EFFECTS OF LIGHT AND TEMPERATURE ON BASIDIOSPORE DISCHARGE IN *SCHIZOPHYLLUM COMMUNE* FR.

by

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Major Professor

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Facilities for this study were provided by the Division of Biology, Kansas State University to which I am very grateful.

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INTRODUCTION

The data and discussions presented below are taken from a series of experiments conduced on Schizophyllum commune Fr. a prominent woodrotting fungus and member of the Hymenomycetes.

The Hymenomycetes are a series of the class Basidiomycetes and include the mushrooms and many of the woodrotting and fleshy fungi. In general, the Hymenomycetes are characterized by the production of basidia on a more or less definite, orderly hymenial layer. The shape and size of the basidiocarps vary greatly, as does the manner in which hymenia are borne.

In S. commune the hymenium is produced on the surfaces of gill-like lamellae that are split longitudinally and that are sensitive hygroscopically to changes in relative humidity. In an atmosphere of high relative humidity, the split gills are appressed to one another in pairs thus exposing the hymenium. The basidiospores which are forcibily discharged from the basidia are then able to fall downward away from the hymenia into the air currents below where they may be dispersed in the atmosphere.

The purpose of this study was to determine the effects that various environmental factors, especially light, have in influencing the pattern of spore discharge during a 24 hr period.

LITERATURE REVIEW

Until the late 1940's and early 1950's, very little information had been published concerning Basidiomycetes, other than the rusts and smuts, as important constituents of the air spora. The reason was 2-fold. First, sampling devices used prior to that time were not designed to operate on a 24 hr basis. For example, basidiospores are generally most abundant in the atmosphere during the early hrs of the morning and therefore were not thought to be a major constituent of the population of air spora by early aerobiologists. Secondly, many of the basidiospores are not easily recognized and as a result were often overlooked.

Early Studies of the Fungal Population of the Atmosphere

Actual collectings of microorganisms of the air had its origin many years earlier. Miquel's (1899) studies on bacteria and molds of the air in France are probably the most sustained series of volumetric measurements of the air spora ever attempted. He observed seasonal variations in both molds and bacteria and noted that, although bacteria were three times more prevalent in the summer than winter, molds showed little seasonal fluctuation. Diurnal periodicity was also found to be a characteristic of the mold population. Miquel described two daily maxima in fungal concentrations, one at 8 AM and the other at 8 PM.

Meier (1935) reported that Lindbergh in his Atlantic survey flight of 1933 presented an opportunity to obtain significant data concerning long-distance aerial movement of spores and pollen. Twenty-six collections of material were made in the summer of that year by exposing petroleum-coated microscope slides to the atmosphere while in flight. Meier (1936) also collected several species of fungi, mostly imperfects, from winds while

flying over the Caribbean Sea. Newman (1948) reported collecting bacteria and fungal spores on commercial air routes. Pady, et al. (1948, 1950, 1953), have done much to increase the knowledge of the fungal content of the atmosphere in flights over the arctic as well as other airways (Pady & Kelly 1953, 1954, 1955). Most of these early works, however, show little recognition of the collection of basidiospores but rather emphasize collection of bacteria and fungal types that occur predominantly during the daylight hrs.

Seasonal Studies

Riley (1952) was one of the first to observe and record observations on seasonal patterns in the release of basidiospores in his work with Fomes ignarius L. ex Fr. Sporulation in this species was continuous from spring to autumn and was influenced by rain, temperature, and relative humidity. Pady (1957) using a Pady-Rittis sampler, began extended studies in 1953 of the fungal content of the air in Kansas. Although basidiospores were seasonal, higher concentrations occurred following rains. Since this time many seasonal studies have been carried out by aerobiologists throughout the world.

Hyde and Adams (1960) showed that basidiospores occurred throughout the year at Cardiff, England with the highest incidence during the months of August and September. Hamilton (1959) did an extensive study of the air spora at Rothamsted in 1954. Her conclusions on the seasonal occurrence of basidiospores was similar to the findings of Hyde and Adams (1960). She also found that rain did not affect the count of hyaline basidiospores, but decreased colored spores. Total counts were increased with rising humidity and decreased with rising wind velocity. Brown basidiospores increased with

temperature but yellow and hyaline basidiospores were not affected. She also identified numerous genera such as Coniophora, Ganoderma, Lactarius, Russula type, Nolanea, Psilocybe, Thelephora, Sporobolomyces, and Tilletiopsis.

Lacey (1962) studied seasonal periodicities of 26 catagories of fungus spores including several genera of Basidiomycetes. She found that at two different sites, 57% and 49% of the total spores counted were Basidiomycetes. Sporobolomyces comprised the highest numbers of spores of the fungi studied reading a maximum concentration of 25,000/m^3 which was reached during the month of July.

Kramer et al. (1959), and Pady and Kramer (1960) studied the seasonal variations of all groups of fungi from September of 1956 through August of 1958. Collections were made on adhesive coated slides as well as nutrient media to allow for colony development by the spores collected. Definite seasonal patterns of basidiospores were found to occur with maximum numbers occurring in June and July. The amount of precipitation also seemed to be an important factor when considering concentrations of basidiospores. Numbers were increased by rain, particularly a wet period of 24 hrs or more. The most abundant basidiospore that was found was the white-spored agaric type with the Clavaria type also appearing quite frequently. When nutrient plates were exposed to the atmosphere, Sporobolomyces was the most frequent type isolated, but five other genera were also identified: Polyporus versicolor L. ex Fr, Polyporus hirsutus Quel., Trametes suaveolens (L. ex Fr.) Fr., Tilletiopsis washingtioensis Nyland, and species of Coprinus.

Adams (1964) worked on the year to year variation of fungus spore content. She found that basidiospores constituted a large portion of the spores collected (hyaline 17.5, colored 7.3, and Sporobolomyces 8.8%). Several genera were identified from the colored and hyaline groups. The

highest concentrations occurred from June to November and declined rapidly during the winter months.

In 1961 Davies (1962), when sampling atmospheric pollen and spores in London, found that basidiospore populations started to rise in the latter part of August, reached a peak in late October and declined during the winter months. Sporobolomyces, however, reached a maximum concentration of $3000/m^3$ during June and July and diminished to very low concentrations ($500/m^3$) by October. Davies (1963) also studied the seasonal occurrence of basidiospores when comparing the summer and autumn air spora at London and Liverpool. Both colored and hyaline basidiospores occurred in higher concentrations at Liverpool and reached a seasonal peak in late September. Ganoderma spores were also collected and found to reach seasonal peaks in the autumn.

Adams, et al. (1968), have sampled the woodlands as a possible source of allergens. Special reference was made to basidiospores, which occurred in much higher concentrations in woodland areas than in Cardiff, a nearby town. The average number of fungal spores at two woodland sites were 205 and 211% higher than at Cardiff while basidiospores were 533 and 376% higher in the woodlands.

Diurnal Studies

With the development of a volumetric sampler that would operate automatically over a 24 hr period (Hirst 1953), the scope of aerobiology widened. It was this device that enabled aeromycologists to study the diurnal patterns of fungi effectively. Gregory (1952) in his work at Rothamsted was one of the first to study, over a 24 hr period, the hourly variation in the basidiospore population of the atmosphere. He found basidiospores to occur predominantly at night. Both colored and hyaline

spores were reported with the latter being low in concentration during the day, but reaching high concentrations (7000/m^3) during the early hrs of the morning. Most of the hyaline spores were Sporobolomyces spp. that seemed to be discharged when dew was present.

Further studies by Gregory and Hirst (1952) and Hirst (1953) revealed similar data of basidiospore discharge. The former paper also reported that high concentrations of basidiospores were found in the air several hrs after a rain had washed pollen and other spora from the atmosphere. A maximum concentration (30,000/m^3) was reached at about 2 AM. Colored spores were mostly Agaricaceae with spores of the following genera being identified: Panaeolus, Hypholoma, Bolbitius and Ganoderma.

Pady and Kramer (1962) and Kramer, et al., (1964) have obtained similar patterns of basidiospore release. Further studies by Kramer and Pady (1963) revealed maximum basidiospore concentrations occurring in October with a similar nocturnal periodicity. High concentrations (286/ft^3) of basidiospores were collected as late as December 31.

Hirst (1953) reported that basidiospores were at times three times as numerous as Cladosporium spores in the atmosphere. Gregory and Hirst (1957) estimated that hyaline basidiospores accounted for 31% of the total air spora. Panzer, et al. (1957), when experimenting with a new spore collector, and Sreeramulu and Seshavataram (1957) found similar patterns of basidiospore discharge. Gregory and Sreeramulu (1958), reported similar findings and added that high humidity and low windspeed favored high concentrations (one million/m^3). They also reported Tilletiopsis as being another abundant member of the hyaline group besides Sporobolomyces.

The only contradiction to the diurnal pattern with night time maxima observed by the above authors was reported by Gregory and Stedman (1958)

from a study done in an orchard near London in 1953. They found that the basidiospore population displayed a daytime pattern of spore release. They attributed this, however, to low temperatures which possibly checked spore liberation.

Numerous other authors (Adams 1964, Hamilton 1959, Lacey 1962) have also found the peaks of spore release in Basidiomycetes to occur at various times at night, usually between midnight and dawn.

Diurnal Studies of Specific Fungi

From the works on the diurnal fluctuations of the fungal populations of the fungal populations of the atmosphere away from initial sources of the spore components, there came an increased interest in the diurnal patterns of spore release of specific fungi with special attention being given to the environmental effects that influence these patterns. The list of people who have worked on the periodicity of spore release in specific fungi is extensive. For this reason, only authors that worked on Hymenomycetes will be cited.

Riley (1952) observed two peaks and two depressions of daily sporulation in Fomes igniarius (Neuman) Campbell. The peaks occurred in the morning and evening, and the depressions occurred during the day and during the night. Relative humidity and temperature were thought to be influencing factors on sporulation.

Carpenter (1949), when working with Pellicularia filamentosa (Pat.) Rodgers, found that basidiospore production and discharge appeared to be a "periodic function which may be strongly influenced by the presence or absence of light." He studied sporulation under natural light, prolonged light, and prolonged darkness. Zoberi (1964), when doing preliminary work on S. commune indicated no light response by this organism.

Apart from Bullers' (1909) studies on Lenzites betulinus (L.) Fr., there is little information on the effect of temperature and relative humidity on basidiospore discharge. Zoberi (1964 and 1965) has shown a definite correlation between temperature and relative humidity and spore discharge in S. commune and Lepiota konradi Huijsman ex P.D. Orton. Sinclair (1963) reported a diurnal periodicity in the airborne spores of Ganoderma applantatum Per. ex Fr. He found that this species had a nocturnal pattern with a maximum daily peak ($21,000/m^3$) occurring at 1 AM. Relative humidity was thought to be the important factor.

Pady et al. (1967), when studying diurnal periodicities of fungi in an orchard, found that mushrooms of the genus Coprinus reached a peak of $1800/ft^3$ at 4 AM. Sporulation began as the basidiocarp reached maturity between 8 and 10 PM, progressed to a peak during the night and gradually decreased with disintegration of the basidiocarp.

Haard (unpublished) probably has done the most comprehensive field studies of the Basidiomycetes to date. He recorded studies of some 30 species of Hymenomycetes from 19 genera and described five patterns of spore release, as well as environmental influences on spore release.

Studies of Basidiospore Discharge Under Controlled Conditions

Virtually no work has been published concerning spore release patterns of Hymenomycetes under controlled conditions. Wood, et al. (1966) observed three fruiting bodies of Fomes annosus (Fr.) Cooke in controlled environmental chambers and found that the greatest spore release occurred at night (12 AM. to 6 AM) and fell off rapidly during daylight hrs.

Zoberi (1964) found that slight changes in relative humidity made spore numbers fall off rapidly in Sporobolomyces, Schizophyllum and Polyporus.

With the studies discussed in this paper, it is hoped that a better

understanding will be possible of the environmental factors that influence spore release in some of the Hymenomycetes, as well as the factors that may influence spore production. Controlled environmental studies should be the logical way to approach this problem.

MATERIALS AND METHODS

Specimens of S. cummune used in the present study were found growing on pieces of decorticating wood in the vicinity of Manhattan, Kansas. The wood sections were brought into the greenhouse and placed in plastic trays partially filled with moist peat moss (Fig. 1). The trays were then placed in a mist chamber constructed of a redwood framework and covered with transparent plastic to allow sunlight in during daylight hrs (Fig. 2). Good basidiocarp growth was initiated in the mist chambers by using cool mist vaporizors to maintain high humidity. When basidiocarp growth seemed to be exuberant, the specimens were brought into the laboratory and studied in environmental chambers.

Most of the studies done on these fungi were carried out in ISCO Model E-1 Environmental Chambers in which environmental conditions such as light, temperature, and relative humidity could be controlled (Fig. 3). Automatic controls made it possible to program the chamber for specific sequences of environmental conditions. By changing only one variable at a time, and maintaining all other conditions constant, it was possible to study the effects of the various environmental factors on the diurnal pattern of spore discharge.

High relative humidity was found to be best for basidiocarp growth and sporulation. For this reason the ISCO chambers were programed to the maximum humidity that they could obtain. This was 90-93% at 70 F which was the temperature used in all studies except where specifically indicated. Humidity varied slightly when the lights went on or off and also when the chamber doors were opened to service samplers and change slides. This variation, however, was not significant enough to influence spore release in the species studied.

Lights could be programed to go on or off automatically at anytime during

Fig. 1 *S. commune* in plastic sampling trays.

Fig. 1

Fig. 2 Redwood mist chamber in greenhouse.

Fig. 2

Fig. 3 ISCO Model E-1 Environmental Chamber and vacuum pump for K-C sampler.

