.

In the unautoclaved soil all four organisms showed a marked decrease in number of colonies present in the plates from the soil samples at the end of 30 days. A somewhat similar situation occurred with the unautoclaved soil on top of the compost except T. koningii maintained about the same number of colonies.

TABLE 7.--Survival of four mushroom fungal pathogens in casing soil.

TREATMENT	Number of fungal colonies/ g dry soil (10 ³)						
	Days 0	after 2	soil 4	infestation 8	16	30	
Autoclaved soil	<u>M. perniciosa</u>	8	3	1	1	2	1
	<u>V. malthousei</u>	11	7	6	9	6	4
	<u>T. koningii</u>	14	20	16	19	23	25
	<u>T. lignorum</u>	16	15	13	21	22	20
Autoclaved soil on top of compost	<u>M. perniciosa</u>	9	8	9	5	2	1
	<u>V. malthousei</u>	14	15	11	17	18	13
	<u>T. koningii</u>	17	19	23	28	36	49
	<u>T. lignorum</u>	15	16	16	20	26	32
Unautoclaved soil	<u>M. perniciosa</u>	3	1	1	0	0	0
	<u>V. malthousei</u>	7	3	3	2	1	1
	<u>T. koningii</u>	11	8	8	7	5	3
	<u>T. lignorum</u>	12	5	4	4	3	2
Unautoclaved soil on top of compost	<u>M. perniciosa</u>	4	2	3	1	1	0
	<u>V. malthousei</u>	7	5	6	4	4	3
	<u>T. koningii</u>	10	8	11	7	6	9
	<u>T. lignorum</u>	13	12	8	5	3	4

The effect of the combination of some mushroom fungal pathogens and their survival in casing soil. Conidial suspensions of M. perniciosa, V. malthousei, T. koningii and T. lignorum, as indicated in the previous experiment, were used to infest casing soil to study their survival throughout the mushroom growing period of 30 days. One half of the inoculum for each of 2 organisms was used to study the combined effect of M. perniciosa and T. koningii, M. perniciosa and T. lignorum, V. malthousei and T. koningii, V. malthousei and T. lignorum, and M. perniciosa and V. malthousei on mushroom production. By using 1/3 of the standard volume of inoculum for each of 3 organisms, the combined effect of M. perniciosa, V. malthousei and T. koningii, and M. perniciosa, V. malthousei and T. lignorum was studied. As controls, 100 ml of sterile distilled water were poured into each one of 4 trays with either autoclaved or unautoclaved soil and compost. Four replicates were used per treatment.

The number of fungal colonies recovered from the casing soil at 0, 2, 4, 8, 16 and 30 days after the inoculation is showed in Table 8. Also the total average production of mushrooms for each of the treatments is included.

The number of colonies of M. perniciosa or V. malthousei remained rather stable up to the 8th day after inoculation. An increase in the numbers was observed on the 16th and 30th, at which time symptoms of both diseases were conspicuous. Only a low number of colonies of M. perniciosa were recovered

TABLE 8.--Effect of combination of some mushroom fungal pathogens, after inoculation into sterile casing soil, and their survival in casing soil and on total production of fruiting bodies.

TREATMENT	Recovered fungal colonies / g dry soil (10 ³)						Mean of Total Production in 30 days (g)
	Days after soil infestation						
	0	2	4	8	16	30	
<u>Mycogone perniciosa</u>	8	9	8	7	11	13	209 ^C
<u>Verticillium malthousei</u>	13	11	10	11	14	23	201 ^C
<u>Trichoderma koningii</u>	18	19	22	30	32	43	186 ^C
<u>Trichoderma lignorum</u>	15	14	18	21	28	36	204 ^C
<u>M. perniciosa</u> + <u>T. koningii</u>	3-11	1-10	2-9	1-7	2-11	4-21	129 ^b
<u>M. perniciosa</u> + <u>T. lignorum</u>	2-9	0-8	1-6	3-11	3-14	5-19	131 ^b
<u>V. malthousei</u> + <u>T. koningii</u>	7-14	8-14	11-15	7-18	6-22	8-24	84 ^a
<u>V. malthousei</u> + <u>T. lignorum</u>	10-14	4-14	7-19	6-15	8-18	11-29	123 ^b
<u>M. perniciosa</u> + <u>V. malthousei</u>	4-9	3-11	2-9	2-13	3-14	7-14	71 ^a
<u>M. perniciosa</u> + <u>V. malthousei</u> + <u>T. koningii</u>	2-6-10	0-3-12	0-6-15	2-6-20	4-11-21	4-12-30	120 ^b
<u>M. perniciosa</u> + <u>V. malthousei</u> + <u>T. lignorum</u>	2-9-13	1-7-15	0-8-16	0-8-19	2-10-25	3-12-28	121 ^b
Unautoclaved soil	21	22	18	23	20	14	307 ^d
Autoclaved soil	0	0	0	1	3	7	315 ^d

Each figure is a mean of soil samples from 4 trays and 4 plates per sample. Means of total production followed by different letters differed statistically at the 5% level of significance.

from the soil when combined with either T. lignorum or T. koningii, but a slightly higher number of colonies was obtained by day 30 when combined with V. malthousei which may be influenced by the amount of inoculum.

Colonies of V. malthousei, when combined with either of the two species of Trichoderma, were recovered in reduced numbers when compared with the recovery of this fungus inoculated alone. Also, reasonably good recovery of this fungus was obtained when combined with M. pernicioso.

The number of recovered colonies of the Trichoderma isolates when combined with the other fungal pathogens was lower when compared with the recovery of these fungi when acting independently. Nevertheless, their recovery from soil was greater in number than the other fungal isolates used in this experiment.

A significantly low production was obtained from trays with casing soil inoculated with M. pernicioso combined with V. malthousei. Similar results were obtained when V. malthousei was combined with T. koningii.

Mushroom production from any of the treatments was significantly lower than the controls using unautoclaved or autoclaved casing soil.

Study of the interaction between three Penicillia isolates with Mycogone pernicioso and Verticillium malthousei. Penicillium corylophilum Dierek and Penicillium aspersorum G. Smith were isolated rather frequently from casing soil in

commercial mushroom houses. They showed a significant growth inhibition to a number of other fungal colonies in agar medium. The two species of Penicillium show no apparent fungal pathogenicity for mushrooms.

The purpose of this experiment was to investigate the possible inhibitory action of the above Penicillium isolates, and Penicillium chrysogenum Q16, an antibiotic producer, against Mycogone pernicioso and Verticillium malthousei. Each of the Penicillium isolates was grown on PDA plates with either M. pernicioso or V. malthousei. After 10 days of incubation at room temperature, a clear zone of growth inhibition was visible between each of the Penicillium isolates and the two mushroom pathogens. The inhibitory action of P. corylophilum against M. pernicioso and V. malthousei is shown in Fig. 6a and b.

Biological control of two mushroom fungal pathogens by three strains of Penicillium. An attempt was made to provide some means of biological control of M. pernicioso and V. malthousei by inoculating the casing soil with conidial suspensions of one of the 3 Penicillia: P. corylophilum, P. asperosporum or P. chrysogenum.

Fifty ml of the standardized conidial suspensions of either M. pernicioso or V. malthousei were used to infest the casing soil of each tray combined with 50 ml of conidial suspension of one of the three Penicillium isolates. In other cases a volume of 100 ml was used per tray. The

Figure 6a.--Growth inhibition of Penicillium corylophilum against Mycogone perniciosa on PDA plates after 10 days of incubation at room temperature.

Figure 6b.--Growth inhibition of Penicillium corylophilum against Verticillium malthousei on PDA plates after 10 days of incubation at room temperature.



suspension contained about 1.5×10^5 conidia/ml for each of the three Penicillium isolates used. Four soil samples were collected at 0, 3, 8, 16, and 28 days after soil inoculation. Trays with only autoclaved soil were used as controls. The number of M. perniciosa colonies recovered from casing when inoculated independently increased continuously after the 8th day (Fig. 7). Similar results were observed in the case of V. malthousei (Fig. 8). However, the number of colonies of V. malthousei recovered from soil when inoculated in combination with any one of the 3 Penicillium isolates usually decreased throughout the 28 day period (Fig. 8). A slight increase in the number of colonies of M. perniciosa was observed 28 days after soil inoculation, when inoculated with either P. asperosporum or P. chrysogenum (Fig. 7). Symptoms of "wet bubbles" caused by M. perniciosa in the soil were very severe but decreased about 50% when this pathogen was combined with P. corylophilum in the soil (Fig. 9a, b, c, and Table 9). A 25% reduction in the symptoms caused by V. malthousei was observed when this pathogen was in the soil with the same species of Penicillium (Table 9). Penicillium asperosporum and P. chrysogenum were less effective in controlling the severity of the diseases caused by the above two pathogens (Table 9).

The number of colonies of the three Penicillium isolates recovered from soil, when inoculated in combination with either M. perniciosa or V. malthousei, increased continuously

Figure 7.--Number of colonies of Mycogone perniciosa recovered from casing soil when inoculated independently or in combination with one of three Penicillium isolates namely, P. corylophilum, P. asperosporum and P. chrysogenum.

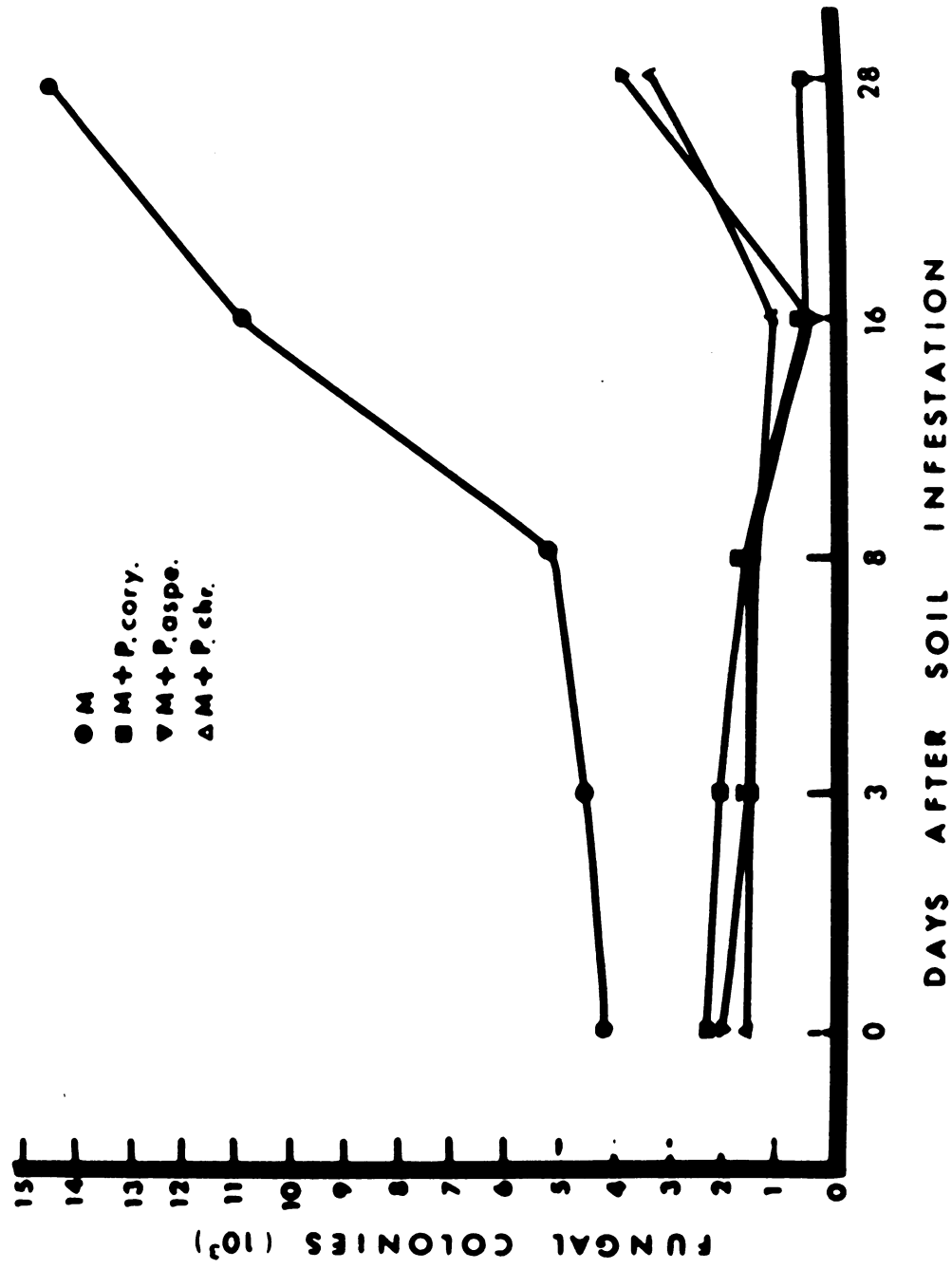


Figure 8.--Number of colonies of Verticillium malthousei recovered from casing soil when inoculated independently or in combination with one of three Penicillium isolates namely, P. corylophilum, P. asperosporum and P. chrysogenum.

