Environmental Factors Influencing Basidiospore Discharge by

Polyporus tomentosus Fr.

by

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ABSTRACT

Basidiospore discharge rates by sporophores of *P. tomentosus* were studied in relation to the influences of light, relative humidity, and temperature in both the field and the laboratory. Discharge rates were reduced abruptly when relative humidity was reduced to approximately 75% under conditions of continuous darkness and constant temperature. Above 75 to 85% relative humidity, discharge rates were not affected by changes in relative humidity. In darkness and high relative humidity, discharge rates were highly correlated with temperature in the range of 35 to 75F. The introduction of light disrupted both these relationships and under natural conditions, the periodicity of discharge rates was not correlated with the periodicities of any of light, relative humidity, or temperature, but appeared, nevertheless, to be exogenous and modified by the interaction of the above three factors. Spore discharge rates were negatively correlated with light intensities above 190 ft-c; below this intensity light had no effect. *P. tomentosus* sporulated well on cloudy days when temperatures were moderate, however, its highest rates of discharge occurred in the evening hours due to rising relative humidity and declining light intensity. Discharge declined in the early morning hours due to falling temperatures. *P. tomentosus* is considered as chiefly a nocturnal sporulator.

Other factors such as sporophore size, growth rate, and moisture content, and soil moisture and nutrient content were examined in relation to basidiospore discharge. None of these factors were related to changes in spore discharge rates provided that all were at a level for basidiospore discharge to occur. Sporophores required at least 180% moisture content to discharge spores at optimal rates. Unexplained variability in spore discharge rates occurred in some sporophores.

An important phenomenon observed during this study was that the developmental rate of the hymenium was not uniform over the whole surface. This was supported by two observations; spores from two sides of the same sporophore were discharged at different rates, and spores from the same area of the hymenium were deposited in different patterns from hour to hour under uniform conditions of light, temperature, and relative humidity.

The University of Manitoba

Environmental Factors Influencing Basidiospore Discharge by *Polyporus tomentosus* Fr.

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INTRODUCTION

Polyporus tomentosus Fr. is a major cause of a stand-opening disease in forests of white spruce (*Picea glauca* (Moench) Voss) in Saskatchewan and Manitoba (Whitney, 1962). This stand-opening disease is characterized by patches of dead or dying trees, some of which have been windthrown, and results in an open area in the usually dense spruce stand. The fungus grows in the roots and butts of the trees and advances slowly upwards causing a severe white-pocket rot. The importance of *P. tomentosus* as a causal agent in the stand-opening disease-complex and the pathogenicity of the organism has been thoroughly studied by Whitney (1962, 1963, 1964, and 1966b).

P. tomentosus produces annual sporophores which first appear near diseased trees in the stand-opening area about the third week of July at Candle Lake, Saskatchewan, where the present studies were undertaken. Sporophores continue to appear until late September or early October, depending upon climatic conditions. In the button stage, the sporophores are tan-colored, but become darker as they expand. A typical healthy, mature sporophore has a tomentose upper surface and a white hymenial surface. Whitney (1962) has suggested that the white color of the mature hymenial surface is probably due to the accumulation of basidiospores which are white in mass. Other characteristics of healthy sporophores include

a pore surface which has uniformly sized pores, a thick white pileus margin, and a high spore discharge rate under suitable environmental conditions. Mature sporophores are usually stipitate and are always attached by mycelial strands to a diseased root; these roots may be up to 15 cm. below the soil surface. The sporophore stipe is normally central, although it may be excentric or lateral when the sporophore grows adjacent to a tree, stump or log. In overmature sporophores, the hymenial surface becomes brown, the pores coalesce, and the sporophore is usually attacked by insects or other micro-organisms.

Sporophores have been observed growing directly from the trunk of a tree, or from exposed roots, and they frequently appear in soil which has been disturbed at least one year earlier. Diseased roots always occur nearby, providing a point from which the sporophores may develop. Patton and Myren (1970) used sporophores as boundary indicators of diseased areas in their studies on the development of root rot caused by P. tomentosus in pine and spruce plantations in Wisconsin. They found that root infections extended no further than three feet beyond the limit of the infected area as delineated by the occurrence of sporophores.

Whitney (1962) gave convincing evidence that root rot caused by P. tomentosus may be spread as a consequence of root contact between healthy and diseased trees. He found that the fungus does not grow in the soil and, from this locus, invade tree roots. Attempting to explain the role of basidiospores in the spread of the disease, he inoculated

basidiospore suspensions into small spruce roots, thereby successfully causing infection (Whitney, 1963). Only wounded roots became infected. The above facts suggest that roots must be exposed and wounded for basidiospore infection to occur or that basidiospores are washed through the soil to the wounded roots by rain. There is also the possibility that mice, squirrels, rabbits or other small animals carry basidiospores and thus serve as vectors of P. tomentosus. The growth habit of this organism at the ground level would be conducive to the various methods of dissemination outlined.

Since basidiospores of P. tomentosus are capable of initiating root infections in wounds (Whitney, 1963), they may be an important factor in the spread of the disease, since it would seem that any long-distance spread of the fungus would require the existence of an air-borne inoculum. Basidiospores of Fomes annosus (Fr.) Cke. have been shown to initiate infection of wounded pine roots and stumps (Rishbeth, 1951; Wallis, 1961) and, while few investigations have been completed, it may be that basidiospores are an important source of inoculum in diseases caused by root-rotting fungi.

This study was undertaken primarily to investigate the influence of relative humidity, temperature, and light on the rate of spore discharge from sporophores of P. tomentosus and, secondarily, to investigate those aspects of sporophore development which may be pertinent to spore discharge. Also, the effects of other factors such as rainfall, soil moisture, soil nutrients, as well as sporophore size, growth, and mois-

ture content, were examined in relation to spore discharge. Little attention has been directed to relationships between sporophore development and spore discharge in Basidiomycetes by other workers. Studies were therefore conducted on sporophore characteristics such as growth rates, developmental stages, and on pecularities of individual sporophores that influence spore discharge. Preliminary investigations indicated that a complex relationship existed and it was necessary to employ both field and laboratory investigations to properly interpret the response of P. tomentosus to its environment. Therefore, spores were collected from sporophores maintained under a variety of environmental conditions in both the field and the laboratory.

LITERATURE REVIEW

Since this study is concerned with three main environmental factors; light, temperature, and relative humidity[1] and their effects on spore discharge, only those references pertaining to this aspect of spore discharge will be discussed. Although the report deals with a Basidiomycete, some literature dealing with other groups of fungi will also be reviewed briefly, since most of the existing information on the influence of environmental factors on spore discharge in fungi deals with non-Basidiomycetes. This review groups classes of fungi, rather than environmental factors since the literature on the Basidiomycetes is considered in detail, while the literature review of the non-Basidiomycetes is only intended to give examples of the response of these other fungi to their environment. A section is not devoted to the relationship between aspects of sporophore development and spore discharge, as there were few references to this topic. Spore samplers and sampling have been considered in a separate section for reasons described in that section.

Spore discharge in non-Basidiomycetes.

Among the Phycomycetes, the few studies which have been reported show that the periodicities of spore discharge in these fungi are exogenous and usually in response to a light

[1]Hereafter, relative humidity will be referred to as R.H.

stimulus.[1] Periodicities of spore discharge in Pilobolus sp. (Buller, 1934; McVickar, 1942), Peronospora tabacina Adam (Hill, 1960), Conidiobolus coronatus (Cost.) Srin. & Thirum. and Basidiobolus ranarum Eidam (Callaghan, 1969) were conditioned by light. Yarwood (1937) reported that spore discharge in the downy mildews was favored by high R.H. and it was this, not darkness, which accounted for their nocturnal pattern of spore discharge.

Spore discharge in the Ascomycetes has been more extensively studied than spore discharge in the Basidiomycetes, and this is likely due to the ease with which many Ascomycetes may be cultured and, consequently, manipulated. Under controlled conditions, diurnal periodicities in response to isolated environmental factors have often been illustrated in the perithecial Ascomycetes.

Light has received the greatest attention as a conditioning factor in the periodicity of spore discharge in the Pyrenomycetes. For example, Podospora curvula de Bary discharged spores with a diurnal periodicity and highest discharge rates occurred during light periods (Ingold, 1928, 1933; Page, 1939). Sordaria fimicola (Rob.) Ces. & de Not. exhibited a periodicity with maximum discharge occurring in the afternoon in response to light (Ingold and Dring, 1957; Ingold, 1965), and observations of spore discharge in Pleurage setosa (Wint.) Kuntze showed that a periodicity existed with maximum dis-

[1] In considering this and other groups of fungi, the reviewer is interested in the general properties of light, so that studies dealing solely with light quality are not considered.

charge occurring in the light periods. However, in continuous darkness this periodicity ceased, indicating the absence of an endogenous rhythm (Callaghan, 1962). Light also stimulated discharge in Sordaria macrospora Auersw. (Walkey and Harvey, 1967), Pilobolus sphaerosporus Palla. (Ingold, 1965), and in Ascobolus sp. (Ingold and Oso, 1969).

Light does not always have a stimulatory effect on spore discharge in the perithecial Ascomycetes and spore discharge in Daldinia concentrica (Bolton ex. Fr.) Ces. & de Not. (Ingold, 1965), Sordaria verruculosa (Jensen) Sacc. and Hypoxylon fuscum (Pers.) Fr. (Ingold and Marshall, 1963), appeared to be suppressed by light since maximum spore discharge rates occurred at night. Bombardia fasciculata Fr. exhibited an endogenous rhythm with highest ascospore discharge occurring in the darkness in both field and laboratory studies (Pady and Kramer, 1969). Hypochrea gelatinosa (Tode ex Fr.) Fr. also showed a diurnal periodicity with peaks in the darkness (Kramer and Pady, 1968). The periodicity was maintained in continuous darkness, but was less pronounced in continuous light. Several other Pyrenomycetes studied by Hodgkiss and Harvey (1969) were also observed to have diurnal rhythms of spore discharge with nocturnal peaks. Ingold (1965) suggested that the nocturnal discharge observed in some Pyrenomycetes might be due to either a direct inhibition of spore discharge by light or its stimulus by light with a long interval between the stimulus and the max-

imum response.

Other factors which have been found to influence spore discharge by various Pyrenomycetes are rainfall and R.H. For example, Ingold (1953, 1957) cites several examples of drought-tolerant Ascomycetes which only commence spore discharge when wetted by rain. Some of these species (e.g. D. concentrica) may discharge spores in dry conditions using moisture stored in the fruiting body. Rainfall and high R.H. stimulated ascospore discharge in Nectria galligena Bres. in the field, and laboratory studies confirmed the correlation with R.H. (Lortie and Kuntz, 1962). In a series of papers by Walkey, Harvey and Hodgkiss (1968a, 1968b, and 1969), rainfall was observed to have the greatest influence on spore discharge rhythms in some Pyrenomycetes, but in their studies, R.H. had no effect on spore discharge under field conditions. Reduced R.H. stimulated spore discharge in S. fimicola (Ingold and Marshall, 1962), a fact confirmed by Austin (1968a) who found that a drop in the R.H. from 95 to 75% led to an immediate, but temporary increase in discharge rate.

Little information is available on the effect of temperature on spore discharge in Ascomycetes, but Ingold (1953) suggests that discharge is probably arrested in most species below 5C. However, Lortie and Kuntz (1963) found that temperature fluctuations had no effect on ascospore discharge in N. galligena in the field and spores were released near the freezing point, although in the laboratory, higher temperatures, up to 20C, stimulated spore release. Spore discharge

in many Pyrenomycetes studied by Hodgkiss and Harvey (1969) were not affected by temperature fluctuations in field studies.

Few studies have been undertaken on the factors influencing ascospore release in Discomycetes. Smerlis (1968) reported that Scleroderris lagerbergii Gremmen was dependent on 100% R.H. for spore release with maximum discharge occurring at 17C. Skilling (1969), studying the same organism, found that rainfall which lasted for more than 4 hours, at temperatures from 17 to 25C, resulted in maximum ascospore release, but Skilling did not note the strong relationship with R.H. that Smerlis reported.

Few studies on spore discharge in the Fungi Imperfecti have been completed; most studies dealing with spore production in culture. Nevertheless, field studies have shown that R.H. and rainfall are important for spore discharge by some members of this group. For example, Meredith (1961a) found the number of spores of Deightoniella torulosa (Syd.) Ellis in the atmosphere was higher following rainfall. High R.H. and rainfall were also favorable for spore release in Pyricularia oryzae Cav. (Barksdale and Asai, 1961) and P. grisea (Cke.) Sacc. (Meredith, 1962). Other studies showed that light has no effect on spore discharge in D. torulosa (Meredith, 1961b), while light was favorable for spore discharge in Cladosporium sp. (Pady, 1969).

Spore discharge in Basidiomycetes.

The experiments reported on by Buller (1909) were among the first extensive studies of basidiospore discharge. Many of his studies dealt with Polyporus squamosus Micheli ex Fr. and he determined the presence of discharged spores in the air around the sporophore by the beam of light method. By this method, a concentrated beam of light passing through a cloud of spores renders them easily visible, and this enabled Buller to establish several relationships which are pertinent to this study, but some of which are herein contradicted. His main premise was that spore discharge was continuous once initiated and, provided the sporophore was sufficiently moist, discharge occurred regardless of atmospheric moisture content and light conditions. He observed that dry air caused a slower rate of spore fall, but did not link this with a slower discharge rate.

Buller's contention that atmospheric moisture conditions do not affect basidiospore discharge rates received little support from later workers. The moisture content of the atmosphere has been shown to be a regulator of spore discharge rates in other classes of fungi, as well as the Basidiomycetes (e.g. Zoberi, 1964). However, in 1958, Parmasto's observations led him to agree with Buller that R.H. does not greatly affect spore discharge from woody fruit bodies of poroid fungi on living trees. In 1953, Ingold, mainly on the basis of Buller's studies, published his agree-

ment with the fact that discharge occurs irrespective of rain or R.H. provided that the water supply to the fruiting body is sufficient. He stressed that this is particularly true among the perennial polypores. McCracken and Toole(1969) observed that variations in spore discharge by Polyporus hispidis (Bull.) Fr. did not appear to be correlated with changes in temperature or R.H.

The literature concerning F. annosus shows that there is disagreement as to the influence of R.H. on spore discharge in this species. Rishbeth (1959) observed that heaviest spore deposition occurred after prolonged rains. He had previously shown (Rishbeth, 1951) that spore discharge decreased during dry weather. Sinclair (1963) observed a definite seasonal pattern of spore production and dispersal. During midsummer, when the temperature was at its highest, spore discharge was lowest. During the spring and early summer, discharge increased with increase in temperature. A diurnal pattern was also observed by Sinclair (1964) in which the lowest rate of deposition occurred during the night and the highest during the afternoon and he felt these fluctuations were correlated with temperature changes and were not influenced by R.H. However, Stambaugh (1962), measuring spore deposition on freshly cut pine stumps, observed that the greatest spore load was deposited at night. He felt that R.H. did act on spore release, low R.H. being most favorable, although this does not agree with his findings of a greater nocturnal spore

load. Rogers (1963) stressed the importance of moisture as an influence on basidiospore discharge and reported that sporophores of F.annosus moistened by rain readily released basidiospores. Schmidt and Wood (1965), studying sporophores maintained in environmental chambers, the greenhouse and in the laboratory, found maximum spore release occurred under conditions of high R.H. and a nocturnal peak was observed in the greenhouse. Wood (1966) confirmed Stambaugh's findings that maximum spore discharge occurred at midnight and the minimum at noon.

The relationship between R.H. and spore discharge has been studied in other Basidiomycetes. For example, Carpenter (1949) observed that high R.H. favored spore discharge in Pellicularia filamentosa (Pat.) Rogers. Degroot (1960) observed that basidiospore discharge in Fomes igniarius (L. ex Fr.) Kickx was positively correlated with R.H. Zoberi (1964) showed that optimum spore discharge rates in Schizophyllum Fr. occurred under conditions of high R.H. and he observed the same behaviour with sporophores of Polyporus brumalis Pers. ex Fr. In the latter species, the rate of spore discharge fell immediately when the R.H. was changed from 96 to 78%. Zoberi (1965) also showed that spore discharge by the agaric, Lepiota conradii Huijsman ex Orton, increased with increased R.H. and the numbers of spores discharged by Cronartium fusiforme Hedgc. & Hunt ex Cumm. were similarly related to R.H. (Snow and Froelich, 1968).

Many Basidiomycetes exhibit a nocturnal pattern of spore discharge which might indicate a preference for high R.H. Ganoderma applanatum (Pers. ex Wallr.) Pat. exhibited maximum spore discharge rates during the period from 2200 to 0400 hours and a minimum rate from 0800 to 1800 hours (Sreeramulu, 1963) and this diurnal periodicity was only disrupted by rain wetting the fruiting body. A nocturnal periodicity was also observed in the rust, Puccinia malvacearum Bert. (Carter and Banyer, 1964), and Exobasidium vexans Massee (Shanmuganathan and Arulpragasam, 1966). Using a Hirst spore-trap, DeGroot (1968) found that a diurnal cycle with nocturnal peaks occurred among the forest fungi he studied. One species studied by DeGroot, Fomes fomentarius (L. ex Fr.) Kickx, showed two peaks; one after sunset and one early in the morning, a situation similar to that reported by Riley (1952) for F. igniarius.

Additional fungi which have recently been reported to have nocturnal spore discharge patterns are: Polyporus schweinitzii Fr. (Moser, 1970); Fomes everhartii (Ell. & Gall.) von Schrenck and Spaulding, P. fissilis Berk. & Vurt., Irpex mollis Fr., and F. marmoratus (Berk. & Curt.) Cooke (McCracken, 1970) and Armillaria mellea (Vahl ex Fr.) Quél (Mistretta, 1970). The predominance of nocturnal patterns is in accordance with air spora studies of Hirst (1957) and the abundance of examples cited leads one to suspect that high R.H. at night has a favorable influence on spore discharge by Basidiomycetes.

Temperature has received little attention as a factor influencing basidiospore discharge rates. Buller (1909) found that Lenzites betulina (L. ex Fr.) Fr. shed spores at 0C. Spores were discharged slowly at 29C, but not at 33C and above. Spore discharge in P. brumalis (Zoberi, 1964) ceased at 25 to 27C, while temperatures below 14.5C prevented or reduced spore discharge in C. fusiforme (Snow and Froelich, 1968). Sinclair (1963) observed a diurnal periodicity in spore discharge in F. annosus which he felt was conditioned by temperature.