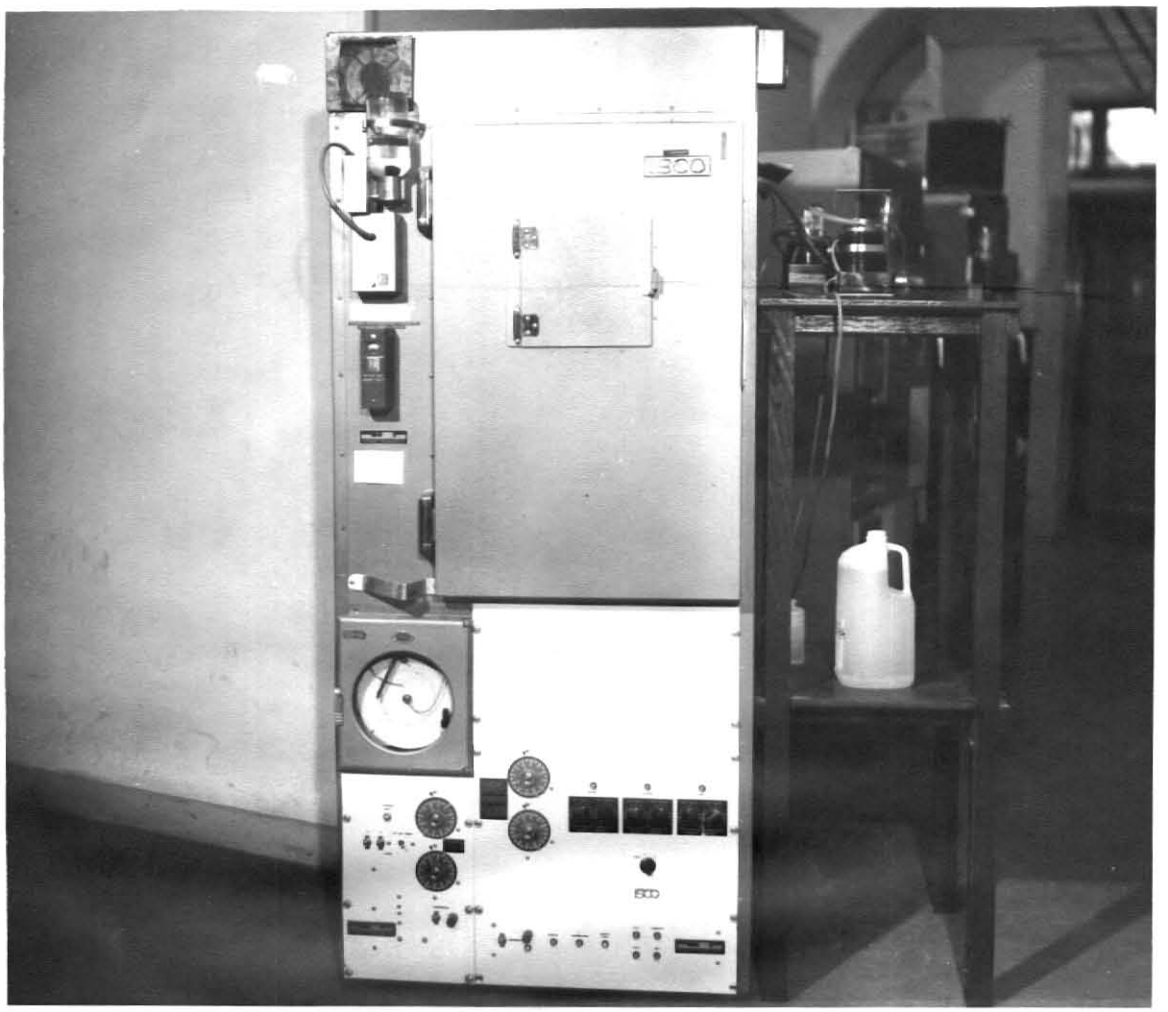


Fig. 3

a 24 hr period. Light intensity was equivalent to 1500 ft candles and remained at this level in each series of experiments where light was needed.

In order to study the hourly variations of spore release in these fungi, Kramer-Collins (K-C) spore samplers (Kramer & Pady 1966) were used. These are automatically operated, slit-type, impinging samplers that deposit spores on glass slides in hourly bands. The slides are moved by a clock mechanism that is designed to advance the slide 2 mm every hr through a 24 hr period. Slides were changed every 24 hrs.

The clock timing mechanism on the sampler used was equipped with a one-point cam that activated the vacuum pump once each hr for a 10 min period. The slide advance mechanism was also equipped with a one-point cam to advance the slide once each hr. Thus spores were deposited in hourly bands on the silicone coated glass slides.

The sampler was placed within the ISCO chamber along with the specimen being studied (Fig. 4) while the vacuum pump was placed outside (Fig. 3). The two were connected by means of rubber tubing through a port in the chamber wall. The inlet tube of the K-C sampler was placed close to basidiocarps being studied. Spore samples were then taken under a given sequence of environmental conditions for at least 5 days. At times, spore release was so great from a particular specimen that it was necessary to make minor adjustments to the previously described method in order to reduce spore numbers per band. This involved either cutting down the amount of air that was sampled or, when sampling the fungus on wood, moving the inlet tube various distances from the sporocarp. Although moving the inlet tube may have had an influence on patterns obtained over a 5 day series, it would not be a factor for any one day since the inlet tube, if moved, was moved only once per day, at the time of servicing of the samplers.

Fig. 4 K-C sampler in operation within the ISCO chamber.

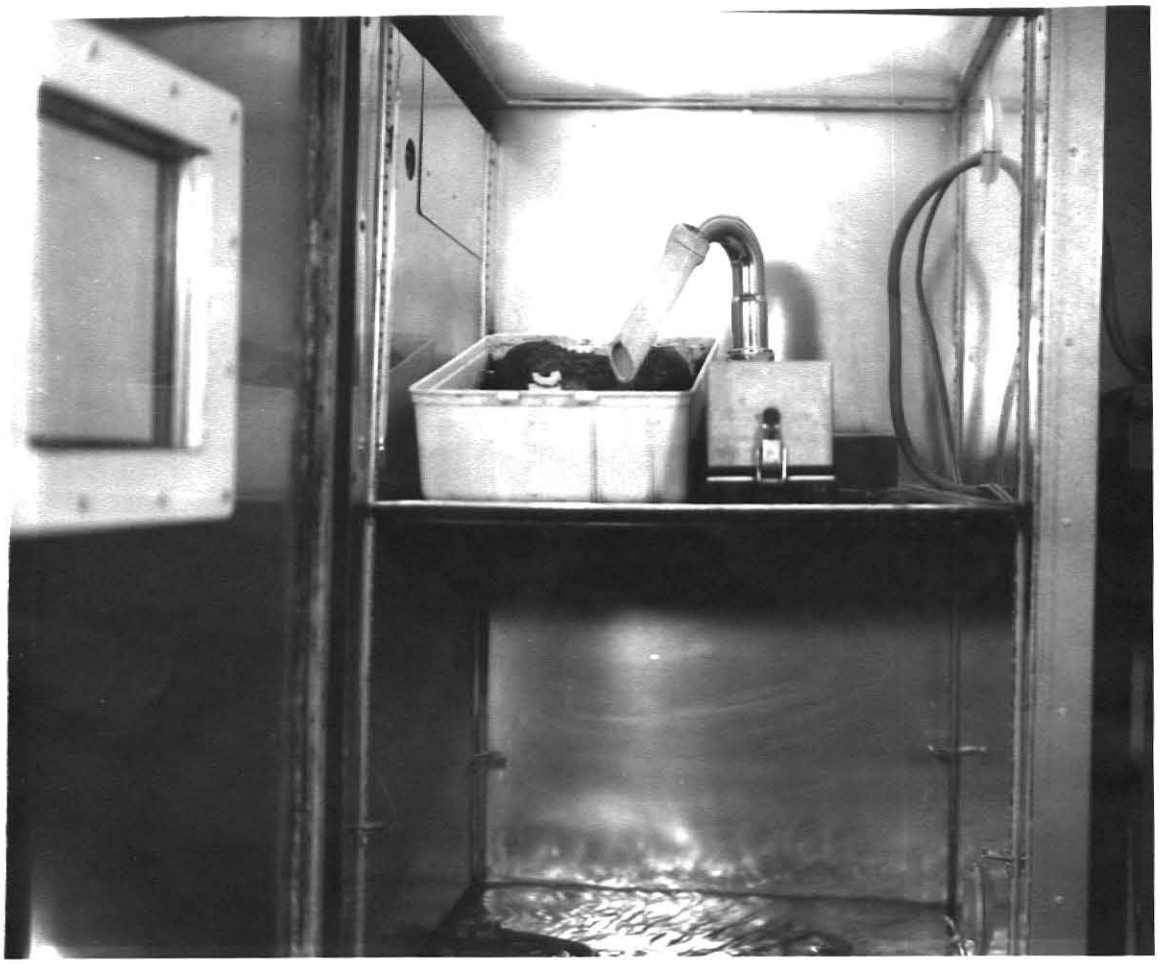


Fig. 4

S. commune was studied both from specimens developed from decorticating wood as described above, and from specimens grown in culture on artificial medium (Fig. 5). The medium used was that recommended for their growth by Raper and Krongelb (1958) and consisted of: 20.0g dextrose, 2.0g peptone, 0.5g $MgSO_4$, 0.46g KH_2PO_4, 120 µg of vitamin B, (thiamine HCl) and 20.0g agar per liter of water.

In the summer of 1968, cultures were obtained from mass spore isolates. These fruited readily on the medium described above (Fig. 5). Cultures were grown in 500 ml flasks to which an exit tube (6mm inside dia) was affixed 3 cm from the base. The flasks were filled with culture medium to just below the exit tube. Basidiocarps were usually developed and ready for study in 10 to 14 days when incubated at room temperature. At this time sporulation was evident by a moist, cream-yellow spore deposit below the basidiocarps. Specimens were then taken to the ISCO chambers and sampled under desired environmental conditions.

The detachable portion on the inlet tube of the K-C sampler was removed and a one-holed rubber stopper was fitted into the stationary portion of the inlet tube. A 3 in piece of glass tubing was fitted into the hole of the stopper and joined to the exit tube of the 500 ml flask by means of rubber tubing. Relative humidity in the flask was not the same as the relative humidity in the chamber. For this reason, a bubbler consisting of a 1 liter flask partially filled with distilled water was connected to the flask being sampled by means of rubber stoppers and glass tubing (Fig. 6). A porcelain bulb was attached to the glass tubing and submerged in the water of the bubbler. When the vacuum pump was activated each hr, air was first pulled through the bubbler to raise the relative humidity to approximately 98% before passing through the culture flask to pick up the basidiospores as

Fig. 5 Basidiocarps of S. commune growing on artificial media in 500 ml flasks.

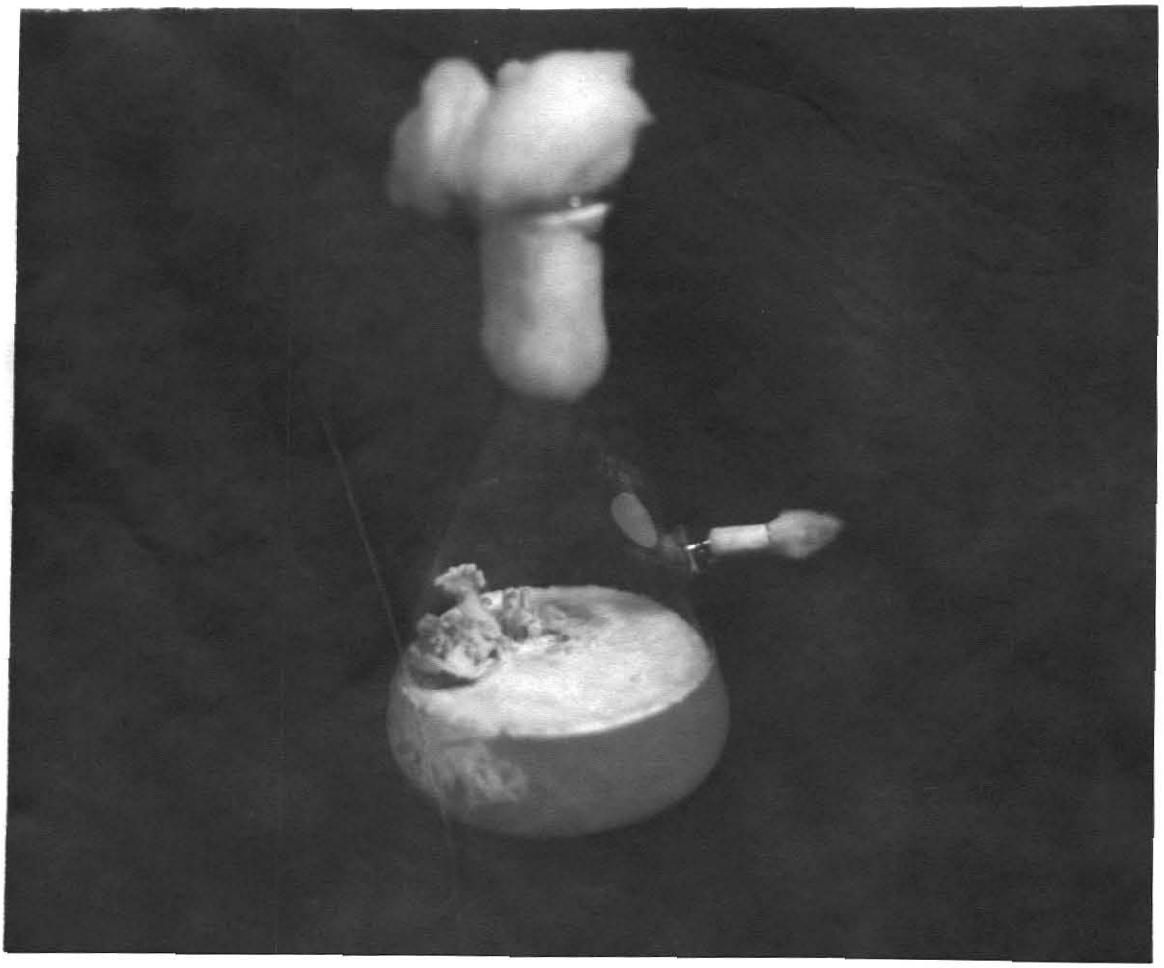


Fig. 5

Fig. 6 Set-up for bubbler and K-C sampler when sampling S. commune in culture.

