STUDIES OF THE FUNGUS CONTENT OF THE AIR

by

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INTRODUCTION

Fungi are disseminated mostly in the form of various types of air-borne spores and to some extent in the form of hyphal fragments. Their concentration in the air may vary according to season in a year, during a season from day to day and from hour to hour on any one day.

Considerable work has been done on the seasonal as well as daily variations of the various air-borne fungus flora (9, 15, 17, 43). This has provided much information in understanding the spread and distribution of various plant pathogens and allergens. However, very little is known about their hourly variations in the air.

Hirst's automatic volumetric spore trap (29) showed the possibilities for detailed study of hourly variations in the number of air-borne fungi over a period of several days. By applying that technic he demonstrated a distinct diurnal pattern for various types of fungus spores in the air and in certain cases demonstrated various meteorological factors governing their occurrence in the air in England (30).

Work conducted in Manhattan during the last few years has shown a distinct seasonal and daily occurrence of various important plant pathogens and allergens in the air. From the previous information obtained here, it was felt desirable that the study be extended to observe the hourly variations in the concentration of air-borne fungus flora. With this end in view, a new slit type continuous spore sampler was designed which could sample the air continuously for 24 hours (42). Present work covers the data obtained on hourly variations of various air-borne fungus flora by sampling air from May through September, 1957, in the new slit type continuous spore sampler. While particular emphasis was on the study of diurnal periodicity of air-borne fungi, some data was obtained regarding the percentage of viable spores and hyphal fragments in the air.

REVIEW OF LITERATURE

Dissemination of fungi through air has long been known and their relation to epiphytotics and allergens has been well established.

Micheli in 1729, as cited by Keitt (33) first observed that fungus spores may be carried in air, liberated by the fruiting bodies in the form of spore dust.

Prevost (55) recognizing the common occurrence of fungus spores in the atmosphere adopted certain precautions to protect his water cultures of the bunt pathogen from contamination. He also pointed out that the bunt spores might be disseminated by the wind at the time of threshing.

In 1840 Pasteur (9) filtered the air-borne micro-organisms by employing nitrocellulose filters and observed the micro-organisms thus collected, directly under the microscope.

Pouchet (9) in 1840 was the first to pass a known volume of air through a funnel, that gave a spore deposit on the glycerine coated slide.

Ward (69) was apparently the first to demonstrate the action of winds in distributing plant pathogens. He exposed glycerine coated slides at various distances (12 feet, 20 feet, 25 feet) from the nearest tree affected with coffee rust (HEMILEIA VASTRATRIX BER. & CR.) and found the spores of this rust on those slides.

At Saint Paul, Minnesota, Freeman & Johnson (20) by exposing gravity slides in the summer, obtained rust spores prior to any infection in the neighboring fields. They also demonstrated the action of winds in carrying the rust uredo-spores from the south.

Stakman et al. (64) in 1921 were the first to make use of the airplane for the survey of upper air fungus flora. They found a large number of fungus spores up to the elevation of 11,000 feet with some spores up to 16,500 feet and showed the possibility of carrying various fungi over large distances by the upper air currents. Stem rust (Puccinia graminis Pers.) was found viable on silicone slides up to the height of 7,000 feet, while Alternaria was found viable as high as 10,000 feet. During their flight from Fort Sill, Oklahoma, to Denison, Texas, they also found a reduced number of uredo-spores with the increase in distance from the source of infection.

Wolf (70) during his aerial survey from 1,000 to 10,000 feet over Nashville, Tennessee, found the number of micro-organisms decreasing with the increase in height. He also discovered a biological stratum between 3,000 and 4,000 feet, as reported earlier by Procter (57).

Meier (36) exposed spores of seven different fungi to an altitude of 72,395 feet and found that for at least four hours, low humidity, high intensity of light and low temperature had very little effect upon the viability of most of the spores. However, they observed a complete loss and partial loss of viability in the case of Hysterium sp. and Cladosporium sp. respectively.

Stephens and Woolman (65) counted the spores of wheat bunt deposited on the leaves, from Pendelton to Lagrande and found that the spores decreased from the source of dissemination and concluded that the quantity of spores is inversely proportionate to the distance from the source.

Duke and Durham (14) were apparently the first to demonstrate a seasonal pattern for the air-borne fungus flora. They observed in the air over Kansas City, Mo. rust uredo-spores from May through September and found a distinct seasonal pattern with a peak in the month of July.

At Chicago, Feinberg and Little, cited by Durham (18), by exposing nutrient plates daily for 15 minutes through the period of one year found a distinct seasonal pattern for various fungi. Later Prince and Morrow (56) by employing the same technic made a seasonal survey at Galveston City (a coastal city of Texas) and isolated about 90 different species of fungi, however they were unable to find Alternaria in that atmosphere.

Durham (15) from his preliminary studies throughout the United States found Alternaria to be a seasonal fungus flora occurring mostly in the months of May through November. He concluded from his geographical distribution studies that the largest Alternaria belt extends from the Rocky to the Appalachian Mountains and found both the coasts and deep south comparatively free from Alternaria. By the observation of various slides exposed at various places he was also able to trace an unusual shower of fungus spores during a wind storm of October 6 and 7, 1937 (16). According to his conclusions the shower originated from southern Minnesota and went as far as New York City.

Since then considerable work has been done on the ecological, seasonal and various other aspects of air-borne fungus flora, as has been excellently discussed by Craigie (11), Harris (27), Durham (17), Keitt (33), Christensen (10) and Stakman and Christensen (63).

In Kansas, Pady and Johnston found a distinct correlation between the development of rusts in the fields, meteorological data (temperature, rainfall, and wind) and the number of spores on gravity slides. In 1954 due to optimum weather conditions, high infection of rusts was recorded in the fields and the maximum number of spores on 24 hour gravity slides during that season was recorded as 167,000/sq.ft. and 239,000/sq.ft. for leaf and stem rust respectively (44). In 1955 due to high temperature and dry weather conditions, the development of rusts was considerably checked and maximum number recorded on gravity slides was only 53,000/sq.ft. and 110,000/sq.ft. for leaf and stem rust respectively (45). Extremely low infection of rusts was observed in the field during 1956 (46) and the gravity slides also showed a low number i.e. 3,195 and 43,000/sq. ft. for leaf and stem rust respectively. During this period of study they also observed that the south and north winds were mainly responsible during spring and fall respectively for bringing primary infection into the Kansas fields.

Pady in 1957 by employing an improved slit sampler (41) demonstrated (43) a distinct seasonal variation in the number of air-borne fungus flora over Manhattan, Kansas. Maximum spores in general were recorded in the months of July and August, with a low from December through March. He also found a direct correlation of air-borne fungus flora with the increase in vegetation in the Kansas soils, reaching a peak in July and August and declining through September and October.

In Canada, Walton and Dudley (68) after a seven year survey of air-microflora of Manitoba, demonstrated a distinct seasonal as well as geographical occurrence for various types of fungi. In general they found higher numbers of fungi in the areas south of Winnipeg as compared with the places in the north.

Pady (40) in 1947 was the first to obtain the cultures of air-borne fungi over arctic regions by exposing nutrient plates from the aeroplane.

Kelly and Pady (34) using aeroplanes over some nonarctic regions of Canada observed higher numbers of fungi in the air during June, July and August, than the rest of the year. They were apparently the first to provide quantitative data of the hyphal fragments in the air.

Pady and Kelly (52) at Churchill, Manitoba, found a distinct daily and seasonal variation, with the high number of spores in the summer and the low in the winter, which were generally affedted by various air masses. Different types and varying ratios of viable and non viable spores that were obtained, helped to trace the history of different air masses. In general higher numbers of air-borne spores were associated with Tropical and Continental air masses, while pure Maritime or Continental air-masses were very low in spore counts (50).

In England, Hyde and Williams (31) found a distinct daily and seasonal variation in the number of air micro-flora. Richards (59) by exposing nutrient plates found higher numbers of Cladosporium from June to August and lower in October to April; later by exposing nutrient plates at various places in the country (60) found no difference in the type but a distinct difference in the quantity. Cladosporium and Alternaria, out of various fungi, were found higher in the cities than the urban areas.

Gregory and Hirst (26) in 1952 demonstrated a high number of Alternaria spores in the air during the month of August, which was mostly encountered after dry periods followed by rain. They also observed colored basidiospores during summer, which were most common during warm spells with substantial rains.

Cammack (8) in Nigeria observed leaf rust of maize (Puccinia polysora Underw.), Nigrospora sphaerica (Sacc.) Mason, and Cladosporium spp. throughout the year. Leaf rust was more prevalent in wet seasons, N. sphaerica in dry and Cladosporium spp. in both wet and dry seasons.

Various fungi were found to be associated with the deterioration of certain textile products. Rajan et al. (58) in India by exposing nutrient plates in the open air found a distinct seasonal pattern of various fungi associated with the textile industry. He found maximum and minimum numbers of fungi during the months of November and December respectively. Low fungus counts in summer (July through September) were attributed mostly to rains or nocturnal condensations.

Distinct seasonal and geographical variations have also been observed in New Zealand (19, 39). Occasional spore showers during spring, summer and fall were recorded by Dye and Vernon (19), which were found responsible for sudden fluctuations in the atmospheric mold flora.

Neill and Armstrong (39) by employing a selective method for the isolation of Gloeotinia (Phialea) temulenta found a distinct seasonal pattern in the atmosphere. The number of ascospores in the air was found proportional to the number of open perithecia in the field, and the maximum infection, to cause blind seed disease of rye grass, was correlated with the high number of ascospores in the air.

Yarwood (66) in 1933 was apparently the first to study the variations in the number of air-borne fungus flora over short periods of the day. By placing adhesive coated slides on the ground for different periods (2-15 hours) of the day, he collected the spores of Erysiphe polygonii D. C. and found that the maximum number of spores present in the air were at about 12:00 noon.

Alvarez and Fossas (3) in Havana, Cuba, made an atmospheric spore study in relation to hourly weather changes for six continuous days. At an hourly interval they exposed nutrient plates and kept an hourly record of the temperature, relative humidity, wind velocity and direction and the barometeric pressure through the period. They obtained higher numbers of fungi from 10:00 A.M. to 6:00 P.M., which they found directly correlated to high wind velocity, high barometeric pressure and high temperature. The fungi found in order of frequency were Hormodendrum (Cladosporium), Mucor, Penicillium and Alternaria. Later, in 1952 Alvarez and Castro (2) by repeating the similar experiment but minimizing the effect of wind, obtained just the opposite results i.e. low number of fungi during the day and high during the night.

Myers (37) in Honolulu, Hawaii, by eliminating the effect of wind, obtained similar results to those of Alvarez and Castro (2), although he found very low numbers of fungi during the night.

Gregory (23) by employing a Cascade Impacter sampled the air for six continuous, four-hour periods during 24 hours and found a distinct variation in the number of various air-microflora over short periods of the day. He also found rust uredospores most abundant from 1:00 to 5:00 P.M. An interesting effect of rain upon the number of Cladosporium spores (24) was noticed when during rain the spore count rose from 50,000 to 100,000/cu.m. within 15 minutes. The rise in the number was thought to be possibly associated with the upward movement of a spore rich layer from near the surface due to the first showers of rain.

Hirst (29) in 1952 in England designed an Automatic Volumetric Spore Trap which could sample the air continuously for 24 hours, giving a deposit of 48 x 14 mm. on an adhesive (vaseline) coated slide. The main parts of the trap were (1) an impactor unit, (2) Wind Vane, which kept the orifice into the wind stream, and (3) a Vacuum pump, with a suction rate of 10 L./minute, fitted in a separate housing and attached to the impactor unit through a rubber tube. By applying this apparatus Gregory and Hirst (25) demonstrated a distinct variation in the number of various types of basidiospores and Cladosporium spores both diurnally as well as due to rain. They found basidiospores mostly during early hours of the morning and a few during noon hours. Also, they encountered a high number of basidiospores in the atmosphere shortly after rain, which after five-six hours were replaced by Cladosporium.

Cammack (8) in 1953 in Nigeria by employing Hirst's Automatic Volumetric Spore Trap demonstrated a distinct hourly variation for Puccinia polysora Underw., Nigrospora sphaerica (Sacc.) Mason and Cladosporium spp. In general he found all these spores showing peaks at noon, during both wet and dry seasons, but a few hours earlier during the dry season which he attributes to the low humidity and high temperature in the dry season.