Although few studies have been made, Ingold (1953) felt that light exerted little direct influence on spore liberation in the Basidiomycetes. As outlined in this review, non-Basidiomycetes and particularly Ascomycetes, are highly sensitive to light conditioning. Buller's studies of P. squamosus led him to conclude that light did not influence spore discharge. Snell (1922) observed that the spores of Trametes serialis Fr. and Lenzites saepiaria Fr. were discharged both in continuous darkness and continuous light. Spore discharge in P. filamentosa was strongly influenced by light with most spores being discharged during the night from 1800 hours to 0600 hours, while a smaller number were discharged in the daytime, with sunny days suppressing discharge the most (Carpenter, 1949). Light had no effect on spore discharge rates in Zoberi's studies (1964) of S. commune. Shrum and Wood (1968) observed that spore discharge

in Fomes rimosus (Berk.) Cke. was regulated by light; the fungus exhibiting a diurnal periodicity with peaks occurring in the dark hours.

A notable study is that of Gay, Hutchinson, and Taggart (1959) in which they showed that inherent rhythms of spore discharge exist in Trametes gibbosa Fr. with dense spore deposits recurring every 30 to 90 minutes. P. filamentosa also exhibited an endogenous rhythm of spore discharge (Carpenter, 1949). No other examples of inherent periodicities in the Basidiomycetes were reported.

Few studies have been made on spore discharge in P. tomentosus, but Whitney[1] observed that low numbers of spores were discharged from sporophores during hot and dry conditions; i.e. temperatures from 21 to 27C and R.H. from 20 to 50% in the afternoon. Discharge rates were particularly low when the temperature reached 27C or higher. Myren (1969) disclosed a reversible halt in basidiospore discharge at 30C and a permanent cessation at 36C, but spores were cast at all temperature levels from 4 to 26C. Myren also observed the unsuitability of low R.H. for optimum spore discharge and found that 0 and 32% R.H. were insufficient to maintain a long spore-casting period. Hagenmueller (1970) reported a periodicity in spore release of P. tomentosus with maxima in the early afternoon and late evening hours.

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[1]Unpublished data of R.D. Whitney.

Spore sampling and spore samplers.

Since a new sampler is described herein and this was used in the present investigations, and since the author feels that sampling techniques have been an important influence on the conclusions reached by many workers, and has probably misled some, it seems pertinent to briefly review spore sampling and spore samplers.

Results of air spora studies may not be indicative of spore discharge rates, although generalizations concerning the periodicity of spore discharge in particular organisms have been concluded from the examination of atmospheric spore content. Several factors must be considered in choosing a sampling instrument. Such factors are: the size of the spores, the efficiency of the sampling instrument to trap these spores, the force with which the spores are discharged, and the amount of air turbulence. Ingold (1957) suggested that the periodicity observed with a trap such as the Hirst spore-trap, which is normally operated at one meter above the ground, does not necessarily reflect a corresponding rhythm in spore liberation. Nevertheless, a study giving evidence that the sampling technique may not be important should be mentioned. An examination of spores of plant pathogens in the air by Sreeramulu (1959), particularly those of G. applanatum, showed that the number of spores collected during air-sampling techniques were comparable with those dis-

charged directly from the sporophore. This was the only such study reported.

Hirst's (1957) studies showed that all groups of spores exhibited a typical diurnal periodicity, with ascospores and basidiospores comprising what he called the 'damp air' spora. He suggested (1959) that the development of a thick boundary layer of air under calmer night conditions is favorable for the appearance of spores in air-sampling traps. At night, the comparatively small, fragile basidiospores are not as easily transported to great heights nor are they as subject to dessication. Thus, the sampling technique may definitely influence the numbers and kinds of spores trapped.

Spore sampling instruments fall into two categories; those whose purpose is to sample air spora content of air flowing through the instrument in such a way that spores are impacted on a surface which is usually coated with vaseline, silicon, or glycerin, and those instruments used for selective spore trapping where the investigator is interested in the numbers of a specific kind of spore, or spore discharge rates from a specific organism. The latter may depend on air-volume sampling or on gravitation.

Of the first type of sampler, Hirst's (1952) automatic volumetric spore sampler is well-known and frequently used. Its main drawbacks are that it requires an external power supply and is relativly non-portable. Gregory developed a

portable volumetric spore trap in 1954, which was not a continuous sampler. Pady (1959) described the Pady-Rittis sampler which is a portable, continuous, volumetric spore trap. Other instruments which sample air are essentially based on the same principle as Hirst's spore trap (e.g. Livingston, 1963; Kramer and Pady, 1966; Powell and Morf, 1967; Pathak and Pady, 1965; and Schenck, 1964).

In the second category of samplers, studies have usually been made by simply placing slides beneath the sporophore. Few other samplers for collecting spores immediately upon discharge from the fruiting body have been developed. Ingold (1963, 1967) used a 'spore clock' in his studies of discharge from organisms in culture, while Wood and Schmidt (1966) described a spore trap which may be placed one centimeter below the tube layer of a sporophore. The latter is automatic and spores are deposited on a 35 mm. photographic film strip which is motionless at each sampling area. Since the bulk of this instrument must be beneath the fruiting body, it is not suitable for studying stipitate sporophores such as P. tomentosus. The instrument used in this study is described later in this report.

MATERIALS AND METHODS

Description of the site

This study was undertaken at Candle Lake, Saskatchewan and in the laboratory of the Winnipeg Federal Forestry Department. In Saskatchewan, on the site where field experiments were conducted, *P. tomentosus* causes a white-pocket rot in the roots and butt of white spruce (*P. glauca*) and black spruce (*P. mariana* (Mill.) BSP.). The site was about two acres in area and has been examined for several years because of the annual appearance of sporophores of *P. tomentosus* and the presence of a large stand-opening (Whitney, 1962) (Fig.1). The vegetation consisted mainly of a mixture of 60 to 80 year-old white and black spruce and the area was at the edge of a poplar stand (*Populus tremuloides* Michx); consequently there was some overlapping of the three species. Where these species formed dense stands, sphagnum moss grew in a continuous layer and was the predominant ground cover associated with occasional herbaceous species.

In areas where the disease was particularly severe, and there were dead and fallen trees, much sunlight reached the forest floor providing atypical ground condition (compared to that occurring in a closed spruce stand). In these open areas the ground cover contained less sphagnum moss and a greater variety of herbaceous species and shrubs were found including wintergreen (*Pyrola sp.*), bunchberry (*Cornus cana-*

Fig. 1. Stand-opening caused by P. tomentosus root rot.

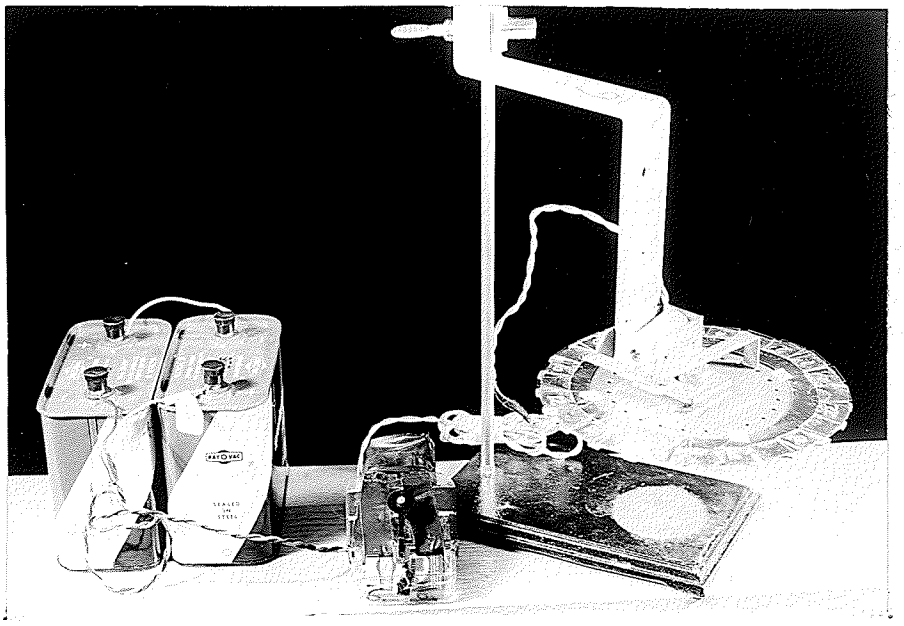


Fig. 2. Spore sampler. 1/6 nat. size.

densis L.), Labrador tea (Ledum groenlandicum Oeder), twinflower (Linnaea boreale L.), and cranberry (Vaccinium oxycoccus L.). Sporophores of P. tomentosus appeared to grow best in shaded areas where sphagnum moss occurred. They also grew in sunny, drier surroundings, but were susceptible to desiccation in such areas.

The soil had a low pH, typical of diseased areas (van Groenewoud, 1956) and was of the grey-wooded type. Detailed analyses of the soils during this study showed that the pH of the humus layer ranged from 3.7 to 5.5, the pH of the A horizon from 3.6 to 5.2, and the pH of the B horizon from 4.7 to 5.6. The soil texture varied from silty sand to sandy loam. The percentage of organic matter in the humus layer ranged from 51 to 92%, while that of the mineral layers ranged from 0.7 to 2.5%. The soil contained low amounts of soluble salts typical for coniferous soils in that area.

As soon as sporophores were observed on the study area, their location was marked with wooden stakes, and the size, date first seen, and location of each sporophore were recorded. Daily examination of the site revealed certain groupings of sporophores which were suitable for experimentation. These groupings were selected so that at least four sporophores grew within an area of approximately 3 feet by 4 feet. This was to ensure that there would be sufficient sporophores to allow for the loss of at least one and that all sporophores would be enclosed in the plastic tents built for experimentation.

The method of spore collecting.

The spore samplers used in this study were designed by R.D. Whitney and built by D.G.H. Ray. A brief description follows: a 1.5 volt battery-operated transistorized clock drove a minute hand to which was attached a magnet. Once each hour, the magnet came into contact with a switch, closing it, and thus completing a circuit powered by two 6-volt batteries. This circuit drove a small lever forward instantly, which was then pulled back by a spring when the circuit was broken. The lever fitted into a notch on a rigid 7-inch aluminum disc which was attached to the apparatus by a magnet. The disc revolved freely and once per hour was pushed forward, so that it completed a revolution in 24 hours. The apparatus was clamped to a ring-stand and positioned so that the edge of the disc was directly beneath the hymenial surface. A foil disc, found most suitable because its shape was not changed by moisture conditions, was taped to the metal disc and twenty-four 18 mm. square coverslips were glued symmetrically to the foil disc (Fig. 2). In this way, 24 one-hour spore deposits were obtained from each sporophore each day. The discs, with coverslips, were easily removed and replaced when necessary.

A foil template was fitted to the sporophore so that only 18 square mm. of hymenial surface were exposed and the discharged mucilaginous spores adhered firmly to the glass coverslips. The distance between the pore surface and the collecting disc was 1-2 mm., although this distance sometimes increased to 5 mm. because of the slanted nature of

some of the sporophores.

All exposed foil discs were preserved in herbarium boxes and numbers of spores counted in the laboratory. Each coverslip was examined under phase contrast at a magnification of 312X. The basidiospores of P. tomentosus have no distinguishing characteristics, being hyaline, smooth-walled, colorless and measuring 3-4μ by 5-6μ (Fig.3). The method of counting was to scan the coverslip, search for the densest area and count the number of spores in a 5X5 grid which covered an area of 0.04 square mm. Four additional areas were counted at even intervals around this first area. Coverslips which had been examined, were transferred to heavy cardboard sheets and glued over black carbon paper which rendered the spore deposits easily visible and allowed comparison of spore deposition patterns.

Field Experiments

In the field, spore discharge was observed under; a. natural conditions, b. natural light and temperature fluctuations with artificially maintained high R.H., c. natural temperature fluctuations with constant darkness and high R.H., d. natural temperature fluctuations with constant light and high R.H., and e. natural temperature fluctuations with alternate light-dark periods and high R.H.

Although light and R.H. were easily controlled, preliminary trials showed that a constant temperature was dif-

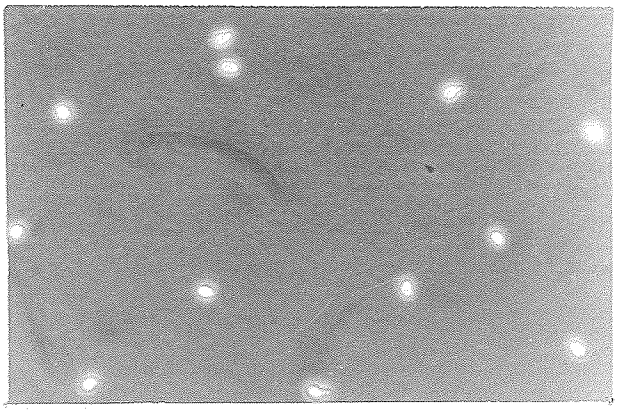


Fig. 3. P. tomentosus basidiospores under phase contrast. 600X.

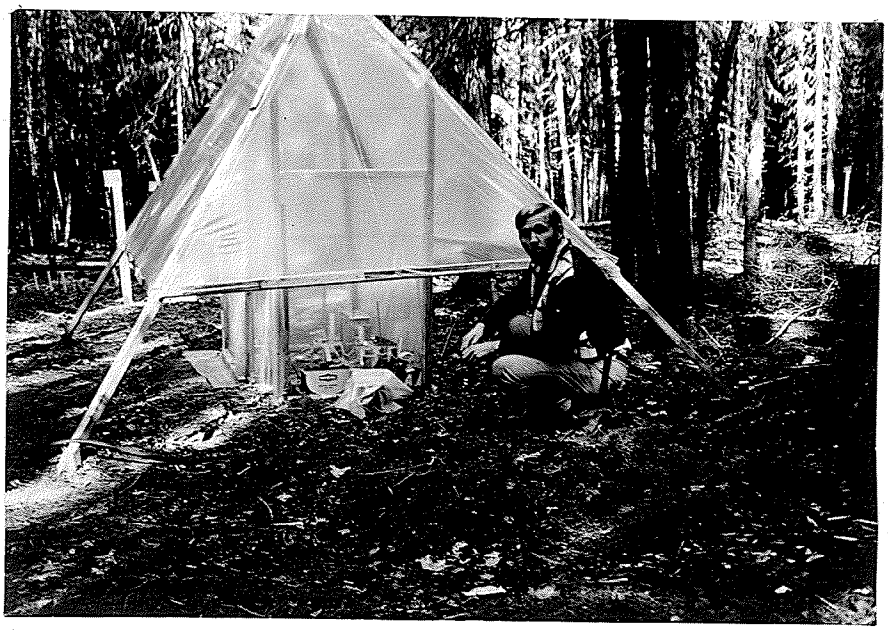


Fig. 4. Tent A, 1968, in which spores were discharged under natural fluctuations of temperature, R.H. and light.

ficult to maintain in the field and, consequently, this factor could only be investigated as it fluctuated naturally. The reason for this difficulty is that the sporophores must be maintained in spaces with little or no air circulation if representative spore deposits are to be collected, and most temperature-regulating instruments depend on air circulation.

In 1968 and 1969, each plot (or tent) contained at least four sporophores initially. If possible, more were included due to the unpredictable rapid exhaustion of some of the sporophores. When the sporophores were discharging spores, as determined by placing a 22 by 50 mm. coverslip beneath the sporophore and examining it microscopically later, they were subjected to various sets of environmental conditions in groups. In 1969, due to unfavorable fruiting conditions, groups of sporophores were covered with plastic sheets thereby creating an atmosphere of high R.H. and promoting initial spore discharge. In the same year, due to poor quality and low numbers of sporophores, some sporophores were transplanted so that each group consisted of four sporophores.

In 1968, four sets of conditions were investigated, and for each set, a tent was built completely around the group of sporophores to maintain the desired set of conditions and to eliminate air currents which might easily have carried away the basidiospores. The four sets of conditions investigated

follow.

A. Natural conditions: The tent was constructed of clear plastic to allow natural light fluctuations. It consisted of an inner roofless chamber covered by a raised canopy. This created a space between the two enclosures, permitting air exchange, but allowing no strong currents to reach the sporophores (Fig. 4). Comparison of hygrothermograph readings taken within the tent with those obtained outside the tent showed that this tent successfully allowed normal temperature and R.H. changes.

B. Darkness and naturally fluctuating R.H.: This tent was constructed of black plastic and it was attempted to maintain the sporophores in darkness while allowing normal temperature and R.H. fluctuations. Due to poor air circulation, the treatment was unsuccessful with the R.H. remaining continuously high rendering the treatment similar to that in tent D. This tent and the following two tents were constructed in the shape of a square, with no outer canopy.

C. Naturally fluctuating light and high R.H.: The tent was made of clear plastic to allow natural light fluctuations and high R.H. was maintained continuously by watering the surrounding soil.

D. Darkness and high R.H.: The tent was constructed of black plastic and high R.H. was also maintained continuously by watering the surrounding soil.

All tents were constructed of 6 mil plastic sheeting

stapled to wooden frames. At the ground level, the plastic was carefully tacked to the duff so that air and rain could not enter. In tents where dark humid treatments were applied, B and D above, an additional dark 'room' was built so that no light entered as the door was opened to examine the sporophores and the equipment. A penlight flashlight was the sole light source and this was usually not on for more than ten minutes. The average space surrounding the sporophores was about 3 feet wide, 4 feet long, and 3 to 4 feet high (Fig. 5). A hygrothermograph and a tensiometer were set beside the sporophores inside each tent (Fig. 6) and one hygrothermograph and one tensiometer were set outside the tents. A thermistor psychrometer was used to supplement and check the hygrothermograph readings.

Although the 'control' tent (A) allowed natural R.H., temperature, and light fluctuations, rainfall was eliminated. This was compensated for by daily examination of a rain guage and a tensiometer outside the tent which indicated when additional moisture (from a nearby stream) should be added directly to the sporophores as well as the humus.