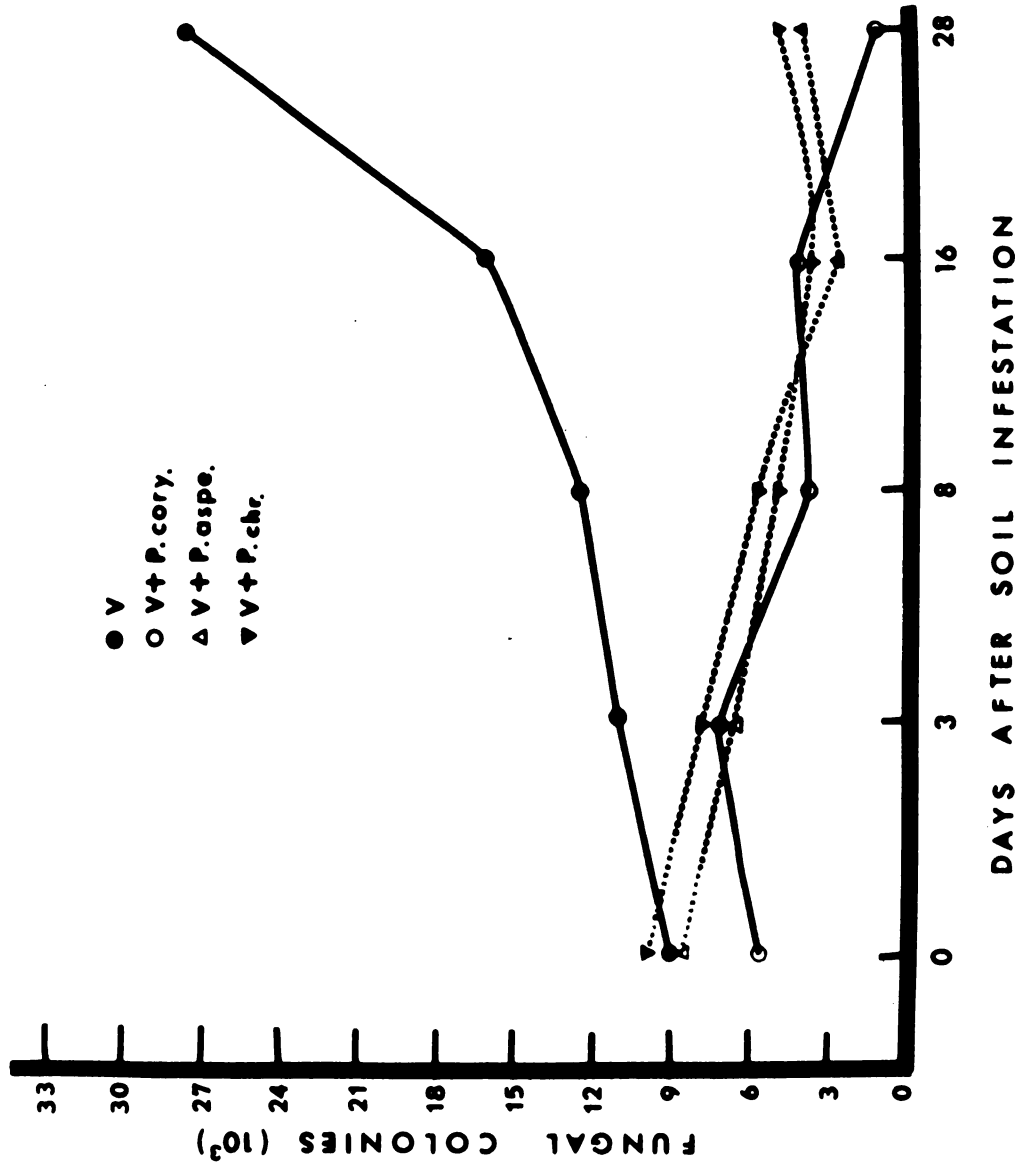


Figure 9a.--Severe symptoms of "wet bubbles disease" obtained by inoculation of the autoclaved casing soil with Mycogone perniciosa.

Figure 9b.--Healthy mushroom cluster obtained when the autoclaved casing soil was inoculated with both Mycogone perniciosa and Penicillium corylophilum.

Figure 9c.--Healthy mushrooms obtained when only 100 ml of sterile water were added to the autoclaved soil by the casing time.



TABLE 9.--The effect of incidence of disease and mushroom production when casing soil was inoculated by Mycogone perniciosa and Verticillium malthousei in combination with 3 Penicillium isolates.

TREATMENT	Fruit bodies with Symptoms %	Mean of production 1200 g dry wt. compost in 28 days
<u>Mycogone perniciosa</u>	100	186 ^a
<u>M. perniciosa</u> + <u>P. corylophilum</u>	50	265 ^b
<u>M. perniciosa</u> + <u>P. asperosporum</u>	75	179 ^a
<u>M. perniciosa</u> + <u>P. chrysogenum</u>	75	178 ^a
<u>Verticillium malthousei</u>	75	170 ^a
<u>V. malthousei</u> + <u>P. corylophilum</u>	25	250 ^b
<u>V. malthousei</u> + <u>P. asperosporum</u>	50	149 ^a
<u>V. malthousei</u> + <u>P. chrysogenum</u>	50	163 ^a
Control		340 ^c

Each figure is mean of the mushroom production of 4 trays. Means of total production followed by different letters differed statistically at the 5% level.

throughout the growing period, but a higher recovery was obtained in the case of P. corylophilum (Fig. 10).

The average mushroom production from the trays inoculated with either M. perniciosus or V. malthousei and P. corylophilum was statistically higher, at the 5% level of significance, than the production for the rest of the treatments, except for the control (Fig. 11). The above findings were confirmed by repetition of the experiment.

Effect on mushroom production of chemical treatment in trays for wood preservation. The decrease in availability and increase in cost of cypress lumber used for the mushroom industry along with the difficulty in cleaning beds, makes it necessary to develop suitable means of wood preservation and prevention of strong adherence of the mycelium of the wood for easier cleaning.

Trays, with standard dimensions, were built using yellow pine and plywood. At the Agricultural Engineering Department, a pressurized treatment with copper-8-quinolinolate was used on the trays. Two levels of the chemical were used, F (full strength), 1/2 strength (1 to 1 parts of sealer and turpentine), and C (control) without any treatment. Two boards of the same material, treated in the same way, were buried into the compost of each tray, in order to observe the mycelium growth on and into the surface of the wood. Four trays were used per treatment and unautoclaved soil was used for casing.

Figure 10.--Number of colonies of Penicillium corylophilum,
Penicillium asperosporum and Penicillium chrysogenum
recovered from casing soil when inoculated with either
Mycogone perniciosa or Verticillium malthousei.
Control (contaminants).

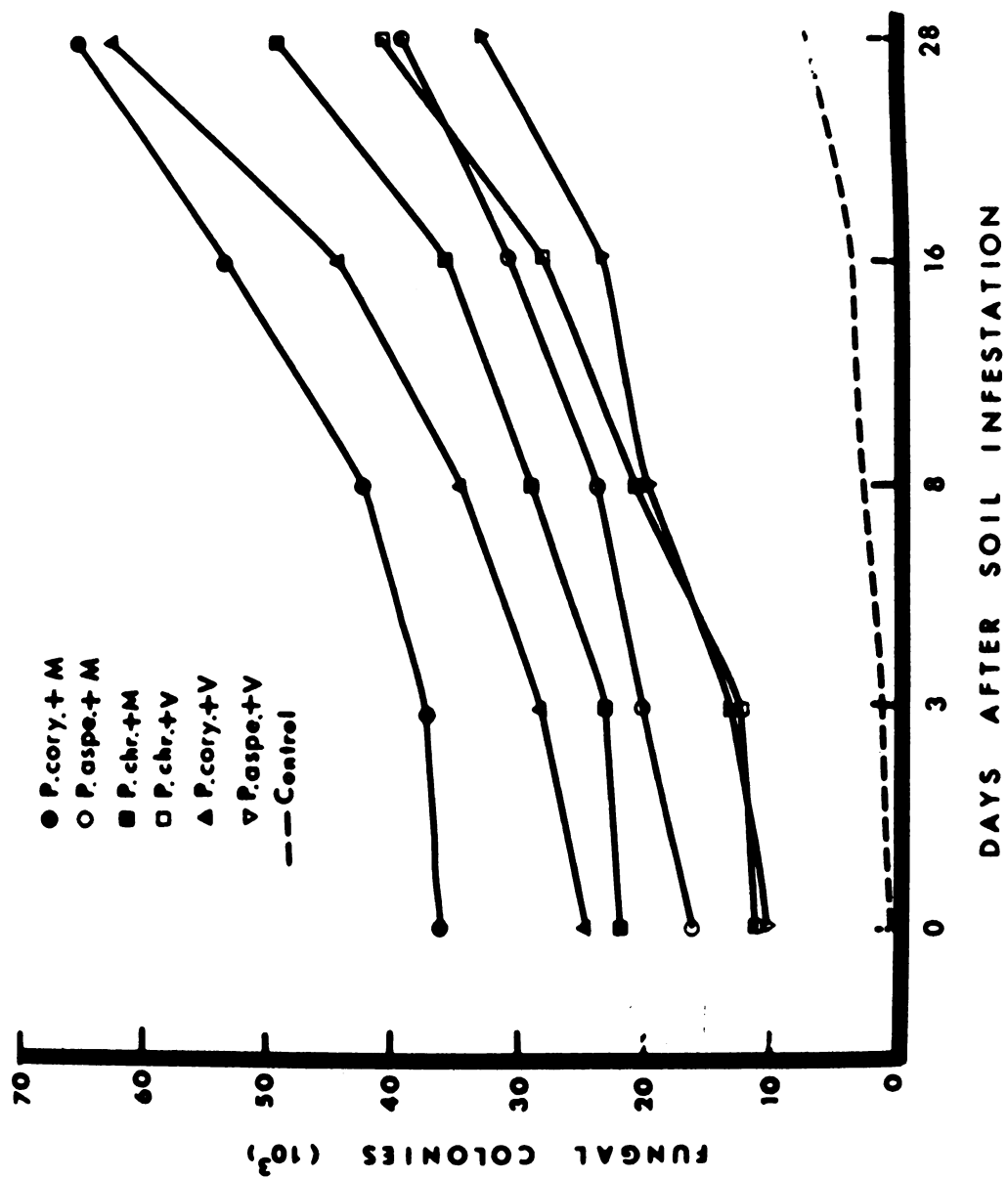
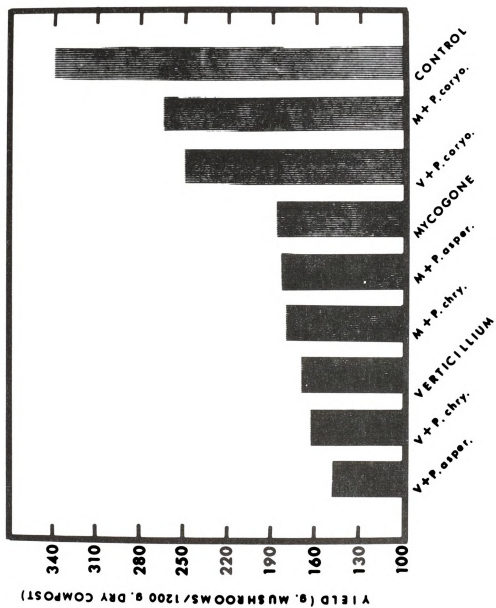


Figure 11.--Mushroom production when casing soil is inoculated with Mycogone perniciosa or Verticillium malthousei and Penicillium spp. Treatments underscored by a line did not differ statistically at the 5% level of significance.