Pady and Kelly (52) in 1953 during a series of aerobiological studies in the Arctic found a distinct variation in the number of fungi and bacteria over short periods of the day. They found some evidence of higher numbers of fungi during 2:00 to 4:00 P.M. on certain days, although considerable hourly fluctuations were observed from day to day; and various air masses were considered to be one of the factors responsible for this variation (50).

Panzer et al. (54) in 1957 designed a new 24 hour Slide Spore Collector; the main features of which were the deposit of fungus spores and other organisms on a greater area (2.5 cm.) as compared to the dense deposit of other volumetric spore traps. They however, employed 24 slides, one slide for each hour of the day and a long (12.5 cm.) suction tube, with an orifice of 6.25 mm. (The internal end of the tube was kept at a distance of 3.1 mm. from the exposed surface of the slide.) By applying this apparatus they demonstrated diurnal variation for various air-borne fungi. Alternaria and Piricularia oryzae were found to be day and night flora respectively.

Pady (42) in 1957 designed a New Slit Type Continuous Spore Sampler, which could sample the air for 24 hours continuously in the form of 24 distinct bands (14 x 2 mm.) on one three x one inch microscopic slide kept at a distance of two mm. from the orifice. The main features of the sampler were the direct attachment of a pump (Dayton blower) to the sampling column and an electrically controlled timing device which moved the slide a little distance after each hour. The orifice (14 x 2 mm.) was always kept into the wind stream by the help of a wind vane, and was always protected from rain by a metal rain shield.

MATERIALS AND METHODS

Silicone Slides

Samples were obtained by exposing adhesive coated slides in the New Slit Type Continuous Spore Sampler (42) mounted on the roof of Willard Hall (Kansas State College Campus), approximately 150 feet above ground. The slides for exposure were prepared by coating a thin film of already tested (51) silicone grease (DC-4) on 5/6 portion of the microscopic slide; the noncoated part of the slide contained the serial number.

The slides were changed daily between 8:30 and 10:00 A.M. except Sundays. It was set in the machine about one cm. past the orifice in such a way that the coated surface remained towards the aperture. The first and the last bands were not complete because of the changing process and owing to this, data between 9:00 and 10:00 A.M. are likely to be less accurate.

Examination of Bands. Each band was scanned along its long axis, under low power of the microscope (10x) covering 98 percent of the band area. For counting different types of spores a hand operated counter was used. With that five different types of spores could be read simultaneously. Because of the length of time required to read a single slide only those slides were studied which had the complete and uniform bands. The fungi studied were *Alternaria*, *Helminthosporium*, *Cercospora*, leaf rusts (*Puccinia recondita* Rob. ex. Desm., *P. striiformis* West., and *P. coronata* Cda.), stem rust (*P. graminis* Pers.), long hyaline and brown ascospores and fragments of hyphae.

Numbers of spores and the hyphal fragments were estimated on a cubic foot basis, which was calculated by the following formula:

$$\frac{\text{No. of spores per band}}{\text{Time of exposure, (60 min.) x Flow rate, (0.4/cu.ft./min.)}}$$

<u>Detailed Analysis of One Slide Under High Power</u>. In order to find out a suitable technic and to study spores other than those previously mentioned, one slide, number 50B, which already had been studied under low power, was selected for detailed study under high power of the microscope. A drop of lactophenol, cotton blue and a cover slip were added to the portion of the slide to be studied. The cover slip covered two to three bands at one time. Each band was studied at 10 places which covered about a 22.8 percent area of the band. Numbers of spores were estimated as described by Pady (41).

Viability Studies

Viability studies of fungus spores and hyphal fragments were conducted by exposing sterile microscope slides (3 x 1 inch) containing a thick layer of two percent water agar, in the Pady-Rittis sampler (41) and observing the germination directly under the microscope. The following material was employed in the process of making agar slides.

1. Water agar (two percent)
2. Microscope slides (3 x 1 inch)
3. Glass tubing (nine inches in length)

Slides were put in petri dishes and sterilized in the oven for an hour at 160°C., agar in the autoclave and glass tubing by flaming just before use.

About three ml. of melted sterilized water agar, taken in a glass tube, were poured slowly into the center of the slide, which was kept in the petri dish. After pouring, the agar covered about 3/4 of the slide. Three slides were made at a time and after this process, were stored in the refrigerator until their use. The slides were exposed between 8:00 and 10:30 A.M. daily from one to three minutes. Each slide was incubated at 73°F. for about 24 hours, after which lacto-phenol with cotton blue and a cover slip were added to the slide. Viable and nonviable units were recorded by observing germination under low (10x) and sometimes under high power (44x) of the microscope. Most of the recognizable spores were recorded, however, spores under five u were omitted.

EXPERIMENTAL RESULTS

Hourly data of the number of various air-borne fungus flora were recorded on 58 days from May through September, 1957. Some of the data are presented on a daily basis for several days, to determine whether there was a diurnal pattern of the various types of spores on different days. Part of this data is presented for each of 24 hours on a monthly average basis, to find out if there exists any definite kind of diurnal cycle for the various air-borne fungus flora.

Fungi Imperfecti

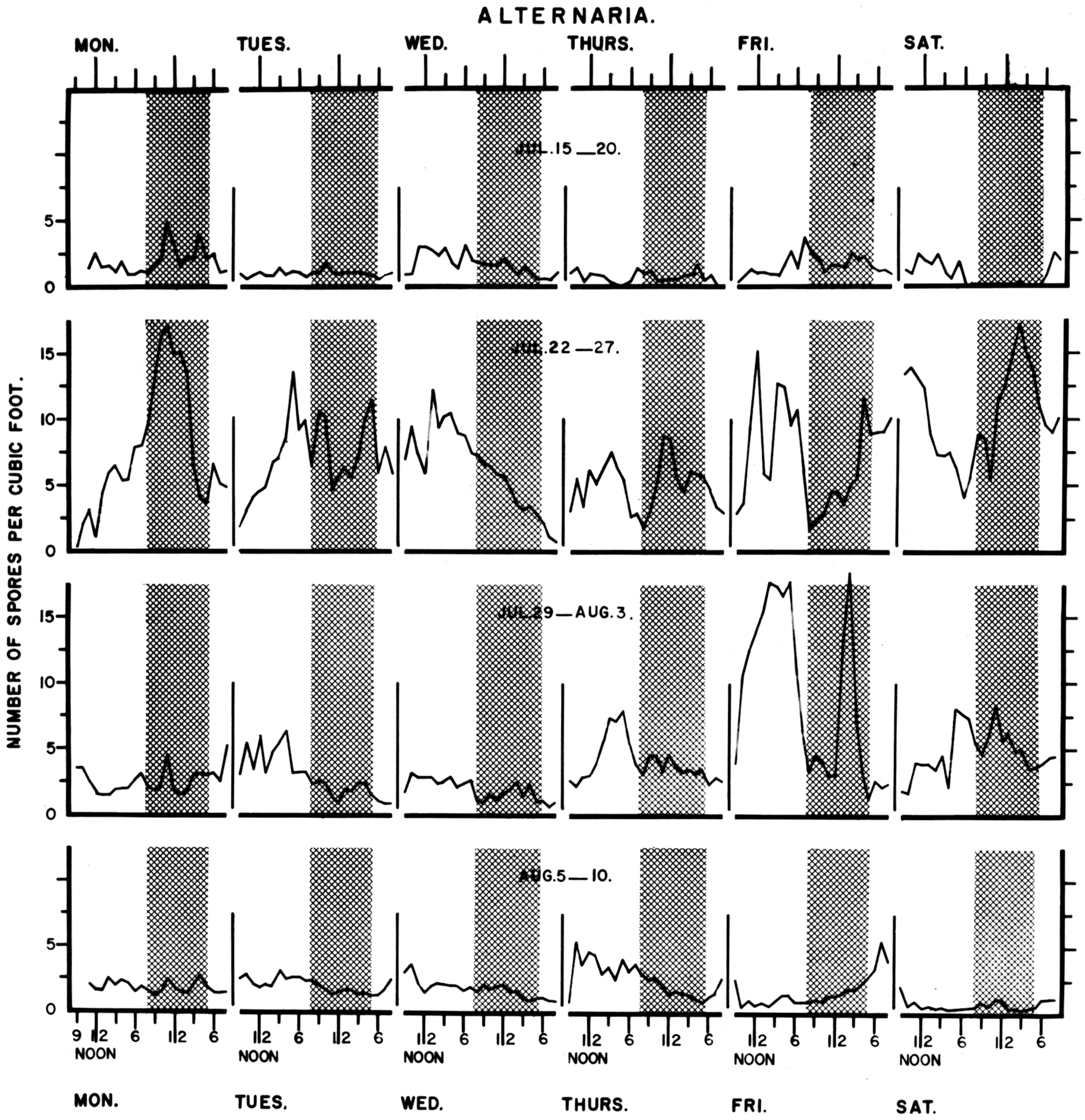
Alternaria. These spores mostly included club shaped, dark, muriform conidia. Alternaria and Stemphylium look very much alike under low power of the microscope and it is very likely that a few spores of Stemphylium, which are present in the atmosphere in small numbers, may be included in this type.

Hourly data on a daily basis are presented from July 15 to August 10, while on a monthly average basis are presented for all the months from May through September, 1957. Hourly studies indicate a distinct fluctuation during 24 hours of the day (PLATE I). No two days were found showing a similar pattern, throughout the period of this study. Sometimes the maximum number of spores was recorded during the day, sometimes during the night, some days showed two or more peaks while on certain days no definite pattern was noticed.

EXPLANATION OF PLATE I

Hourly variations in the number of *Alternaria* spores, July 15 through August 10, 1957.

PLATE I


ALTERNARIA.
MON.
TUES.
WED.
THURS.
FRI.
SAT.
JUL. 15 — 20.
JUL. 22 — 27.
JUL. 29 — AUG. 3.
AUG. 5 — 10.
NUMBER OF SPORES PER CUBIC FOOT.
0
5
10
15
9
12
6
NOON

One distinct peak during the daylight hours was recorded on July 24 and on August 1, 2, 6, 8, and 9, while a peak during the night was recorded on July 22 only. Two or more peaks both during day and night were observed on July 15, 16, 17, 23, 25, 27, 29, 30 and on August 2, and 3. No distinct pattern was observed on July 20, 31 or August 5, 6, 7, and 10.

At 6:00 P.M. on Saturday, July 20, a sudden decline of spores to 0.04/cu. ft. was recorded following 0.87 inch of rain between 5:00 and 6:00 P.M. The count remained low until the next day at 7:00 A.M., when it rose to 2.5/cu.ft. Following the rain on Saturday there was evidence of further rise in the number of spores in the atmosphere showing a peak of 17 spores/cu.ft. on Monday at midnight. In general the rest of the days during that week (July 22-27) showed high concentration of <u>Alternaria</u> spores with distinct peaks varying at various times of the day. The number of spores during the next two weeks remained low except on Friday, August 2, when two distinct and similar peaks with high counts (18 spores/cu.ft.) were recorded at 2:00 A.M. and 2:00 P.M. respectively.

While daily data do not show any definite pattern, monthly averages for May, June, July and September suggest a distinct diurnal pattern with a peak at 2:00 to 4:00 P.M. and a small one at midnight (PLATE II). In August the pattern, however, was reversed when a peak of 4.9 spores/cu.ft. was recorded during the night at 2:00 A.M. while a smaller peak with a count of 4.5/cu.ft. was recorded at 3:00 P.M.

The effect of rain in washing the spores from the atmosphere was apparent on July 25 at 6:00 P.M.

The present data on a daily basis do not show any consistent pattern, however, on a monthly average basis, there is some suggestion of a consistent pattern.

EXPLANATION OF PLATE II

Hourly variation during 24 hours as obtained on the basis of monthly averages, May through September, 1957.

Fig. 1. *Alternaria* spores.

Fig. 2. Hyphae (undifferentiated).