The moisture content of sporophores, humus , and mineral horizons of the soil in the tent were determined every second day. Samples were oven-dried and moisture content was measured as a percentage of the oven-dry weight. A sample of soil from each plot was subjected to a detailed analysis of pH, texture, conductivity, percentage organic matter, and

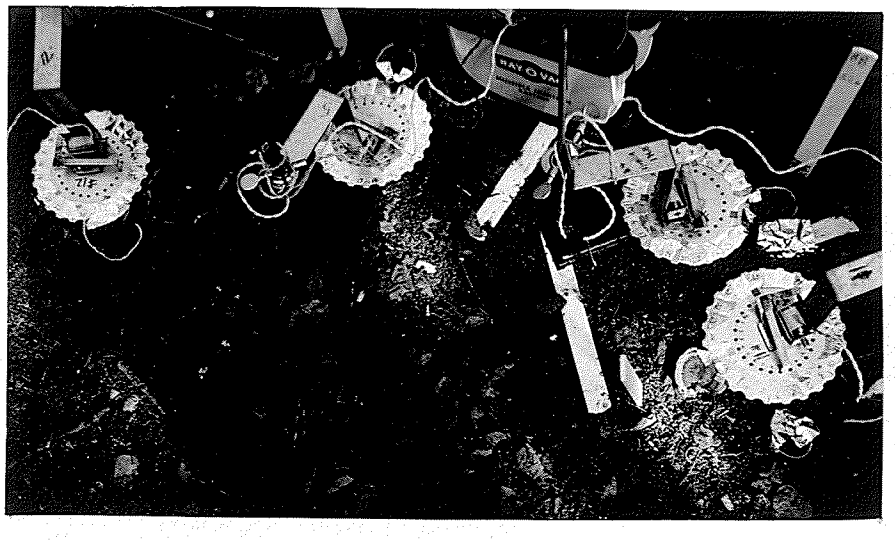


Fig. 5. Interior of tent A, 1968, showing typical spacing of P. tomentosus sporophores and instruments.

Fig. 6. Hygrothermograph and tensiometer within tent A, 1968.

amounts of soluble salts, in the laboratory in Winnipeg.

Other frequent recordings included light readings at the sporophore level which were measured three to four times daily with a Weston light meter, as well as the measurements of the increase in size of the pileus of each sporophore every two to three days. A brief weather synopsis was recorded daily.

In 1969, because of the results obtained in earlier field experiments and laboratory studies carried out during 1967 and 1968, greatest emphasis was placed on the effect of light. The sporophore crop was poor and abnormal growth greatly affected the studies. Seven sets of conditions were applied in 1969, two sets each being applied in three of the tents (B, C, and D). Description of the four plots (including the seven sets of conditions) follow.

A. Natural conditions: This tent was constructed exactly as in 1968.

B. Light and high R.H.: The tent was constructed of clear plastic. For the first half of the sampling period, natural light fluctuations prevailed. In the second half, natural light was supplemented with continuous light from flourescent tubes of approximately 500 ft-c[1] intensity at the sporophore level.

C. Alternate light-dark periods and high R.H.: This tent was constructed of black plastic. For the first half of the

[1]Foot-candles.

sampling period, light intensity of approximately 500 ft-c at the sporophore level was applied from 0700 to 1900 hours. In the second half, the same intensity of light was applied from 1900 to 0700 hours.

D. Alternate light-dark periods and high R.H.: The tent was also constructed of black plastic and the spores were collected in continuous darkness for the first half of the sampling period. In the second half, light intensity of approximately 600 ft-c at the sporophore level was applied from 0700 to 1900 hours.

All factors were measured as in 1968, except that light readings in tents A and B were taken more frequently and with different equipment. Photocells were attached directly above each sporophore (Fig. 7) and all photocells led to a multi-channel digital data recorder. Readings were taken once per minute and the instrument was battery-operated. Unfortunately, the spore discharge rates were so consistently low in tents A and B that the light readings could not be utilized.

In those tents where artificial light was supplied, the light source consisted of two banks of fluorescent lights (each bank containing four 40-watt cool white flourescent tubes) suspended at a suitable height above the sporophores. Reference to laboratory experiments indicated that light intensities of from 400 to 600 ft-c inhibited spore discharge, and therefore, the height of the light banks above the sporo- varied so that intensity was fixed in the above range. Light intensities were measured with a Weston light meter at the

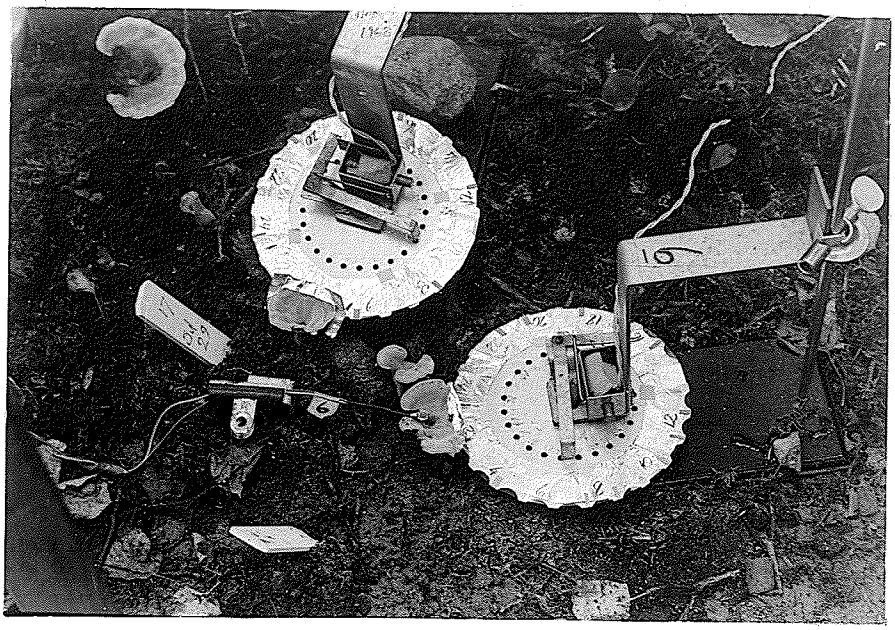


Fig. 7. Photocell clamped into position above P. tomentosus sporophore.

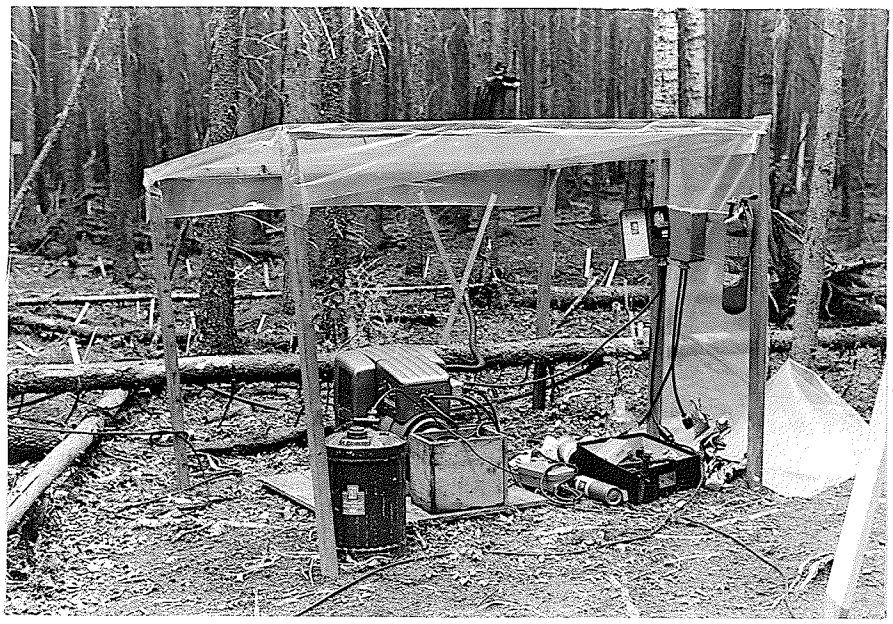


Fig. 8. Gasoline-operated generator with attached wiring and time switches under plastic shelter.

level of the sporophores. The light banks were powered from a gasoline-operated portable generator and time switches were connected to the light banks to effect automatic and accurate light changes (Fig.8).

Laboratory experiments.

Laboratory experiments were conducted during the winters of 1967-70. Suitable sporophores were selected from the field, stored for several months, and successfully revived in the manner described by Whitney and Bohaychuk (1969). During experiments, the sporophores were maintained in growth cabinets measuring 4 by 2 by 2 feet which were devoid of any automatic control of temperature and R.H. because of the necessity of maintaining calm air. A hygrothermograph was used to measure temperature and R.H. Although control of temperature was difficult, R.H. changes were easily effected by opening the doors of the chamber a measured distance. Where a particular light intensity was required, light banks of the same type used in field experiments were placed above the chambers and readings taken with a Weston light meter. High R.H. was maintained by adding water to vermiculite which had been placed in the bottom of the chamber.

In the laboratory, spore discharge was observed under; a. continuous darkness and fluctuating R.H., b. continuous light and fluctuating R.H., c. continuous darkness and high R.H., d. continuous light and high R.H., and e. high R.H. and fluctuating light.

RESULTS

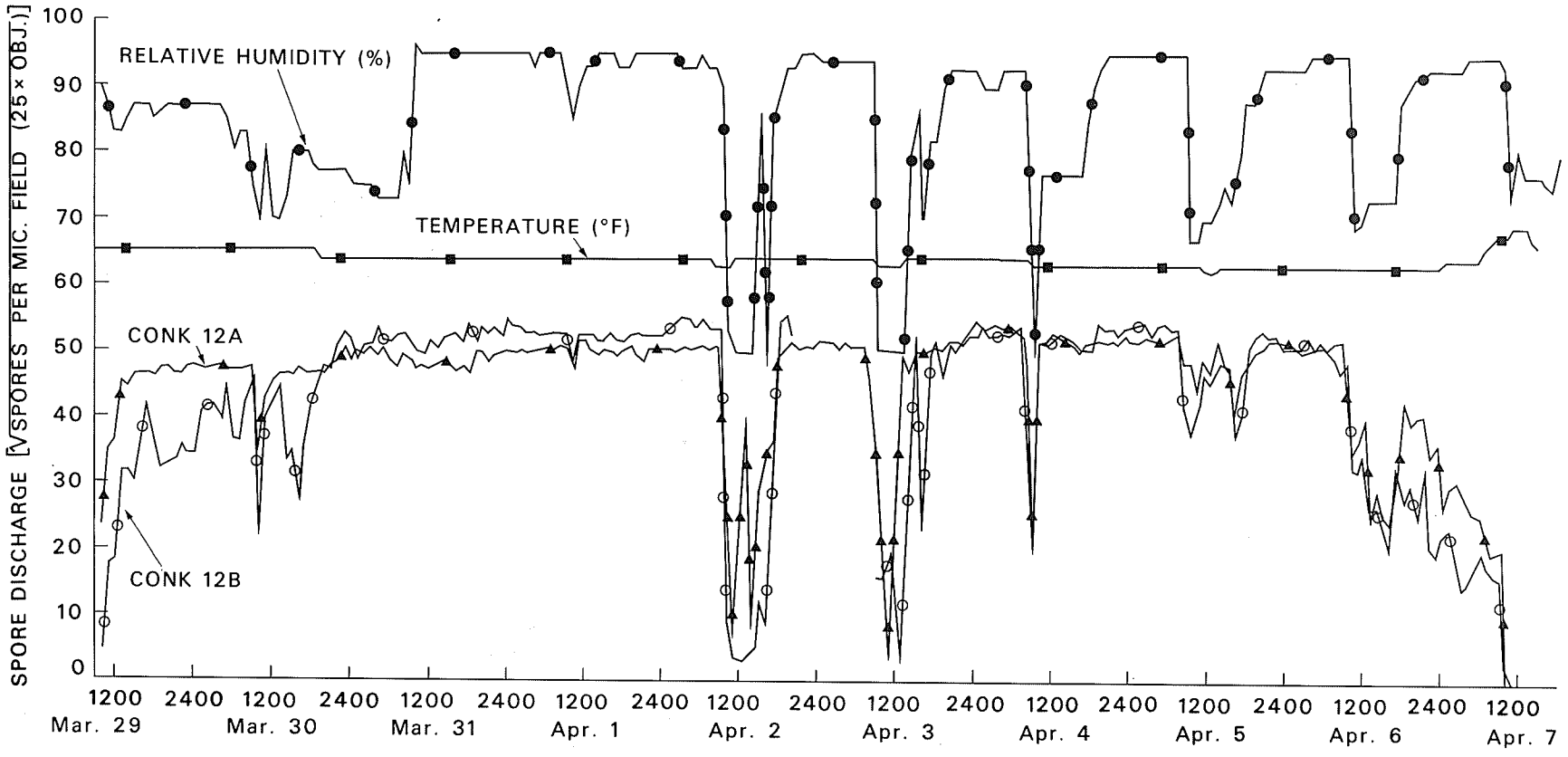
Spore discharge under controlled conditions in the field and the laboratory.

Effects of R.H.

In the laboratory, maximum spore discharge rates were obtained in darkness, when R.H. was at saturation levels and temperature was constant. Sporophore L-12[1] was sampled on two sides, A and B, and both sides showed the same response to R.H. fluctuations (Fig. 9). After a period of about 48 hours during which the sporophore was recovering from the effects of being in storage, the sporophore indicated a high sensitivity to R.H. When the R.H. was reduced from saturation to approximately 50% between 0900 and 1000 hours on April 2, the spore discharge rate of sporophore L-12B decreased from about 2500 spores per microscope field to about 60 or 70 spores per microscope field during the same one hour interval. Between 1400 and 1500 hours on the same day, the R.H. was increased again to 85% during which time the spore discharge rate increased rapidly from the above low rate back up to about 2500 spores per microscope field. Similar instantaneous reactions of spore discharge rate to R.H. changes occurred on April 3 and 4. It is impor-

[1]The sporophores are referred to as conks in the figures, since the shortness of the latter word facilitated placement in the diagrams.

Fig. 9. The relationship between fluctuating R.H. and spore discharge rates by two sides, A and B, of sporophore 12 at constant temperature in darkness in the laboratory. (Each point on all graphs depicting spore discharge rates is an average of the five readings on each hourly spore deposit).


SPORE DISCHARGE [√SPORES PER MIC. FIELD (25× OBJ.)]
100
90
80
70
60
50
40
30
20
10
0
RELATIVE HUMIDITY (%)
TEMPERATURE (°F)
CONK 12A
CONK 12B
1200 2400 1200 2400 1200 2400 1200 2400 1200 2400 1200 2400 1200 2400 1200 2400 1200 2400 1200
Mar. 29
Mar. 30
Mar. 31
Apr. 1
Apr. 2
Apr. 3
Apr. 4
Apr. 5
Apr. 6
Apr. 7

tant to note that lowering the R.H. to 70% caused little and often no change in the spore discharge rate (March 31, April 4, 6, and 7). This will be discussed in relation to light influences on spore discharge rates. The same response to R.H. was confirmed in two other experiments involving three additional sporophores in laboratory studies.

The pore surface of sporophore L-12 was steeply inclined from the outer edge of the pileus to the stipe, was cream-colored, and consisted of pores of uniform size. The sporophore was free of insects and other micro-organisms.

When R.H. and spore discharge rates were compared statistically, there was a high degree of positive correlation (Table I). The author feels it is valid to compare statistically only those values within the hours shown since, as will be discussed, spore discharge appears to be unaffected by environmental conditions when it is initially recovering from storage. In most examples illustrated in this report, spore discharge rates are shown from the time spore discharge commences to the time it ceases and it seems justified to ignore the initial and terminal lags in spore discharge rates when making statistical analyses.

Sporophores 1 and 2, also studied in the laboratory, did not discharge spores as rapidly as sporophore L-12 even under optimal conditions and were more sensitive to the lowering of R.H. to 70% (Fig. 10). Both sporophores were rapidly exhausted after two days of discharging spores. Such poor

Table I. Correlation between R.H. and spore discharge by four sporophores of P. tomentosus maintained in darkness at 70F in the laboratory. (See Figs. 9 and 10)

Sporophore	Readings From	To	df	Correlation Coefficient
12A	1200 hrs. March 31	1200 hrs. April 6	144	0.699**
12B	1200 hrs. March 31	0900 hrs. April 6	129	0.857**
1	0100 hrs. Dec. 15	1300 hrs. Dec. 18	74	0.838**
2	1000 hrs. Dec. 15	2400 hrs. Dec. 17	58	0.670**

** Significant at the 1% level.

Table II. Correlation between temperature and spore discharge by sporophores of P. tomentosus under controlled conditions in the field (treatment D, 1968). (See Fig. 11)

Sporophore	Readings From	To	df	Correlation Coefficient
39	1100 hrs. Aug. 17	2400 hrs. Aug. 28	274	0.658**
102	1100 hrs. Aug. 17	2400 hrs. Aug.28	244	0.629**

** Significant at the 1% level.

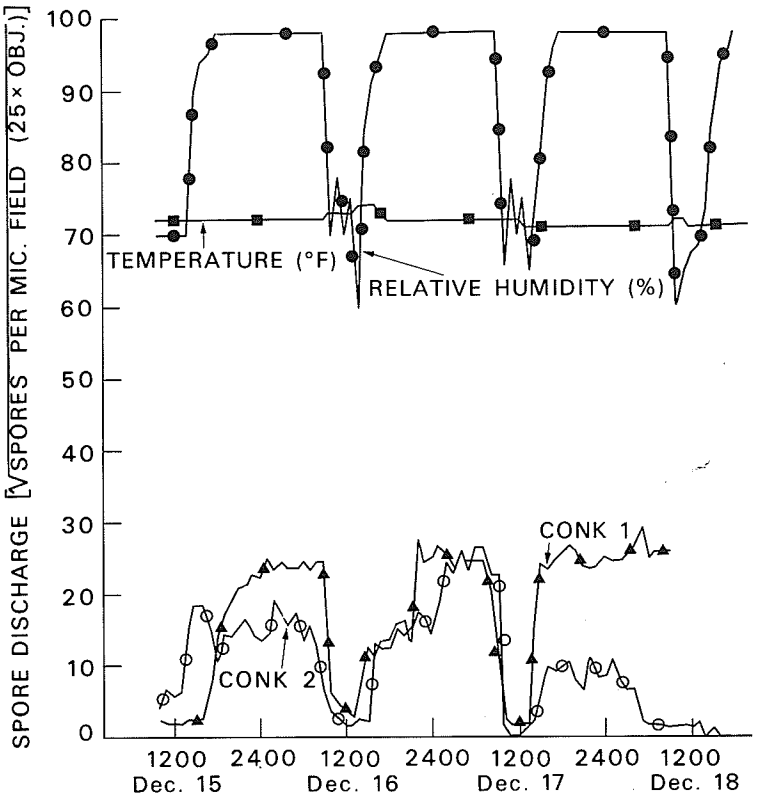


Fig. 10. The relationship between fluctuating R.H. and spore discharge rates by sporophores 1 and 2 in darkness at constant temperature in the laboratory.

quality sporophores probably do not as accurately reflect the typical behaviour of this organism.

Effects of temperature.