The average production from the trays receiving F, 1/2 and C treatments for the 29 day growing period was 154, 140 and 149 g, respectively. No statistical difference was obtained. The fruiting bodies in each of the treatments boxes were normal, and a similar pattern of distribution on the casing soil was observed (Fig. 12a, b, c).

The vigor of mycelial growth was similar in each treatment as observed in the profiles and on the pieces of board that were buried (Fig. 13a, b, c). No deleterious effect on the fungus growth or fruit bodies was observed.

Studies of the relationship of carbon dioxide and ethylene to fructification of *Agaricus bisporus* (Lange) Sing. The purpose of these experiments was to detect any relationship between the carbon dioxide, ethylene or a combination of these two gases above and below casing soil on fruit body formation. A preliminary experiment was designed to assure suitable functioning of the growth chamber for study of gases in relation to fructification of *Agaricus bisporus*. Low air flows of 80, 100, and 150 ml air/minute were provided, after casing, to a heavy mycelial growth of fungus on 150 g dry weight basis of compost. Water was supplied to the growth chambers when required, and the temperature was held at 60°F. A heavy mycelial growth on the casing layer was observed in all the chambers provided with the above air flows at the end of 26 days. Primordial formation was observed after 26 days from the casing time only

Figure 12a.--Mushroom production for one break in a tray
treated with copper-8-quinolinolate (full strength).

Figure 12b.--Mushroom production for one break in a tray
treated with copper-8-quinolinolate (1/2 strength).

Figure 12c.--Mushroom production for one break in a tray
which was not treated with copper-8-quinolinolate.



Figure 13a.--Vigorous mycelial growth of Agaricus bisporus observed in the profile left by the buried piece of board treated with copper-8-quinolinolate (full strength), after removal.

Figure 13b.--Vigorous mycelial growth of Agaricus bisporus observed in the profile left by the buried piece of board treated with copper-8-quinolinolate (1/2 strength) after removal.

Figure 13c.--Vigorous mycelial growth of Agaricus bisporus observed in the profile left by the buried piece of board, which was not treated with copper-8-quinolinolate, after removal.



with flow rates of 150 ml air/min. The fruiting bodies, mostly with malformations, reached partial maturation 13 days later.

Gas samples were collected from the atmosphere above the casing layer. The carbon dioxide concentrations of the samples ranged from 0.16% to 1.21% throughout the growing period. The oxygen concentrations remained rather consistent, fluctuating between 18.2% and 21%. The maximum concentration of ethylene, 0.35 ppm., was obtained at the end of the growing period.

From the above preliminary experiment, it was observed that the air flows rates selected were not suitable for fructification. Only mycelial growth on top of the casing soil was observed with the two lower flow rates and mostly abnormal fruiting bodies were formed at a flow of 150 ml air/min. The primordial initiation and development of carpophores took much longer in comparison to commercial conditions. The ethylene concentration went over 0.1 ppm, a concentration high enough to be physiologically significant to higher plants under these growing conditions.

An experiment was designed to determine the optimum atmosphere for the growing mushrooms and for the study of the role and behavior of the carbon dioxide and ethylene on the fruiting body formation and sporophore development. In this experiment, 250 g dry weight of compost were used per growth chamber and gas samples were collected from the atmospheres above and under the casing layer every other day

throughout the growing period. For each treatment 2 or 3 replicates were used. The flow rates of air used, 100, 200, 300, 400, 500, 1000, 2000, and 2500 ml air/min., were initiated immediately after casing. The average of the replicates for concentrations of carbon dioxide, oxygen and ethylene found in both atmospheres, under and above the casing, are shown for each flow rate used in Figures 14, 15, 16, 17, 18, 19, 20, 21, 22. Similar results were obtained by repetition of these experiments. The carbon dioxide concentrations above and under the casing soil were generally uniform throughout the experiment as seen in Figures 14, 15, 16, 17, 18, 19, 20, 21, 22. Figure 14 and figure 15 show that carbon dioxide concentrations above and under the casing were similar at flows of 100 and 200 ml air/min. But at flows of 300, 400 and 500 ml air/min. (Figures 16, 17, and 18) the carbon dioxide concentrations differed, particularly after 13 days. In general, however, all of the carbon dioxide flow rates through 500 ml air/min. maintained a certain degree of stability around 0.1% carbon dioxide above the casing soil (see Table 10).

A different pattern was observed at 1000 ml air/min. (Figure 19). The carbon dioxide concentrations above the casing from 0.096% to less than 0.02% by the 11th day at which time primordial formation was seen. After that time carbon dioxide values began to increase gradually, reaching a level of nearly 0.1% at the time of harvest on the 21st

TABLE 10.--The relationships between the rate of flow/min. and the concentration of carbon dioxide and ethylene found above and under the casing soil at the time of primordial formation and at the time of the harvest of basidiocarps.

Air flow rate ml. air/ minute	Days after casing until primordia formation	Concentrations on the day primordia first observed				Days from casing to harvest	Concentrations of the day of harvest				Yield (g./250 g. dry wt. compost)
		CO ₂ % Above Casing	CO ₂ % Under Casing	C ₂ H ₄ ppm Above Casing	C ₂ H ₄ ppm Under Casing		CO ₂ % Above Casing	CO ₂ % Under Casing	C ₂ H ₄ ppm Above Casing	C ₂ H ₄ ppm Under Casing	
100	13 ^a	0.40 ^a	0.38 ^a	0.122 ^a	0.026 ^a	24 ^a	0.56 ^a	0.44 ^a	0.272 ^a	0.086 ^a	- ^a
200	18	0.12	0.17	0.016	0.011	28	0.11	0.16	0.017	0.012	8.5*
300	13	0.11	0.18	0.012	0.024	28	0.09	0.16	0.026	0.012	16.0*
400	12	0.12	0.23	0.014	0.014	26	0.13	0.21	0.025	0.026	29.25
500	11	0.18	0.21	0.018	0.015	24	0.22	0.24	0.034	0.066	29.7
1000	11	<0.02	0.12	0.007	0.010	21	0.10	0.26	0.029	0.024	67.0
1500	13	<0.02	0.18	0.022	0.030	24	0.04	0.20	0.030	0.030	37.5
2000	13	<0.02	0.14	0.015	0.023	24	0.03	0.25	0.023	0.023	31.7
2500	13	<0.02	0.12	0.010	0.013	24	0.04	0.25	0.023	0.026	29.5

^aNo primordia formation was observed.

* Abnormal fruit bodies.

Figure 14.--The concentrations of carbon dioxide, oxygen and ethylene above and under the casing soil when the air flow was 100 ml air/min. in the atmosphere. An average of 3 replicates.

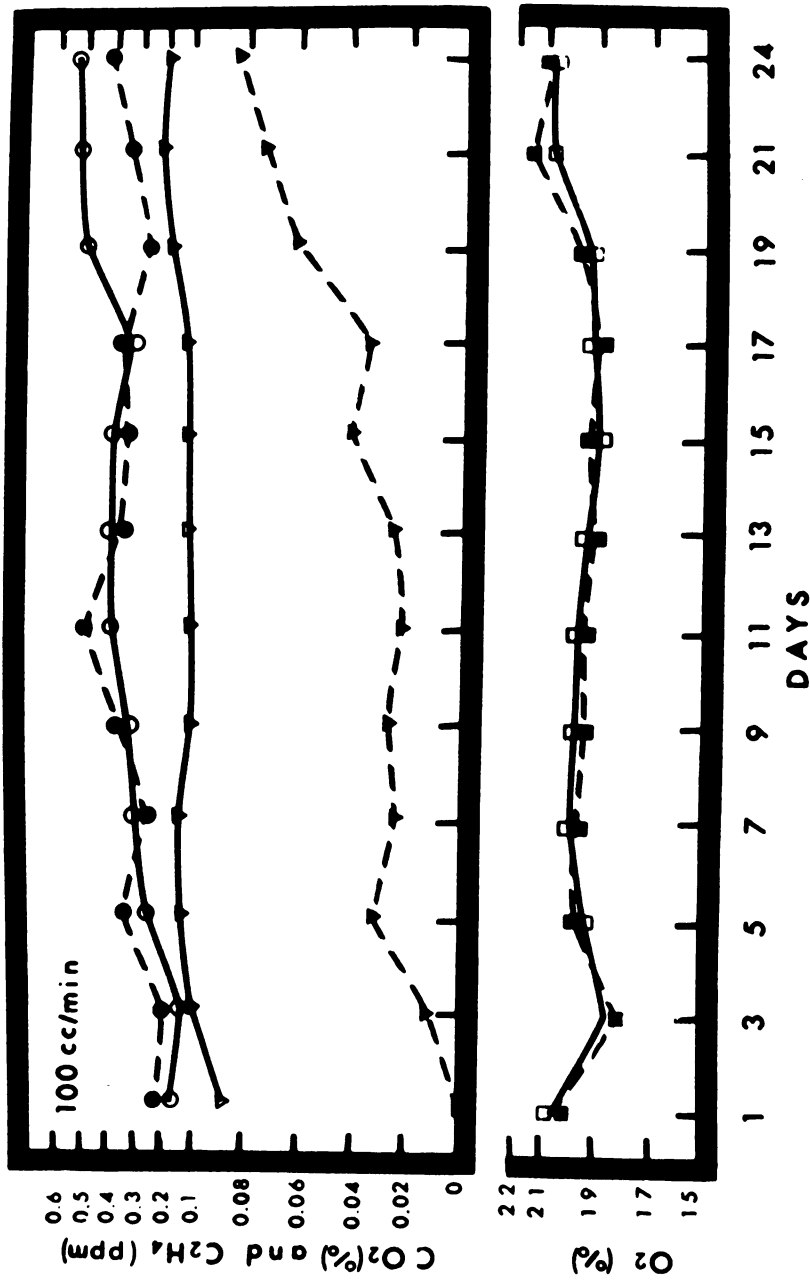


Figure 15.--The concentrations of carbon dioxide, oxygen and ethylene above and under the casing soil when the air flow rate was 200 ml air/min. in the atmosphere. An average of 3 replicates.