PLATE II

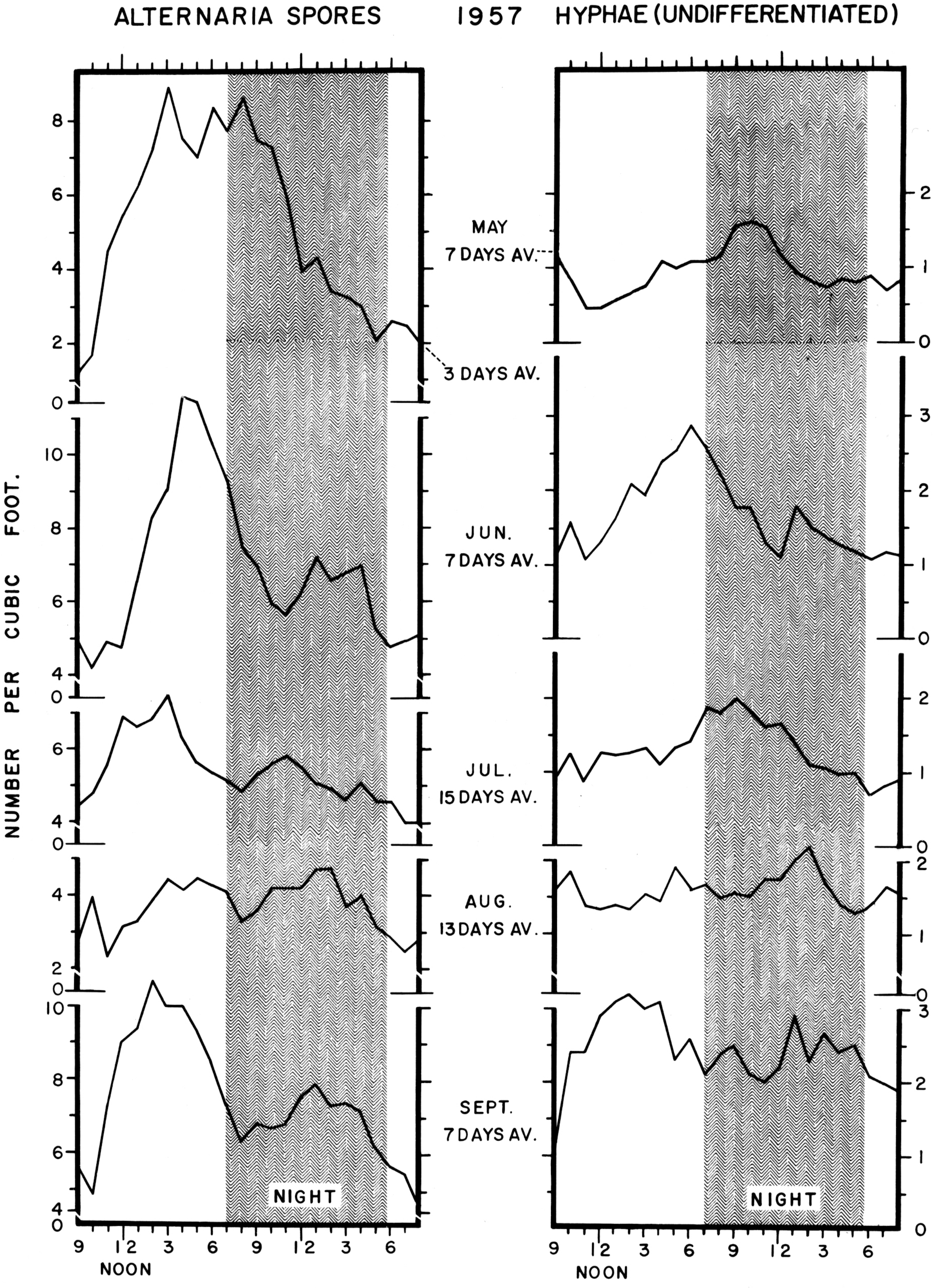


Fig. 1

Fig. 2

Helminthosporium. These spores mostly included long, cylindrical spores with more than four cross walls and dark brown color (21). These were abundant during the month of June, few in number in July and traces in the month of August.

Helminthosporium does not show any definite pattern on a daily basis. Data of two consecutive periods of three days each from the month of June and July respectively are presented (PLATE III). During the period of June 18–20 there is one distinct peak each day at 5:00 P.M., 3:00 P.M. and 5:00 P.M. respectively, while small peaks are also apparent during the night throughout the period. The second period, July 11–13 does not show any consistent pattern while a peak at 7:00 P.M. is apparent on July 12 with a sudden rise at 9:00 A.M. from 0.35/cu.ft. to 6.9/cu.ft. followed by an abrupt drop to 1/cu. ft. at 10:00 A.M. July 13 shows a small peak at about 10:00 P.M.

Helminthosporium also shows an hourly variation throughout this period of study. June 18–20 shows distinct peaks during the afternoon, while July 11–13 does not show any consistent pattern. Average of 11 slides obtained during these two months shows a distinct peak at 3:00 P.M. A sudden rise to 6.9 spores/cu.ft. at 9:00 A.M. on July 13 and an immediate drop to a low of 1 spore/cu.ft. suggests that a small cloud of *Helminthosporium* spores was passing over this area, which remained in this atmosphere for about an hour.

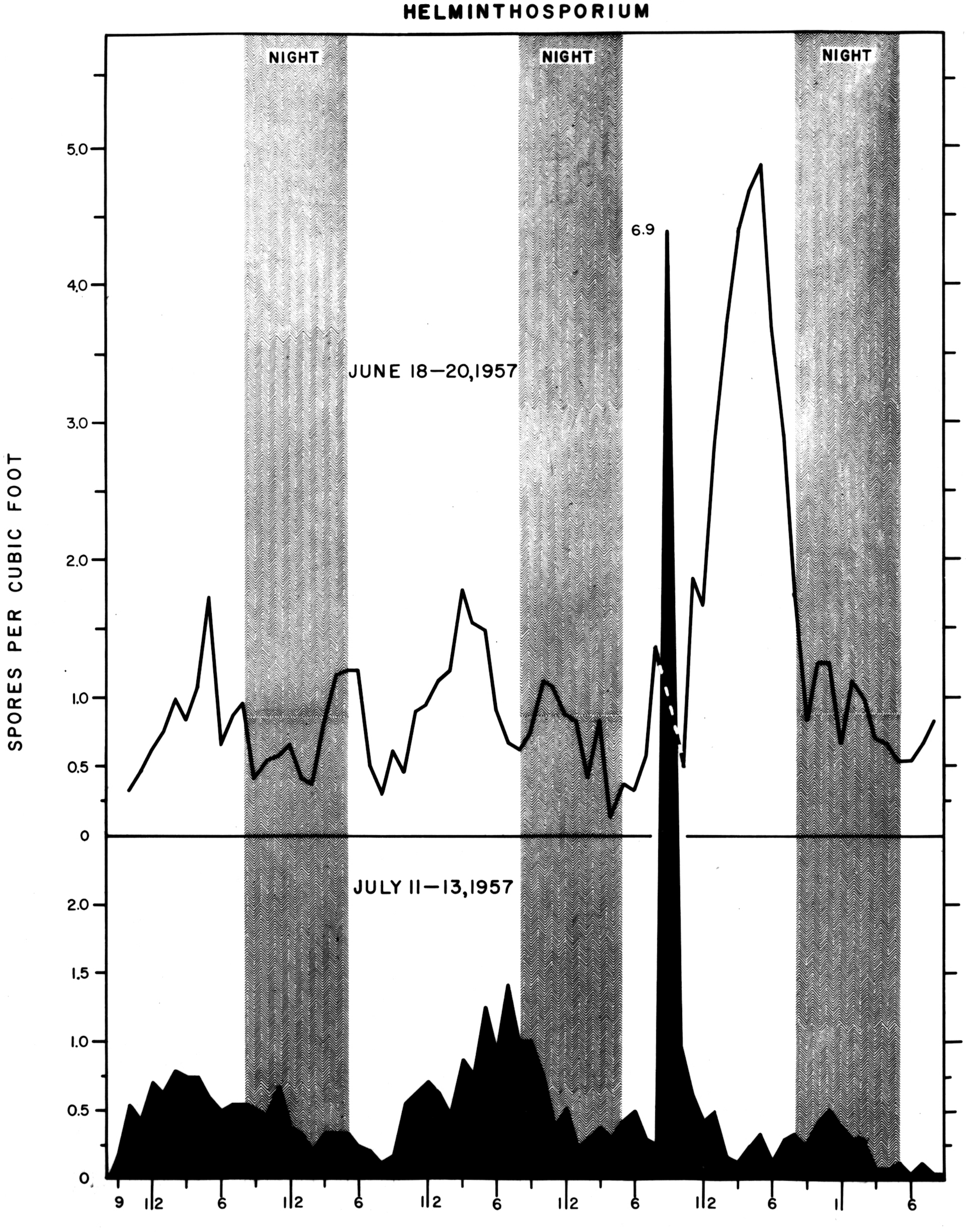
Cercospora. These conidia are characteristically long tapering hyaline, many septate with a distinct scar at the point of attachment at the broad end. They were most abundant during the months of July and August. Daily observations are presented from July 22 through August 3 (PLATE IV) while monthly averages are presented for these two months.

In this case, also, no definite pattern was observed on a daily basis as

EXPLANATION OF PLATE III

Hourly variation in the number of *Helminthosporium* spores, as observed during June 18-20 and July 11-13, 1957.

PLATE III


HELMINTHOSPORIUM
NIGHT
NIGHT
NIGHT
SPORES PER CUBIC FOOT
5.0
4.0
3.0
2.0
1.0
0.5
0
6.9
JUNE 18—20,1957
JULY 11—13,1957
2.0
1.5
1.0
0.5
0
9
12
6
12
6
12
6
12
6
12
6
1
6

EXPLANATION OF PLATE IV

Hourly variations in the numbers of:

Fig. 1. Hyphal fragments — July 15 through August 10, 1957.

Fig. 2. Cercospora spores — July 22 through August 3, 1957.

PLATE IV

HYPHAE (UNDIFFERENTIATED).

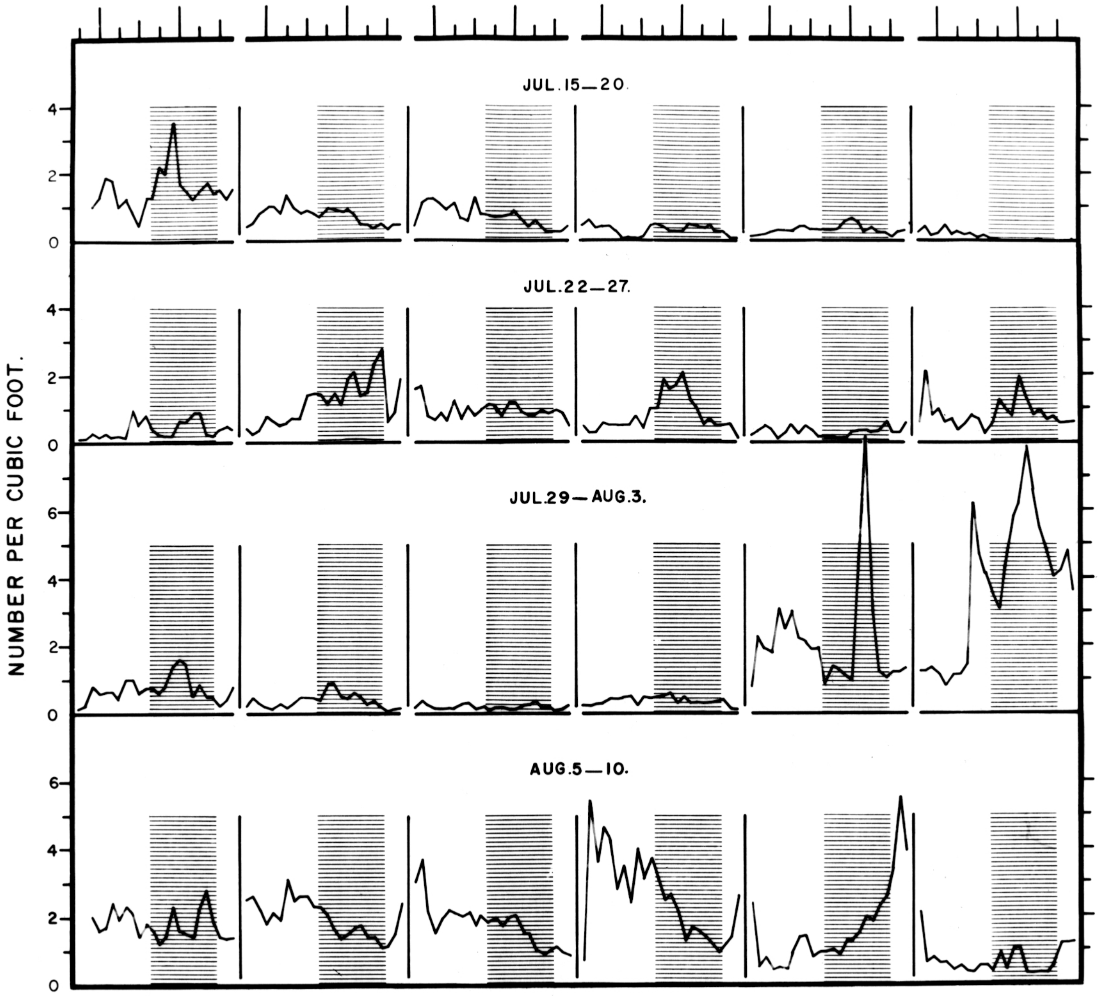


Fig. 1

CERCOSPORA SPORES.