In continuous darkness at constantly high R.H., the periodicity in spore discharge rates coincided with the periodicity of natural temperature fluctuations (Figs. 11 and 12). Figure 12 depicts the results from sporophores maintained in tent B in the field in 1968, in which it was attempted to allow natural R.H. changes. However, the R.H. rarely dropped to below 80% and there is sufficient evidence elsewhere in this report to indicate that R.H. fluctuations above 80% do not produce effects on spore discharge by the majority of sporophores. Three-point moving averages were graphed to emphasize trends in the results. In figure 11 (tent D, 1968), the raw data are shown which emphasize the high correlation between temperature and discharge rates.

The trends in spore discharge rates of sporophores 39 and 102 (Fig. 11) had a significant positive correlation with temperature over an 11-day period (Table II). The table only treats data until August 28 since there is an alteration in spore discharge rates after this date which is probably due to exhaustion of the sporophore.[1] The trends in spore discharge rates of sporophores 27 and 30 (Fig. 12) were positively correlated with temperature on at least 7 out of 12

[1]Exhaustion of sporophores and cycles of exhaustion will be discussed more fully in a later section.

Fig. 11. The relationship between naturally fluctuating temperature and spore discharge rates by sporophores 39 and 102 in darkness at high R.H. in the field (tent D, 1968).

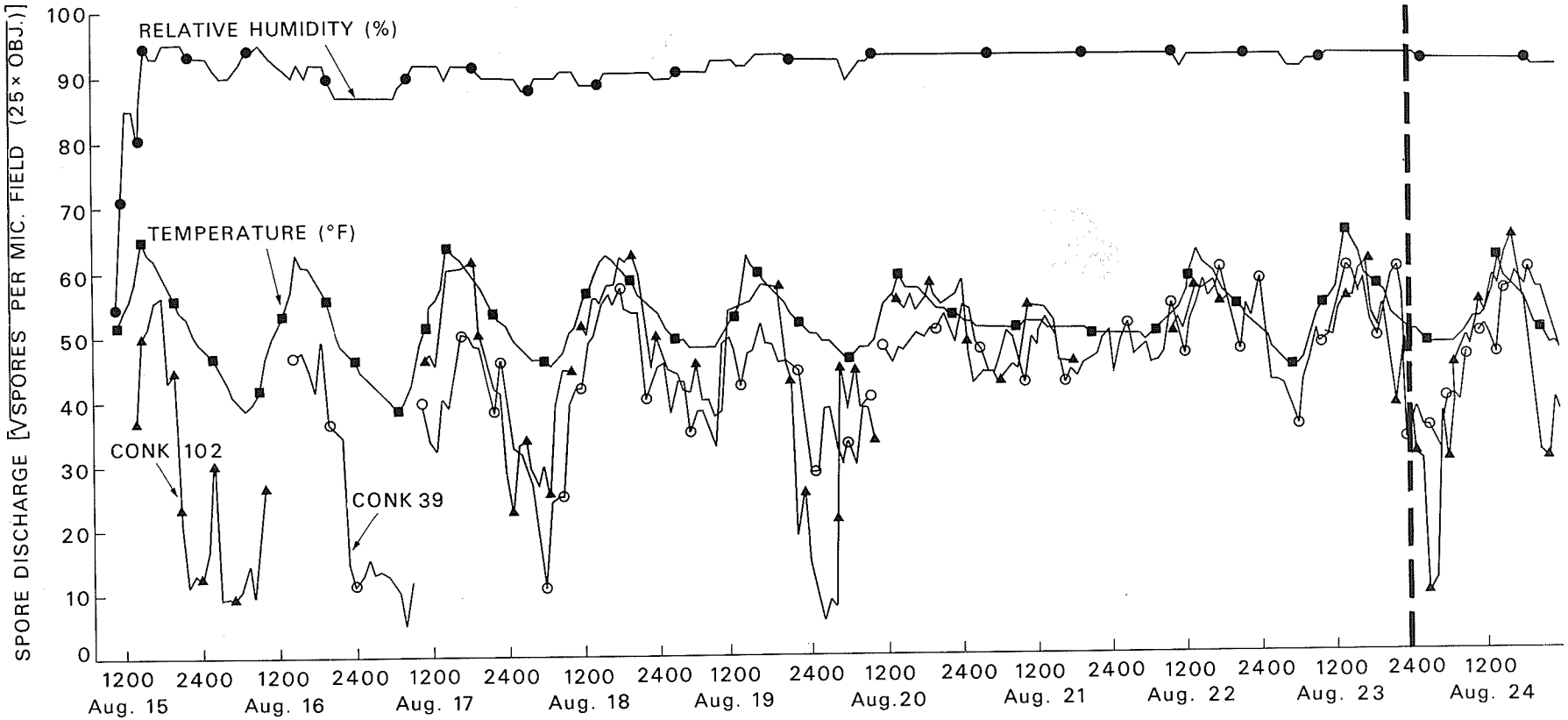
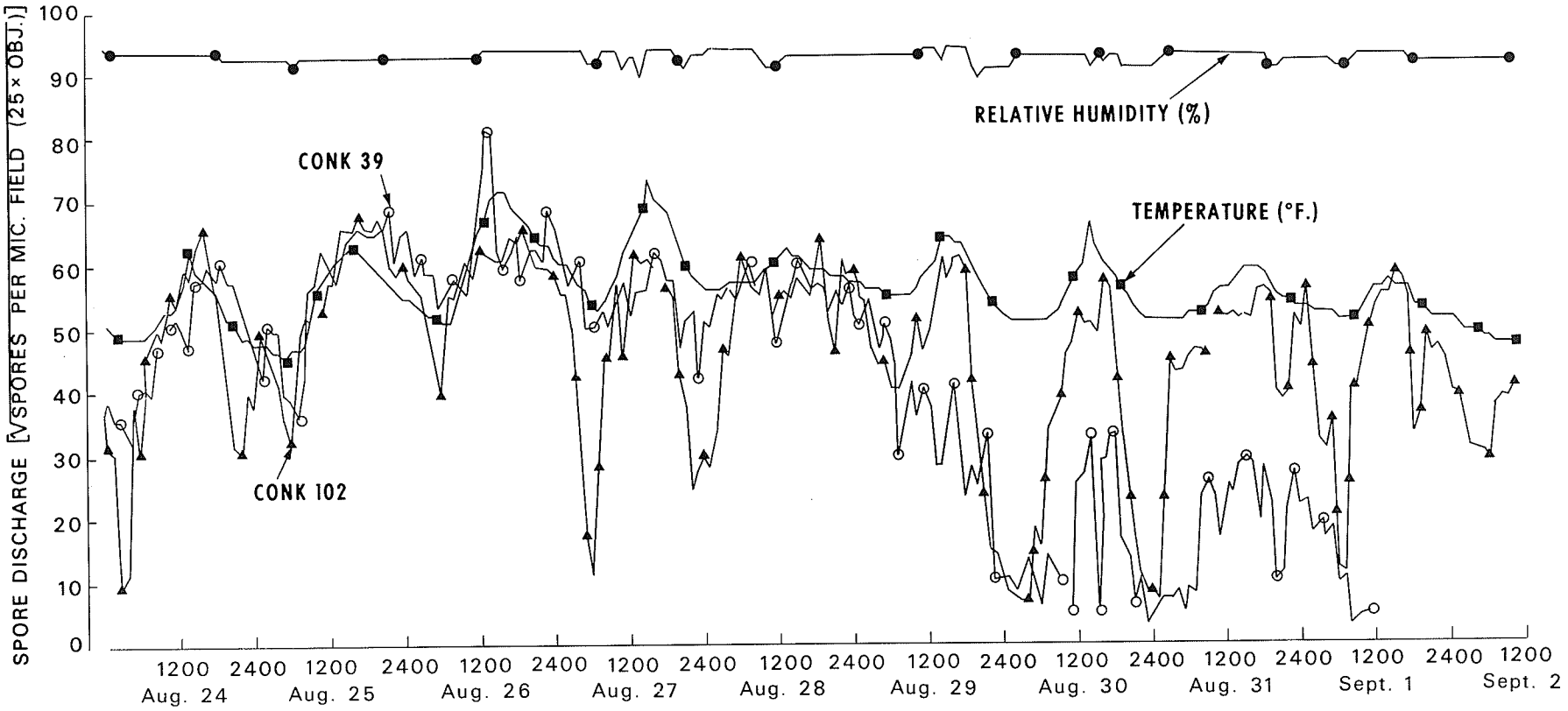

SPORE DISCHARGE [√SPORES PER MIC. FIELD (25× OBJ.)]
RELATIVE HUMIDITY (%)
TEMPERATURE (°F)
CONK 102
CONK 39
100
90
80
70
60
50
40
30
20
10
0
1200 2400 1200 2400 1200 2400 1200 2400 1200 2400 1200 2400 1200 2400 1200 2400 1200 2400 1200
Aug. 15 Aug. 16 Aug. 17 Aug. 18 Aug. 19 Aug.20 Aug. 21 Aug. 22 Aug. 23 Aug. 24

Fig. 11. Continued.


SPORE DISCHARGE [√SPORES PER MIC. FIELD (25× OBJ.)]
100
90
80
70
60
50
40
30
20
10
0
RELATIVE HUMIDITY (%)
CONK 39
TEMPERATURE (°F.)
CONK 102
1200 2400 1200 2400 1200 2400 1200 2400 1200 2400 1200 2400 1200 2400 1200 2400 1200 2400 1200
Aug. 24
Aug. 25
Aug. 26
Aug. 27
Aug. 28
Aug. 29
Aug. 30
Aug. 31
Sept. 1
Sept. 2

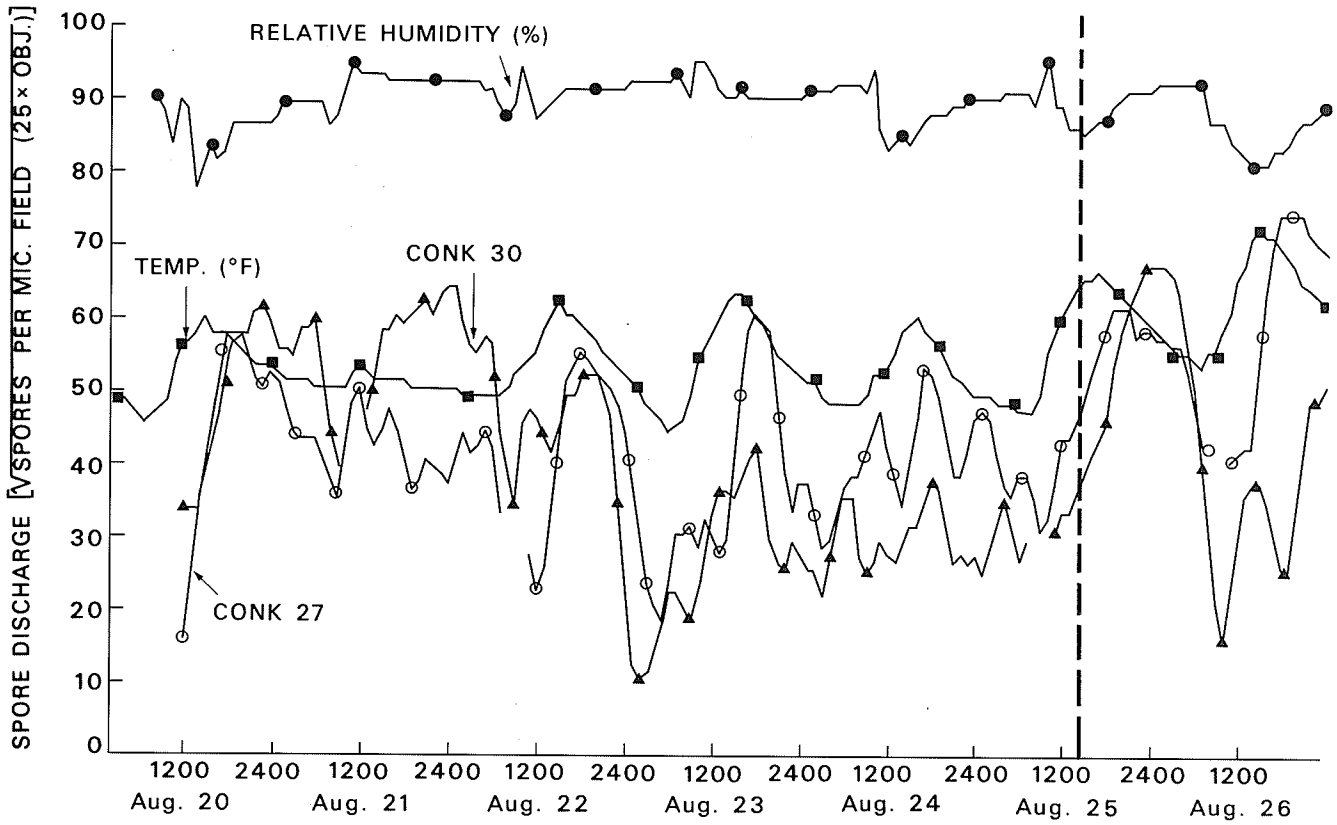


Fig. 12. The relationship between naturally fluctuating temperature and spore discharge rates by sporophores 27 and 30 in darkness at high R.H. in the field (treatment B, 1968).

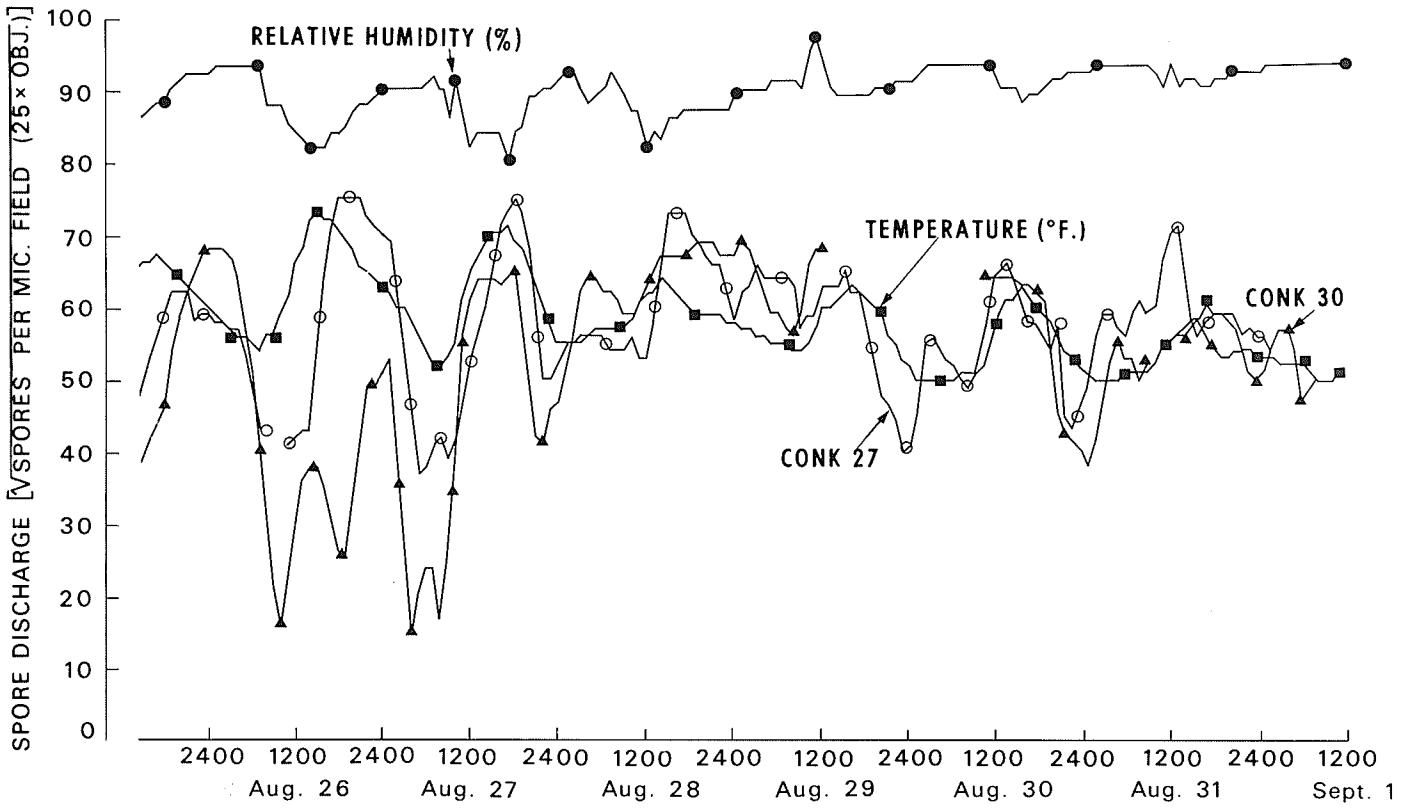


Fig. 12. Continued.

days (Table III). The data were examined on a daily basis because although the trends were similar to those of sporophores 39 and 102, the absolute value of the spore discharge rates showed more variation from day to day, possibly due to the slightly greater R.H. fluctuations.

The sporophores varied in their reaction to a specific temperature in tents B and D. For example, on August 22, 23, and 24, the temperature reached a maximum of approximately 60F on each day, while the maximum spore discharge rate of sporophore 27 was different on each day (Fig. 12). Sporophore 30 exhibited the same behaviour as sporophore 27 on these three days. It is suspected that the sporophores go through cycles of exhaustion when they are subjected to fluctuations in environmental conditions.

There was no evidence of an endogenous rhythm in these investigations and spore discharge trends were mainly in response to temperature. After August 28, the spore discharge rate of sporophore 39 steadily declined and the spore discharge rate of sporophore 102 dropped to a lower level than previously on three consecutive days when the temperature dropped. By September 2, the sporophores were badly overgrown and discharged spores for only 2 to 3 days longer.