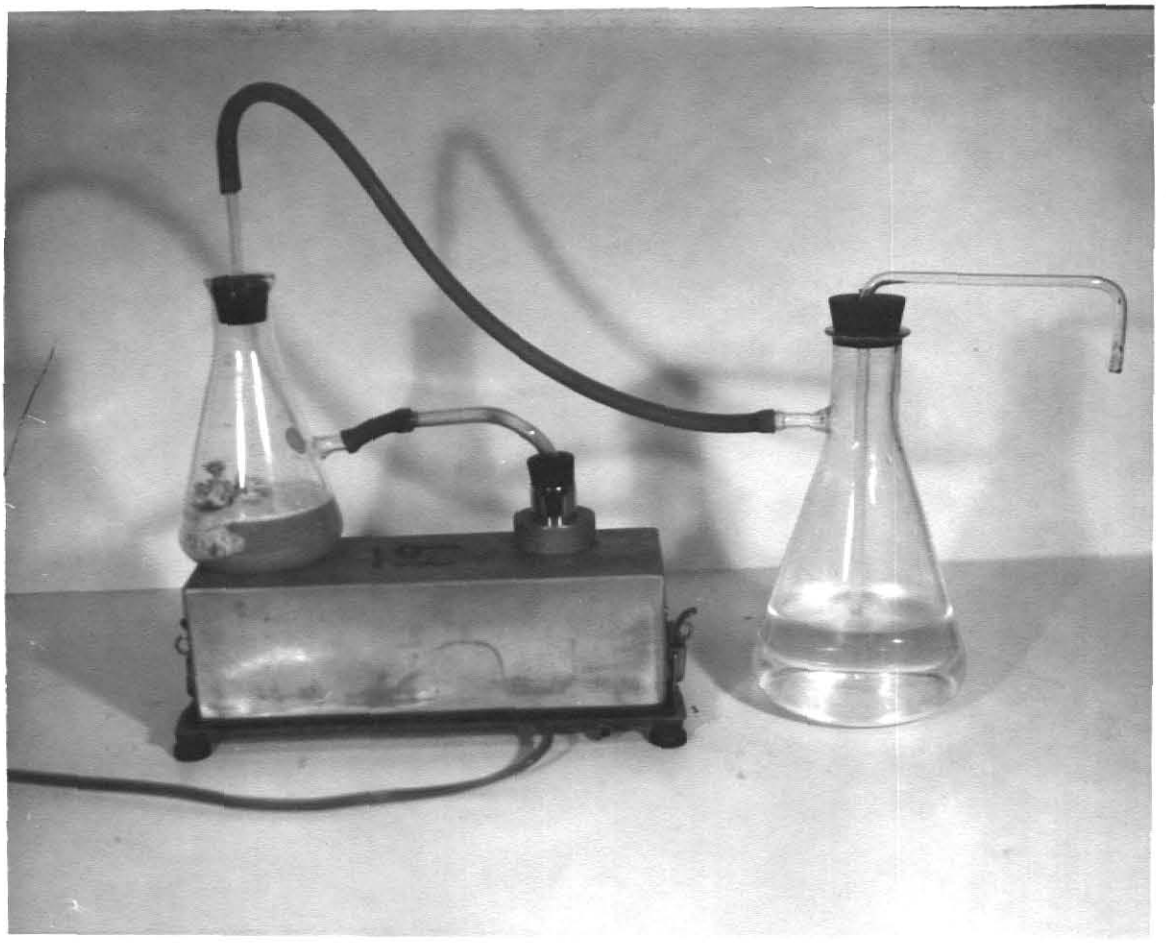


Fig. 6

they were discharged. In this way high relative humidity was maintained in the culture flasks.

Relative humidity was at times a problem when sampling on wood. Even with the relative humidity as high as 90-93%, desiccation would often occur within one or two days. It was this problem that led to another adaption to the K-C sampler.

A cool mist vaporizor, such as used in the mist chambers previously described, was connected to the three-way microswitch of the K-C sampler and placed in the bottom of the ISCO chamber (Fig. 7). The vaporizor and vacuum pump were connected to the microswitch in such a way that only one of them was in operation at a time. The timing was so set that the vaporizor was in operation for 50 min/hr and the sampler for the remaining 10 min period. Thus, mist from the vaporizor was not being produced during the time of sampling and would not cause condensation on the slides. With this device approximately 98% relative humidity was maintained, except during the 10 min sampling periods.

Samplers were serviced and slides changed each day at 1 PM. Whenever new samples were brought in to be studied, the first band was never counted. This was done because of the amount of spores already present in heavy concentrations around the basidiocarps that would be collected during the first sampling period. Servicing and slide changing was done as quickly as possible to prevent any appreciable change in environmental conditions of the chamber which might be caused by leaving the chamber door open.

Spores were counted as time permitted, and as soon as possible after a series of experiments was completed. In all cases Howard counting discs were used to facilitate the counting.

Fig. 7 ISCO chamber showing the cool mist vaporizor adaption for the K-C sampler.

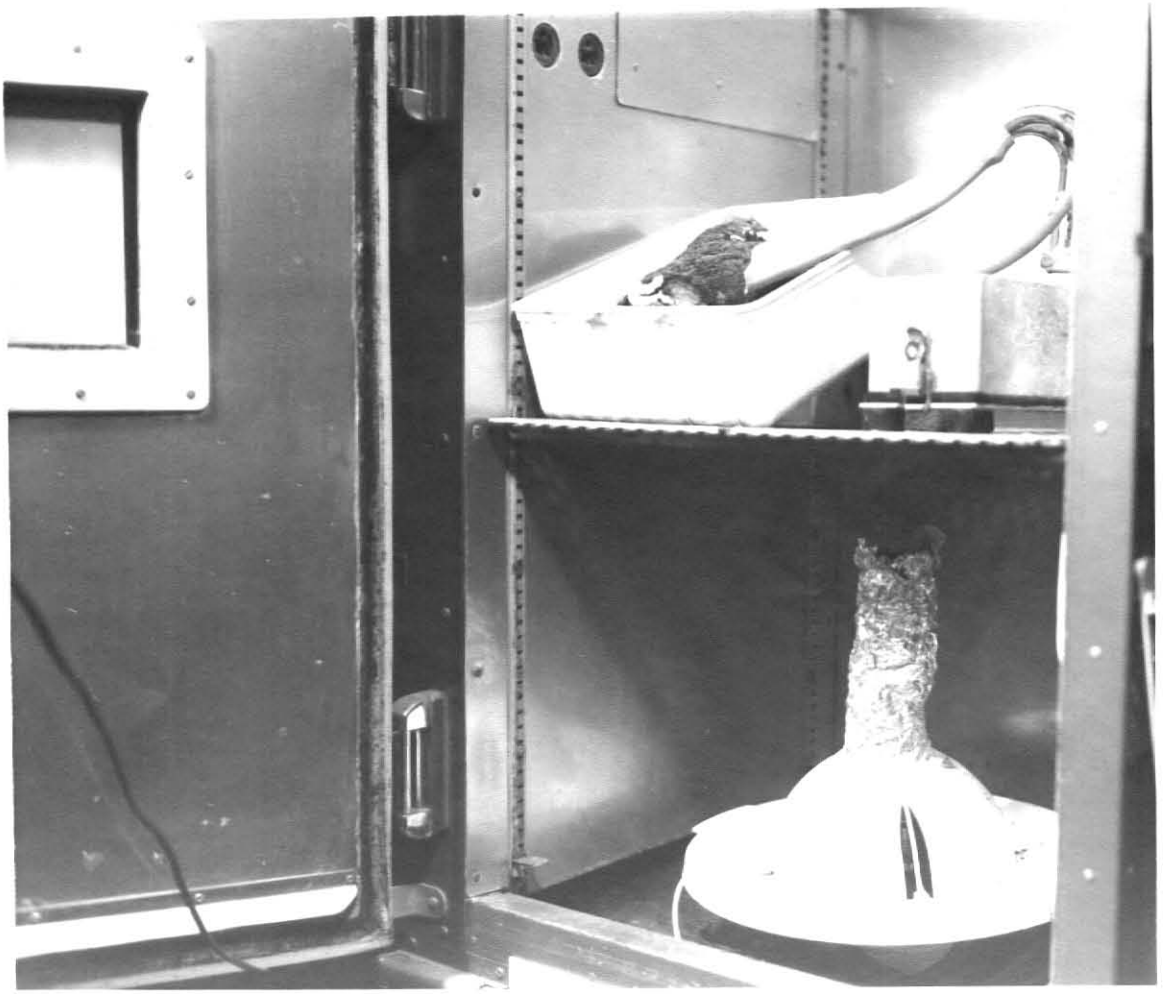


Fig. 7

RESULTS

Sporophores of S. commune that developed naturally on portions of decorticating wood in the field, or in the greenhouse, as well as those that were produced on culture media were studied under controlled conditions to determine the effects of various environmental factors, especially light on spore discharge.

Under conditions of alternating 12 hrs light and 12 hrs dark (12L:12D), and with constant temperature (70 F) and relative humidity (approx. 98%), the spore discharge pattern was similar from sporophores grown either on wood or on culture media (Figs. 8-10). Within the first hr after the lights came on there was an immediate drop in spore discharge. However, within one to three hrs, the rate of spore discharge again reached a comparatively high level where it was maintained throughout the remainder of the light period. When the lights went off at the beginning of the 12 hr dark period, there was often, but not always evident an additional increase in spore numbers to an even higher level during the dark period. This pattern of spore discharge is best illustrated in the graph of Figure 10.

Alternating 18L:6D (Figs. 11-13) showed almost identical results as those discussed under alternating 12L:12D. However, with the longer light and shorter dark periods, the number of spores discharged did not begin to decrease from the nighttime peak until the beginning of the light exposure. This emphasized the effects of the change from dark to light more distinctly than under a 12L:12D alternating condition. When subjecting S. commune in culture to an 18L:6D cycle, a change was made in the photoperiod so that lights came on at 6 AM rather than 8 AM. The drop in spore numbers still occurred only with the change from dark to light and not with the time of day.

To further establish that it was the change from dark to light that caused the temporary decrease in spore discharge, series using alternating

Fig. 8 The periodicity of S. commune in culture under conditions of 12L:12D, 70 F, and 98% RH.

Fig. 8

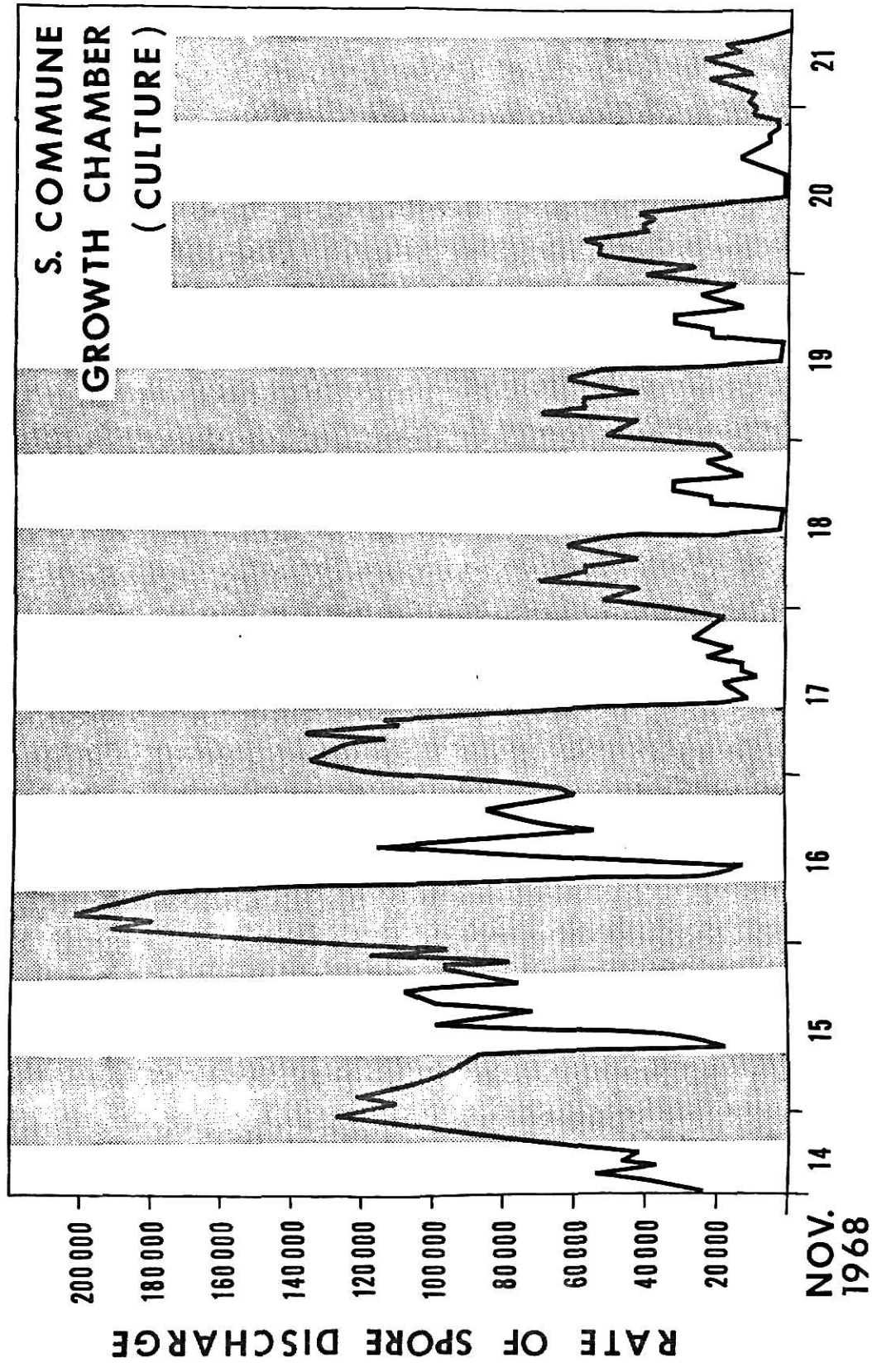

S. COMMUNE
GROWTH CHAMBER
(CULTURE)
RATE OF SPORE DISCHARGE
200 000
180 000
160 000
140 000
120 000
100 000
80 000
60 000
40 000
20 000
NOV. 1968
14
15
16
17
18
19
20
21

Fig. 9 The periodicity of S. commune on wood under conditions of 12L:12D, 70 F, and 98+% RH.