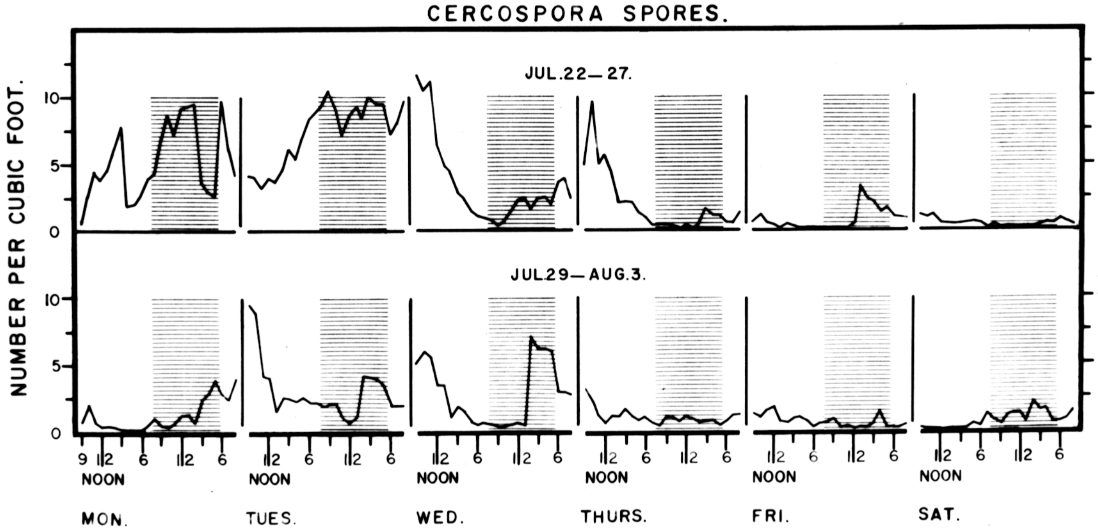


Fig. 2

for Alternaria and Helminthosporium. During these 12 days one distinct peak during light hours was recorded on July 24 and 25. A night peak on July 26 and 29 and two or more peaks were recorded on July 22, 23, 30 and 31, while no pattern was apparent on July 27 and August 1 and 2.

Monthly averages for 10 and 12 days for July and August respectively show some kind of pattern; although no distinct peak at a particular time was observed. In July a high count of spores 3.5/cu.ft. was recorded at 4:00 and 8:00 A.M. while a peak of 3.9 spores/cu.ft. was observed at 10:00 A.M. In August 3.2 spores/cu.ft. were recorded at 2:00 A.M., while a little higher count of 3.5 spores/cu.ft. was noticed at 9:00 A.M. Cercospora also does not show any definite diurnal pattern on a daily basis, while monthly averages suggest higher numbers during night and early morning particularly between 2:00 and 10:00 A.M.

Rusts

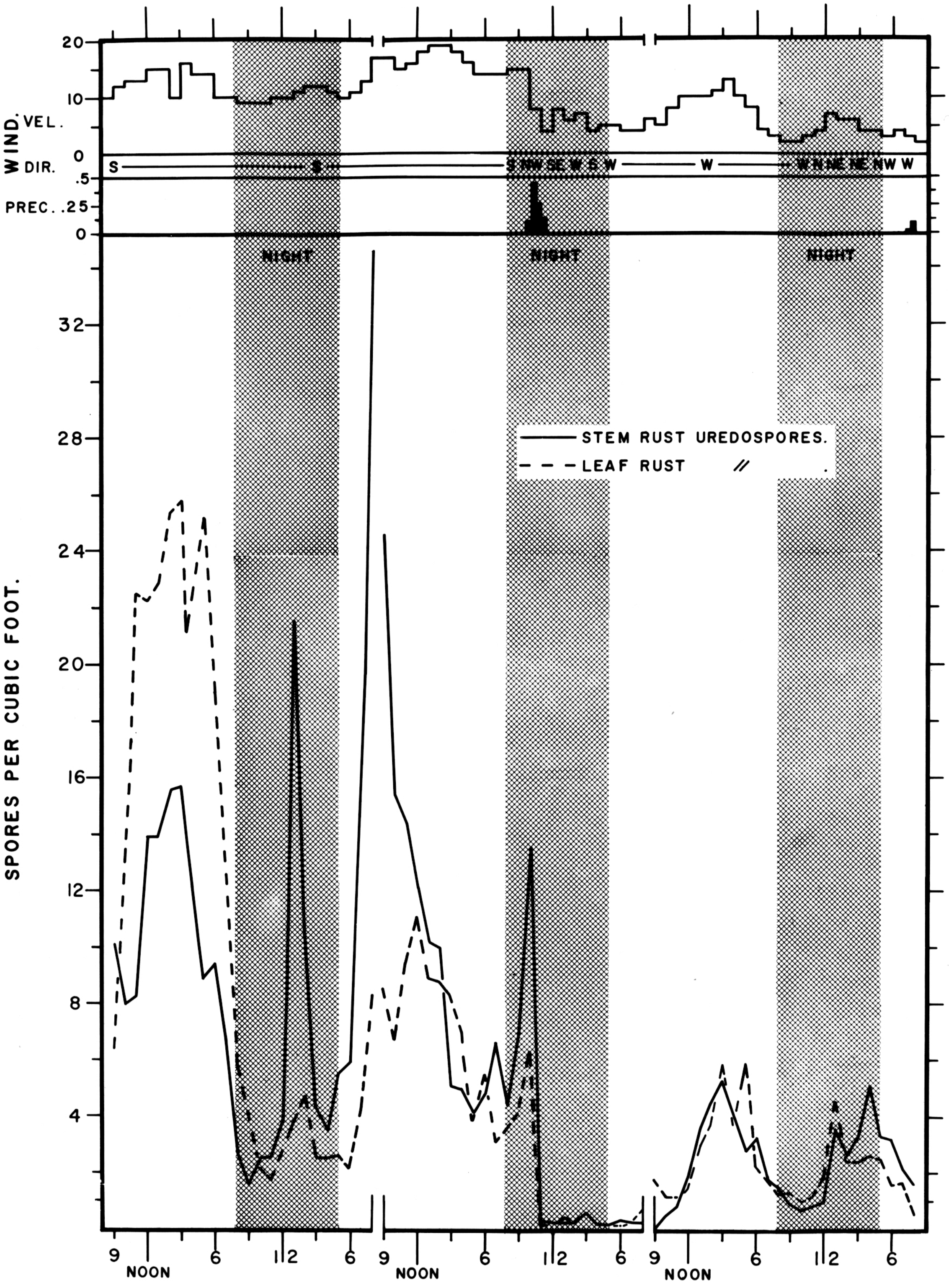
Leaf Rusts. Uredospores of leaf rust could be readily identified under low power of the microscope due to their bright yellow color, size (16-32x12-24 u) and round shape (4). During May the incidence of stripe rust (Puccinia striiformis West.) was also reported on the wheat crop and as the spore morphology of both these rusts is essentially the same (12) it is likely that some spores counted as leaf rust may include some of the stripe rust spores also.

Spores of leaf rust were observed on 27 slides during May 24 through July 17. Of the 27 days studied three consecutive days are selected for illustration (PLATE V). June 20 to 23 shows a higher concentration of spores in the air during light hours with the peaks of 26, 11 and 6 spores/cu.ft. each

EXPLANATION OF PLATE V

Hourly variations in the number of rust spores in relation to various meteorological factors between June 20 and June 23, 1957.

PLATE V



JUNE 20—23, 1957.

day. Correspondingly, the peaks during the night hours were represented by five, six and five spores/cu.ft. respectively. Further it was noticed that high concentrations of spores in the air were directly correlated with higher wind velocity throughout this period, except on June 21 at 11:00 A.M. when a decline in the spore count, 0.04/cu.ft. was recorded coinciding with 0.04 inch of rain, which was probably responsible for the sudden fall in the spore count. The spore count which remained low (0.04/cu.ft.) throughout the rest of the night, began to rise the next day and showed two distinct peaks of 5/cu.ft. each at 3:00 and 5:00 P.M. respectively.

It is also of interest to note that when the spore count was high on June 21 and 22, the wind was from the south. On June 22, the wind was W, NW or NE, and the spore count was recorded low.

Maximum numbers of spores were observed on June 6 and 7 when about 41 and 55 spores/cu.ft. were recorded at 1:00 A.M. and 5:00 P.M. respectively, while on several days the count was found to be almost zero. Wind velocity and direction were found to have a profound effect. High winds with a southern direction probably carry more spores.

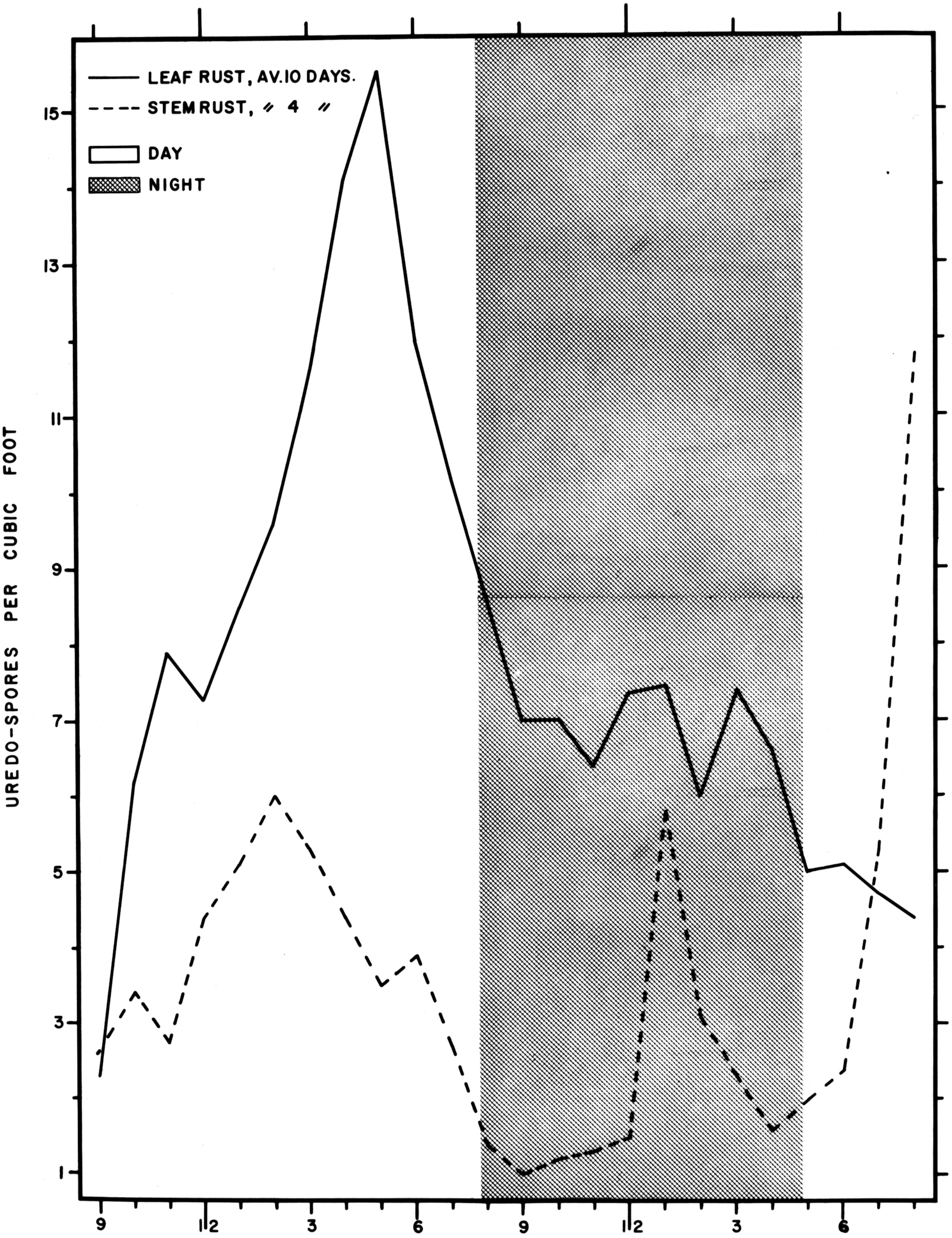
The average of 10 days shows a distinct peak of about 16 spores/cu.ft. and then two small peaks at night, at about 12:00 o'clock and 3:00 A.M. with almost an equal spore count. A distinct diurnal pattern is apparent in the case of leaf rust spores, with a prominent peak at 5:00 P.M. (PLATE VI).