Sporophores 27 and 30 (Fig. 12), while showing a close relationship to temperature, exhibited much greater hourly fluctuation in spore discharge rates than the sporophores in tent D. Sporophores 27 and 30 might have been sensitive to small R.H. changes because of other moisture conditions

Table III. Correlation between temperature and spore discharge by sporophores of P. tomentosus maintained in darkness and high R.H. in the field (treatment B, 1968). (See Fig. 12)

Sporophore	Readings From	To	Date	df	Correlation Coefficient
27	1100 hrs.	2400 hrs.	Aug.20	13	0.031
27	0100 hrs.	2400 hrs.	Aug.21	23	0.731*
27	0100 hrs.	2400 hrs.	Aug.22	22	0.230
27	0100 hrs.	2400 hrs.	Aug.23	23	0.663*
27	0100 hrs.	2400 hrs.	Aug.24	23	0.479**
27	0100 hrs.	2400 hrs.	Aug.25	23	0.707*
27	0100 hrs.	2400 hrs.	Aug.26	23	0.394
27	0100 hrs.	2400 hrs.	Aug.27	23	0.826*
27	0100 hrs.	2400 hrs.	Aug.28	23	0.535*
27	0100 hrs.	2400 hrs.	Aug.29	23	0.396**
27	0100 hrs.	2400 hrs.	Aug.30	23	0.574*
27	0100 hrs.	2400 hrs.	Aug.31	23	0.125
30	1100 hrs.	2400 hrs.	Aug.20	13	0.434
30	0100 hrs.	2400 hrs.	Aug.21	22	0.270
30	0100 hrs.	2400 hrs.	Aug.22	23	0.067
30	0100 hrs.	2400 hrs.	Aug.23	23	0.778*
30	0100 hrs.	2400 hrs.	Aug.24	23	0.279
30	0100 hrs.	2400 hrs.	Aug.25	22	0.513**
30	0100 hrs.	2400 hrs.	Aug.26	23	0.352
30	0100 hrs.	2400 hrs.	Aug.27	23	0.766*
30	0100 hrs.	2400 hrs.	Aug.28	23	0.527*
30	0100 hrs.	2400 hrs.	Aug.29	13	0.674**
30	0100 hrs.	2400 hrs.	Aug.30	14	0.701**
30	0100 hrs.	2400 hrs.	Aug.31	22	0.426**

** Significant at the 1% level
* Significant at the 5% level

which were comparatively low in this tent. For example, the average daily moisture content of the humus in tent B was 91.6%, while that in tent D was 155.0%. However, the moisture contents of the sporophores in tent B were higher than in tent D, so that factor could not have been limiting. Although it was not the intention of this study to examine the immediate microclimate of the sporophores, it must be admitted that such information might have been useful in explaining some of the unaccountable variations observed.

Effects of light.

With the majority of sporophores studied, spore discharge rates were highest under conditions of continuous darkness when temperature and R.H. were maintained at optimum levels for spore discharge. This is illustrated by figure 13 and portions of figures 9, 11, 16, and 17 over periods of at least 48 hours. Illumination caused a disruption in the patterns of spore discharge observed in darkness. Controlling the environment as was attempted in this study, made it difficult to maintain both R.H. and temperature at constant levels while regulating the light intensity. The results from six separate studies (Figs. 13 to 18), plus the results from three other studies not included, serve to illustrate the effects of light on spore discharge. They also show the variability of light effects on the sporophores and results of all six experiments are shown because of gaps in

the record for individual sporophores due to problems with the equipment.

The discharge rates of sides A and B of sporophore L-10 which had a thick white margin around the pileus and a white hymenial surface with distinct pores are shown in figure 13. Side A of this sporophore discharged spores at a high rate for five consecutive days under conditions of continuous darkness, high R.H., and temperatures of 60 to 70F. This sporophore may be compared to sporophore L-12 (whose discharge rates are shown in figure 9) since they both have high rates of spore discharge and are healthy, and it would be expected that sporophore L-10 would be as insensitive as sporophore L-12 to a slight decrease in R.H. The high rate of discharge by side A of sporophore L-9 was reduced only during exposure to light. If the reduction were due to R.H., it is unlikely that the discharge rate would recover before R.H. returned to its high level. The second exposure to light quickly inhibited spore discharge 3 to 4 hours after the light period was begun.

The data on spore discharge by sporophore L-1 (Fig. 14) are incomplete due to technical difficulties. It appears that light of 120 ft-c had little effect on spore discharge rates, while light intensities of 490 and 900 ft-c reduced spore discharge and this reduction carried into the ensuing periods of darkness. After January 14, the sporophore ceased discharging spores.

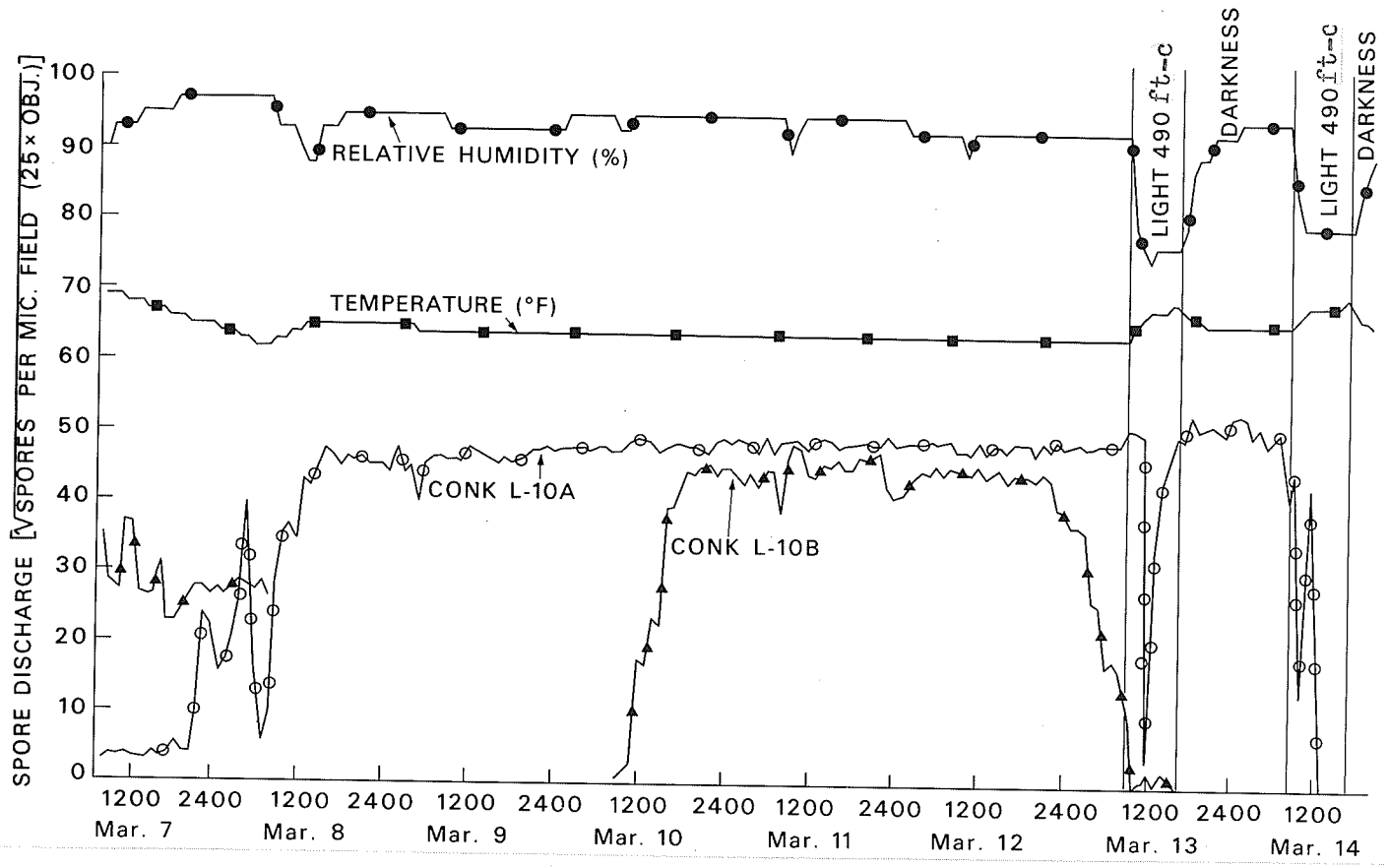


Fig. 13. The relationship between light (490 ft-c) and spore discharge rates by opposite sides, A and B, of sporophore L-10 at constant temperature and high R.H. in the laboratory.

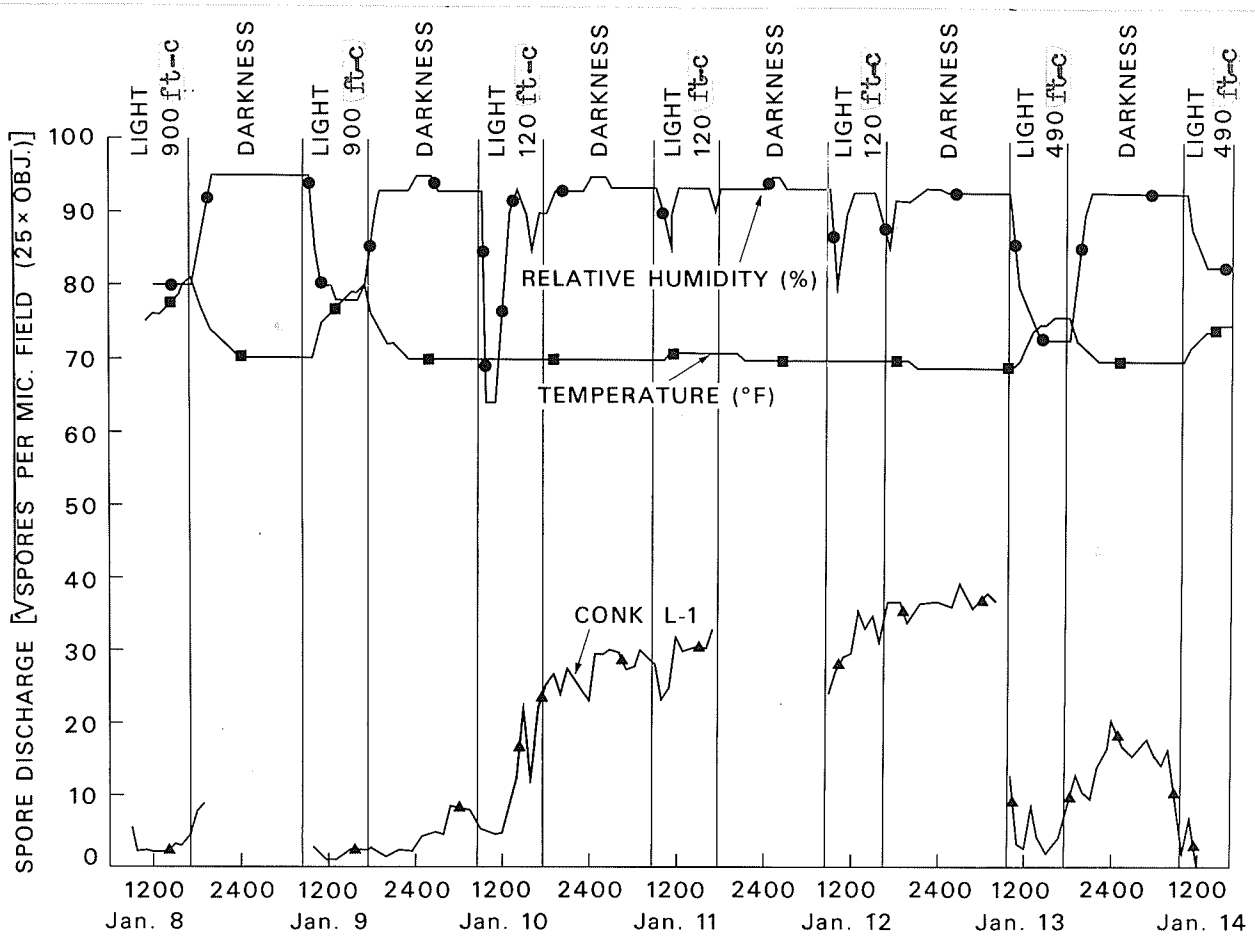


Fig. 14. The relationship between light (120, 490, and 900 ft-c) and spore discharge rates by sporophore L-1 at constant temperature and high R.H. in the laboratory.

Sporophores L-2 and L-3 showed a similar response to light (Fig. 15). Exposure to light of 490 ft-c intensity resulted in a drop in spore discharge rates. Possibly light and slightly reduced R.H. have an additive effect in the suppression of spore discharge rates, but it is unlikely that the lowered R.H. alone caused the reduction in discharge rates, since the drops in R.H. were slight during periods of light application, reaching 60% only briefly on January 17. Sporophore L-2 was not affected by light intensity of 190ft-c, but was affected by an intensity of 490 ft-c. Sporophore L-3 only reached two peaks of spore discharge, both in darkness, and showed consistently low rates of discharge at other times.

Sporophore 13 was a healthy sporophore with small uniform pores and the spore discharge rates are shown in figure 16. The discharge rate did not seem to be affected by a light intensity of 120 ft-c nor the accompanying R.H. changes. Sporophore 8, another healthy specimen, did not seem to be affected by light intensity of 120 ft-c until June 17, when the changes in R.H. were probably responsible for the lower discharge rate. Drops in R.H. from 97% to 80% did not affect the spore discharge rate during continuous darkness on June 14 and 15, and therefore the reduced discharge rates on June 16, 18, and 19 were probably not solely due to R.H. changes, although R.H. may have influenced the response. Neither sporophore regained its original high

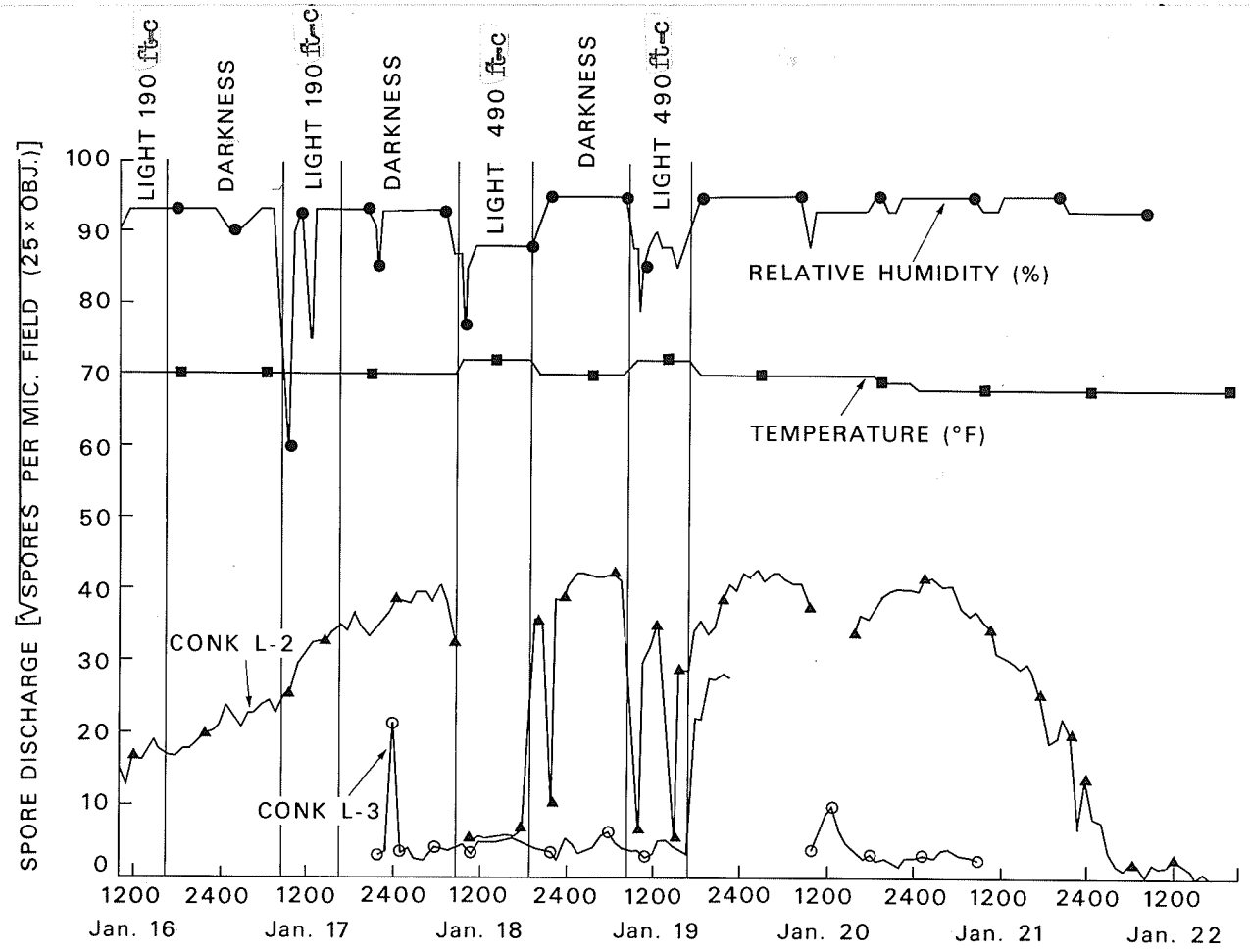
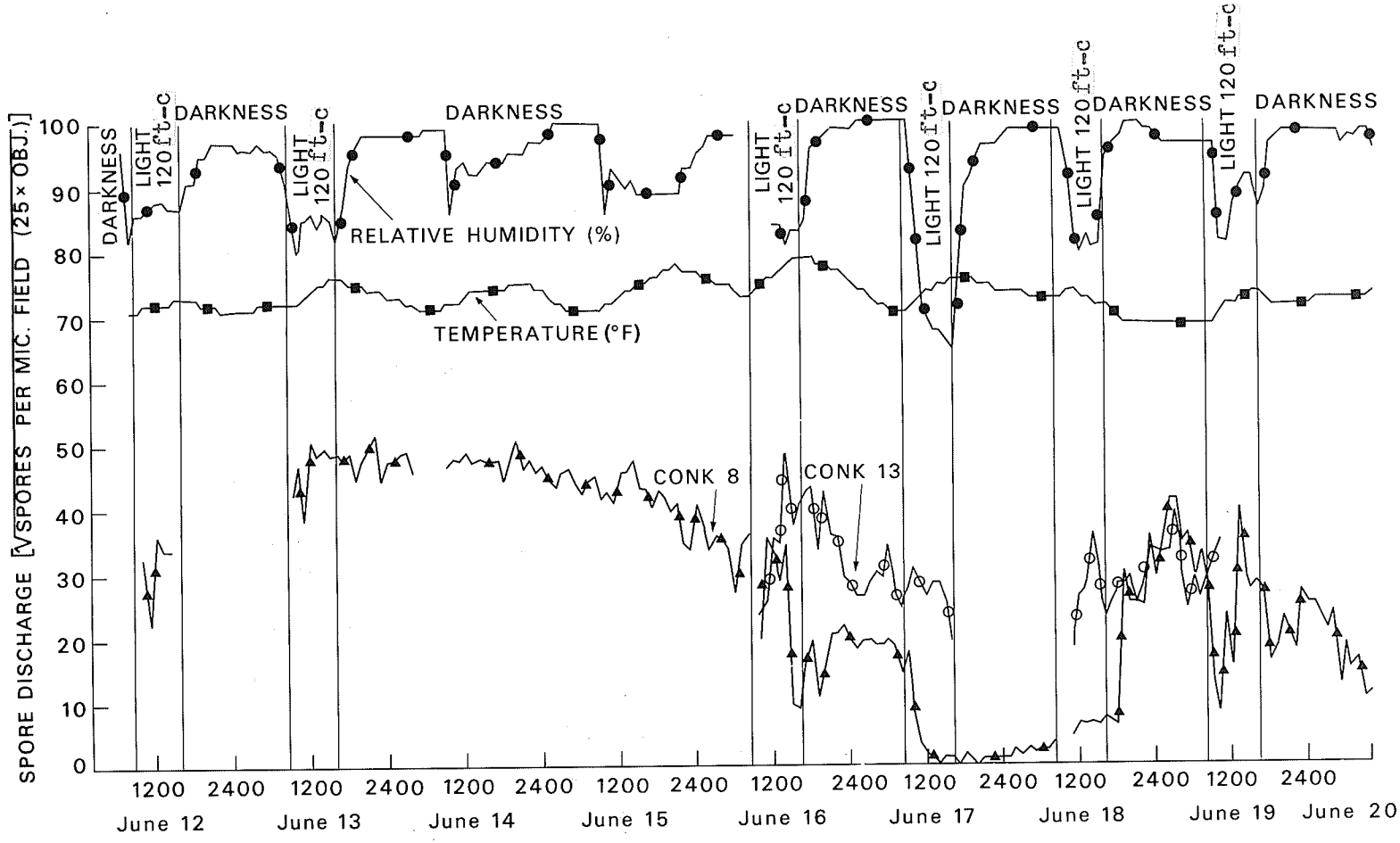


Fig. 15. The relationship between light (190 and 490 ft-c) and spore discharge rates by sporophores L-2 and L-3 at constant temperature and high R.H. in the laboratory.