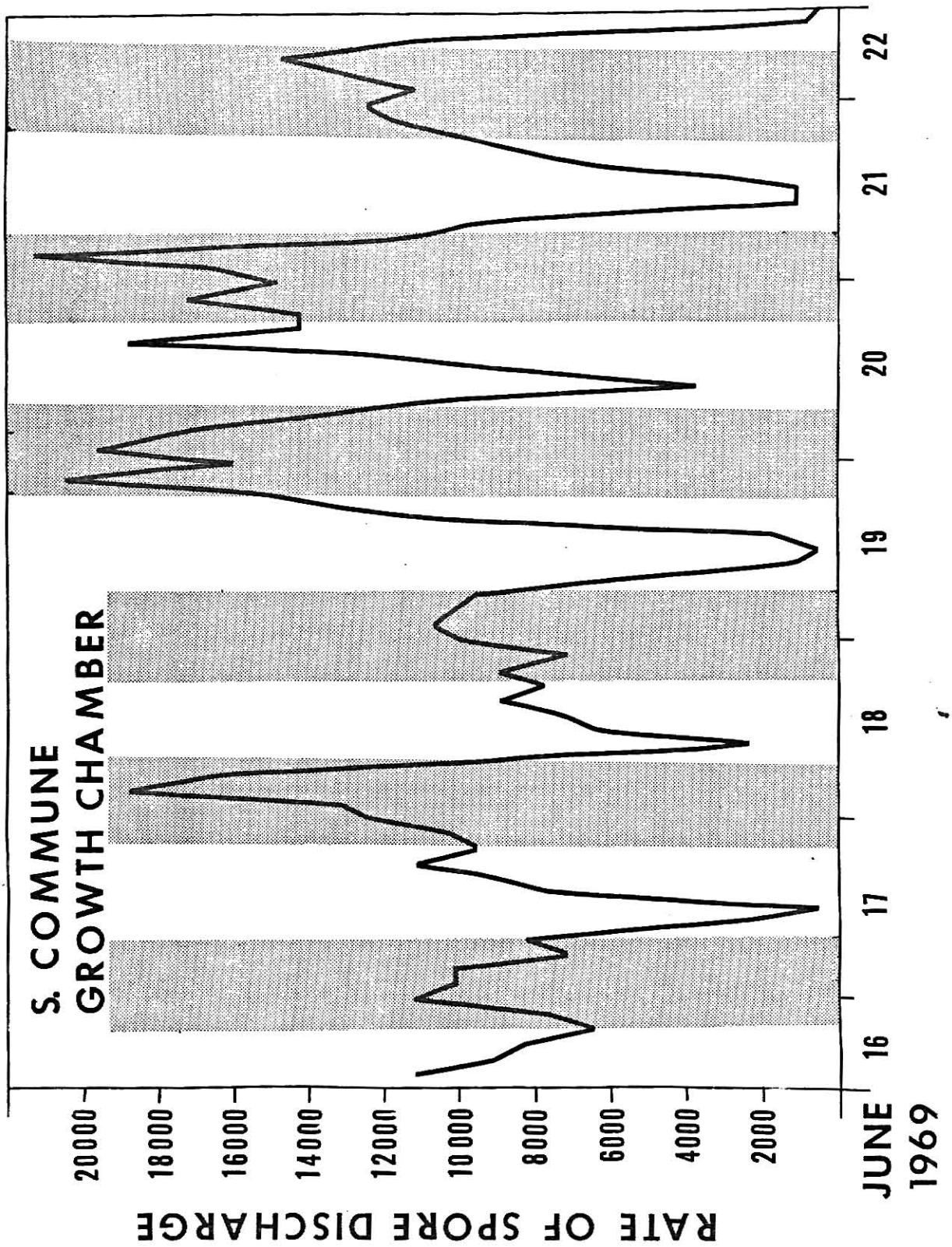

S. COMMUNE
GROWTH CHAMBER
RATE OF SPORE DISCHARGE
20000
18000
16000
14000
12000
10000
8000
6000
4000
2000
16
17
18
19
20
21
22
JUNE
1969

Fig. 10 The periodicity of S. commune when averaging each hr of a 24 hr period of the 12L:12D series of specimens growing on wood and in culture.

Fig. 10

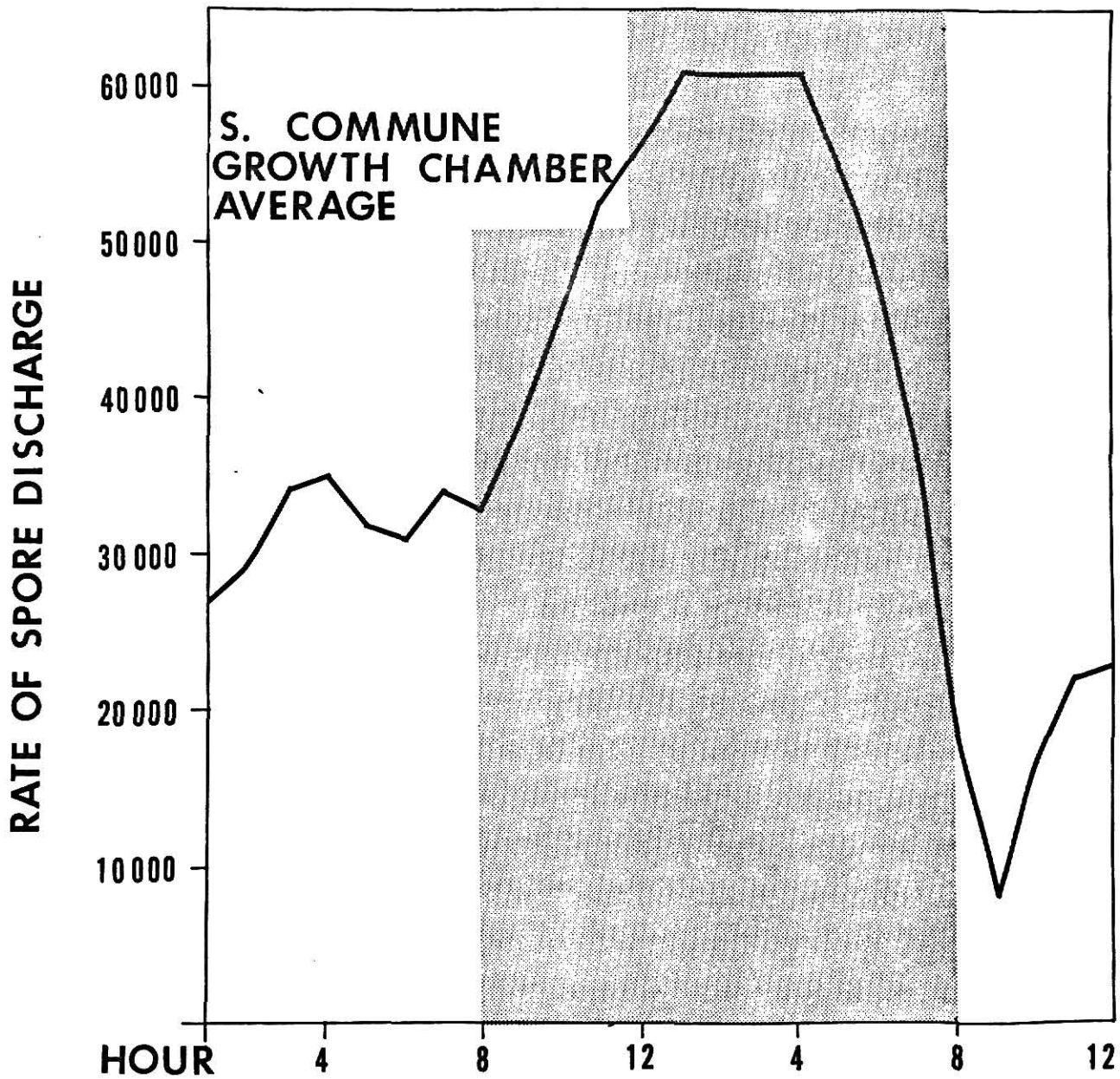

S. COMMUNE
GROWTH CHAMBER
AVERAGE
RATE OF SPORE DISCHARGE
60 000
50 000
40 000
30 000
20 000
10 000
HOUR
4
8
12
4
8
12

Fig. 11 The periodicity of S. commune on wood under conditions of 18L:6D, 70 F, and 98+% RH.

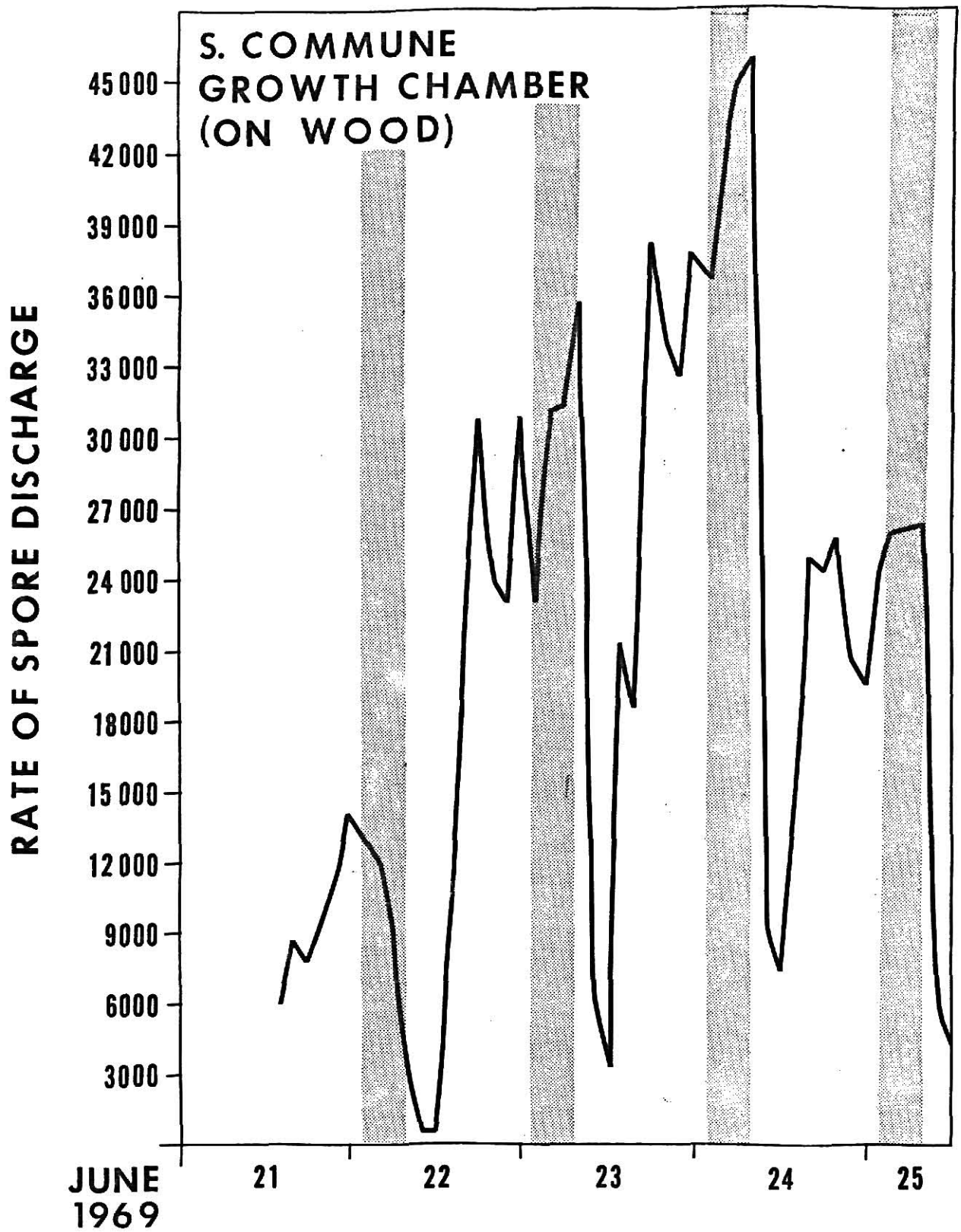

S. COMMUNE
GROWTH CHAMBER
(ON WOOD)
RATE OF SPORE DISCHARGE
45 000
42 000
39 000
36 000
33 000
30 000
27 000
24 000
21 000
18000
15 000
12 000
9000
6000
3000
JUNE
1969
21
22
23
24
25

Fig. 13 The periodicity of S. commune when averaging each hr of a 24 hr period of the 18L:6D series of specimens growing on wood.

Fig. 12

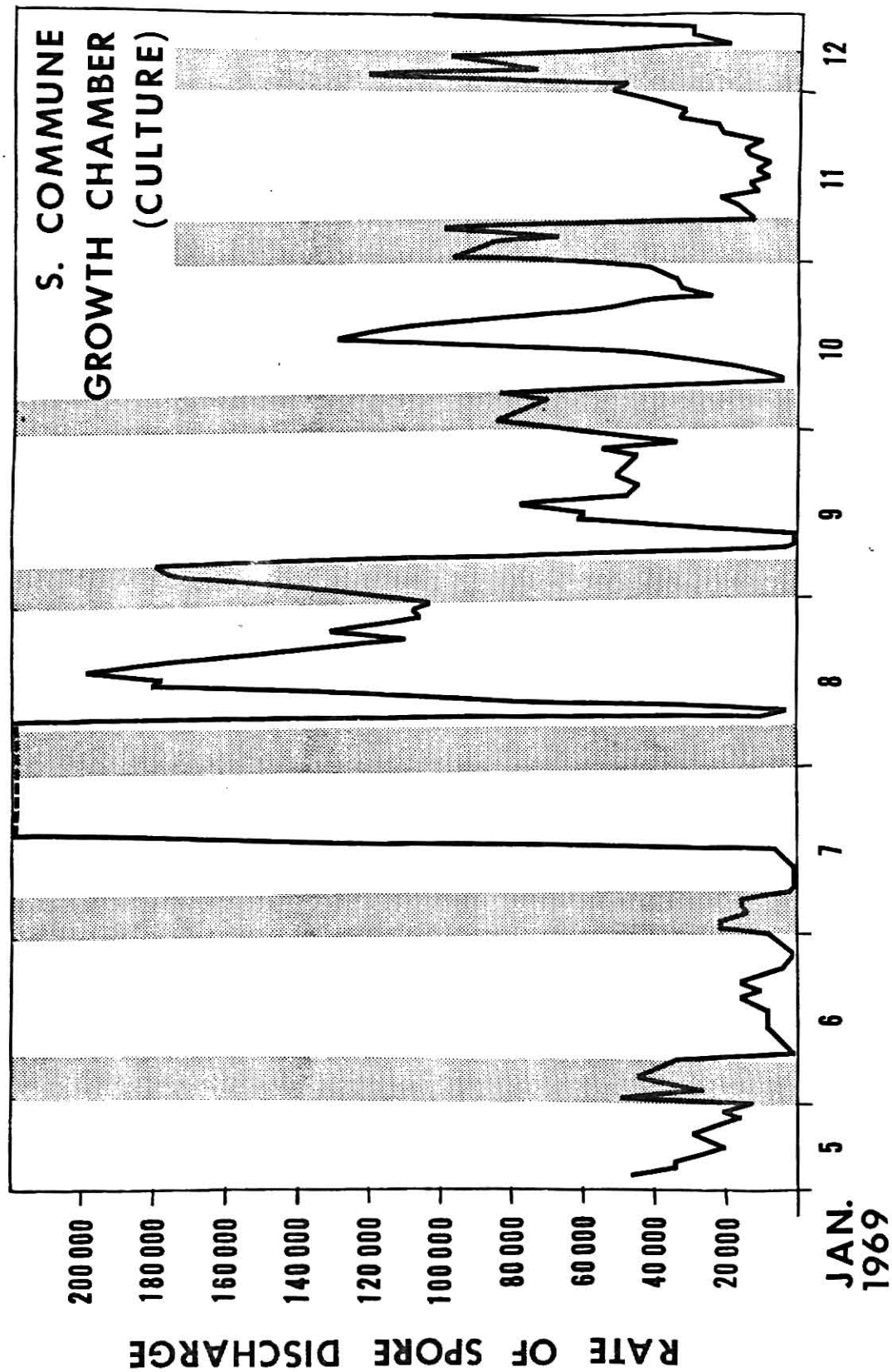

S. COMMUNE
GROWTH CHAMBER
(CULTURE)
RATE OF SPORE DISCHARGE
200 000
180 000
160 000
140 000
120 000
100 000
80 000
60 000
40 000
20 000
5
6
7
8
9
10
11
12
JAN.
1969