Stem Rust. These spores were identified on the basis of their yellowish color, size (21-42x13-24 u), oblong shape, and golden brown wall, and the distinct echinulations at each end (4). These were observed on 22 slides from June 10 through July 22, and the data here presented are of three consecutive days, June 20-23 as an illustration (PLATE V). Stem rust was found to follow

EXPLANATION OF PLATE VI

Diurnal pattern of leaf and stem rust spores as obtained from the averages of 10 and 4 days respectively.

PLATE VI


LEAF RUST, AV. 10 DAYS.
STEM RUST, " 4 "
DAY
NIGHT
UREDO-SPORES PER CUBIC FOOT
15
13
11
9
7
5
3
1
9
12
3
6
9
12
3
6

a similar pattern to that for leaf rust except that there were additional peaks during night hours. On June 20 a small, although distinct peak, 16/cu. ft., was observed at 4:00 P.M. followed by a higher peak, 21/cu.ft. at 2:00 A.M. and a still higher, 34/cu.ft. at 8:00 A.M. The next day on June 21, the pattern was almost similar to leaf rust, but with a higher concentration in the atmosphere. On June 22, the pattern was again similar and the number of spores was almost the same, also.

Winds were found to have a direct correlation with the number of spores in the atmosphere. Effect of rain in reducing the number of spores in the atmosphere was apparent on June 21 at 11:00 P.M. Maximum number of spores was observed on June 21, which amounted to 34/cu.ft. at 8:00 A.M. while a low count up to zero was recorded on several days.

Four days average during this period of study show three distinct peaks (PLATE VI), one peak, (6/cu.ft.) at 2:00 P.M. followed by a similar peak at 1:00 A.M. and then a still higher peak of 12/cu.ft. at 8:00 A.M. While the average data are based upon only four days, it may not be representative and still more data are needed to form any conclusion.

Stem rust on a daily basis showed a distinct pattern during this period of study, with the high mostly during the day time.

Wind velocity and direction appear to have a profound effect upon the number of stem rust spores in the atmosphere. Rainfall was found to wash the spores from the air.

Long Hyaline and Brown Ascospores

This group included readily recognizable hyaline or light brown filiform, multiseptate spores. These are probably ascospores and are difficult to differentiate under low power of the microscope. Owing to their uncertain classification these are grouped together under one heading.

Behavior of the group is characterized by a sudden appearance, lasting a few hours and then an abrupt disappearance from the atmosphere. Their number in the atmosphere is known to be associated with high humidity or rainfall (27). The relevent data for six days are presented in PLATE VII.

On Sunday, May 12, high moisture due to 0.46 inch of rain was recorded at 3:00 to 5:00 A.M. The first slide exposed at 9:00 A.M. on Monday, May 13, showed a high concentration, 2/cu.ft., on the first hour which was recorded declining to the minimum of 0.2/cu.ft. at 4:00 P.M. A maximum of 15.6/cu.ft. was recorded following an onset of a shower of rain at about 7:00 P.M. and again an immediate decline was recorded following that peak. On May 24, 0.15 inch of rain was recorded at 7:00 P.M. and at the same time these spores appeared in the atmosphere rising to a maximum of 4/cu.ft. at 8:00 P.M. and a heavy rain followed by a decline in the ascospores was recorded. A similar pattern was apparent on May 29 with the onset of rain at 10:00 P.M., followed by a peak of 7.5 spores/cu.ft. at 1:00 A.M.

While the previous days, May 13, 24 and 29 are not consecutive, but had rain each day, the data presented from June 11 to 13 is of three continuous days with only high humidity* but no rain. During this 3-day period, a sudden rise in the spore content, with the presence of high moisture and then an abrupt drop following the sunrise was recorded. However, a small appearance of spores on June 12, at noon with no high humidity in the atmosphere was recorded while high humidity on June 13, during light hours was not found

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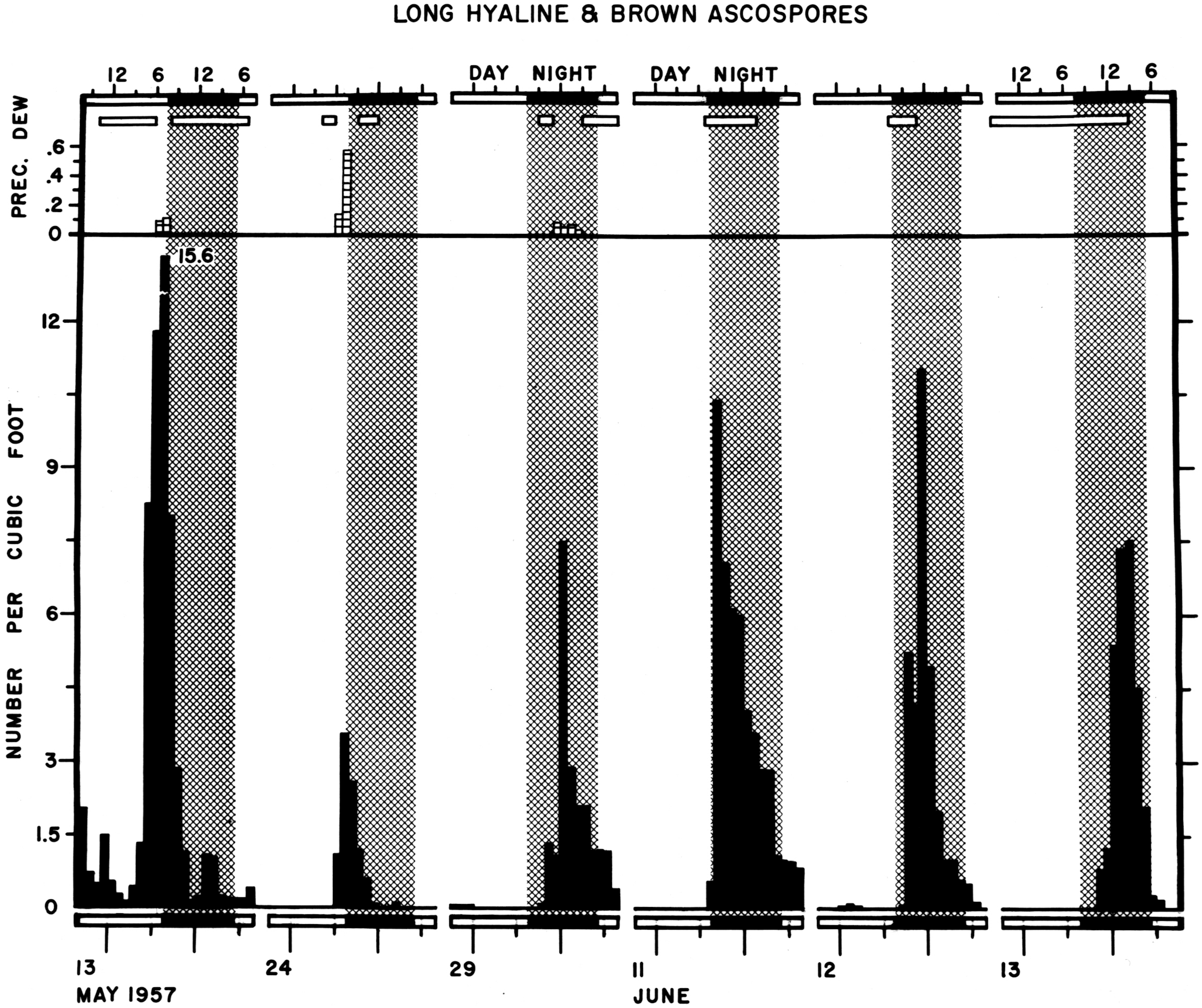
* High humidity in the atmosphere as observed on the 24 hours band slide, in the form of small droplets and shown in PLATE VII as dew. No quantitative data are available.

EXPLANATION OF PLATE VII

Observations on the number of long hyaline and brown ascospores as recorded on particular days.

PLATE VII


LONG HYALINE & BROWN ASCOSPORES
12
6
12
6
DAY
NIGHT
DAY
NIGHT
12
6
12
6
PREC.
DEW
.6
.4
.2
0
15.6
12
9
6
3
1.5
0
NUMBER PER CUBIC FOOT
13
MAY 1957
24
29
11
JUNE
12
13

followed by any appearance of spores in the atmosphere.

These ascospores were mostly recorded following high humidity or rain, which mostly occurred during night, however, on May 13 and 24, these even appeared during light hours when high humidity was recorded during the daytime.

Hyphal Fragments

These included mostly brown to dark brown, thick walled, septate fragments of hyphae, consisting of one to several cells. Conidiophore tips were also common and sometimes a cluster of several conidiophores was also recorded. The fragments mostly represented Alternaria, Cladosporium, Helminthosporium, Penicillium and Aspergillus as judged from their morphologic characters.

Hourly data on a daily basis are presented from July 20 through August 10, and show no consistent pattern throughout the period of study (PLATE IV). One distinct peak during daytime was observed on four days during this period of study and was recorded on July 16 and August 6, 7 and 9. It was recorded at night on seven days, July 15, 19, 23, 25, 29 and 30 and on August 2. Two or more peaks were observed on six days, July 22, 27, August 3, 5, 8 and 10 and no pattern was observed at all on seven days falling on July 17, 18, 20, 24, 26, 31 and August 1.

No hyphal fragments were recorded in the air following 0.87 inch of rain on Saturday, July 20 at 6:00 P.M. and in the ensuing hours the number remained low and fluctuating from hour to hour.

Hourly data on the monthly average basis show some kind of pattern which was recorded during the months May through September, 1957 (PLATE II). In general, the peak during the day was observed during the month of June while the peak during the night was observed in May and July and three and two distinct peaks were observed in the months of August and September respectively.

Comparison of the Studies Conducted by Different Methods

In order to find out a suitable technic for the study of 24 hour band slides a comparative study of one slide both under low and high power magnification was made. It was found that although there were some noticeable differences in the total number of spores under two magnifications, yet the diurnal pattern was found to be similar. It will be seen from Table 1, that for Alternaria the total number of spores counted under two magnifications shows a similar upward trend, reaching the maximum between 10:00 P.M. and 12:00 midnight, followed by a parallel decline touching a minimum at 10:00 A.M. Cercospora spores exibited almost a similar pattern with a high concentration between 10:00 A.M. and 12:00 noon. Diurnal pattern for hyphal fragments obtained under two magnifications was not as uniform as that obtained for Alternaria or Cercospora. Still the counts under two magnifications show a broadly similar pattern.

Keeping in view the economy of time and efficiency, the 24 hour band slide could also be studied once only, along its long axis, under high magnification, that covered about 2.2 percent of the exposed area. To find out its efficiency its comparison with the average of 10 sweeps was made, that covered about 22 percent of the area which should be more accurate as compared to the study of one sweep only.