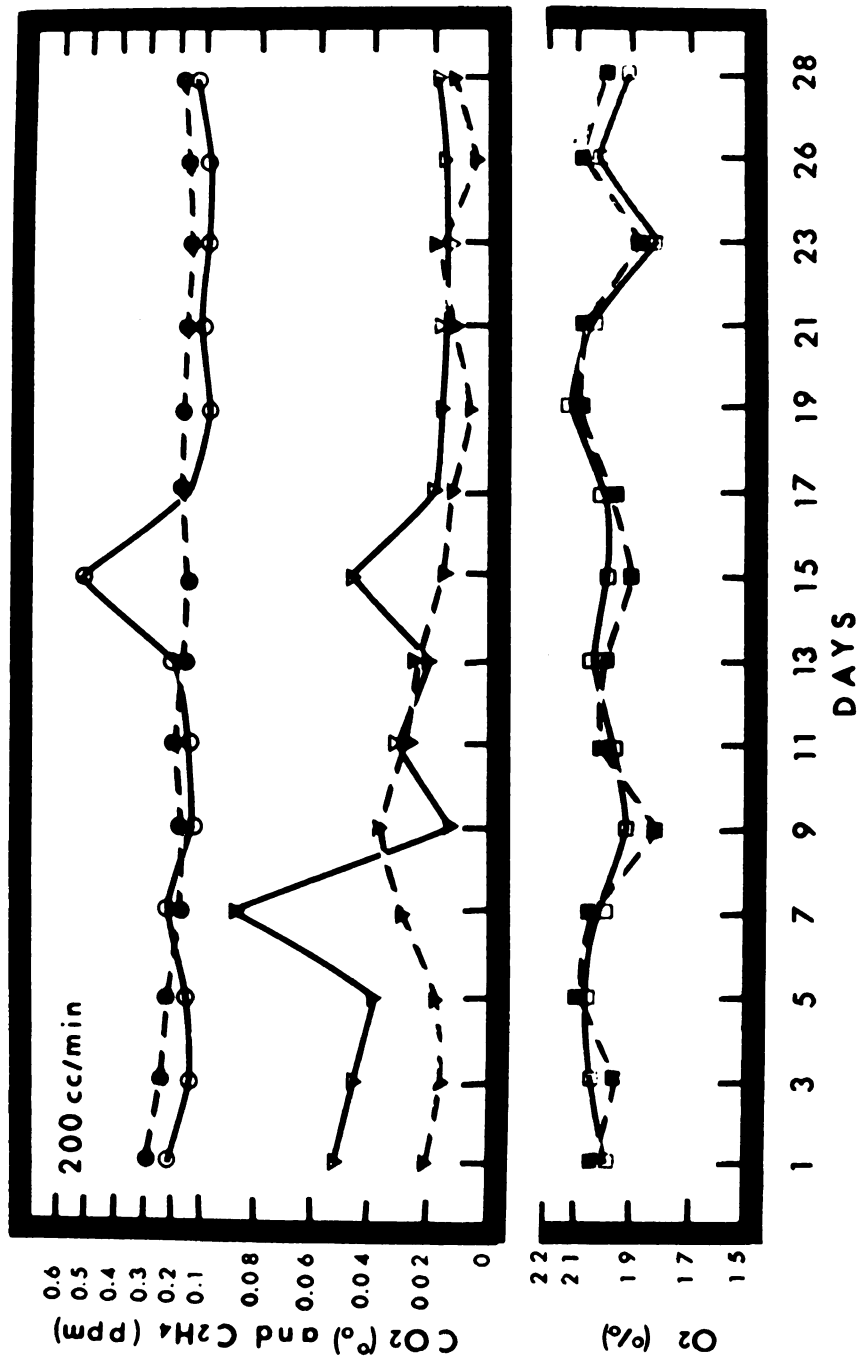


Figure 16.--The concentrations of carbon dioxide, oxygen and ethylene above and under the casing soil when the air flow rate was 300 ml air/min. in the atmosphere. An average of 3 replicates.

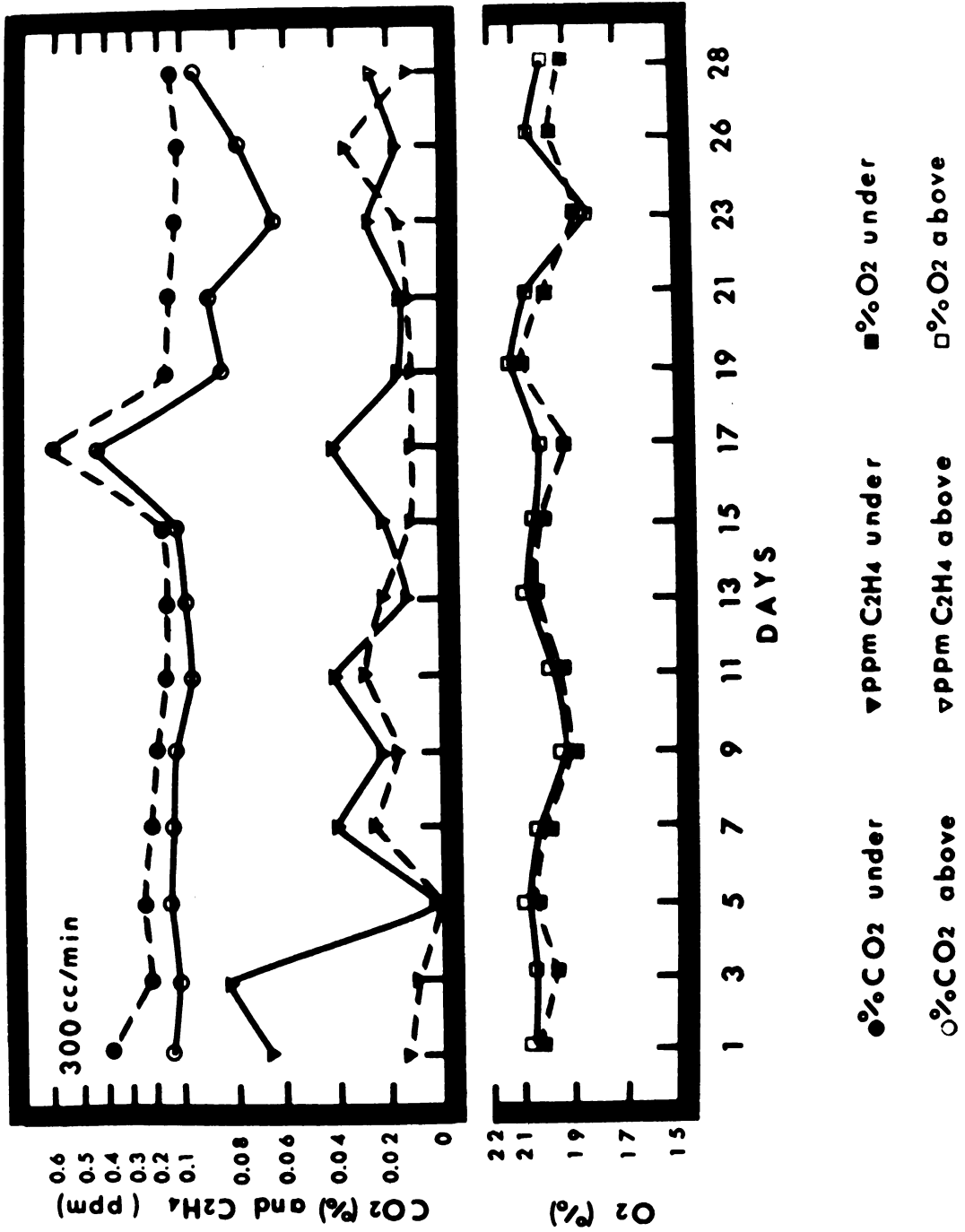


Figure 17.--The concentrations of carbon dioxide, oxygen and ethylene above and under the casing soil when the air flow rate was 400 ml air/min. in the atmosphere. An average of 3 replicates.

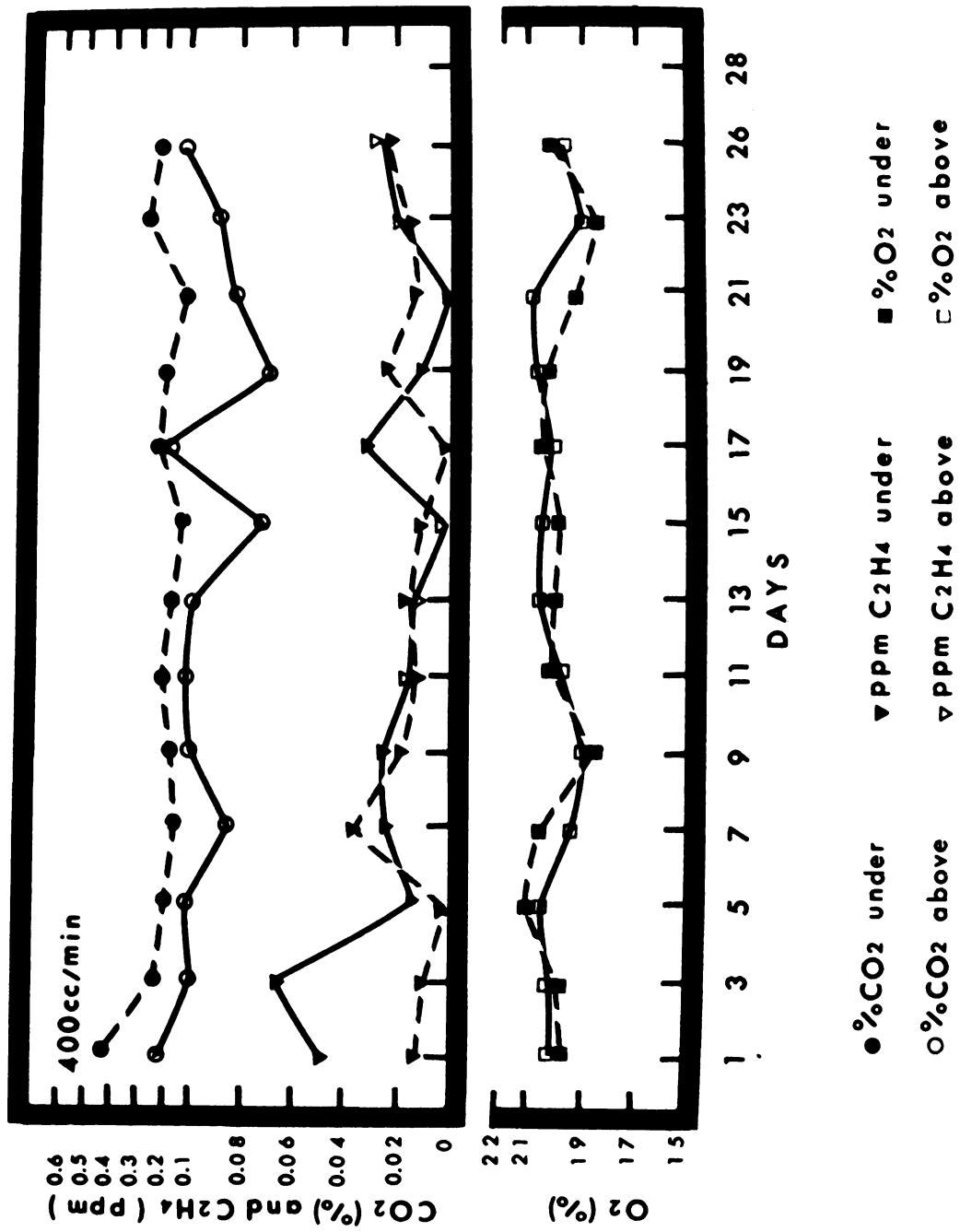


Figure 18.--The concentrations of carbon dioxide, oxygen and ethylene above and under the casing soil when the air flow rate was 500 ml air/min. in the atmosphere. An average of 3 replicates.