Fig.]3

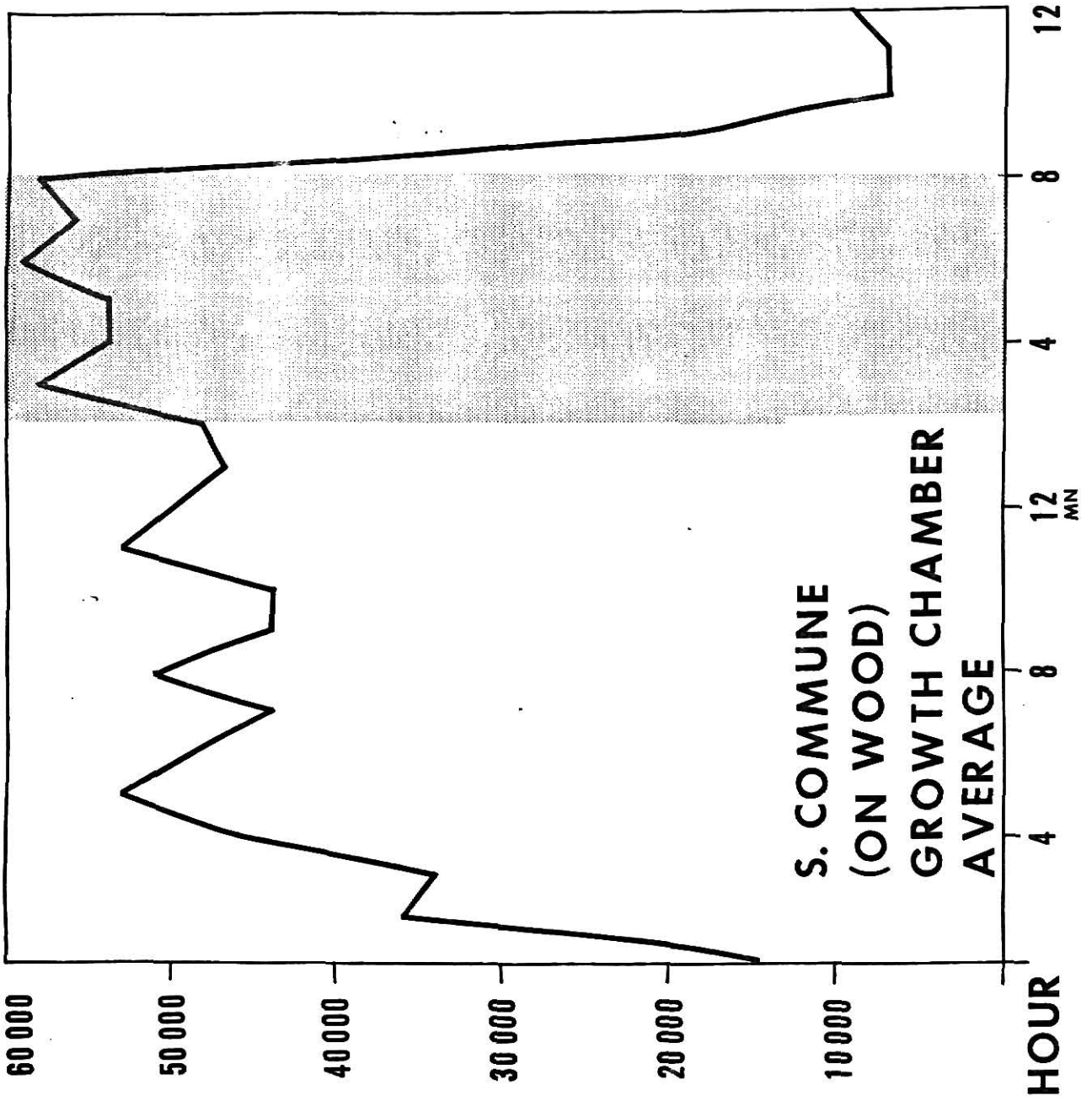

60 000
50 000
40 000
30 000
20 000
10 000
HOUR
4
8
12
MN
4
8
12
S. COMMUNE
(ON WOOD)
GROWTH CHAMBER
AVERAGE

Fig. 12 The periodicity of S. commune in culture under conditions of 18L:6D, 70 F, and 98+% RH.

periods of 12L:36D and 36L:12D were made (Figs. 14-16). As before, regardless of the length of time of the dark and light periods (12L:12D, 18L:6D, 12L:36D, or 36L:12D), there was a distinct but temporary decrease in spore release with each change from a dark to a light period.

When a dark period was omitted from a regularly alternating 12L:12D sequence, a decrease in spore numbers was also omitted from the spore discharge pattern during that 24 hr period (Fig. 17).

After conditioning in alternating light/dark, and subjecting to continuous light, spore discharge seemed to show no evidence of a diurnal pattern (Fig. 18). This is particularly evident when averaged hourly for a 24 hr period (Fig. 19). However, over a 5 day period there was a gradual flucuation in the rate of spore discharge corresponding with the productivity of the sporocarps with some young ones beginning to sporulate and others becoming senescent (Fig. 18).

When a similar study was made in continuous dark after conditioning in alternating light/dark, spore discharge was again continuous (Fig. 20). On some days there was an indication of a depression corresponding to the previous light period, however, when the data of the 5 day series were averaged, these depressions were not evident (Fig. 21).

In an attempt to determine if temperature might have an effect on spore discharge, the temperature was dropped from 70 F for 6 hr period every 24 hrs during continuous light conditions (Fig. 22). This was done in continuous light because it had become apparent that spore discharge (and spore production) was continuous in constant light. Spore discharge was found to decrease with the drop in temperature and remain low throughout the six hr minimum. However, as temperature was again raised to 70 F, spore numbers also rose to levels of concentration approximately equal to those prior to the drop. Figure 23 shows an hourly average for each day of the 5 day series.

Fig. 14 The periodicity of S. commune in culture under conditions of 12L:36D, 70 F, and 98% RH.

Fig. 14

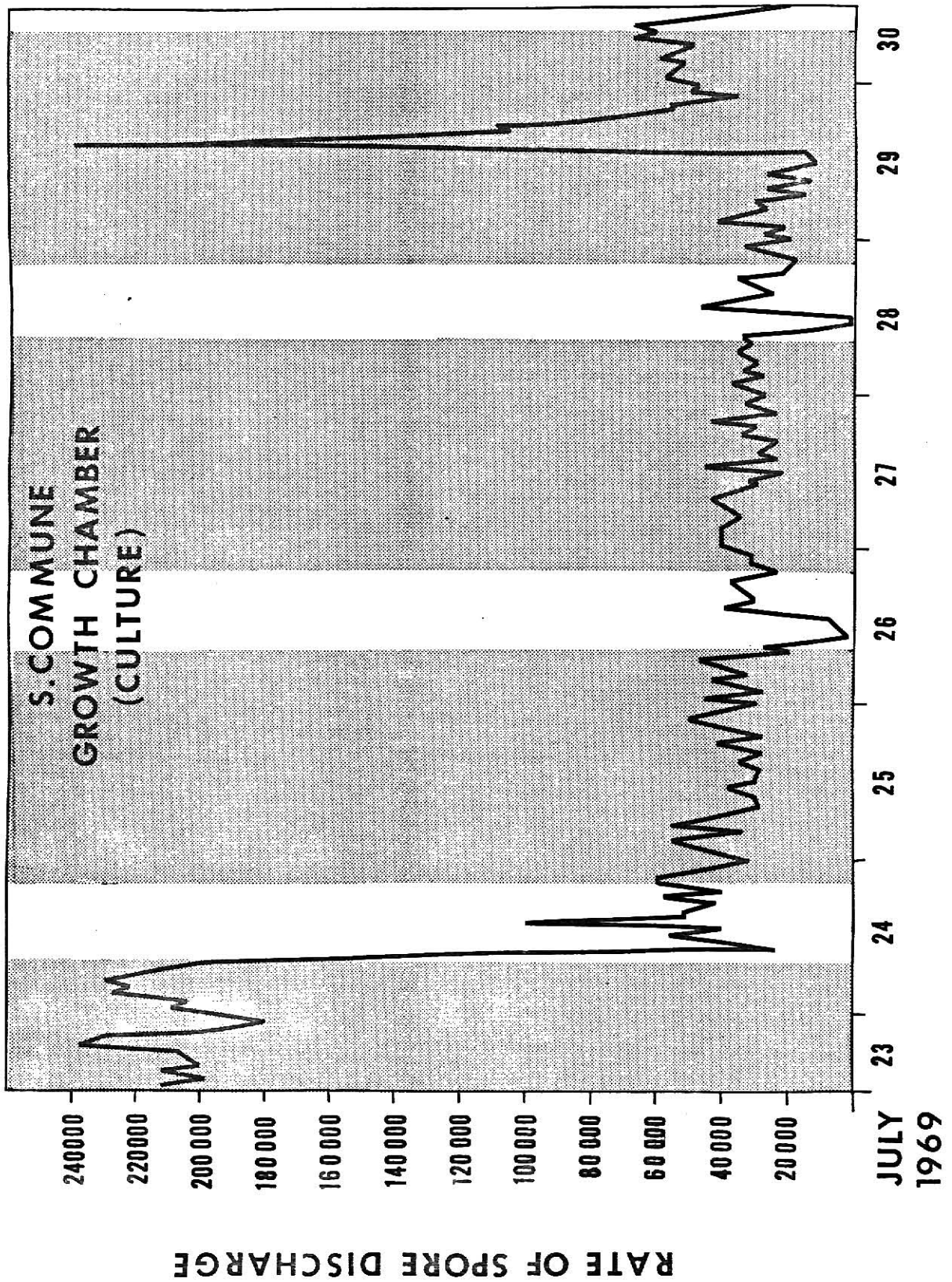

S.COMMUNE
GROWTH CHAMBER
(CULTURE)
RATE OF SPORE DISCHARGE
240000
220000
200000
180000
160000
140000
120000
100000
80000
60000
40000
20000
23
24
25
26
27
28
29
30
JULY
1969

Fig. 15 The periodicity of S. commune on wood under conditions of 12L:36D, 70 F, and 98+% RH.

Fig. 15

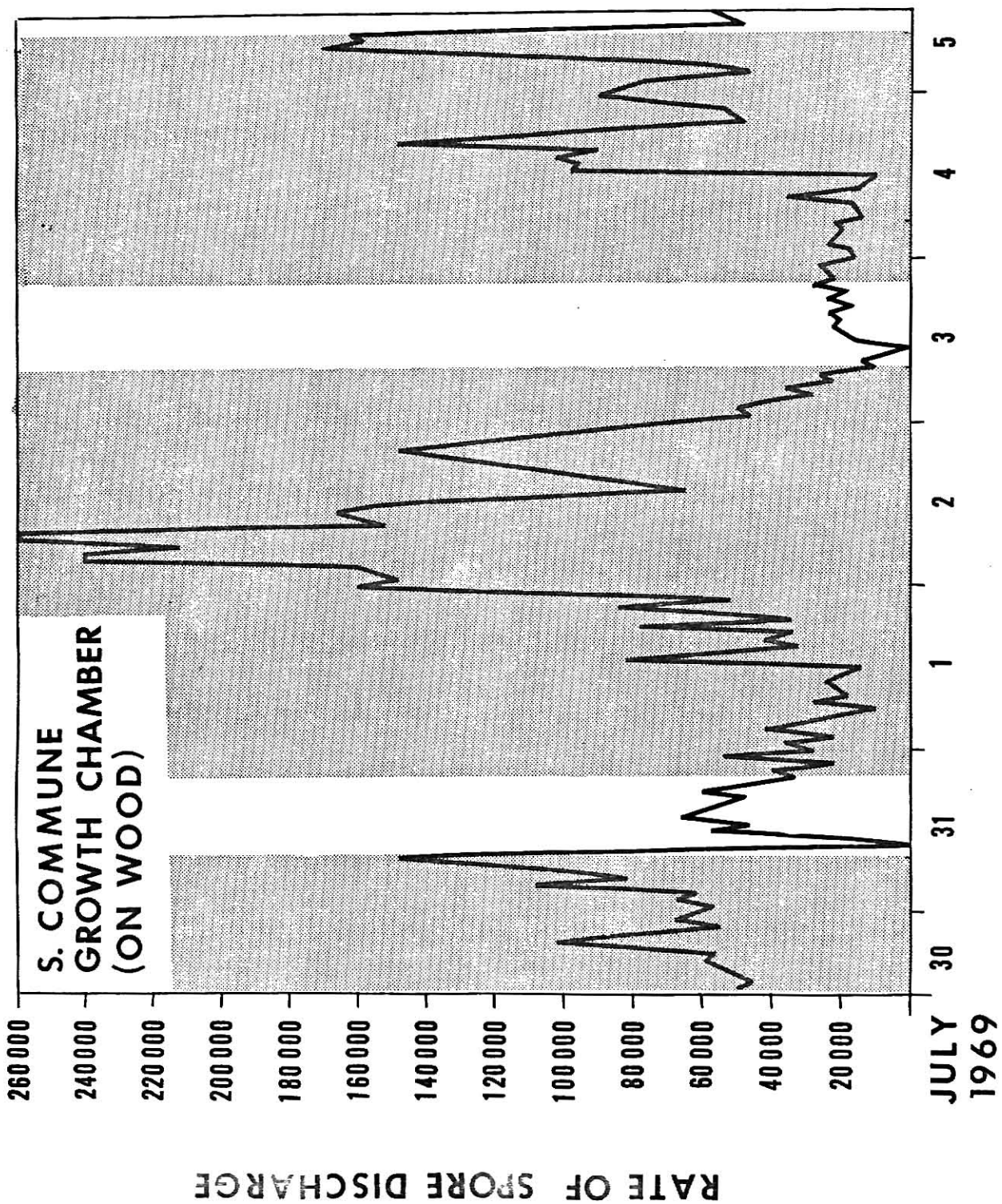

S. COMMUNE
GROWTH CHAMBER
(ON WOOD)
RATE OF SPORE DISCHARGE
260 000
240 000
220 000
200 000
180 000
160 000
140 000
120 000
100 000
80 000
60 000
40 000
20 000
JULY
1969
30
31
1
2
3
4
5

Fig. 16 The periodicity of *S. commune* in culture under conditions of 36L:12D, 70 F, and 98% RH.