Fig. 16. The relationship between light (120 ft-c) and spore discharge rates by sporophores 8 and 13 in darkness and slightly fluctuating temperature in the laboratory.

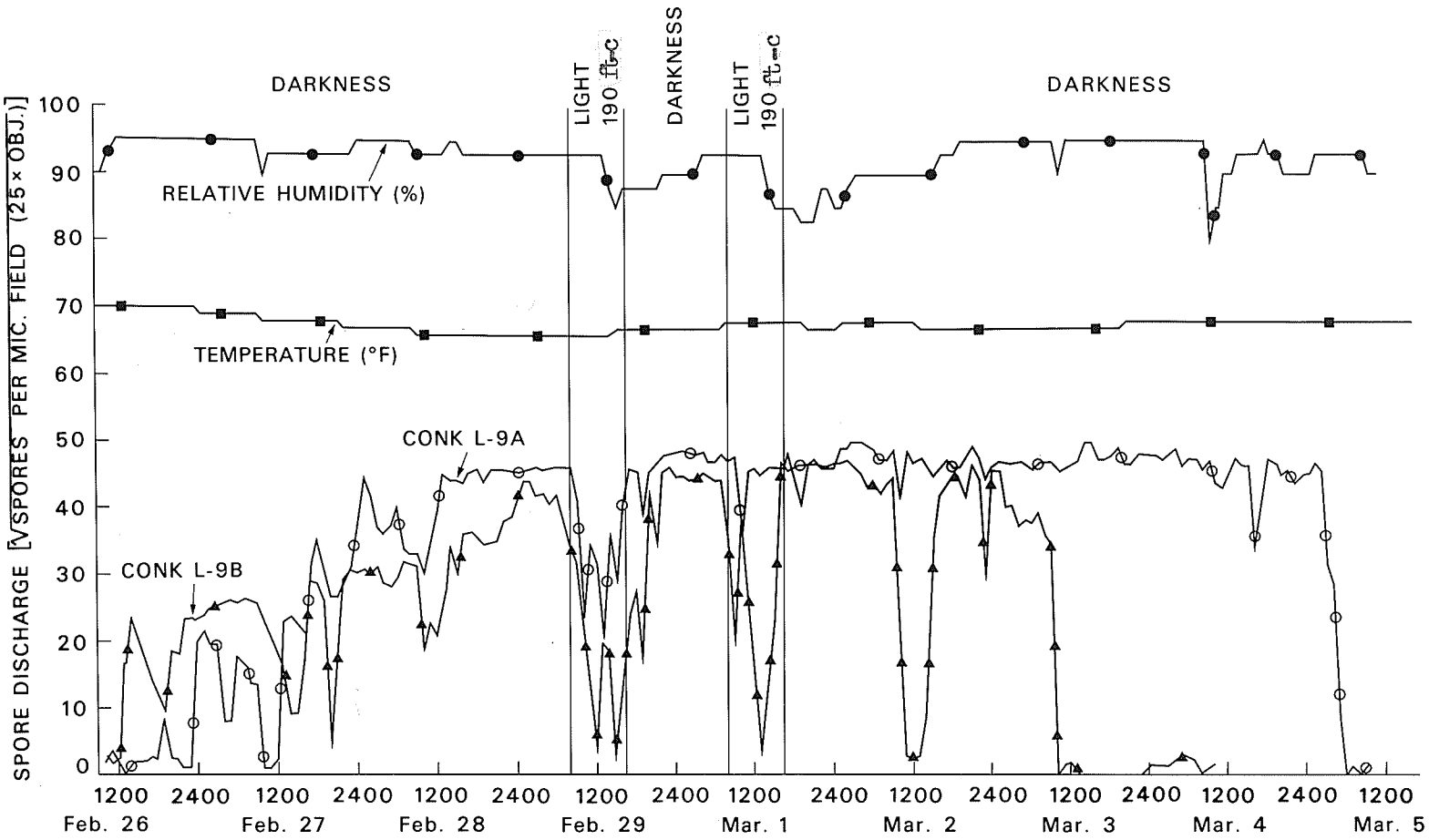

SPORE DISCHARGE [VSPORES PER MIC. FIELD (25× OBJ.)]
100
90
80
70
60
50
40
30
20
10
0
DARKNESS
LIGHT 120 ft-c
DARKNESS
LIGHT 120 ft-c
DARKNESS
LIGHT 120 ft-c
DARKNESS
LIGHT 120 ft-c
DARKNESS
LIGHT 120 ft-c
DARKNESS
LIGHT 120 ft-c
DARKNESS
RELATIVE HUMIDITY (%)
TEMPERATURE (°F)
CONK 8
CONK 13
1200
2400
June 12
June 13
June 14
June 15
June 16
June 17
June 18
June 19
June 20

rate of spore discharge after exposure to low R.H. (60%) for four hours, and appeared thereafter to be more sensitive to light intensity of 120 ft-c.

Sporophore L-9 was a healthy sporophore with a firm white margin and a steeply inclined white pore layer with uniformly spaced pores. Excepting 48 hours for recovery from storage, spore discharge rates reached a high level under optimal conditions of darkness, high R.H., and a constant temperature of 60 to 70F (Fig. 17). Both sides of sporophore L-9 were sensitive to light intensity of 190 ft-c. When light intensity of 190ft-c was introduced at 0900 hours on February 29, the spore discharge rate of sporophore L-9B dropped from about 900 spores per field to approximately 10 to 15 spores per field by 1200 hours, even though neither R.H. nor temperature had changed. A similar reduction occurred with sporophore L-9A. A reduction in spore dischare rate also occurred with sporophore L-9B when light was introduced on March 1. The spore discharge rate dropped from approximately 1800 spores per microscope field to about zero per field before the R.H. dropped at all. R.H. probably had no influence on the reduction in spore discharge rates on March 1, since the R.H. was still dropping slightly at 1530 hours when the lights were turned off, and the spore discharge rate had returned to its high level by this time.

It is interesting that the spore discharge rate of side B of sporophore L-9 was reduced to the same low level on each of the next two days after March 1, even though light was

Fig. 17. The relationship between light (190ft-c) and spore discharge rates by two sides, A and B, of sporophore L-9 at constant temperature and high R.H. in the laboratory.


DARKNESS
LIGHT 190 ft-c
DARKNESS
LIGHT 190 ft-c
DARKNESS
RELATIVE HUMIDITY (%)
TEMPERATURE (°F)
CONK L-9A
CONK L-9B
SPORE DISCHARGE [√SPORES PER MIC. FIELD (25× OBJ.)]
100
90
80
70
60
50
40
30
20
10
0
1200 2400 1200 2400 1200 2400 1200 2400 1200 2400 1200 2400 1200 2400 1200 2400 1200
Feb. 26
Feb. 27
Feb. 28
Feb. 29
Mar. 1
Mar. 2
Mar. 3
Mar. 4
Mar. 5

not applied. This was the only example throughout this study of an apparently conditioned rhythm of spore discharge persisting beyond the existence of the conditioning factors. The majority of the sporophores of P. tomentosus exhibited periodicities of spore discharge which were completely exogenous and dependent on environmental factors.

The spore discharge rates of sporophores 11 and 14 were reduced by illumination by light of 760 ft-c intensity (Fig. 18). The light also appeared to retard recovery of the sporophores, since they did not resume high rates of spore discharge until after two to three hours in darkness.

Interactions

The effects of interacting factors on spore discharge rates can be seen in figures 19 to 21, showing studies which were all undertaken in the field. In 1968, tent C (Fig. 19) allowed examination of the interactions between temperature and light where these factors were undergoing natural fluctuations, although light quality and intensity were slightly altered due to passage through plastic. R.H. was held continuously at the saturation level. Until August 28, discharge rates were positively correlated with temperature on only four days and not the same four days for each sporophore (Table IV). After August 28, the spore discharge pattern was altered with high discharge rates occurring at low as well as high temperatures. Comparison of these data with

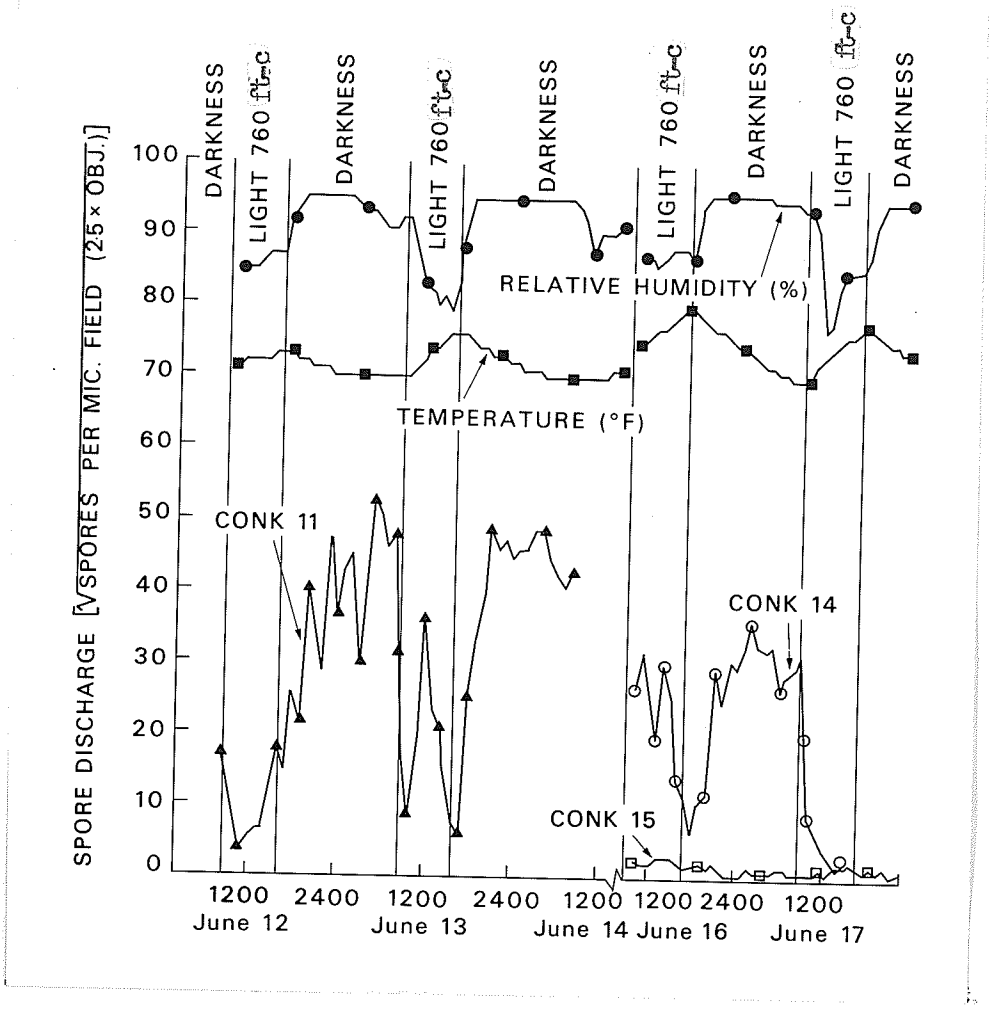


Fig. 18. The relationship between light (760 ft-c) and spore discharge rates by sporophores 11, 14, and 15, at high R.H. and slightly fluctuating temperature in the laboratory.

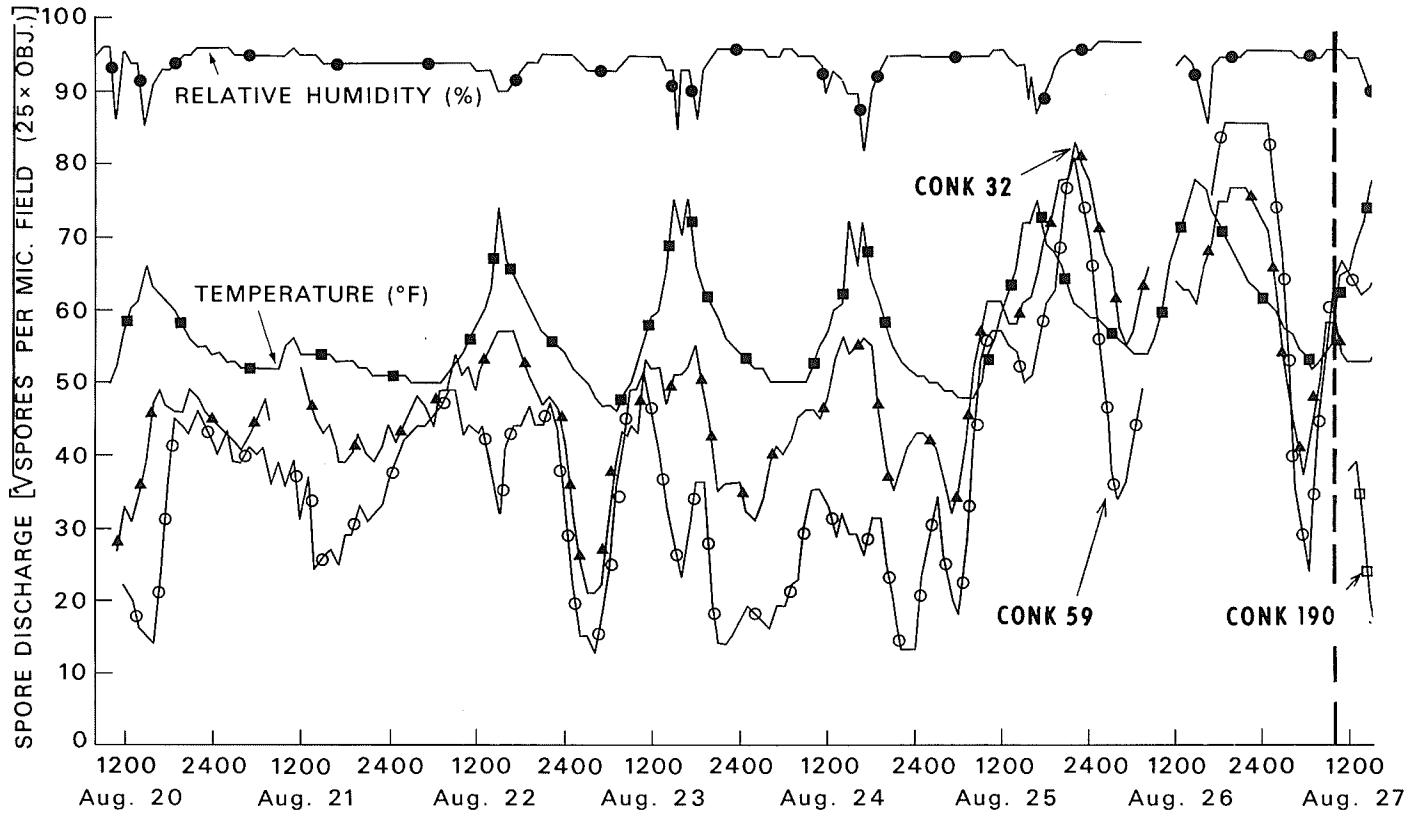


Fig. 19. The relationship between fluctuating temperature and light, and spore discharge rates by sporophores 32, 59, and 190, maintained at high R.H. in the field (treatment C, 1968).