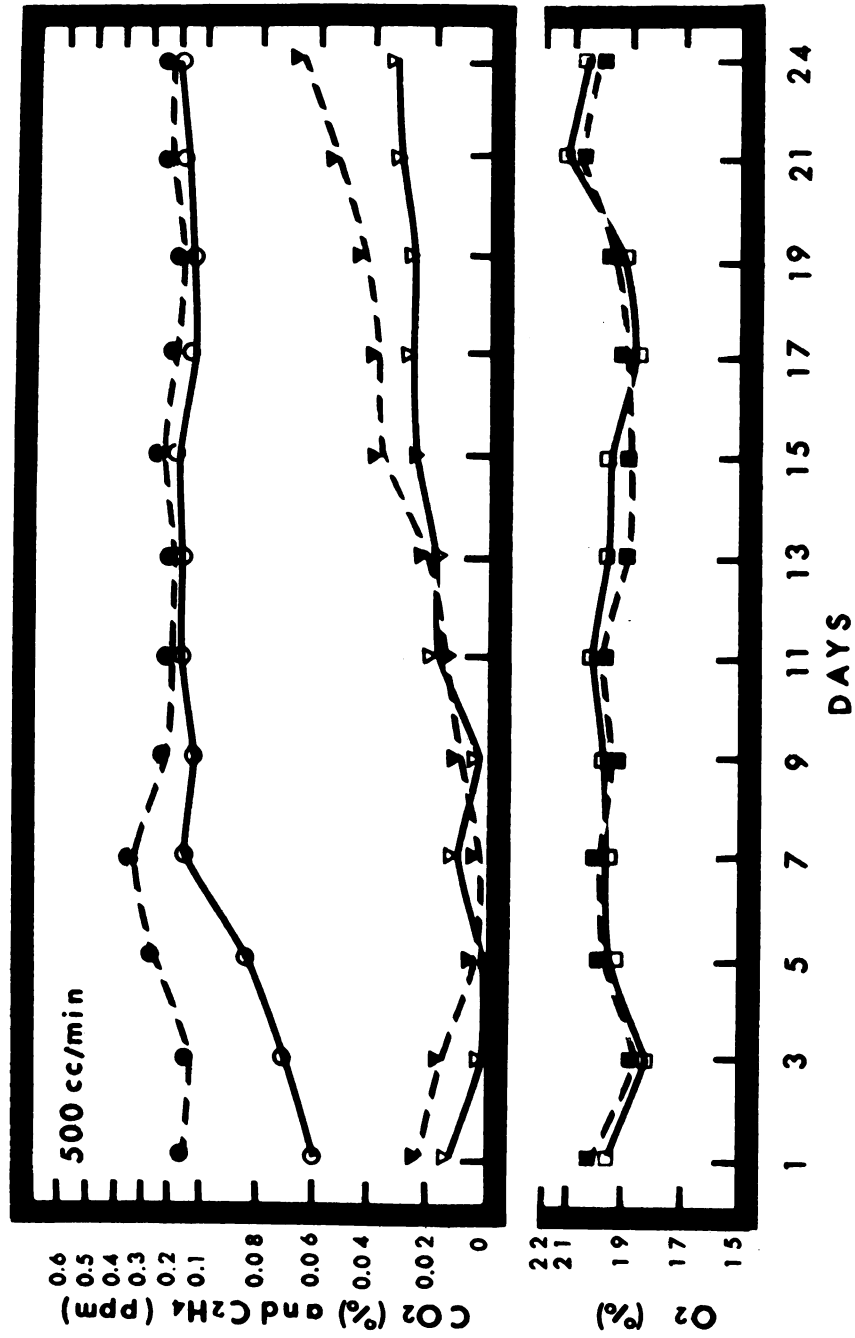


Figure 19.--The concentrations of carbon dioxide, oxygen and ethylene above and under the casing soil when the air flow rate was 1000 ml air/min. in the atmosphere. An average of 3 replicates.

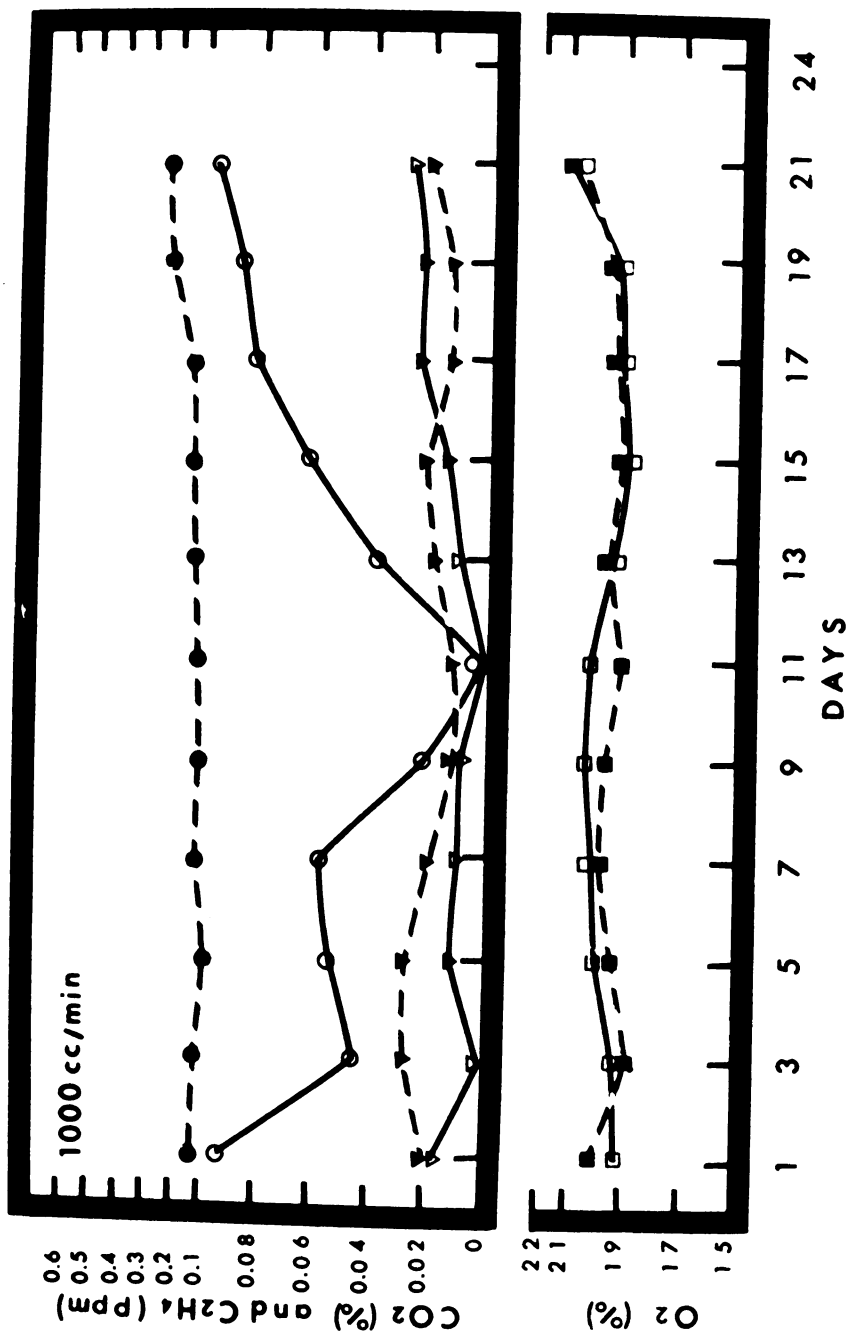


Figure 20.--The concentrations of carbon dioxide, oxygen and ethylene above and under the casing soil when the air flow rate was 1500 ml air/min. in the atmosphere. An average of 3 replicates.

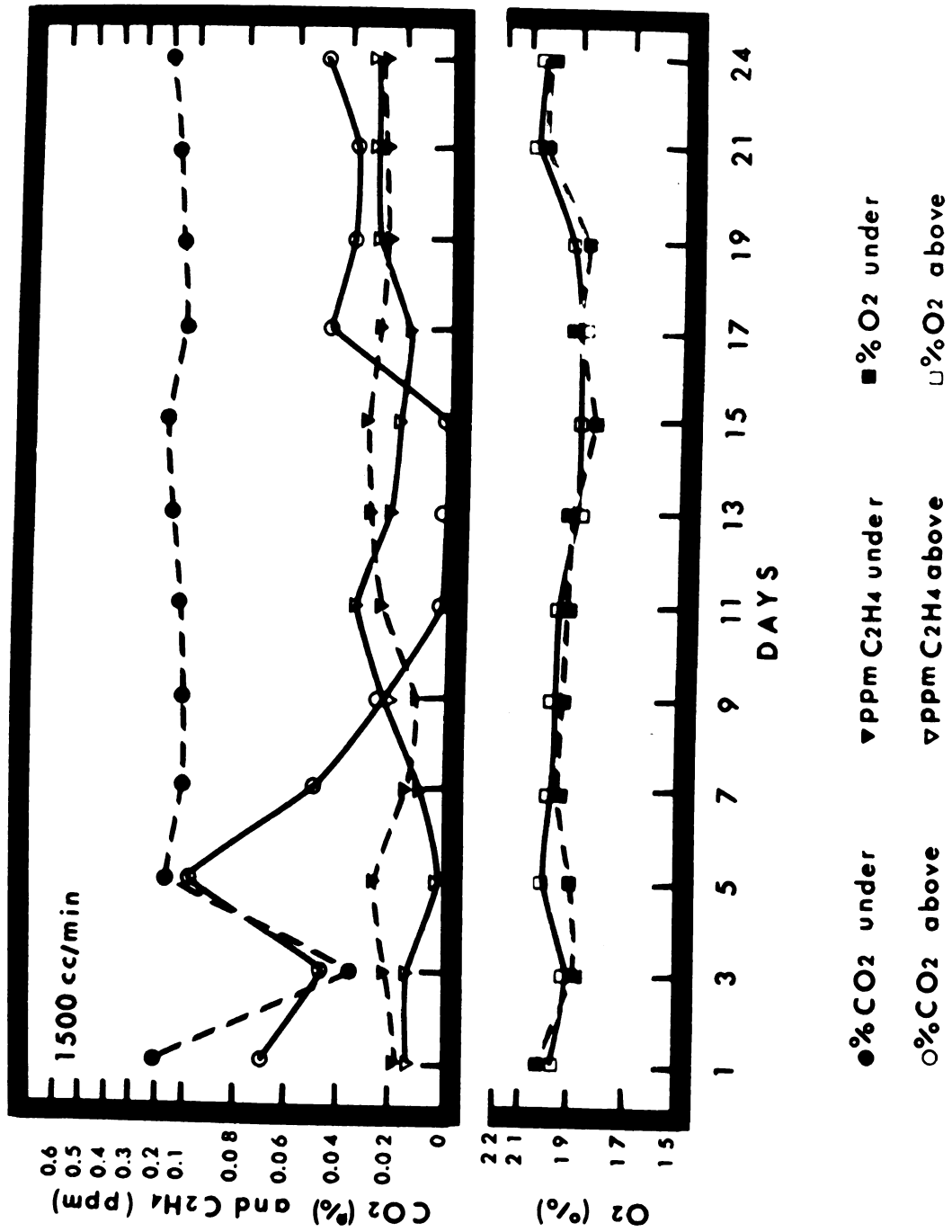


Figure 21.--The concentrations of carbon dioxide, oxygen and ethylene above and under the casing soil when the air flow rate was 2000 ml air/min. in the atmosphere. An average of 3 replicates.