Fig. 16

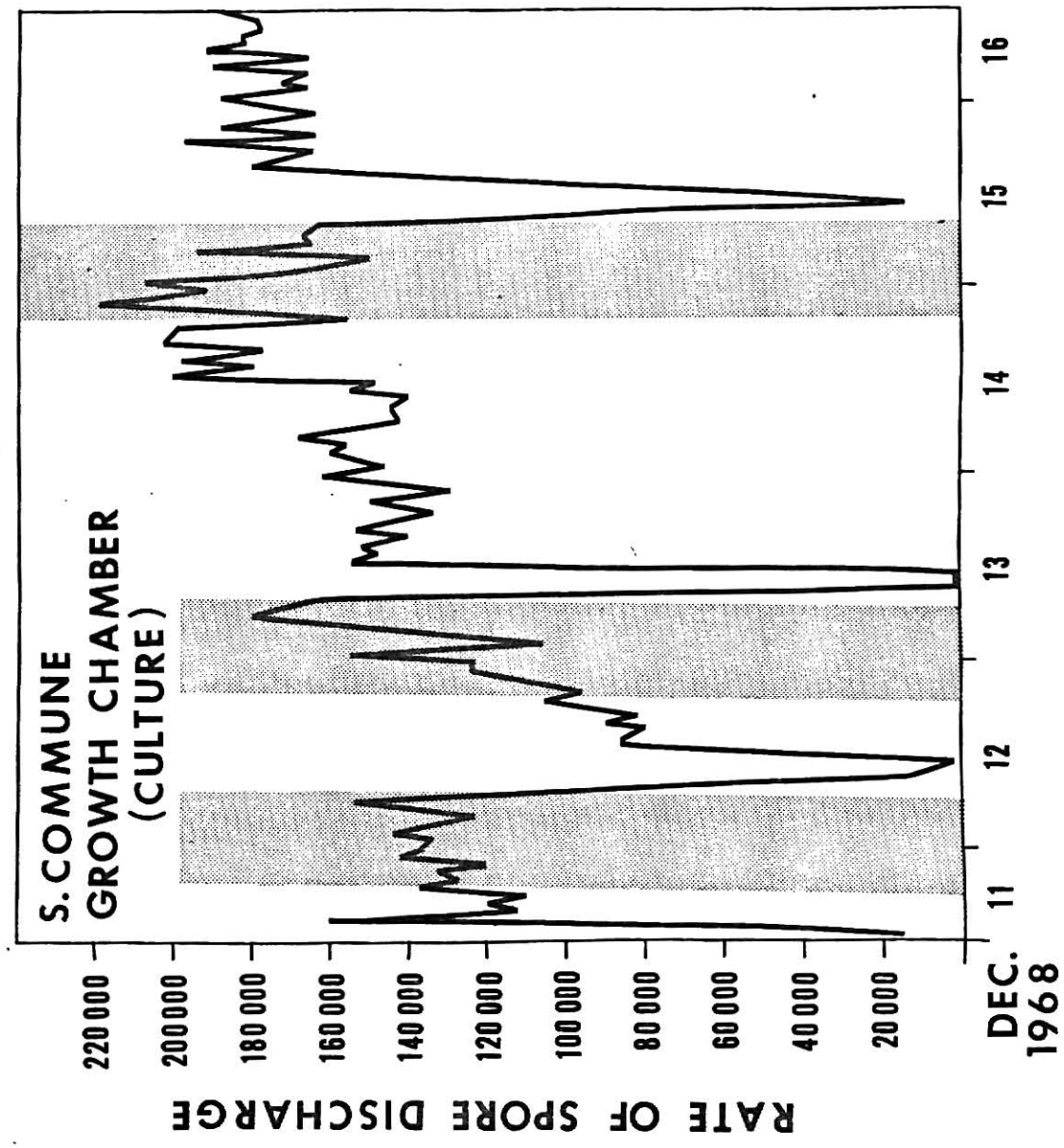

S.COMMUNE
GROWTH CHAMBER
(CULTURE)
RATE OF SPORE DISCHARGE
220 000
200 000
180 000
160 000
140 000
120 000
100 000
80 000
60 000
40 000
20 000
11
12
13
14
15
16
DEC.
1968

Fig. 17 The periodicity of S. commune in culture when a dark period was omitted from a regularly alternating 12L:12D cycle.

Fig. 17

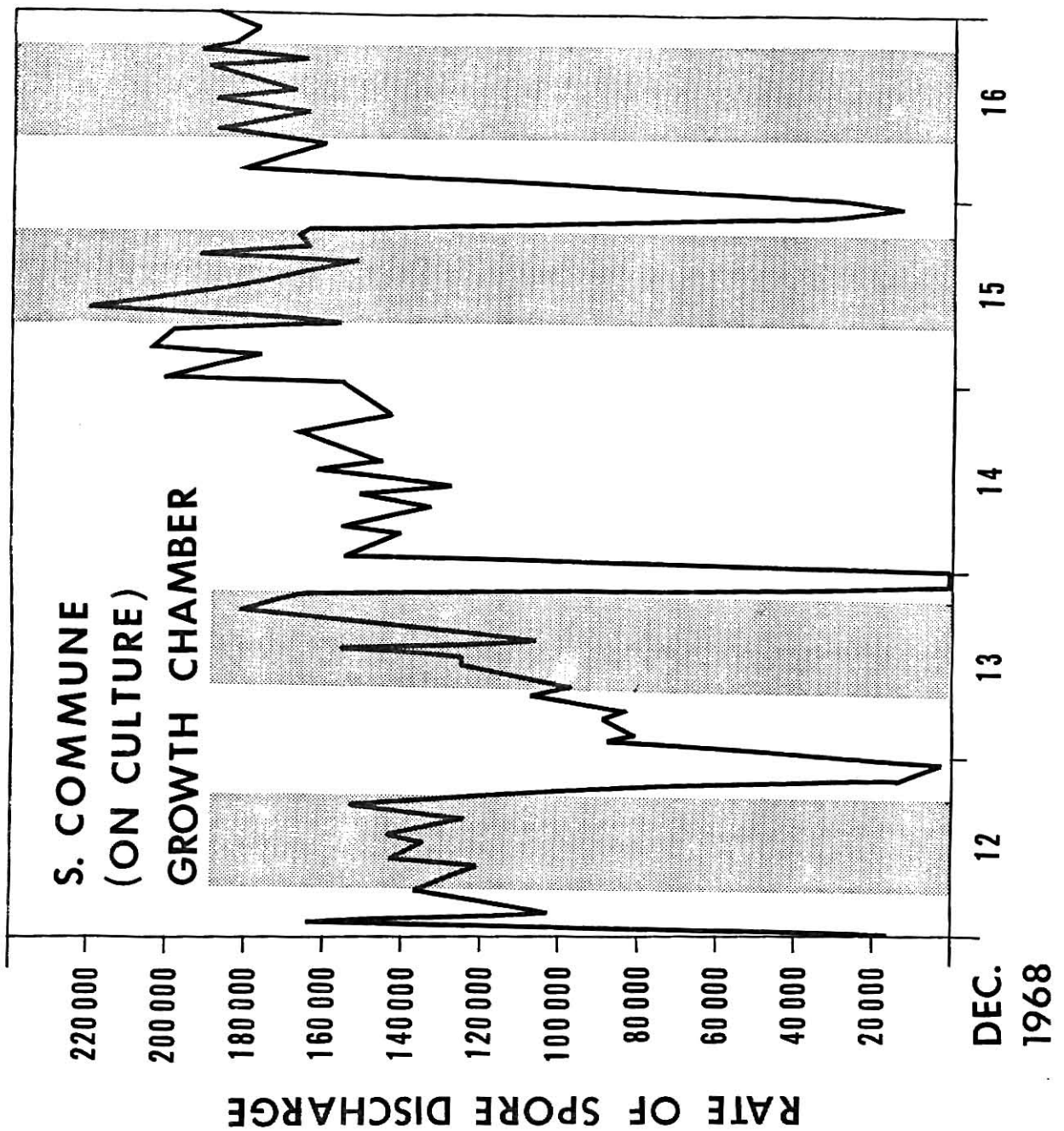

S. COMMUNE
(ON CULTURE)
GROWTH CHAMBER
RATE OF SPORE DISCHARGE
220 000
200 000
180 000
160 000
140 000
120 000
100 000
80 000
60 000
40 000
20 000
12
13
14
15
16
DEC.
1968

Fig. 18 The periodicity of S. commune on wood under conditions of continuous light, 70 F, and 98+% RH.

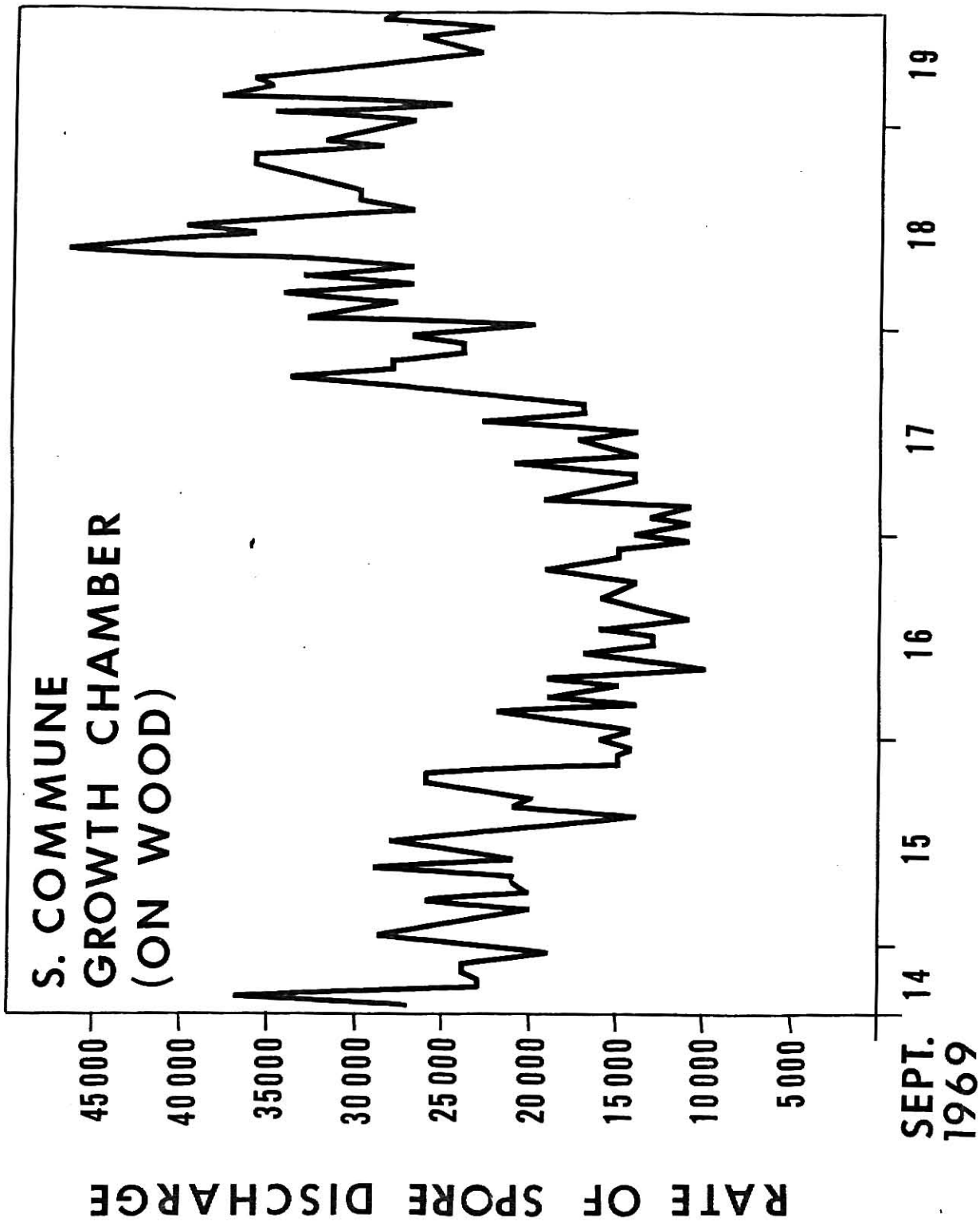

S. COMMUNE
GROWTH CHAMBER
(ON WOOD)
RATE OF SPORE DISCHARGE
45000
40000
35000
30000
25000
20000
15000
10000
5000
SEPT. 1969
14
15
16
17
18
19

Fig. 19 The periodicity of S. commune on wood when averaging each hr of a 24 hr period of the continuous light series.