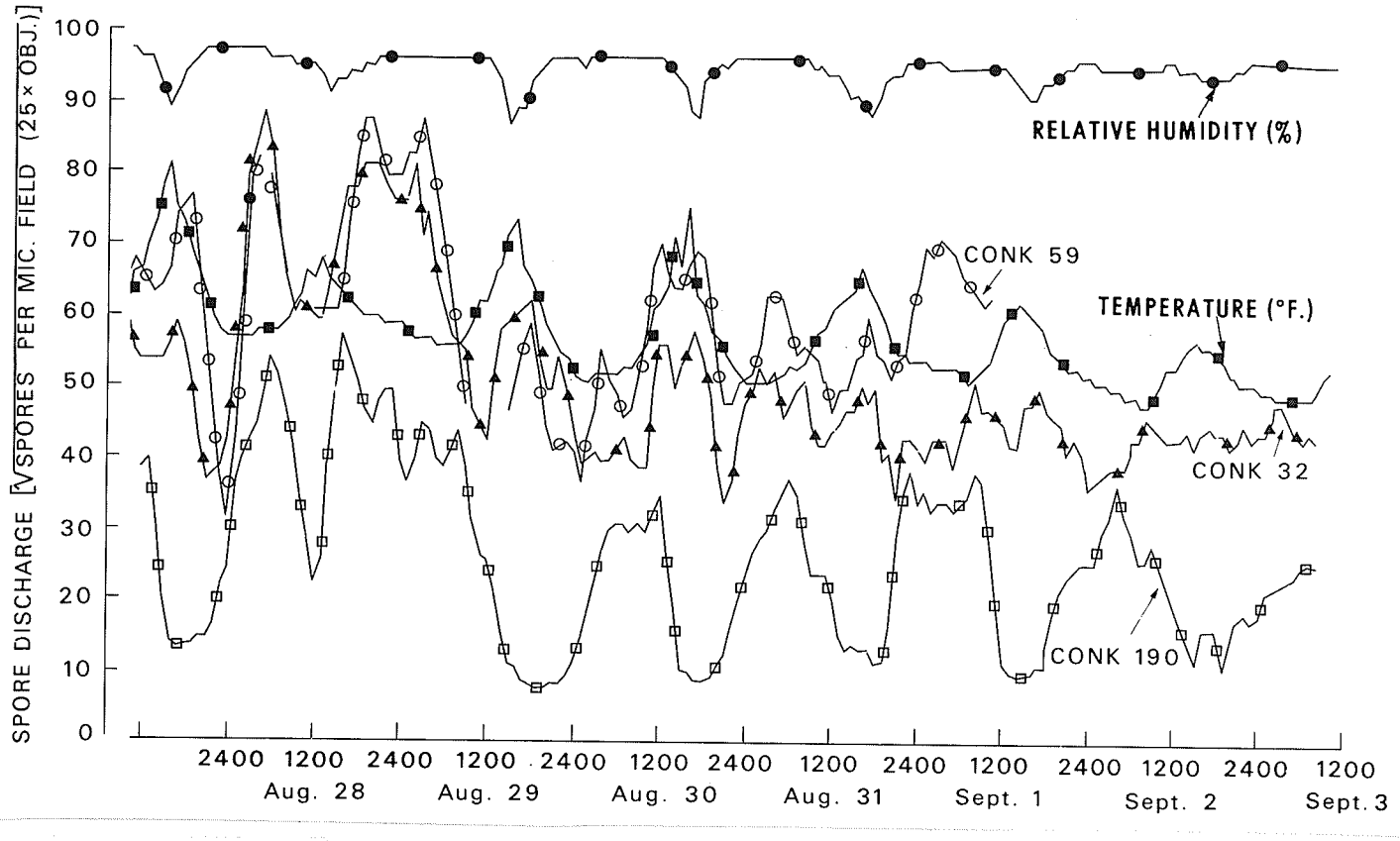


Fig. 19. Continued.

Table IV. Correlation between temperature and spore discharge by sporophores of P. tomentosus in the field, in an enclosure allowing natural fluctuations of temperature and light (treatment C, 1968). (See Fig. 19)

Sporophore	Readings From	To	Date	df	Correlation Coefficient
32	0100 hrs.	2400 hrs.	Aug.22	23	0.531**
32	0100 hrs.	2400 hrs.	Aug.23	23	0.595**
32	0100 hrs.	2400 hrs.	Aug.24	23	0.628**
32	0100 hrs.	2400 hrs.	Aug.25	23	0.605**
32	0100 hrs.	2400 hrs.	Aug.26	22	0.205
32	0100 hrs.	2400 hrs.	Aug.27	11	0.460
59	0100 hrs.	2400 hrs.	Aug.22	23	0.202
59	0100 hrs.	2400 hrs.	Aug.23	23	0.061
59	0100 hrs.	2400 hrs.	Aug.24	23	0.467*
59	0100 hrs.	2400 hrs.	Aug.25	23	0.567**
59	0100 hrs.	2400 hrs.	Aug.26	17	0.694**
59	0100 hrs.	2400 hrs.	Aug.27	11	0.697**

** Significant at the 1% level.
* Significant at the 5% level.

spore discharge rates from sporophores in tents B and D of 1968 (Figs. 11 and 12), where conditions were essentially the same except the sporophores in the latter two tents were in continuous darkness, revealed greater variation in periodicity of spore discharge rates in the light. It might be concluded that the introduction of light changed the pattern of spore discharge in tent C. In 1969, a similar treatment (the first half of the collection period in tent B) produced the same results, although levels of spore discharge were lower.

In tent C of 1968 (Fig. 19), spot light readings on cloudy overcast days (which included the entire days on August 20, 21, 31, September 1, 2, and 3; the mornings of August 22, 23, and 24; and the afternoons of August 26, 28, and 29) showed that the sporophores were exposed to light intensities of from 100 to 250 ft-c. During periods of sunlight, intensities from 250 to 2200 ft-c were recorded. An even rate of spore discharge was shown by sporophore 32 on the days with total cloud cover which might have been due to low light levels and the slighter than normal fluctuations in temperature. August 25 and 26 were the only entirely sunny days and, on both these days, the sporophores showed a drop in spore discharge rate commencing at approximately 1200 hours, followed by a peak in spore discharge which lagged approximately four hours behind the temperature peak. The mornings of August 26, 28, and 29 were sunny, and spore discharge peaks lagged behind temperature peaks on August 26 and

28, while a depression in spore discharge was observed at noon on August 28 and 29. On the three days with sunny afternoons only, (August 22, 23, and 24), a depression or lag in the spore discharge rate was not apparent. It appears that the effect of natural light added to a natural temperature regime was to delay by 4 to 6 hours the maximum spore discharge rate which coincides with the temperature peak in darkness. On cloudy days, light effects were negligible.

The spore discharge pattern of sporophore 190 was almost directly opposite to that produced by other sporophores in tent C (Fig. 19). Since the drop in spore discharge rate began shortly before or after mid-day and continued into the afternoon, it might be concluded that this sporophore was particularly sensitive to light. It consistently exhibited this behaviour throughout the period of observation.

Figure 20 depicts spore discharge rates from sporophores to which light was applied during the period of temperature increase, 0700 to 1900 hours. Although high R.H. and increasing temperature induced a high rate of spore discharge in p previous studies in darkness, exposure to light while other conditions were favorable resulted in depressed spore discharge rates. Maximum discharge occurred in periods of darkness and minimum discharge in the light periods.

Maximum discharge rates also occurred in dark periods in tent C of 1969 from August 22 to 26 (Fig. 21), in which light was applied while the temperature was rising (0700 to

1

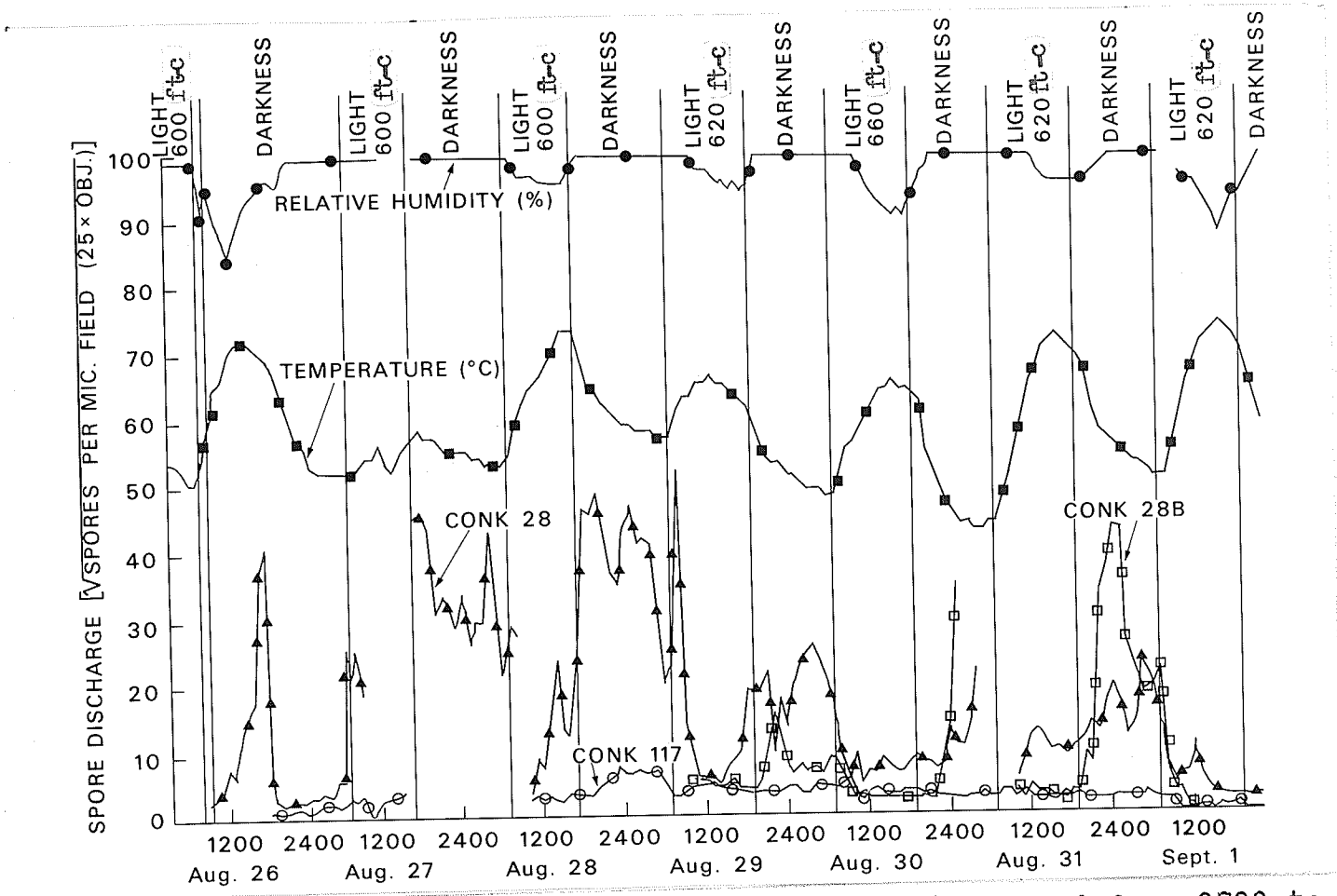


Fig. 20. The relationship between light (600 ft-c) applied from 0700 to 1900 hours and spore discharge rates by sporophore 117 and by opposite sides, A and B, of sporophore 28 at high R.H. and naturally fluctuating temperature in the field.

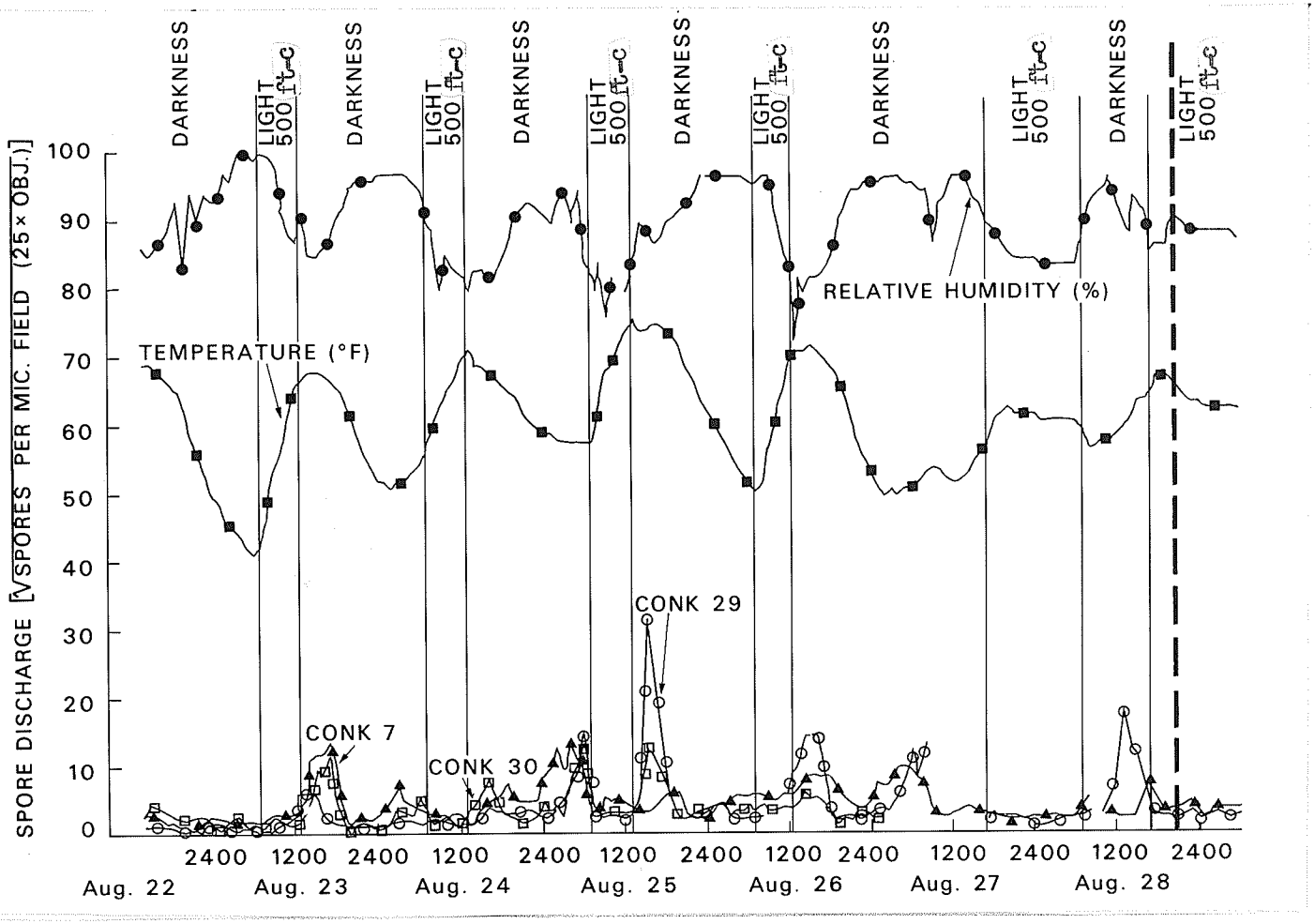


Fig. 21. The relationship between light (500 ft-c) applied from 0700 to 1300 hours and from 1900 to 0700 hours, and spore discharge rates by sporophores 7, 29, and 30 at high R.H. and naturally fluctuating temperature in the field.

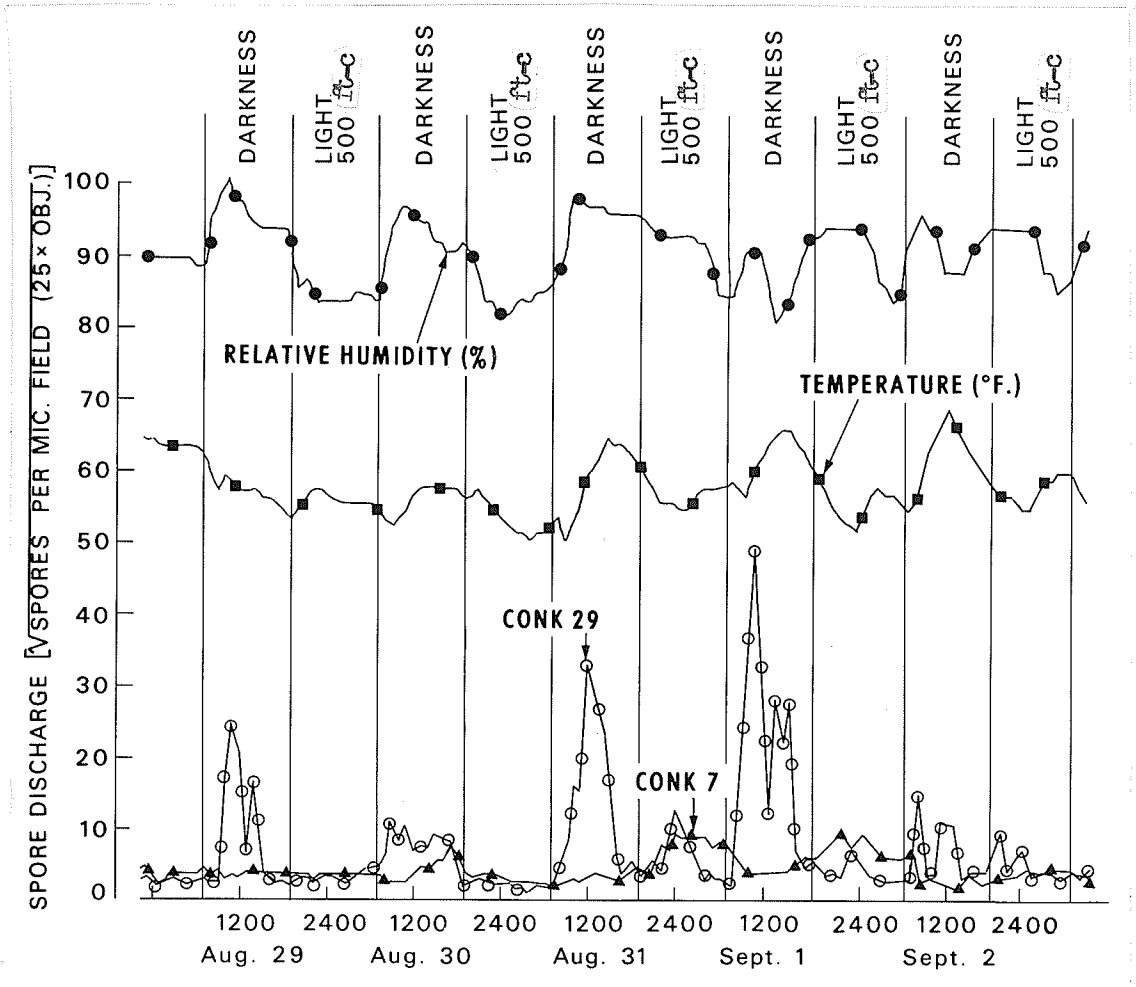


Fig. 21. Continued.

1300 hours), and the R.H. was favorably high. Spore discharge rates were consistently lower during the six-hour periods of light application. A rise in spore discharge rate occurred immediately after the lights were turned off, although the discharge rate was high only briefly, possibly due to the low total potential for high spore discharge rates which was typical of all sporophores examined in 1969, and possibly due to the falling temperature. From August 27 onwards, darkness was maintained during natural daylight hours (0700 to 1900 hours). Sporophore 29 discharged spores during these periods of darkness showing that reversal of the natural light cycle can reverse the spore discharge cycle.

Spore discharge under natural conditions.

Figure 22 shows the spore discharge rates of sporophores maintained under naturally fluctuating conditions in 1968 (tent A). From August 22 to 29, an even periodicity was exhibited by four sporophores, in which maximum spore discharge occurred from 4 to 8 hours after the temperature had reached a peak. The R.H. had been rising from its lowest point for a similar period. Arrival of maximum spore discharge was delayed the most on August 25, during which clear skies prevailed, and on August 26, which was clear only in the morning. On August 27, which was also a completely clear day, the onset of the peak was not as delayed as on August 25, but a secondary peak occurred approximately 6 hours after the initial

Fig. 22. The relationship between naturally fluctuating light, temperature, and R.H. , and spore discharge rates by sporophores 24, 25, 26, and 88 in the field (tent A, 1968).