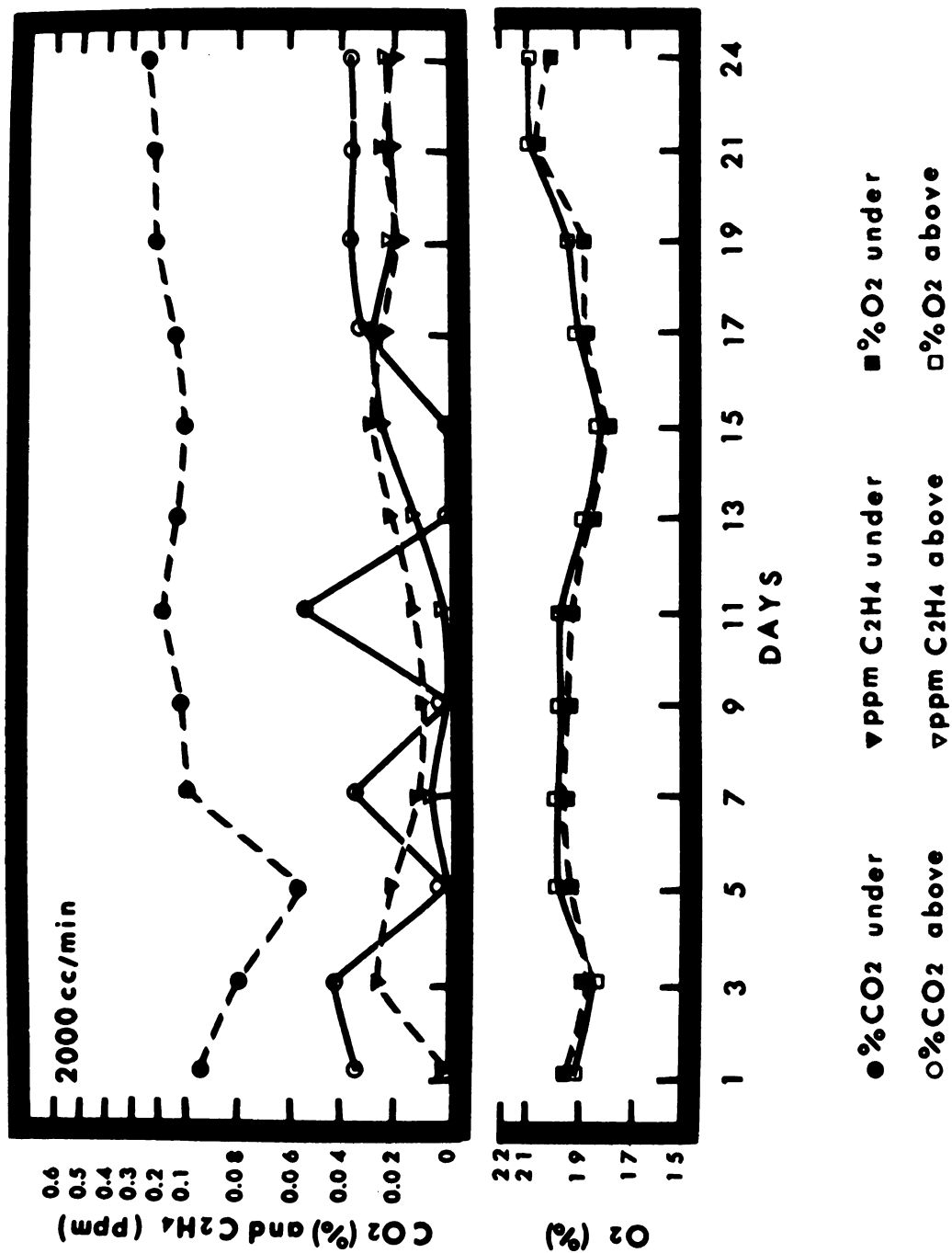
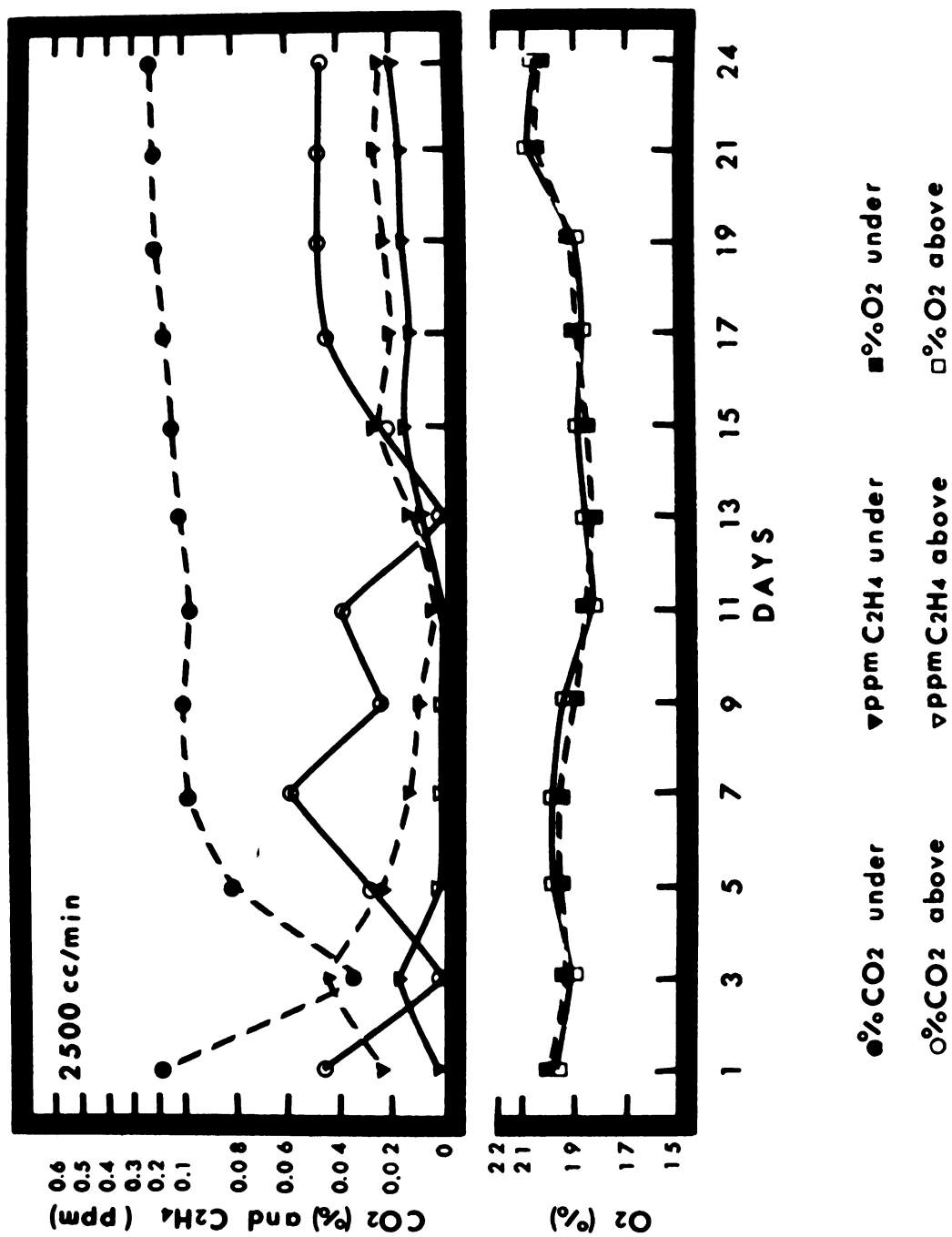


Figure 22.--The concentrations of carbon dioxide, oxygen and ethylene above and under the casing soil when the air flow rate was 2500 ml air/min. in the atmosphere. An average of 3 replicates.



day. Carbon dioxide concentrations under the casing showed little variation remaining around 0.1%.

In the case of higher air flows of 1500, 2000 and 2500 ml air/min. (Figures 20, 21, and 22) considerably lower concentrations of carbon dioxide were recorded above the casing soil, sometimes less than 0.02%. All 3 air flow rates had the lowest concentrations between day 11-13 at the time of primordial formation. A small increase in this gas to about 0.04% was noticed toward the end of the growing period, at the time of mature fruiting bodies.

The ethylene concentrations (Figures 14, 15, 16, 17, 18, 19, 20, 22) in both above and under casing soil atmospheres were similar in each treatment with one exception, but in every case the concentrations were lower than 0.1 ppm (see Table 10). The one exception occurred when an air flow of 100 ml/min. was used (Figure 14). In this case, ethylene concentration above the casing was fairly constant at a level slightly more than 0.1 ppm. The ethylene concentration was lower in one case when primordia first formed at 11 days with an air flow rate of 1000 ml air/min. (Figure 19).

The oxygen concentration in both above and under casing soil atmospheres was fairly constant for all air flow treatments (Figures 14, 15, 16, 17, 18, 19, 20, 21, 22). Although some minor fluctuations were observed throughout the growing period, the concentrations ranged from 18%-20.9%.

The relationships between the rate of flow and the concentration of carbon dioxide and ethylene above and under the casing soil at the time of primordial formation and at the time of the harvest of basidiocarps are summarized in Table 10. The highest production, 67 g of sporophores was obtained with a flow of 1000 ml air/min. (Figure 23 a, b). The earliest primordial formation occurred at 11 days after casing with an air flow of 500 ml air/min. and 1000 ml air/min. Thirty seven and one-half grams of mushrooms were obtained under a flow of 1500 ml air/min. (Figure 24), while primordial formation occurred 2 days later and the growing period took 3 more days than the previous treatment.

Very similar yields of mushrooms, 29.25 g., 29.7 g., 31.7 g., and 29.5 g., were obtained under flows of 400, 500, 2000, and 2500 ml air/min. respectively (Table 10, Figure 25).

Mushroom fruit bodies obtained under air flows of 500 and 2500 ml air/min. are shown in Figures 26 and 27.

With air flows of 200 and 300 ml air/min. 16 g and 8.5 g were obtained. This weight was obtained from very poorly developed fruiting bodies. Only mycelial growth on top of the casing was obtained in growth chambers under a flow of 100 ml air/min. after 24 days from casing (Figure 28).

In a separate experiment air flows of 50 and 200 ml air/min. were provided to growth chambers containing only commercially pasteurized compost, during a period of 24 days. At the lower air flow, carbon dioxide concentrations increased

Figure 23a.--Fruiting bodies that developed 21 days after casing with an air flow rate of 1000 ml air/min. through the growth chamber.

Figure 23b.--Enlarged view of the fruiting bodies obtained in 23a.



Figure 24.--Fruiting bodies that developed 24 days after casing with an air flow rate of 1500 ml air/min. through the growth chamber.



Figure 25.--The yield of mushrooms when the air flow rate of 100, 200, 300, 400, 500, 1000, 1500, 2000, or 2500 ml air/min. passed over the casing soil in the growth chambers.

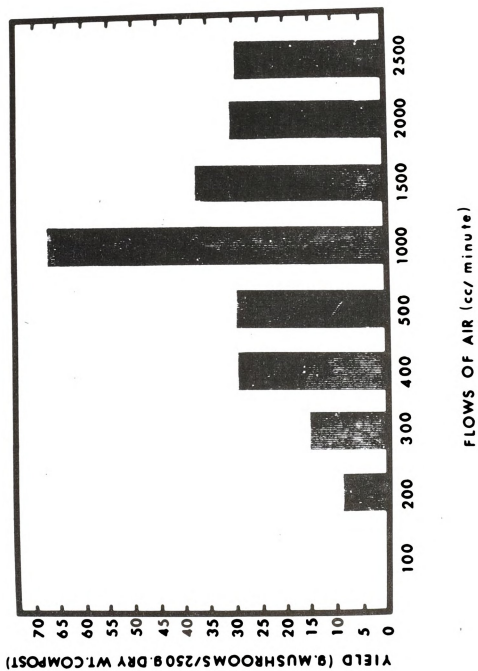


Figure 26.--Fruiting bodies that developed 24 days after casing with an air flow rate of 500 ml air/min. through the growth chambers.