Fig. 19

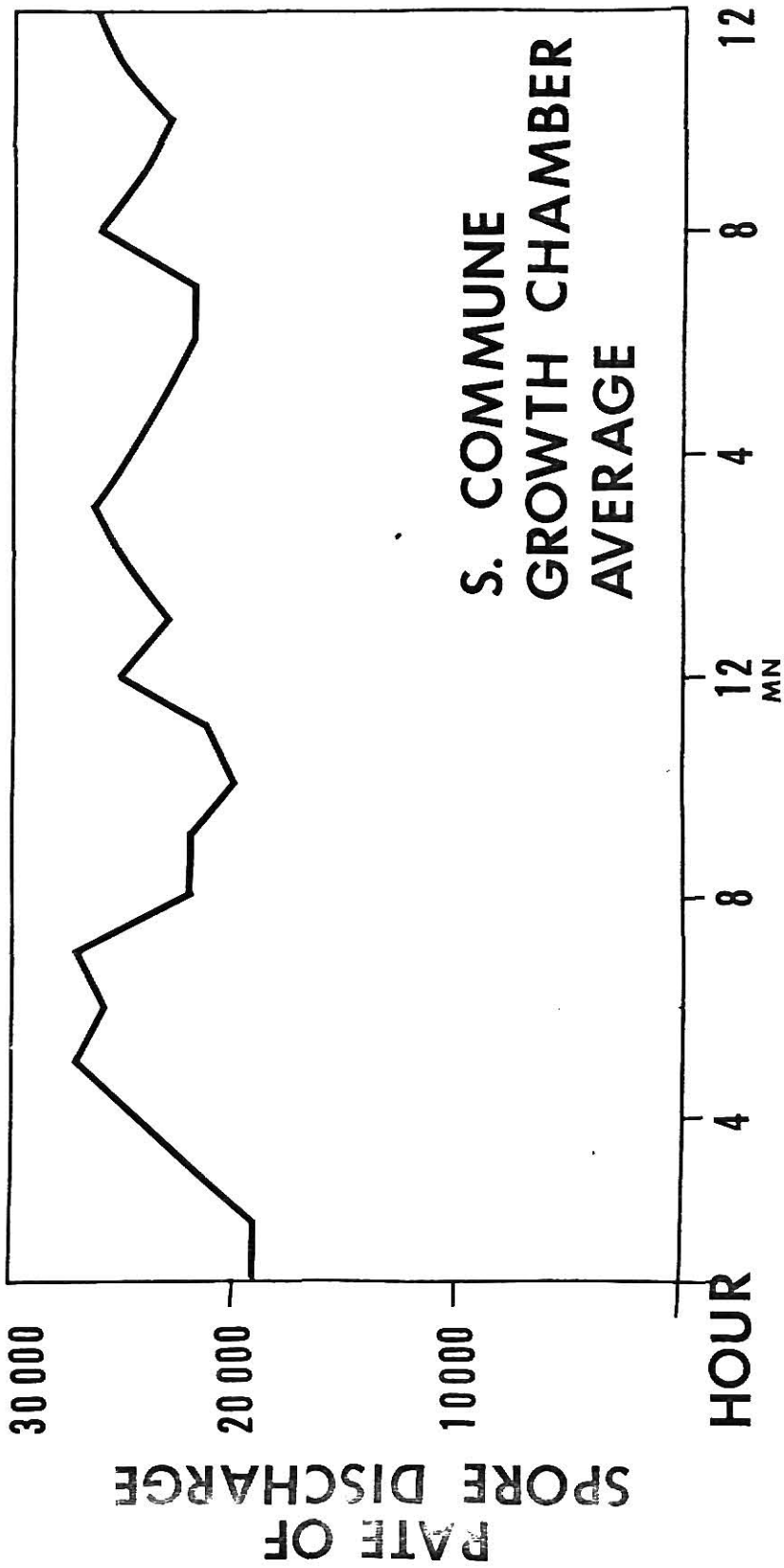

S. COMMUNE
GROWTH CHAMBER
AVERAGE
RATE OF
SPORE DISCHARGE
30 000
20 000
10 000
HOUR
4
8
12
MN
4
8
12

Fig. 20 The periodicity of S. commune in culture under conditions of continuous dark, 70 F, and 98% RH.

Fig. 20

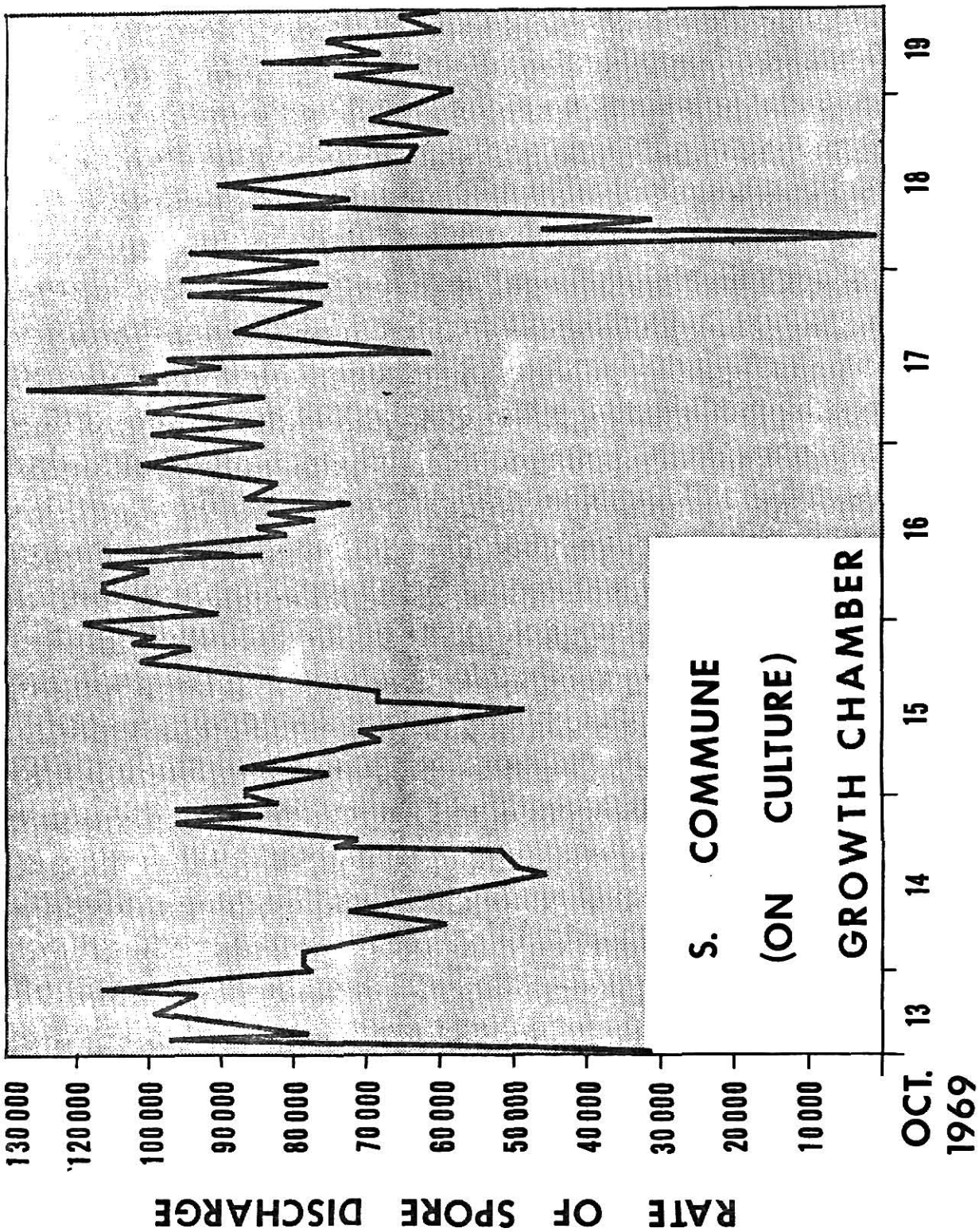

RATE OF SPORE DISCHARGE
130 000
120 000
100 000
90 000
80 000
70 000
60 000
50 000
40 000
30 000
20 000
10 000
OCT. 1969
13
14
15
16
17
18
19
S. COMMUNE
(ON CULTURE)
GROWTH CHAMBER

Fig. 21 The periodicity of S. commune in culture when averaging each hr of a 24 hr period of the continuous dark series.

Fig. 21

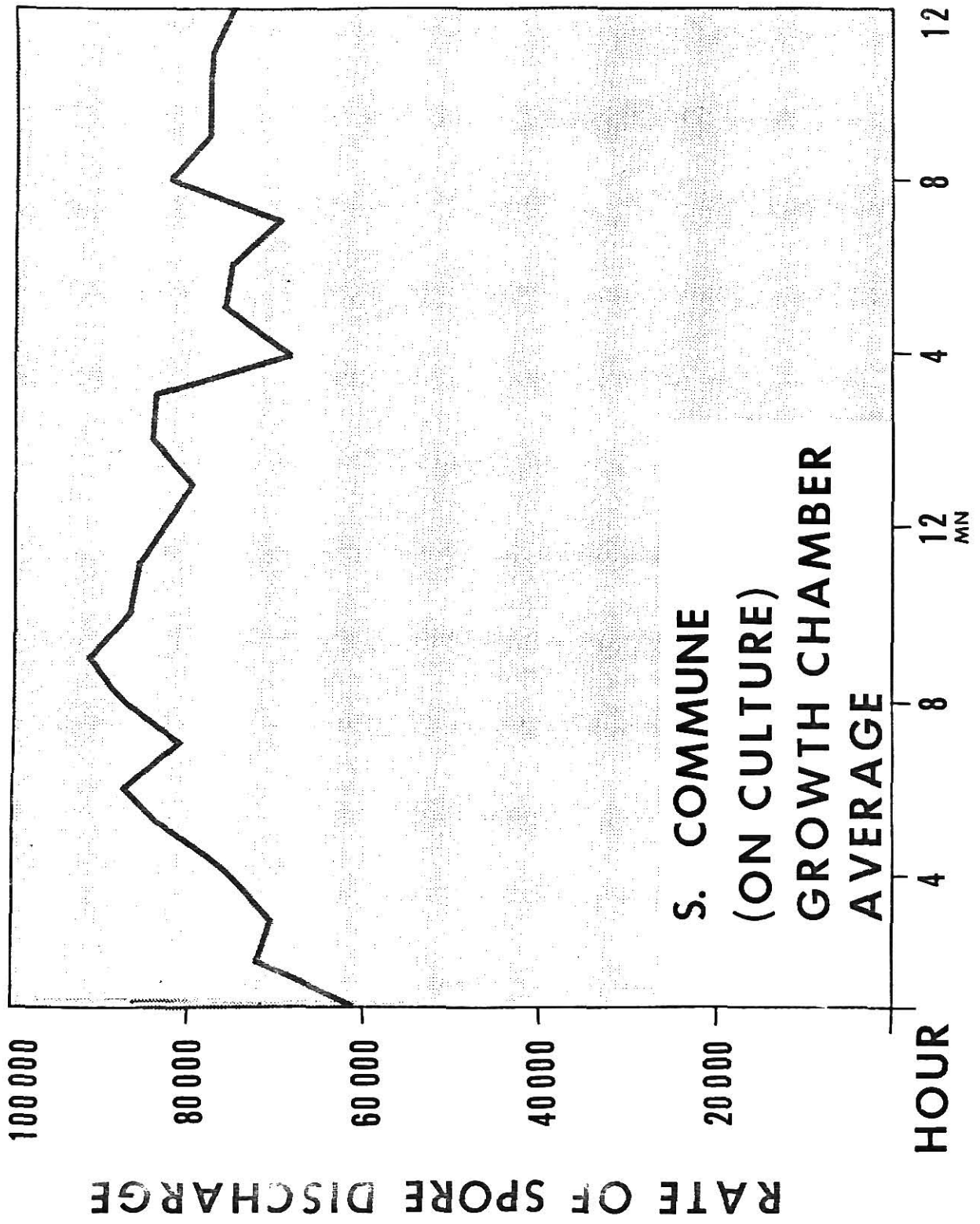

RATE OF SPORE DISCHARGE
100 000
80 000
60 000
40 000
20 000
HOUR
4
8
12
MN
S. COMMUNE
(ON CULTURE)
GROWTH CHAMBER
AVERAGE
4
8
12

Fig. 22 The periodicity of S. commune in culture under conditions of continuous light and 98% RH. Temperature is dropped from 70 F to 50 F from 12 MN to 6 AM.