The results show that the pattern obtained by the single sweep and 10 sweep method were similar only in a very broad manner (Table 2). The curve obtained by the single sweep method ran roughly parallel to that given by 10 sweep method, yet was characterized by a lack of smoothness and presented sudden fluctuations all the way along its length. So much so there was lack of uniformity within any one band, as is clear at 1:00 A.M. (Table 2), that

Table 1. Comparison of the number of spores and hyphal fragments observed on 24 hours slide, 50B, under high power (magnification 440) and low power (mag. 100).

B.No.	Time	Alternaria H.P.	Alternaria L.P.	Cercospora H.P.	Cercospora L.P.	Hyphae H.P.	Hyphae L.P.
1	8:30–9	9.5	8.0	3.8	2.0	2.2	0.6
2	10	9.5	7.7	4.3	2.6	2.2	0.5
3	11	17.1	12.4	3.8	3.0	2.9	0.3
4	12	20.3	15.9	3.1	3.1	2.5	0.5
5	1 PM	20.7	17.7	3.2	2.0	2.7	0.8
6	2	27.0	21.9	3.2	2.6	1.1	0.5
7	3	30.2	24.4	3.2	1.5	2.2	0.2
8	4	27.5	17.5	3.2	0.7	2.2	0.6
9	5	21.1	17.5	2.3	0.7	0.4	0.3
10	6	31.5	23.3	2.2	0.6	1.4	0.4
11	7	22.5	22.3	2.3	0.6	1.4	0.4
12	8	30.6	24.8	2.7	0.2	2.2	0.2
13	9	27.2	21.2	3.2	0.25	2.3	0.25
14	10	21.4	20.2	2.3	0.1	2.2	0.4
15	11	16.2	16.5	2.0	0.25	3.6	0.7
16	12	12.2	10.4	0.7	0.25	2.0	0.4
17	1 AM	15.7	11.5	1*	0.2	3.6	0.7
18	2	11.0	8.3	2.0	0.2	2.9	0.6
19	3	11.3	7.5	2.5	0.25	1.6	0.3
20	4	9.0	7.1	0.9	0.5	2.0	0.5
21	5	4.3	4.1	1.3	0.7	1.1	0.4
22	6	5.8	5.5	1.6	0.5	1*	0.4
23	7	5.8	5.1	1.1	0.3	——	0.2
24	8	4.7	3.0	1.1	0.2	0.5	0.1
25	9	5.6	3.5	0.5	0.5	0.9	0.3
26	10	2.8	1.5	2*	0.3	2*	0.25

H.P. – high power
L.P. – low power
* – actual number of spores observed.

Table 2. **Comparison of the results of a single sweep* to the average of 10 sweeps* as obtained by the study of one slide, number 50B.**

B.No.	Time	1 Sweep Total/cu.ft.	Average 10 Sweeps/cu.ft.	Difference
1	8:30-9	311.4	275.4	+36.0
2	10	392.4	340.6	+51.8
3	11	432.0	351.2	+81.8
4	12	327.6	351.5	-23.9
5	1 PM	246.6	250.9	- 4.3
6	2	253.8	205.7	+48.1
7	3	153.0	137.5	+15.5
8	4	176.4	163.6	+12.8
9	5	149.4	124.6	+24.8
10	6	113.4	109.8	+ 3.6
11	7	120.6	126.8	- 6.2
12	8	91.8	234.4	-142.6
13	9	154.8	130.3	+24.5
14	10	124.2	97.4	+26.8
15	11	136.8	134.6	+ 1.8
16	12	91.8	115.2	-24.4
17	1 AM	**	146.2	
18	2	201.6	198.5	+ 3.1
19	3	212.4	211.1	+ 1.3
20	4	183.6	194.6	-11.0
21	5	167.4	184.0	-16.6
22	6	235.8	238.1	- 2.3
23	7	176.4	214.9	-38.5
24	8	199.8	194.4	+ 5.4
25	9	140.4	163.4	-23.0
26	10	162.0	164.9	- 2.9

* - Sweep: Area covered under high power (44x) of the microscope parallel to the long axis of the slide.

** - No deposit. Debris in slit caused blockage.

no deposit was found due to blockage in some part of the slit. The overall pattern given by the single sweep method consists of sudden fluctuations from hour to hour and still smaller variations representing nonuniformity within the bands.

It was thus concluded that so far as the observation of Alternaria, Cercospora, hyphal fragments and similar large size spores were concerned, counts under low power were satisfactory both as regarding the accuracy and time economy. Moreover, the pattern of deposit on the slide, which was in the form of distinct bands (PLATE VIII) allowed study of each band separately, even under low power, which is probably not possible for the slides having a continuous deposit.

While conducting the studies under high power fifteen additional types of spores and a composite group of pollen grains were recorded (Table 3). These included pollen grains, Cladosporium, colored basidiospores, Fusarium, smuts, Stemphylium, Septoria, Sporormia the spores which were like Septonema, Leptosphaeria and Pleospora, some unidentifiable one and two celled hyaline and one and two celled dark spores.

Maximum number of total spores was observed at 12:00 noon. Pollen grains, Cladosporium, and Fusarium also showed a distinct peak during the day at 12:00 o'clock while smuts were observed maximum at 3:00 P.M. Stemphylium was also found in some number, which showed high number at 11:00 P.M.

Colored basidiospores and one celled hyaline (Sporobolomyces) spores showed peaks at 3:00 and 10:00 A.M. respectively.

Viability Studies

These studies were conducted in order to find out the percentage of

EXPLANATION OF PLATE VIII

Fig. 1. Photograph of 24 hours band slide (exposed 10:00 A.M., July 3, 1957, left hand side to 10:00 A.M. July 4, 1957).

Fig. 2. Photomicrograph of two different fields under high power (44x) of slide 50B, exposed on May 1, 1957

Fig. 3. Photograph of Agar slide, exposed for 3 minutes in Pady-Rittis sampler on July 4, 1957.

PLATE VIII

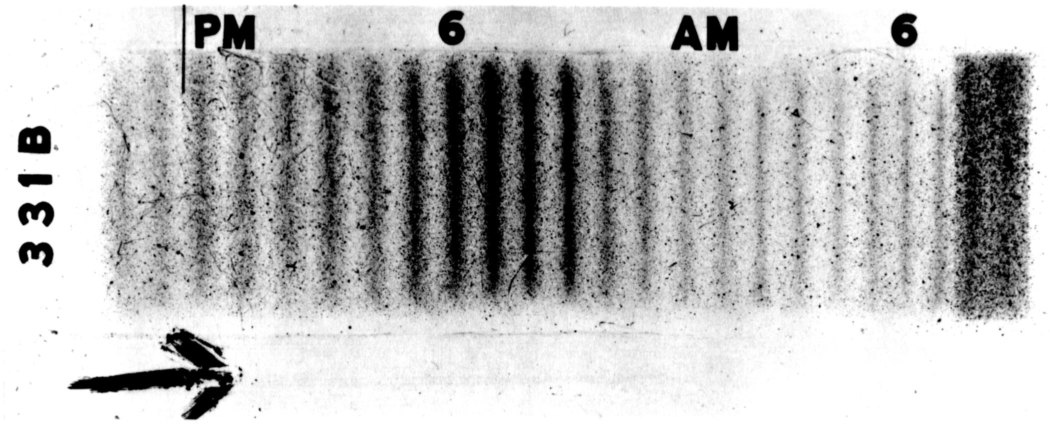
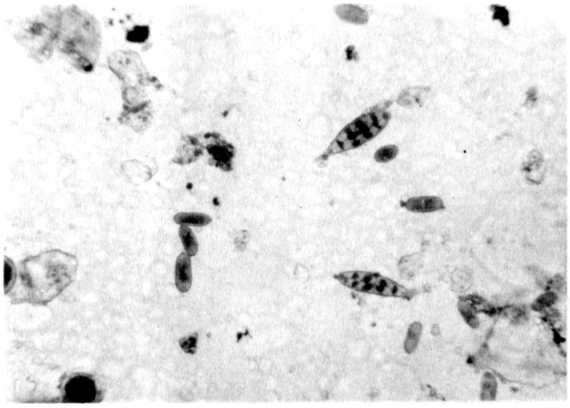


Fig. 1



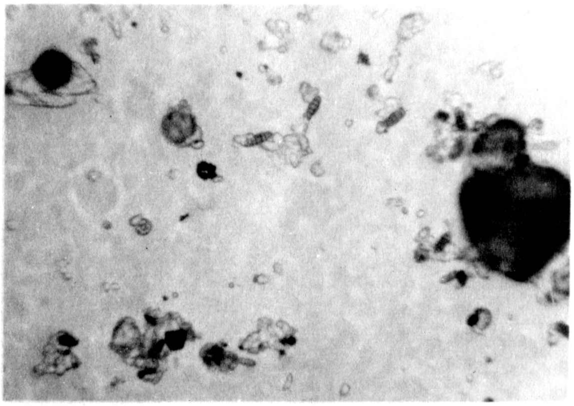


Fig. 2

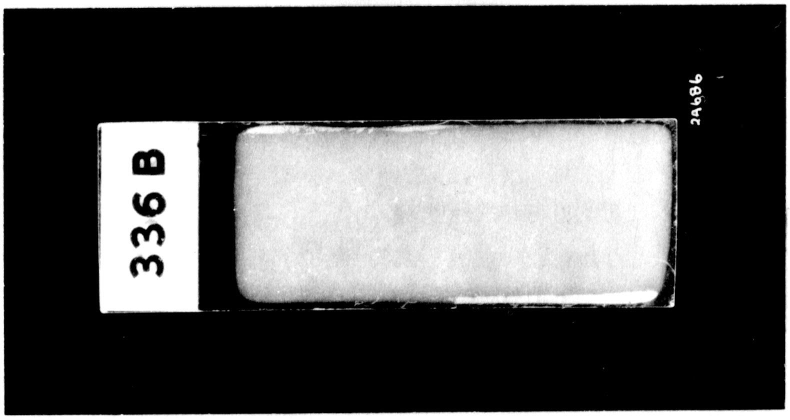


Fig. 3

Table 3. Detailed analysis of 24 hour band slide (50B, exposed May 1, 1957) under high power of the microscope (44x). Data provided on the basis of spores per cu. ft./band.