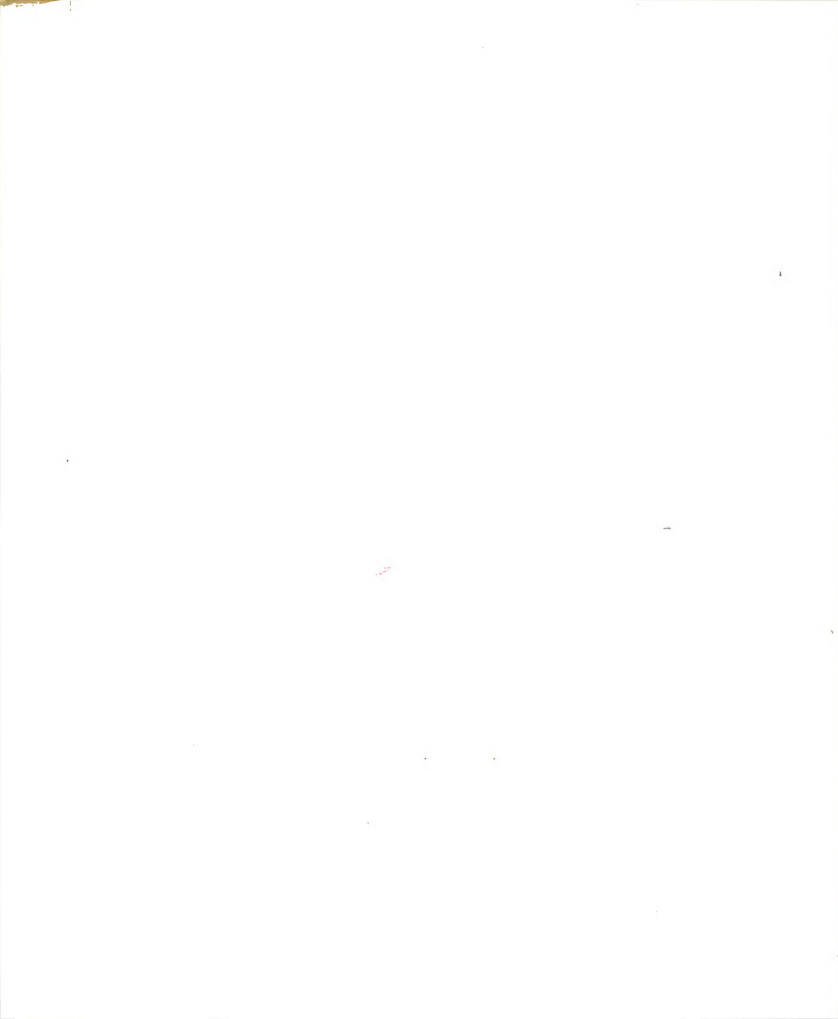
Figure 27.--Fruit body that developed 24 days after casing with an air flow rate of 2500 ml air/min. through the growth chambers.





Figure 28.--Mycelial growth on top of the casing soil 24 days after casing with an air flow of 100 ml air/min. through the growth chamber.





from 0.15% up to 0.45%, but at 200 ml flow rate this concentration was kept lower than 0.1%. Ethylene concentration was lower than 0.1 ppm for all of these treatments. Mild fluctuations of the oxygen concentration, between 18.0-21.5%, were detected.

In another experiment, carbon dioxide concentrations from 0.15 to 0.4% were obtained when air flows of 250 ml/min. were provided to growing mycelium on either autoclaved or commercially pasteurized compost. Ethylene concentration reached a level of physiological significance, 0.2 ppm, by the 10th day of the experiment just from growth chambers containing mycelium growing on pasteurized compost. Again, mild fluctuations on the oxygen concentration, between 18.0-21.5%, were detected. These two last experiments were incubated at 60°F.

DISCUSSION

Little information has been known about the changes that occur in the fungal population, gas exchange above and below the casing soil and the relationship these might have to fructification of mushrooms in mushroom houses. Casing soil studies have shown changes in types and numbers of fungi present during the period of mushroom culture in a mushroom house. Characteristic gas pattern was determined during the casing soil study in relation to primordial formation and fruiting.

The qualitative and quantitative changes were studied in the fungal population in the casing soil throughout 2 different growing periods, winter and summer, in a commercial mushroom house. Quantitative data concerning microorganisms are subject to considerable variation due to several factors such as type of soil, seasonal distribution, ecological conditions and isolation techniques employed (24, 50). The number of fungal colonies from the casing soil, even with the application of zineb, was higher in the summer as would be anticipated. The considerable increase in fungal microorganisms during the summer over the winter growing periods may be due partly to a difference in the source of the soil



and most likely due to air borne contaminants which are higher in numbers in outside air in summer.

The average production from the above mushroom houses during the complete growing periods was 2.14 and 2.23 lbs/sq. ft. for winter and summer respectively. However, in a very few scattered areas of the mushroom beds conspicuous symptoms of Verticillium and Mycogone diseases were observed during both seasons. These two pathogens were isolated from the casing soil prior to the zineb treatments, however, Mycogone was isolated only once 6 days after casing during the winter time, these pathogens appeared to be well controlled by zineb during both growing periods. Near the end of the crop in both winter and summer growing periods, the two fungi appeared again in the casing. Probably the appearance of the pathogens could be from the persistence of the fungus in the soil, or from external sources such as: flies, humans, wind, soil or debris from neighboring farms or soil for casing (14).

Members of the Mucoraceae, such as Zygorynchus sp., Rhizopus sp. and Absidia sp. were not recovered from casing soil during the winter growing period but were in the summer. Only Mucor sp. was recovered once from the casing soil. Latter (42) in a comparative study on the microbiology of four moorland soils found that Mucoraceae and yeast were isolated more frequently in winter while other forms including Penicillium spp. and Trichoderma spp. occurred more frequently in summer. Considering the fast growing ability of the above

Mucoraceae members, the seasonal distribution or some other undetermined condition may be the limiting factor on the appearance of these genera in the casing soil.

Genera of Trichoderma, including T. koningii, T. lignorum and T. viride, were isolated frequently throughout both growing periods. It has been showed by a number of investigators that Trichoderma spp. have a great survival capacity, fast growing ability and have been found as the main colonizers of soil treated with one of several chemicals (7, 8, 10, 16, 47).

Dwivedi and Garrett (16) found that at the highest degree of staled nutrient agar, colonies were produced only by Trichoderma viride and 3 species of Penicillium. They concluded that success of any species in colonization of staled nutrient agar is determined both by its tolerance to fungistatic growth substances and by its inoculum potential in that particular situation. This result emphasized the remarkable tolerance of some Penicillia for habitats unfavorable to most soil fungi which may explain their persistence in the zineb treated soil.

Regarding the diversity of genera of fungal microorganisms found, it might be said that a majority of the species present possessed wide limits of environmental tolerance. This permitted them to grow in varied habitats, although most of the time the environment was adverse in the casing soil if the applications of fungicides were used. In other words, it was observed that the fungal microorganisms did not form

discrete communities nor a pattern of associated distribution. It was interesting to find that several of the genera of fungi found in this study were good antibiotic producers, possibly exerting in this way some ecological effect, expressed in different kinds of antagonism which permitted them to escape from food competition. Hawker (28) pointed out that it is important to know the methods by which fungi and other microorganisms survive under adverse conditions for a reasonable interpretation of the distribution of the species in time and space.

The fungal contamination of the air in a mushroom house and the microbial invasion of the casing soil was studied in a so-called "small mushroom house." Pathogenic species on mushrooms such as Trichoderma koningii and T. lignorum were isolated from the soil and from the air after only one day following casing. Other nonpathogenic species of this genus, such as T. viride, were isolated as well. Since the soil used in this case was treated with chloropicrin and stored for about one year, it is very possible to have a dominant recolonizing effect by this fungus. The same pattern was observed with some members of the Mucoraceae. It was pointed out by Dwivedi and Garrett (16) that Trichoderma viride established itself as a dominant fungus, at least for 6 months, in a soil treated with diluted formalin and in sterilized soil, the first colonizer fungi were fast-growing Phycomycetes and T. viride.

Verticillium sp. was isolated from soil in this "small mushroom house" after one day of casing. Colonies of V. malthousei were isolated from casing soil, from the sterilized soil in the plastic boxes placed in the mushroom beds, and from the air 28 days after casing. This pathogen did not appear among any of the isolates in the Petri plates, in the previously collected samples, about 14 days before. Wind, as a conidial dispersing agent of Verticillium, has been a matter of controversy to account for the presence of this organism. However, colonies of V. malthousei were obtained in the Petri dishes exposed to air inside the mushroom house. Cross and Jacobs (14) mentioned the possible wind dissemination of dust or debris particles to which conidia of Verticillium sp. may have adhered. This may explain the occurrence of conidia of V. malthousei in the external air which moves through air vents to the inside of the mushroom house.

It should be pointed out that severe symptoms of the Verticillium disease were observed during the last 25 days of this growing period, as showed in Figure 3a. On that basis, a high amount of inocula was present throughout the last part of the cropping period enhancing the chance of conidia dissemination. The incidence of Mycogone disease was lower than that of Verticillium disease. The pathogen, Mycogone, was recovered from the soil 42 days after casing, and from the air, and from the soil in plastic trays 12 days later. Since neither V. malthousei nor M. perniciosa were

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recovered from one of the three sources, namely, casing soil, soil from the plastic trays, or air, during the first 3 weeks of the growing period, the source for the infestations by these pathogens may have occurred from the outside of the mushroom house.

A different pattern was observed with Dactylium dendroides. The fungus which was isolated from the air one day after casing, did not appear in the soil until about 3 weeks later. At this time the organism had an abundant growth of mycelium in scattered spots in the house as showed in Figure 3b.

In this study mushroom pathogens namely, T. koningii, T. lignorum, V. malthousei, M. perniciosa and D. dendroides were isolated from the casing soil in plastic trays placed in the beds, at different times during the mushroom growing period. In addition, some other fungal species were recovered from this source such as Penicillium, Rhizopus and Mucor. In general, the number of fungal colonies recovered from the soil in plastic trays increased gradually throughout the cropping period, reaching the highest population at the end as compared to the population in the casing soil of the mushroom beds. Most of these colonies were species of Trichoderma and Penicillium. Even though these two fungi have a higher colonizing capacity, both V. malthousei and M. perniciosa were recovered. This recovery might be explained on the basis of survival capacity in the soil and by effective means of dispersal.

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The number of fungal colonies in the casing soil increased about 3 times as rapidly during the first 2 weeks and then remained rather constant. On the other hand, the number of fungi in the sterilized soil in the plastic trays increased gradually until the number of fungal colonies was higher than that of the casing soil at the end of the growing period, as previously indicated. An increase occurred in the number of bacteria and actinomycetes present in the sterilized soil in plastic trays, but these figures were always lower than for the casing soil.

A similar situation was reported by Kreutzer (39) who found that soil fumigation increases the nutrient status of the soil, largely by the killing of microorganisms with a subsequent release of nutrients bound in microbial protoplasm. The increase in number of the fungi in fumigated casing soil was eventually retarded by competition or by the fast growing mycelium of A. bisporus in that substrate.