Fig. 22

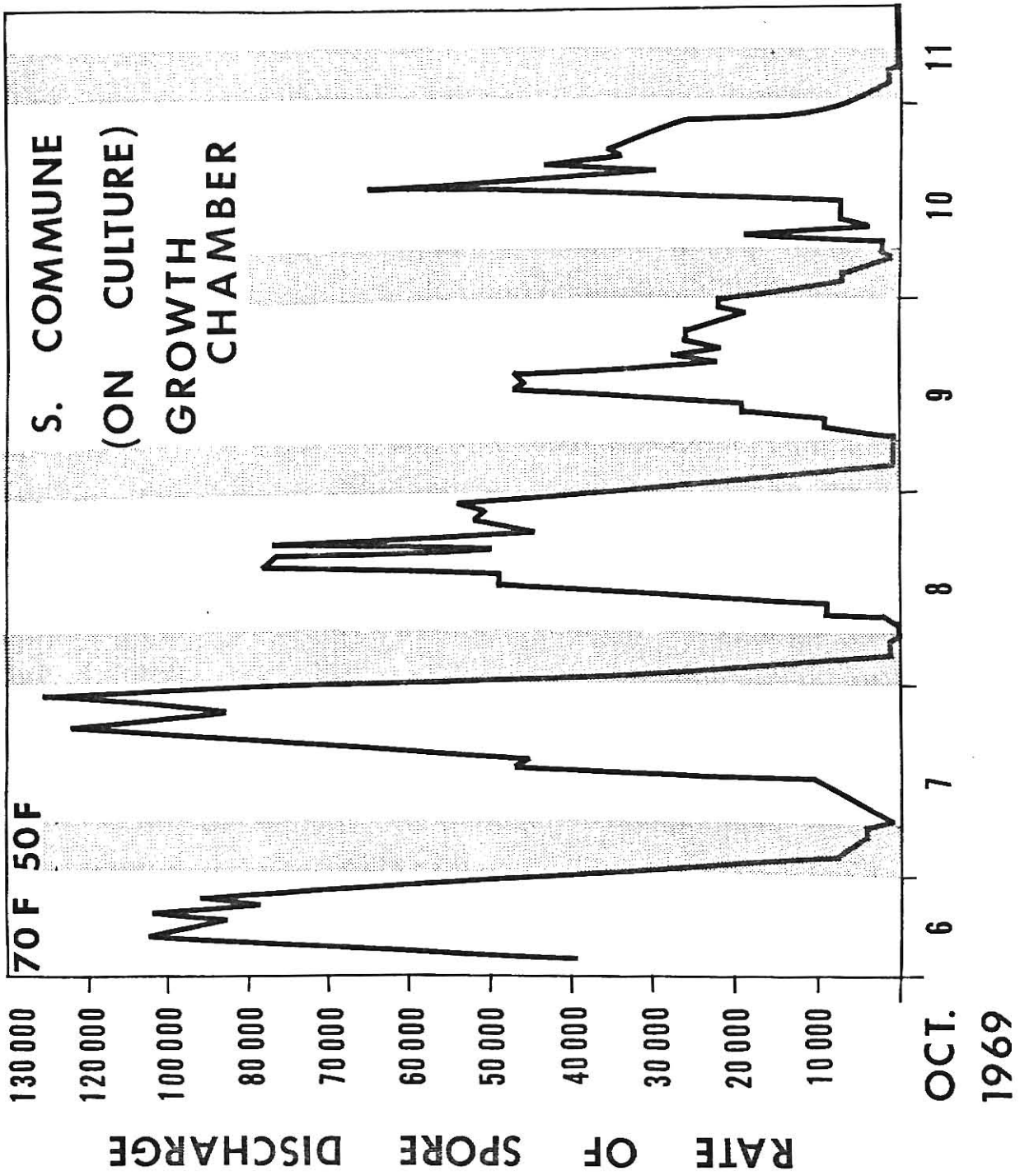
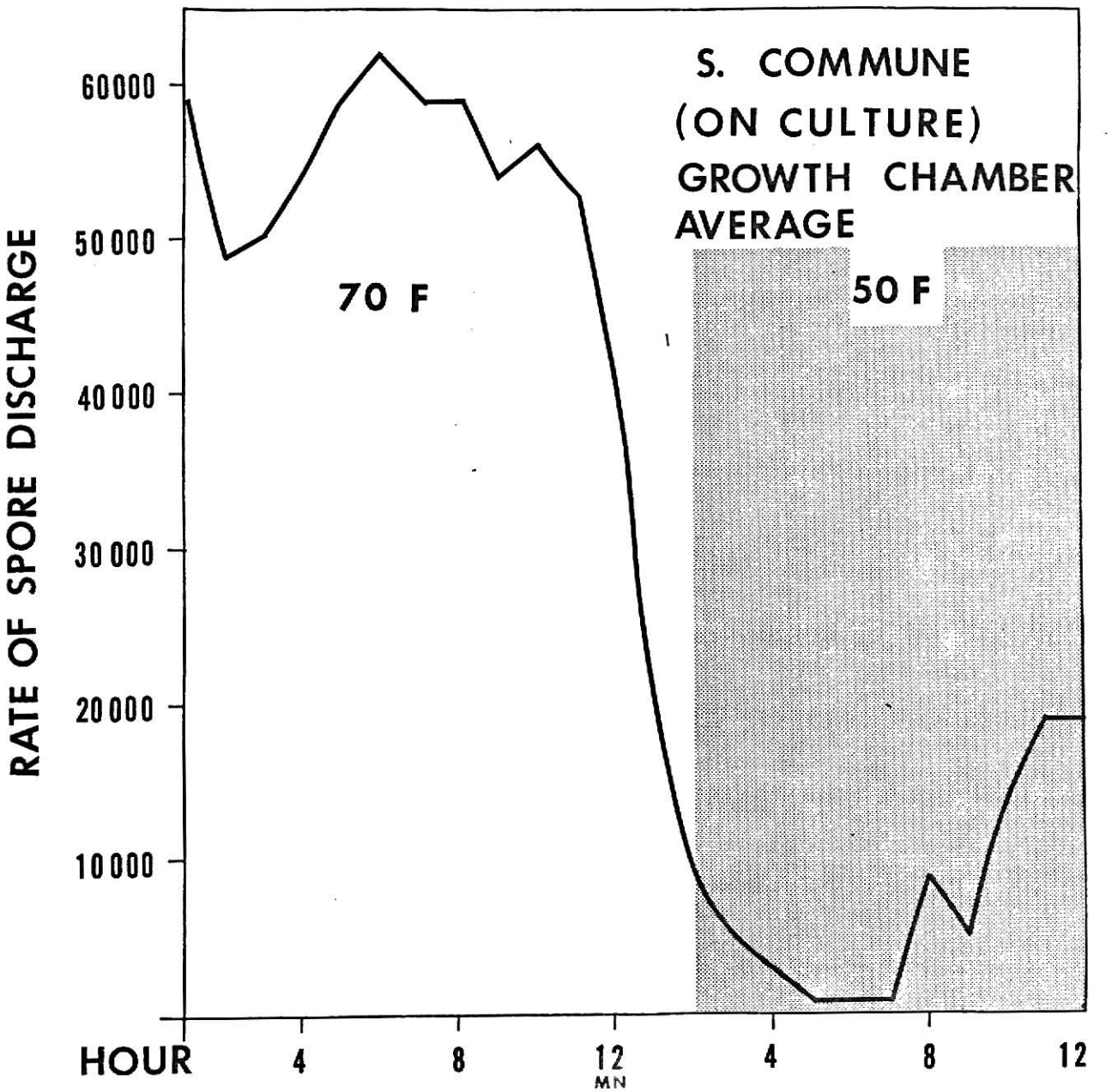

RATE OF SPORE DISCHARGE
130 000
120 000
100 000
80 000
70 000
60 000
50 000
40 000
30 000
20 000
10 000
70 F 50 F
S. COMMUNE
(ON CULTURE)
GROWTH
CHAMBER
6
7
8
9
10
11
OCT.
1969

Fig. 23 The periodicity of S. commune in culture when averaging each hr of a 24 hr period of the temperature drop series.

Fig. 23


S. COMMUNE
(ON CULTURE)
GROWTH CHAMBER
AVERAGE
70 F
50 F
RATE OF SPORE DISCHARGE
60000
50 000
40 000
30 000
20 000
10 000
HOUR
4
8
12
MN
4
8
12

DISCUSSION

A study has been made to determine the effects of external environmental factors, particularly light and temperature, on spore release in S. commune. Relative humidity was also of concern since it has an effect on the heartiness of the basidiocarp and therefore on spore release. Zoberi (1964) has shown that slight changes in the lowering of the relative humidity caused a reduction in spore discharge and that by increasing the relative humidity this effect could be reversed. As has been learned in these studies, when the relative humidity is 90% or less, the split gills fold back in such a way as to conceal the hymenium. It is only in an atmosphere of near 100% relative humidity that the gills remain unfolded exposing the hymenium. Because of this, vaporizors and bubblers were used to insure that the gills remained open throughout the experiments.

Zoberi (1964) stated that in preliminary work on S. commune he witnessed no effect of light on spore discharge. However, in the studies reported here under constant temperature and relative humidity, the number of spores discharged from sporophores growing either or wood or culture media decreased at the beginning of each light period. This decrease was only temporary; within one to three hrs, the rate of spore discharge again rose to a comparatively high level where it was maintained throughout the remainder of the light period. Because of the immediate and temporary effect, it appears to be the change from the dark to light condition rather than the presence of light that results in the temporary decrease in spore numbers.

Although it has not been definitely established whether this decrease in spore numbers at the beginning of light periods was due to an inhibition of spore production or an inhibition of spore discharge, the immediate response to light would indicate the latter. However, if spores were still

being produced at a normal rate during this one to three hr period, it would then be expected that once the inhibition had been overcome, there would be a sharp increase in the spore numbers due to the release of the accumulated spores produced but not released during that period. Spore discharge patterns do not show this to occur. Rather, spore numbers rose gradually over the three hr period. This may imply that the decrease in spore numbers was due either to an inhibition of spore production or to an inhibition of both spore production and spore discharge. Due to the gradual rise this would be a feasible suggestion, particularly if the initial recovery from the inhibition was a gradual rather than an abrupt event.

In an alternating light/dark cycle, the maxima reached during the dark periods seemed to be higher than the maxima reached during the light periods. This coupled with the temporary decrease in the rate of spore discharge that occurred after the beginning of the light periods and the folding back of the gills, may function to increase the chance of survival of the fungus. During the early morning hrs as it is becoming light, the relative humidity is usually comparatively high resulting in the exposure of the gills, and thus active spore release. The probability of spores surviving may decrease if liberated in the early morning because the low relative humidity that usually accompanies the daytime hrs may be detrimental to germination and vegetative growth of the fungus. Temporary inhibition of spore discharge or spore production or both would also conserve cytoplasm and prolong the longevity of the sporocarp.

The pattern of spore discharge expressed in S. commune during alternating light/dark conditions has not been reported in other fungi. However, Carpenter (1949) in preliminary studies on Pellicularia filamentosa (Pat.) Rodgers found spore discharge to be higher during the night than during the

day. Under favorable temperature (20-23C) and high relative humidity, results suggested that the periodic pattern of spore discharge may be strongly influenced by manipulation of the light and dark periods to which the fungus was exposed. However, on continuous light and also continuous dark, spore discharge was not uniform as in S. commune, but rhythmic.

In an extensive study of spore discharge in the field, Haard (unpublished) was of the opinion, based on studies of some 19 genera of Hymenomycetes, that if given a high relative humidity and suitable temperature, many of the species would liberate spores continuously. However, since variation in the environmental factors could not be controlled, it was difficult to determine the effects of alternating light conditions on spore discharge. For this reason, preliminary studies are now being undertaken in our laboratory where diurnal patterns of a number of these fungi are being studied under controlled conditions in environmental chambers. Preliminary studies of Polyporus elegans Bull. ex Fr., P. gilvus (Schco.) Fr., Mycena sp., and several other species of agarics under alternating 12L:12D and constant relative humidity and temperature indicate that spore discharge is continuous with no indication of a diurnal fluctuation. Polyporus dichrous Fr. is of particular interest here. In the few preliminary studies done thus far, there is an indication that it has a pattern of spore discharge similar to that of S. commune under conditions of alternating light/dark.

Preliminary studies in our laboratory on the effect of temperature on spore discharge in Polyporus versicolor L. ex Fr. indicate a temperature sensitivity similar to that reported here for S. commune. When the temperature was dropped from 70F to 50F, spore numbers immediately decreased. As temperature was again raised spore discharge increased. Although the temperature threshold below which spore discharge is inhibited completely,

was not determined in either of these species, it is evident that temperature has a pronounced effect on spore discharge.

Haard (unpublished) also has shown that late season species such as Suillus sphaerosporus (Peck) Smith & Thiers, and species of Panaeolus are able to discharge spores at lower temperatures than the midseason species such as Tylopilus felleus Bull. ex Fr. and Lactarius deceptivus Peck. Cortinarus also seemed to have a low temperature threshold of about 45 F. Varying temperature thresholds were discussed in several other genera of the Hymenomycetes.

SUMMARY

Basidiocarps of Schizophyllum commune Fr. that were developed on wood and in culture were used in a study to determine the effects of various environmental factors on basidiospore discharge. These studies were done under controlled environmental conditions in ISCO Environmental Chambers. Kramer-Collins spore samplers were used to obtain collections of the spores as they were released from the basidiocarps. Under alternating conditions of 12 hrs light and 1rs dark (12L:12D), there was an immediate but temporary decrease in spore numbers at the beginning of each light period. When subjected to other light cycles such as 18L:6D, 12L:36D, 36L:12D, the same decrease occurred with the beginning of each light period. This response was immediate, occurring within the hr and only at the beginning of the light periods regardless of when these light periods occurred. However, the effect was only temporary; within three hrs spore numbers usually returned to a comparatively high level. It is felt that this level of spore discharge is approximately the same as it would be under continuous light conditions when spore discharge is also continuous. Thus, it is felt that the presence of light is not the primary factor involved but rather it is the change from dark to light conditions that is the stimulus that effects this decrease in spore numbers.

Because of the immediate response, it would seem that the change from dark to light has an inhibitory effect on spore discharge rather than spore production. However, it is likely that spore production is also curtailed temporarily since there is never an indication of a peak in spore discharge after the inhibitory effect has been overcome as would be expected due to spores being accumulated during that period.

Under continuous light and constant relative humidity (approx. 98%)

temperature was lowered to determine its effect on spore discharge. When the fungus was conditioned to a temperature of 70 F and then dropped to a low of 50 F for six hrs of every 24 hr period, spore discharge decreased with the six hr minimum but increased rapidly as it was increased to 70 F.

Preliminary studies in the laboratory as well as field studies on various other Hymenomycetes indicated that similar responses to light and temperature to those found in S. commune occur in other fungi.

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EFFECTS OF LIGHT AND TEMPERATURE ON BASIDIOSPORE DISCHARGE IN *SCHIZOPHYLLUM COMMUNE* FR.

by

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B. A., Ottawa University, 1967

AN ABSTRACT OF A MASTER'S THESIS

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MASTER OF SCIENCE

Division of Biology

KANSAS STATE UNIVERSITY
Manhattan, Kansas

1970

Abstract

Schizophyllum commune Fr. is an important woodrotting Hymenomycete that is cosmopolitan in its distribution. Because of its abundance and ability to withstand harsh environmental conditions for long periods of time without injury to the fruiting body, a study was undertaken to determine the effects of various environmental factors on basidiospore discharge.

A series of experiments was made in ISCO Environmental Chambers. Kramer-Collins spore samplers were used to obtain collections of the spores as they were released from the basidiocarps. The effect of light was the factor of primary concern in this experiment. Under alternating conditions of 12 hrs. light and 12 hrs. dark (12L:12D), and constant relative humidity (98^{+}) and temperature (70F), there was an immediate but temporary decrease in spore numbers at the beginning of each light period. When subjected to other light cycles such as 18L:6D, 12L:36D, the same decrease occurred with the beginning of each light period. This response was immediate, occurring within the hour and only at the beginning of the light periods, regardless of when these light periods occurred. However, the effect was only temporary; within 3 hrs. spore numbers usually returned to a comparatively high level. It is felt that this level of spore discharge was approximately the same as it would be under continuous light conditions when spore discharge was also continuous. Thus, it is felt that the presence of light was not the primary factor involved but rather it was the stimulus that resulted in the decrease in spore numbers.

Because of the immediate response, it seems that the change from dark to light had an inhibitory effect on spore discharge rather than spore production. However, it is likely that spore production was also curtailed since there was never an indication of a peak in spore discharge after the

inhibitory effect had been overcome as would be expected due to spores being accumulated during that period.

Under continuous light and constant relative humidity (98^{+}), temperature was lowered to determine its effect on spore discharge. When the fungus was conditioned to a temperature of 70 F and then dropped to a low of 50 F for 6 hrs of every 24 hr period, spore discharge decreased with the drop in temperature. Spore numbers stayed low during the 6 hr minimum but increase reapidly as it was increased to 70 F.

Preliminary studies in the laboratory as well as field studies on various other Hymenomycetes indicated in some cases, similar responses to light and temperature to those found in S. commune.

It is believed that change from dark to light may well be a factor that inhibits spore discharge in S. commune in the early hours of the morning when relative humidity and temperature may still be suitable for high spore discharge. This would greatly reduce the number of spores released in the morning that would not perhaps survive the day.