B.No.	Time	Wind Dir.	Wind Vel.	Temp. °F.	Dust	Pollen	Total	Clad.	Col. Basidiospores	Alt.	Cercos.	Fus.	Smut	Hyph.	Hel.	Stemphyl.	Septor.	Septonema	1.H	2.H	1.D	2.D	Others
1	8:30-9	S	6	68	1	18.1	275.4	196.4	19.1	8.0	2.0	13.3	6.8	0.6			1.1		17.3	6.8			
2	10	NE	6	70	2	26.5	340.6	253.0	19.9	7.7	2.6	10.4	7.6	0.5			1.1		18.2	12.2			
3	11	NE	6	73	2	41.2	351.2	257.4	18.2	12.4	3.0	11.3	7.4	0.3					18.7	9.2	4.9		
4	12	N	6	75	2	88.6	351.5	267.3	11.7	15.9	3.1	12.4	10.6	0.5			0.9		10.4	10.8	3.1		
5	1 PM	E	6	77	2	40.7	250.9	183.2	5.8	17.7	2.0	7.7	8.6	0.8			1.1		8.8	6.5	2.2		
6	2	NE	7	77	2	26.1	205.7	160.6	2.2	21.7	2.6	1.1	6.3	0.5	1.3	1*			2.2	0.9			
7	3	NE	7	78	2	27.1	137.5	97.5	3.8	24.4	1.5	0.4	21.8	0.2						1.3			
8	4	NE	7	77	2	39.8	163.6	120.7	3.6	17.5	0.7	1.6	1.6	0.6	0.9	1*				1.6			
9	5	SE	6	77	2	26.3	124.6	78.8	5.4	17.5	0.7	2.7	5.4	0.3	2*	0.5				3.4			
10	6	S	6	75	2	23.4	109.8	52.0	13.5	23.3	0.6	2.3	5.0	0.4	0.7	1*			0.9	0			
11	7	W	3	73	2	31.3	126.8	77.4	13.9	22.3	0.6	2.5	3.6	0.4	1*				1.4	1*			Spor-
12	8	E	3	70	3	36.4	124.4	72.0	9.4	24.8	0.2	1.1	2.0	0.2	2*	2*		1*	3.4				ormia
13	9	E	2	66	3	41.4	130.3	72.5	13.5	21.2	0.25	2.3	7.2	0.25	1*	0.7		0.5	0.7				1*
14	10	E	1	64	3	41.8	97.4	50.6	12.6	20.2	0.1	3.2	2.3	0.4	1*	0.7		1.3	0.5				
15	11	W	2	64	2	28.1	134.6	61.7	39.2	16.5	0.25	2.5	4.0	0.7		1.1		0.5	2.3				
16	12	W	2	63	2	24.3	115.2	36.7	54.0	10.4	0.25	2.9	2.0	0.4	1*	2*	1*	2*	1.4				
17	1 AM	W	2	63	2	14.2	146.2	44.8	91.1	11.5	0.2	1*	2.5	0.7	0.9	0.7		2*	0.5				
18	2	W	1	62	2	16.2	198.5	49.3	125.1	8.3	0.2	1.6	2.7	0.6	2*	0.7		1*	2.7	1*	2*	1*	
19	3	W	1	61	2	25.0	211.1	37.3	147.2	7.5	0.25	4.0	1.3	0.3	1*	0.5		1.1	4.9		0.7		
20	4	NE	1	61	2**	10.4	194.6	48.3	127.1	7.1	0.5	0.9	0.7	0.5		1*	2*	0.7	4.0		1*		
21	5	W	1	60	2	14.2	184.0	78.1	90.2	4.1	0.7	1.4	1.6	0.4		2*		2*	5.0			0.5	
22	6	W	2	60	2	43.0	238.1	112.7	102.2	5.5	0.5	2.2	1.1	0.4					9.4		0.9		Lepto-
23	7	NE	1	61	2	30.2	214.9	111.2	83.6	5.1	0.3	1.1	1*	0.2				1.1	7.9		1.8		Pleo- sph.
24	8	E	1	62	2	39.4	194.4	114.8	57.4	3.0	0.2	1.8	—	0.1			0.5	1.1	8.3	0.9	2.2	0.5	spora 1.3
25	9	E	2	62	2	13.9	163.4	90.4	24.1	3.5	0.5	6.7	—	0.3			3.8		16.9		0.5	2*	10.4 3.2
26	10	E	4	63	2	10.4	164.9	40.3	20.3	1.5	0.3	8.1	—	0.25			4.5		37.0	13.1	0.7	3.6	26.5 6.8

Legend:
Total – Total spores per band/cu.ft.
Clad. – Cladosporium
Col. Basid. – Colored basidiospores
Alt. – Alternaria
Cercos. – Cercospora
Fus. – Fusarium
Hyph. – Hyphal fragments
* – actual number of spores observed
** – Traces of rain

Hel. – Helminthosporium
Stem. – Stemphylium
Septor. – Septoria
1.H – 1 cell hyaline
2.H – 2 cell hyaline
1.D – 1 cell dark
2.D – 2 cell dark

viable units* in the atmosphere. Data from August 1 to August 15 is presented in Table 4. This period, however, may not be typical of the whole season due to varying atmospheric conditions, but it does show the presence of nonviable spores and viable hyphal fragments in the atmosphere and also the possibility of employing agar slides for observing germination directly under the microscope. Germination of hyphal fragments as found under high power is shown in PLATE IX.

The percent viability of Alternaria spores varied from 0 to 100 percent and was found to be resistant under these environmental conditions. The percent viability varied from day to day. Spores of Helminthosporium were found in small number, and their maximum viability was found up to 43 percent. Smuts were found in large numbers on two days and their maximum percent viability was recorded as 91 percent on August 7. Colored basidiospores were found regularly throughout this period of study, but very few spores were found to be viable. Lowest percent viability was recorded in the case of Cercospora. Only one spore was noticed germinating that was recorded on August 15.

A large number of viable hyphal fragments were also recorded during this period of study. Their viability varied from day to day and ranged from 0 to 80 percent. Some of the germinating hyphae are shown in PLATE IX.

On August 9 it will be seen that no spores or hyphal fragments were viable. From these results it will be seen that there were very few spores or hyphal fragments that could help in dissemination of fungi, on that particular day.

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* Single spore, clump of spores or hyphal fragment, which on germination may produce a single colony, is considered as one unit.

Table 4. Total number of fungal units/cu.ft. (single spore, clump of spores and hyphal fragment, considered as one unit) and their percent germination as obtained from Agar Slides, exposed for two minutes in the Pady-Rittis Sampler at 10:00 A.M. between August 1 and August 15, 1957.

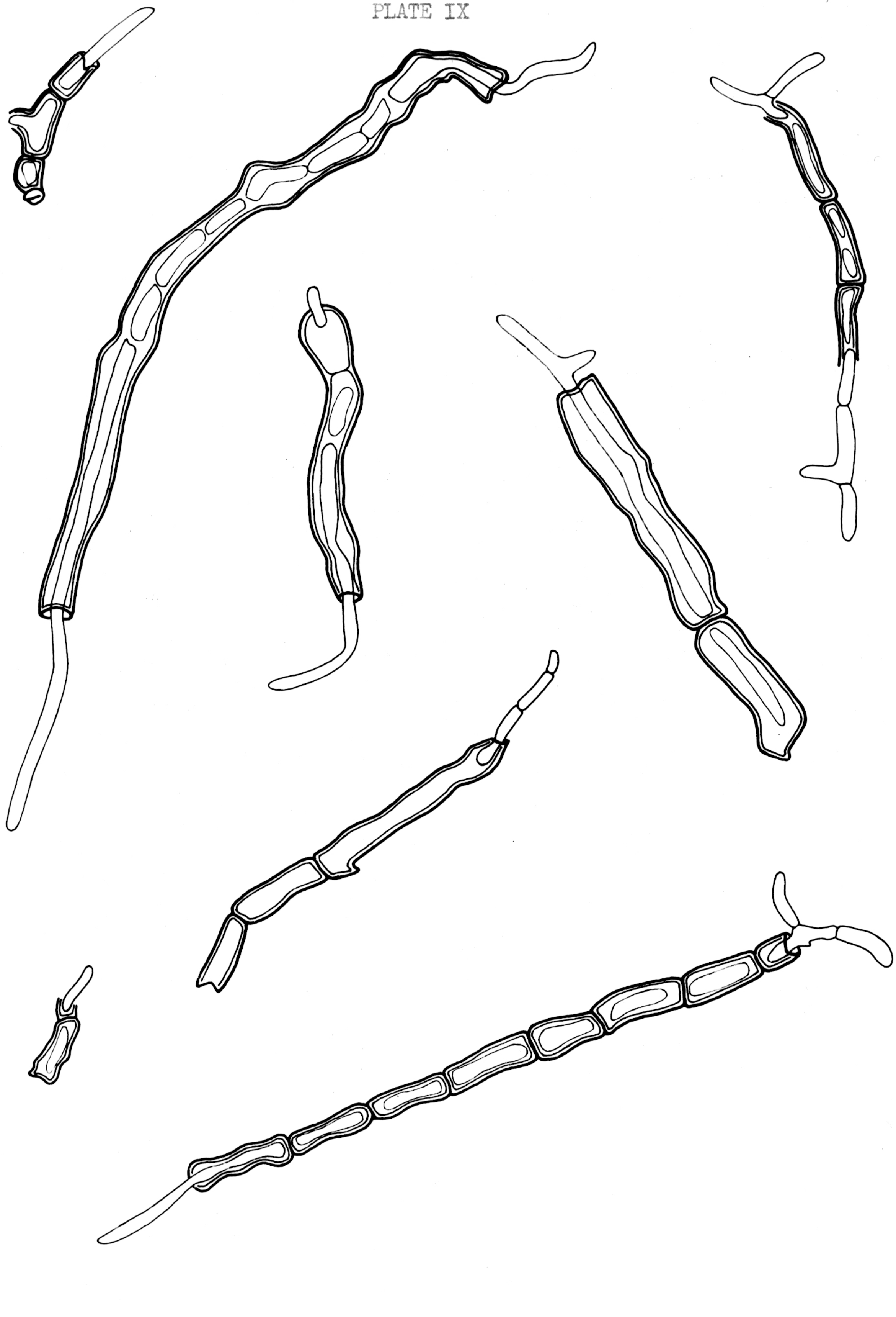
Date	Cladosporium		Smuts		Helminthosporium		Alternaria		Colored Basidiospores		Hyphal Fragments		Cercospora	
	Total	% Germ.	Total	% Germ.	Total	% Germ.	Total	% Germ.	Total	% Germ.	Total	% Germ.	Total	% Germ.
Aug. 1	16.3	11.6	7.7	12.5	2.9	33.3	31.7	84.9	1*	0	9.6	0	3.8	0
2	18.2	63.2	3.8	0	2.9	33.3	29.8	87.0	1*	0	10.7	0	3.8	0
3	28.8	82.2	—	—	—	—	15.4	81.2	2.9	0	8.6	77.7	—	—
5	40.3	26.1	—	—	6.7	43.0	12.5	100.0	4.8	20.0	8.6	33.3	—	—
6	41.3	0.9	4.8	20.0	1*	0	7.6	37.5	8.6	0	8.6	0	1*	0
7	34.6	86.1	22.1	91.3	—	—	14.4	93.3	9.6	10.0	4.8	80.0	1*	0
8	12.5	30.8	11.5	0	3.8	25.0	16.3	41.1	3.8	0	15.4	12.5	2.9	0
9	22.1	0	1*	0	2*	0	6.7	0	2*	0	5.7	0	—	—
10	20.2	9.4	1*	0	—	—	3.8	25.0	2.9	0	3.8	0	2.9	0
12	25.0	7.6	2.9	0	3.8	25.0	8.6	77.7	1*	0	5.7	0	2*	0
13	14.4	46.5	3.8	50.0	2*	2*	9.6	100.0	2*	1*	—	—	—	—
14	17.3	66.4	2*	2*	2*	2*	2.8	100.0	2*	1*	4.8	60.0	—	—
15	4.8	80.0	—	—	2*	2*	7.6	87.5	2.9	66.0	1*	0	2*	1*
Average		76.6		46.5		44.4		37.5		19.6		18.7		6.2

* — Toral spores.

EXPLANATION OF PLATE IX

Hyphal fragments (undifferentiated) showing germination, as observed on Agar slides exposed during August 1957.

PLATE IX



DISCUSSION

With a view to work out an efficient and time saving method of making spore counts, the exposed slides were studied under (a) high power representing 10 sweeps along the length of the slide that covered about 22 percent of the exposed area, (b) high power representing one sweep as in (a) covering 2.2 percent of the area and (c) under low power along the long axis of the band covering about 98 percent of the area. From the data obtained it was concluded that the counting of spores (viz. wheat rusts, Alternaria, and other spores having large diameters) under low power was not only as accurate as that under high power, but it was also found less time consuming and thus all the studies were conducted under low power of the microscope.

These studies show that there were distinct hourly variations both on any one day and from day to day. No two consecutive days were found to have a similar pattern, yet average curves obtained by computing the data for 24 hours (of several days) showed a distinct trend towards a uniform diurnal pattern in certain fungi.

Alternaria showed a distinct hourly variation from day to day throughout the period of study. Sometimes the peak was observed during the daytime, sometimes during the night, and on certain days no pattern at all was observed although most of the days during this period of study showed peaks during afternoon hours. Studies in England (30) show a distinct diurnal pattern for Alternaria with an almost consistent peak during the afternoon hours. These differences may be due to the fairly uniform weather of England, whereas our weather is subject to great fluctuations.

Data based upon two days studies from Maryland, Wis. (54) whowed Alternaria to be a day spora, with the peaks at about 9:00 and 11:00 A.M. which is in agreement with our studies only on certain days eg. August 8 (PLATE I). Monthly averages reduced the meteorological effects to some extent which showed Alternaria to be maximum from 2:00 to 4:00 P.M. throughout the period of study with a small peak during midnight, except that of August, when a little higher peak was observed during midnight as compared to afternoon. A high concentration of Alternaria was recorded during July 22 to 27, after a short spell of dry season followed by rain, the similar rise has been reported from England (31) after similar meteorological conditions.