In summarizing the "small mushroom house" study, several important conclusions were evident. Contamination from the outside of the mushroom house played perhaps the most important role on the high incidence of disease. The technique used in this work showed in most cases a certain degree of correlation between the infestation of the casing soil and the air of the mushroom house as a possible transmitting agent. However, due to the importance of this subject, further investigation of casing soils in other commercial

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houses, and other sampling methods need to be used to provide a better understanding of the relationship of microorganisms in casing soil to their possible importance on yield.

Methyl bromide reduced the number of fungi present when used as a sterilant in soil for casing especially at the higher concentration of 4 lbs/100 sq. ft./1 ft. depth of soil. However, a high number of recolonizing fungi such as Trichoderma spp., Mucor spp. and Penicillium spp., were isolated from the soil treated with the two levels of the pesticide before using it for casing. Mughogho (47) in a study in which soil was treated with one of several chemicals including methyl bromide, found high development of dominant population of Trichoderma in the recolonizing fungal flora. T. viride and T. koningii were included within those dominant species. It seems that recontamination of the soil took place after the treatment, and a fast colonization of this soil was achieved by the above fungal species. During the first 10 days following casing, the fungal population continued to increase until a strong competition, exerted by the fast growing mycelium of A. bisporus, maintained a rather constant fungal population for the rest of the growing period.

Methyl bromide has been reported relatively ineffective against Verticillium spp. (23). However, no colonies of this fungus were observed after the soil treatment.

In a study of the interaction between a saprophytic fungus such as a species of Penicillium and M. pernicios in casing soil, the objective was to determine if the severity of the incidence of disease could be reduced in the commercial mushroom production. The two pathogens in mushrooms, M. pernicios and V. malthousei showed a severe incidence of disease in sterilized casing soil for this study.

One hundred per cent severe incidence of disease was obtained when autoclaved soil was infested soon after casing with a conidial suspension, 4×10^6 /ml of M. pernicios at a temperature of 62°F. According to Fletcher and Ganney (21) the time for appearance of symptoms caused by this pathogen appears to vary with the concentration of the inoculum potential. The appearance of the symptoms suggest that a desirable concentration of inoculum potential was used, and the fungal isolate used in this study was highly pathogenic.

In the case of V. malthousei used in this study, a lower incidence of disease, graded at 75%, was observed. In only a few cases, severe symptoms of the disease developed mainly during the second break, but most frequently mild symptoms were observed. It seems that some other factors, besides the inoculum, might be involved in the expression of pathogenicity of this fungus. Temperatures higher than 62°F might promote more severe disease symptoms. However, in commercial production at a temperature of 60°F very severe symptoms and high incidence of the disease are frequently observed.

After inoculation of autoclaved casing soil with M. perniciosa, V. malthousei, T. koningii and T. lignorum it was possible to isolate them throughout a 30 day period. A considerable increase in numbers of these fungal isolates was obtained when the layer of the autoclaved soil was on top of compost.

Verticillium malthousei was recovered from unautoclaved soil 30 days after soil infestation. Cross and Jacobs (14) reported that the conidia of this fungus can survive for at least 10 months in soil. Mycogone perniciosa was recovered from unautoclaved soil on top of compost 16 days after the soil infestation or over a longer period of time than on soil alone. They also found that the conidia in soil are subject to fungistasis. The results of this study with V. malthousei are similar in casing soil to those reported for natural soil by Cross and Jacobs (13). They reported that the germination rate was low in natural soil and also that the conidia are subject to fungistasis.

Interactions occurring between two pathogenic fungi in higher plants have been found as important factors limiting production (35). In the present work a synergistic effect was produced, reflected by a significantly lower production of mushrooms when the casing soil was infested with V. malthousei plus either M. perniciosa or T. lignorum. Likewise when any combination in pairs of V. malthousei, M. perniciosa, T. koningii and T. lignorum were used, a

significantly lower mushroom production was obtained than when each isolate was inoculated into the soil separately.

Since high populations of Trichoderma spp., including T. koningii and T. lignorum, have been observed rather frequently in casing soils, their association may be a factor influencing the severity of "wet and dry bubbles diseases."

In vitro, Penicillium corylophilum and P. asperosporum were showed to possess an antagonism toward colonies of M. perniciosa and V. malthousei on culture media. In the literature, several cases have been reported of biological control with certain fungi restricting the spread or completely stopping root rot diseases of different vascular plants (11, 19). In this study with the mentioned Penicillium isolates, significant reduction or partial biological control of Verticillium and Mycogone diseases was demonstrated. A significantly higher mushroom production was obtained when either of these two mushroom pathogens and Penicillium corylophilum were inoculated into the soil than when either pathogen was inoculated separately. In addition, the expression of the symptoms of both diseases were less severe under the above combinations. However, a significantly higher mushroom production occurred in the control trays without the fungus pathogens.

Additional saprophytic fungi need to be considered in future studies concerning biological control of pathogenic fungi in mushrooms. Varied combinations of organisms, optimum

inoculum to achieve desired results, strain selection of Penicillium to be more antagonistic or a better competitor, and influence of pH, temperature and type of soil are among the factors that need to be considered in following up the initial attempt to control the mushroom pathogens, V. malthousei and M. perniciosa, biologically.

Data obtained from the present work on the evolution of the carbon dioxide concentration above and under sterile casing soil throughout the growing period of mushrooms show important relationships to the primordia formation and to the carpophore development. No significant variation of carbon dioxide concentration was obtained under the casing layer; it was maintained above 0.1% during the entire growing period at any of the air flows provided. The most suitable growing conditions and highest mushroom production were obtained under an air flow rate of 1000 ml/min. Under such conditions, carbon dioxide concentrations above the casing decreased to less than 0.02% by the time primordia formation was observed. This may indicate a need for exogenous requirement of carbon dioxide by the mycelium for fruiting body formation. This result agrees with those of Long and Jacobs (45) who found a carbon dioxide requirement for sporophore initiation. Barinova (3) has suggested that carbon dioxide functions as a "catalyst" in the fungus metabolism. At air flows higher than 1000 ml/min. the carbon dioxide concentration above the casing was very low

from the beginning of the experiment and perhaps the carbon dioxide required for primordia formation was kept under the optimum level.

After primordia formation and throughout the rest of the growing period, a gradual increase in carbon dioxide concentration reaching 0.1% at harvesting time with an air flow of 1000 ml/min. This may be due to the progressive metabolic activity of the developing carpophores. A similar response was obtained at higher air flows but the highest carbon dioxide level reached was 0.05% at harvest time.

Mushroom production obtained at air flows of 400 and 500 ml/min. was very close to that obtained with flow rates higher than 1000 ml/min. However, with the former conditions, the carbon dioxide concentration above the casing fluctuated around 0.1%. According to Tschierpe and Sinden (65) this condition retards formation and reduces the number of fruiting bodies. On that basis, explanation for a significant decrease in mushroom production at air flows lower than 300 ml/min. can be understood if an increase in carbon dioxide concentration above the casing soil occurs. The data also indicate that this effect was due to the presence of carbon dioxide and not the the dilution of oxygen.

Tschierpe (64) stated that "the object of the casing soil is to create a carbon dioxide gradient from the air in the compost to the air above it, and fructification takes



place in the region of this carbon dioxide partial pressure gradient." Results from the present work agree with this statement. The carbon dioxide concentration, slightly higher than 0.1% was maintained under the casing and fluctuations were observed only above the casing, thus providing the carbon dioxide gradient mentioned by Tschierpe (64). It should be considered that in the commercial production, the casing soil, which is partially or not sterilized, supports a considerable microbial activity that might be considered a possible source of the exogenous carbon dioxide required for the fruiting body formation.

There was significant evolution of carbon dioxide from pasteurized compost with air flows of 50 ml and 200 ml/min. When mycelium of Agaricus bisporus was placed on sterile and pasteurized compost with an air flow of 250 ml at 60°F, the carbon dioxide concentration became slightly higher than 0.1% and remained rather constant for both treatments. This indicates that the mycelium readily grows in the substrate at this carbon dioxide concentration, and competes well with the other microorganisms present in the compost. This may explain why there are so few cases of contamination during the mycelial growth under commercial conditions.

The soil in these experiments was sterile before casing. However, microorganisms including fungi, bacteria and actinomycetes were present before fruiting bodies developed. These casing soil studies however, did not provide any

solution to the conflicting data on whether or not microbial activity is involved in the mechanism of fruiting body formation.

Data obtained from this work showed that concentrations of ethylene above and under the casing were lower than 0.1 ppm, suggesting that no important role is exerted by this gas on the metabolism of mushrooms. Apparently no interaction occurs between this metabolic gas and carbon dioxide.

Quantitative data have been presented on the evolution of carbon dioxide above and under the casing, as well as the role that this gas plays on the primordial formation and carpophore development throughout the growing period.



SUMMARY

A study was made of fungal genera present in the casing soil in a large commercial mushroom house during two different mushroom growing periods, summer and winter. Zineb reduced fungal population although species of Trichoderma and Penicillium continued to be numerous throughout both growing periods. However, a diversity of other genera of fungi were found. These microorganisms did not form discrete communities nor a pattern of associated distribution.

In a small mushroom house, a comparison was made of fungi in casing soil, sterilized soil in a plastic box and air during a mushroom growing period. Verticillium malthousei was detected in the air samples, casing soil, and sterilized soil prior to appearance of disease symptoms on the fruit body of the mushroom.

Methyl bromide reduced but did not completely control the number of fungi present when used as sterilant in soil for casing. Recolonization of the treated soil took place by species of Trichoderma, Mucor and Penicillium.

One hundred percent of disease incidence was obtained when autoclaved casing soil was infested with conidial suspension of Mycogone perniciosa. Lower disease severity

occurred with either Verticillium malthousei or Trichoderma koningii, and the lowest disease incidence was with T. lignorum.

In autoclaved soil a decrease of the survival capacity of M. perniciosa and V. malthousei was observed while an increase occurred with T. koningii and T. lignorum during a 30 day period. A marked decrease in survival capacity for these fungal species occurred in unautoclaved soil. Trichoderma koningii and T. lignorum increased in numbers when the autoclaved soil was on top of compost.

A synergistic effect, reflected by a significantly lower production of mushrooms, was produced when autoclaved casing soil was infested with both M. perniciosa and V. malthousei or the latter with T. koningii. Likewise when any combination in pairs of V. malthousei, M. perniciosa, T. koningii and T. lignorum were used, a significantly lower production of mushrooms was obtained than when each isolate was inoculated into the soil separately.

In vitro, Penicillium corylophilum and P. asperosporum were showed to possess an antagonism toward colonies of M. perniciosa and V. malthousei.

A significant reduction or partial biological control of Verticillium and Mycogone diseases, as reflected by a significantly higher mushroom production and less severe expression of symptoms, was accomplished by Penicillium corylophilum.

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Neither the mushroom production nor the vigor of the mycelial growth of Agaricus bisporus appeared to be affected when grown in wooden trays treated with copper-8-quinolinolate.

Quantitative data on the carbon dioxide evolution was taken above and under the casing soil throughout the growing period of mushrooms. Below the casing soil, the carbon dioxide remained nearly constant at 0.1% at the different flow rates of air from 100 to 2500 ml air/min. Above the casing there was a marked drop in carbon dioxide level at the time of primordial formation to less than 0.02% when the rate of air flow was at least 1000 ml air/min. Higher yields of mushroom occurred under this rate of flow. These results support the theory which states that the casing soil layer creates a carbon dioxide gradient from the air above and under it, as one of the requirements for formation of the fruit bodies. Since ethylene remained below 0.1 ppm, it is suggested that this gas plays no important role on the metabolism of mushrooms and no interaction occurs between this gas and carbon dioxide.

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