Considerable difficulty was experienced in distinguishing Stemphylium spores under low power of the microscope due to their gross similarity to those of Alternaria. In view of this fact it is very likely that on 24 hour band slides where Stemphylium was found (also with Alternaria), spore count obtained for Alternaria might have been increased due to inadvertent inclusion of Stemphylium spores.

Helminthosporium was found to follow a pattern similar to Alternaria, although its spore concentration in the atmosphere was much less throughout the period of study. A sudden rise in numbers due probably to a spore shower was observed on July 12 at 9:00 A.M.; that was followed by an immediate decline (PLATE III). The wind directions at 8:00 and 9:00 A.M. were recorded from East to West and at 10:00 A.M. South to North; this suggests that the spore shower which apparently came from the East remained over this area for approximately an hour and most probably changed its direction to North with the shift of the wind. Such occasional sudden wind changes bringing in spore showers have been reported for Cladosporium and Alternaria over Eastern U. S.; of rust spores in Kansas (45), in the Southern part of the Mississippi Valley (16), and from New Zealand (19) for various other types of fungi.

Cercospora constituted one of the major members of the recorded air-borne fungus flora in the months of July and August, and was mostly recorded as night spora, which is in sharp contrast to the diurnal pattern of *Alternaria* and *Helminthosporium*. Previous studies in Manhattan (43) by exposing 30 minute slides once daily between 8:00 and 9:00 A.M. showed very low number of these spores in the atmosphere, that may be due to the fact that during the previous years mostly dry seasons prevailed, whereas the period of this study (1957) was comparatively wet. On a monthly average basis a definite pattern with a high concentration of *Cercospora* spores at 2:00 A.M. to 10:00 A.M. was recorded.

Rusts also showed a distinct hourly variation from day to day. On the basis of ten days average leaf rust showed a definite peak at 5:00 P.M. while stem rust on the basis of four days average showed three distinct peaks at 2:00 P.M., 1:00 A.M. and 8:00 A.M. Due to the low number of days considered in both cases, this may not be actual pattern for these spores. Studies conducted in England (23) show a rust peak from 1:00 to 5:00 P.M. which in the broad sense is in agreement with the present studies.

High wind speeds are suspected to increase the number of air-borne fungus spores and hyphal fragments and this was apparently true in the case of rusts during this period of study. There is a suggestion also that South winds bring more rust spores than the winds from the other directions which is in conformity with the previous studies (44, 45, 46). Although these data provide some information about the pattern of rust spores in the air and to some extent the effect of weather this is too meager to make any definite conclusion, and more data are needed to understand the nature of such an important pattern in the air.

Long hyaline and brown spores for the sake of convenience were classified together. These are classed as ascospores on the basis that various ascomycetous fungi require a stimulus of water to liberate their spores and as these were recorded mostly after rain or heavy dews, these were considered as ascospores. Moreover, lack of scar at the point of attachment confirmed the possibility of classifying them as ascospores. These were particularly numerous following showers of rain or heavy dews (PLATE VIII) and in general during the night hours. As far as the hourly pattern is concerned, these results show that at least for this period of study they were mostly nocturnal. Hirst (30) in England suggests that these are ascospores and are forcibly discharged from mature asci after wetting by water. These have been recorded in a considerable number in the local atmosphere and attempts are being made to identify these spore types by culture methods.

No definite pattern was recorded for hyphal fragments. Peaks were observed at any time throughout the period of study. There is some suggestion of a diurnal pattern on a monthly average basis throughout the months from May through September although no two months were found alike and the peak varied from month to month. Maximum numbers of hyphal fragments were recorded in June and September and low numbers during May, July and August while previous studies showed a high number from June to September. In general, present studies show low numbers of hyphal fragments, as compared to the previous year's (43) which may be due to the difference in season.

Detailed analysis of one 24 hour band slide showed the presence of a composite group of pollen grains and 14 other types of fungus spores in the air. Hirst (30) in England recorded maximum numbers of pollen grains at about 4:00 P.M., while in our case they were at a maximum at about 12:00 noon. He recorded pollen of Dactylis glomerata and various other grasses, whereas in our case coniferous trees were the main source. The difference in the diurnal pattern may be due to the two different types of plants involved or may be due to varying weather conditions.

Under our conditions *Cladosporium* and smuts were recorded at about 12:00 noon and 3:00 P.M. respectively while in England these both have been recorded an hour later, which keeping in view the results of the studies are in considerable agreement.

Diurnal pattern of *Fusarium* and *Fusarium*-like spores has not been previously reported and it is of interest to know that they occurred in high numbers and showed a peak at 12:00 noon like *Cladosporium*.

Our studies show maximum colored and hyaline basidiospores at about 3:00 A.M. and 10:00 A.M. while the maximum number in England (30) has been reported at 2:00 A.M. and 6:00 A.M. respectively.

Considerable work has been done on the ratio of nonviable to viable spores in the air (49). The usual method is to compare the total fungus colonies per cubic foot on the nutrient plate and the total number of spores per cubic foot on the silicone slides. This may lead to the wrong conclusion due to the sampling of different air masses or unsuitable medium or nonsporulation of various fungi having easily recognizable spores on the silicone slides., like *Alternaria* and *Cladosporium*, and also due to the presence of spores like rusts that will not form colonies on nutrient media. The agar slide technic was found suitable as it was possible to see every individual spore and to observe the germination among the spores deposited. Moreover, by employing this technic, it was possible to observe the germination of hyphal fragments, of which it was not known before whether they could be a source of dissemination.

SUMMARY

Results are reported, obtained from the study of 58 slides, exposed in the New Slit Type Continuous Spore Sampler, May through September, 1957. The studies included the spores of Alternaria, Helminthosporium, Cercospora, leaf and stem rusts, long hyaline and brown ascospores and hyphal fragments.

Data obtained show that there was a distinct hourly variation within any one day and from day to day. The pattern of daily variation for any two consecutive days was never found to be similar. However, curves drawn for 24 hours for Alternaria, Helminthosporium, Cercospora, rusts and the hyphal fragments from the average of several days data gave a fairly uniform pattern.

Alternaria on the monthly average basis showed prominent peaks mostly during the daytime from 2:00 to 4:00 P.M., with a small peak at midnight, except in August, when slightly higher peak was observed during the night. Peaks during the daytime on a monthly average basis showed the spore count as 9.0, 12.0, 7.8, 4.5, and 11.2/cu.ft. while the night peaks had the spore count as 4.5, 7.0, 6.0, 4.9 and 8.0/cu.ft., during the months of May through September respectively.

A high number of Cercospora spores was observed from 2:00 A.M. to 10:00 A.M. during both the months of July and August, peaks were observed at 10:00 A.M. and 9:00 A.M. with the number of spores as 3.9 and 3.6/cu.ft. respectively.

Leaf rust on the basis of 10 days average showed a distinct peak at 5:00 P.M. having 16 spores/cu.ft., with two small but similar peaks, each having 7.5 spores/cu.ft. at midnight. Stem rust on the basis of four days average showed a peak at 8:00 A.M. with 12 spores/cu.ft. Two smaller peaks were observed in this case at 2:00 P.M. and 1:00 A.M. each having 6 spores/cu.ft.

Long hyaline and brown ascospores were observed appearing immediately

after rains or heavy dews mostly during night and disappearing suddenly within a few hours. The maximum number of spores, 15.6/cu.ft. was recorded on May 13 after about 0.2 inch of rain.

Hyphal fragments on a monthly basis showed peaks at 10:00 P.M., 5:00 P.M. and 9:00 P.M. during the months of May, June and July, with the count of 1.75, 3.0 and 2.25/cu.ft. respectively. In August three distinct peaks were apparent, the peak with the maximum number of 2.5/cu.ft., was observed at 2:00 A.M. The month of September showed two distinct peaks, the main peak occurred at 2:00 P.M. with the count of 3.5/cu.ft.

Germination studies show a considerable number of nonviable spores present in the atmosphere. Some hyphal fragments were also found viable in the atmosphere. The total percent viability of different types of fungus spores and hyphal fragments was as follows:

Alternaria	76.6 percent
Smuts	46.5 percent
Helminthosporium	44.4 percent
Cladosporium	37.5 percent
Colored basidiospores	19.6 percent
Hyphal fragments	18.7 percent
Cercospora	6.2 percent.

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STUDIES OF THE FUNGUS CONTENT OF THE AIR

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AN ABSTRACT OF A THESIS

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MASTER OF SCIENCE

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Aerobiological studies conducted in Manhattan during the last few years have shown a distinct daily and seasonal occurrance of various important plant pathogens and allergens in the air. Studies conducted in other parts of the world show that there occurs a distinct hourly variation in the number of various air-borne fungus flora. Present studies were conducted in order to find out whether there exists a similar pattern of hourly variations in the number of various air-borne fungi viz., Alternaria, Helminthosporium, Cercospora, leaf rusts (Puccinia recondita Rob. ex Desm., P. coronata Cda., and P. striiformis West.) and stem rust (P. graminis Pers.), long hyaline and brown ascospores and the hyphal fragments under Kansas conditions.

Fifty-eight slides were studied under low power (10x) of the microscope, obtained by exposing silicone grease coated slides in the New Slit Type Continuous Spore Sampler during the months from May through September, 1957. All the fungi under study showed hourly variations within the day and from day to day. On no two consecutive days was the pattern found alike and no definite pattern could be suggested on the basis of single day data; as a result, monthly averages were made.

Alternaria on the basis of monthly averages showed a consistent pattern, with a peak mostly from 2:00 to 4:00 P.M. except in the month of August when the highest spore count was during the night at 2:00 A.M. Peaks during the daytime showed the spore count as 9.0, 12.0, 7.8, 4.5 and 11.2/cu.ft., while night peaks had a spore count of 4.5, 7.0, 6.0, 4.9 and 8.0/cu.ft., during the months of May through September respectively.

Helminthosporium was mostly found in June, although on a few days it was also recorded in the month of July. Averages of 11 days show a distinct peak at 3:00 P.M. with the spore count of 1.5/cu.ft.

Maximum number of Cercospora spores were recorded in July and August showing peaks at 10:00 A.M. and 9:00 A.M. with a count of 3.9 and 3.6 spores /cu.ft. respectively.

Leaf rust on the basis of 10 days average showed a distinct peak at 5:00 P.M. with 16 spores/cu.ft. and two small but similar peaks at midnight each having 7.5 spores/cu.ft. While stem rust on the basis of four days average showed a peak at 8:00 A.M. with 12 spores/cu.ft. Two smaller and distinct peaks were observed also at 2:00 P.M. and 1:00 A.M. each having six spores/cu.ft.

Long hyaline and brown ascospores were observed appearing immediately after rain or heavy dews and disappearing suddenly after a few hours. The maximum number of spòres, 15.6/cu.ft. was recorded on May 13 after about 0.2 inch of rain.

Hyphal fragments on a monthly average basis showed peaks at 10:00 P.M., 5:00 P.M. and 9:00 P.M. during May, June and July, with the count of 1.75, 3.0 and 2.25/cu.ft. respectively. In August, three distinct peaks were apparent, one peak with a maximum number of 2.5/cu.ft. was observed at 2:00 A.M. The month of September showed two peaks, the main peak occurring at 2:00 P.M. with the count of 3.5/cu.ft.

Observations made on the hourly variations of fungus flora in the air could be correlated to some extent with some of the more obvious meteorological factors like rain and wind. In general rain was found to reduce the number of fungi present in the air, yet the presence of certain other fungi was observed to be directly associated with rain or heavy dews. High winds were usually associated with high spore numbers. Southern winds were probably responsible for increasing the spore load of leaf and stem rust at least on certain days during the period of study.

Germination studies, using agar slides, were made to determine the percentage of viable spores in the air. The viability percentage of different types of fungus spores and hyphal fragments in the air was as follows: *Alternaria* 76.6 percent, smuts 46.5 percent, *Helminthosporium* 44.4 percent, *Cladosporium* 37.5 percent, colored basidiospores 19.6 percent, hyphal fragments 18.7 percent and *Cercospora* 6.2 percent.

In general, present studies indicate a definite hourly variation throughout 24 hours of the day. Sometimes there was a single peak during the day, sometimes at night, occasionally there were two or more peaks and in a few cases many small peaks were observed.