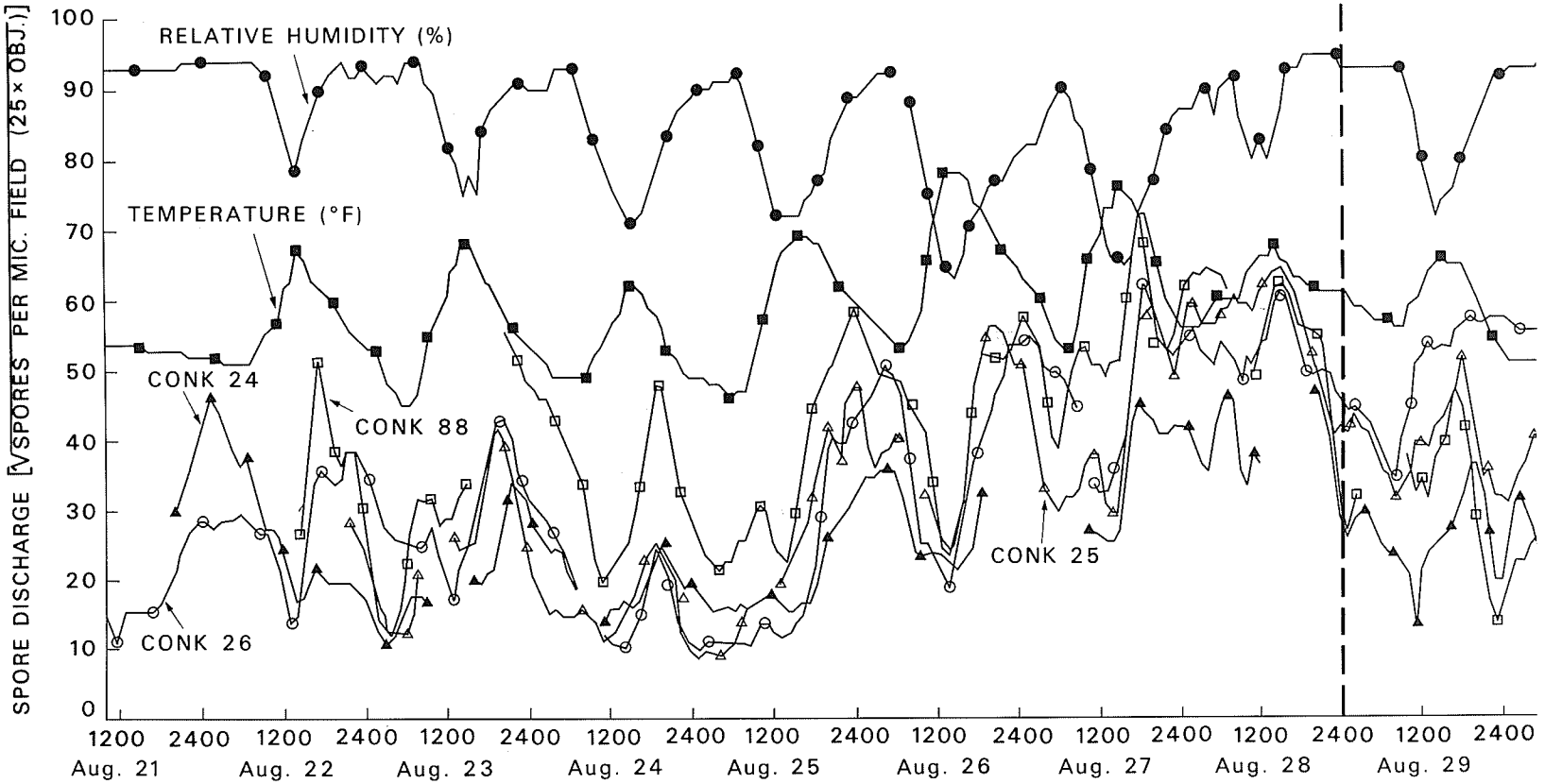
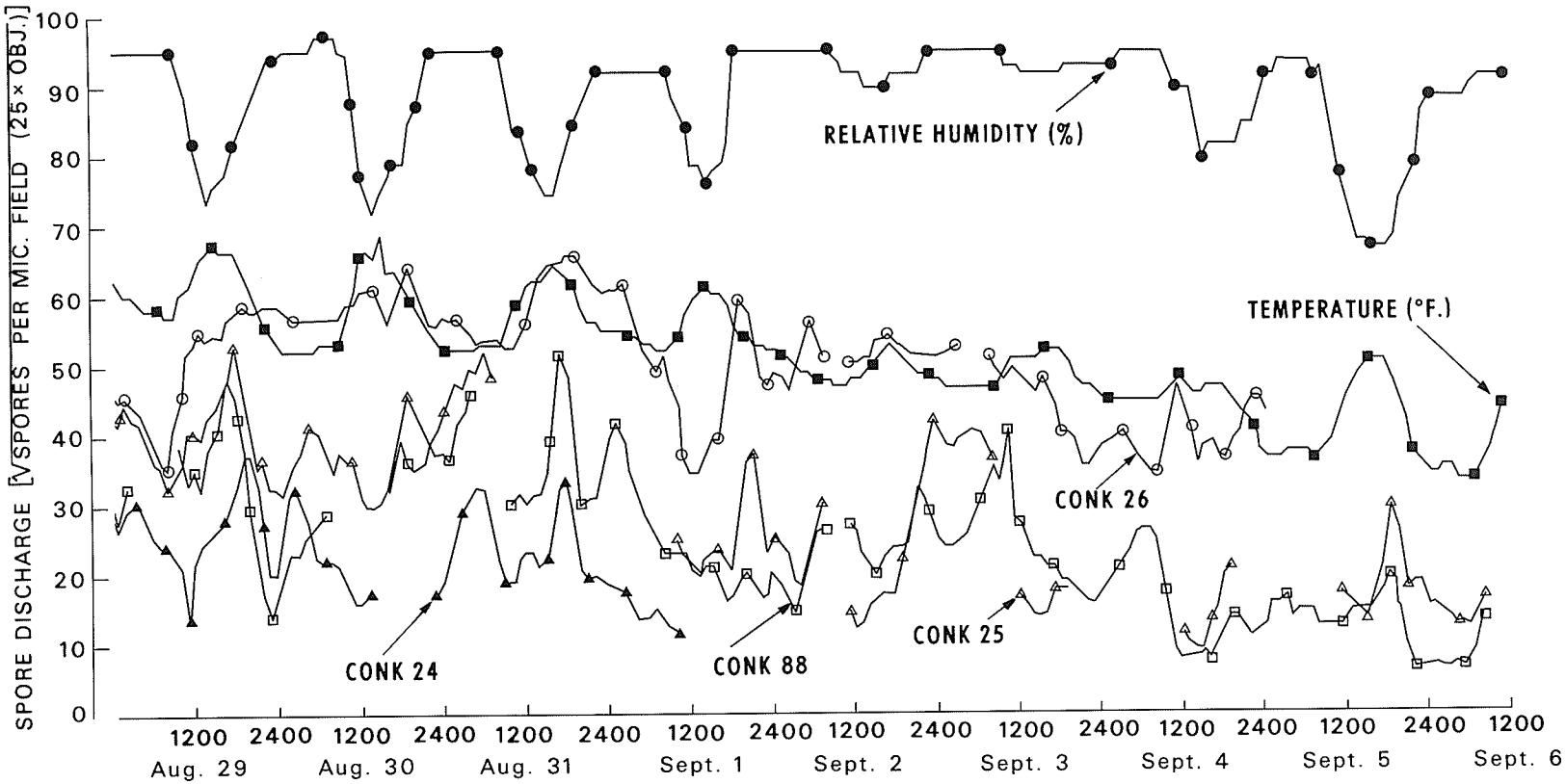

SPORE DISCHARGE [VSPORES PER MIC. FIELD (25× OBJ.)]
100
90
80
70
60
50
40
30
20
10
0
RELATIVE HUMIDITY (%)
TEMPERATURE (°F)
CONK 24
CONK 88
CONK 25
CONK 26
1200 2400 1200 2400 1200 2400 1200 2400 1200 2400 1200 2400 1200 2400 1200 2400 1200 2400
Aug. 21 Aug. 22 Aug. 23 Aug. 24 Aug. 25 Aug. 26 Aug. 27 Aug. 28 Aug. 29

Fig. 22. Continued.


SPORE DISCHARGE [VSPORES PER MIC. FIELD (25× OBJ.)]
100
90
80
70
60
50
40
30
20
10
0
RELATIVE HUMIDITY (%)
TEMPERATURE (°F.)
CONK 24
CONK 88
CONK 25
CONK 26
1200 2400 1200 2400 1200 2400 1200 2400 1200 2400 1200 2400 1200 2400 1200 2400 1200
Aug. 29
Aug. 30
Aug. 31
Sept. 1
Sept. 2
Sept. 3
Sept. 4
Sept. 5
Sept. 6

peak. On August 22 to 24, cloud cover prevailed in the morning and the spore discharge peak was not as delayed on these days as on the sunny days. Beginning on the afternoon of August 29, there was a continuous period of overcast days which ended on September 6. During this time, the periodicity of spore discharge was disrupted. Light levels were so low as to be ineffective in influencing most sporophores and temperature amplitudes were greatly reduced.

The same periodicity that was exhibited by sporophores from August 22 to 29 in 1968, was also observed for sporophores in 1969 for 6 days. These sporophores were similarly examined under natural conditions and periodicity occurred even though the spore discharge rates in 1969 were generally much lower than those in 1968.

Variations and peculiarities of spore discharge.

Certain phenomena were observed during the study which were not related to technical problems and which were important to the interpretation of data on spore discharge rates.

Unaccountable variations in spore discharge rates.

Figures 23 and 24 show two examples of variation in spore discharge rates which was unrelated to any of the factors examined. The constant levels of light, temperature, and R.H. shown in figures 23 and 24 usually elicited a constant spore discharge rate from the majority of sporophores studied

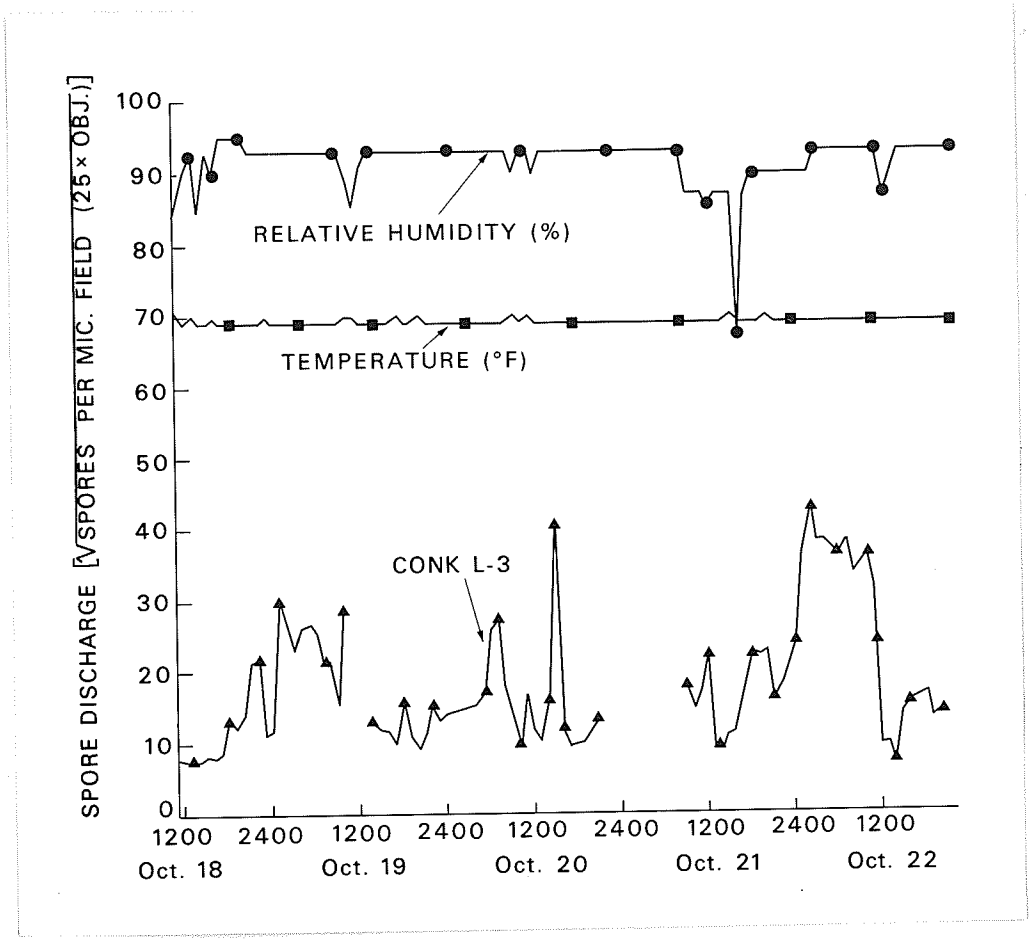
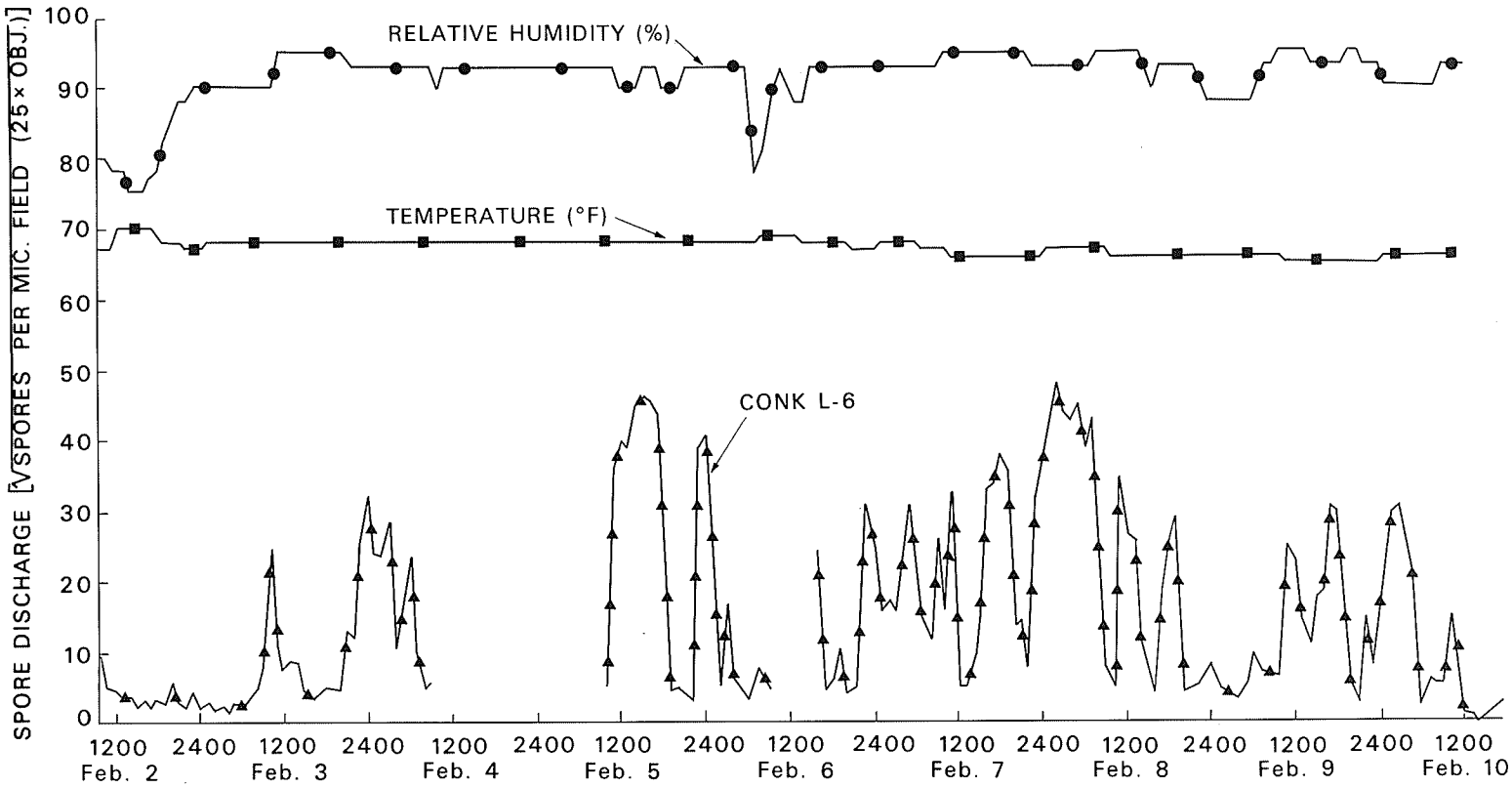


Fig. 23. Unaccountable variation in spore discharge rates by sporophore L-3 in darkness at high R.H. and constant temperature in the laboratory.

Fig. 24. Unaccountable variation in spore discharge rates by sporophore L-6 in darkness at high R.H. and constant temperature in the laboratory.


RELATIVE HUMIDITY (%)
TEMPERATURE (°F)
CONK L-6
SPORE DISCHARGE [√SPORES PER MIC. FIELD (25 × OBJ.)]
100
90
80
70
60
50
40
30
20
10
0
1200 2400 1200 2400 1200 2400 1200 2400 1200 2400 1200 2400 1200 2400 1200 2400 1200
Feb. 2
Feb. 3
Feb. 4
Feb. 5
Feb. 6
Feb. 7
Feb. 8
Feb. 9
Feb. 10

(e.g. portions of figures 9, 11, 16, and 17). The phenomenon was noted early in the study to be comparatively rare and it was compensated for by sampling at least two sporophores in each experiment, and even two sides of one sporophore in some experiments.

Variations in spore discharge rates from different areas of the hymenium.

The sampling equipment used in this study necessitated choosing a mature area of the hymenium which was discharging spores. The response of the area chosen may not have been representative of the total response of the sporophore and this was investigated by sampling two sides of a single sporophore. Examples are shown in figures 9, 13, 17, and 20. The spore discharge rates from two sides of a sporophore were generally similar (e.g. Fig. 9). However, differences were observed; for example, side B of sporophore L-10 ceased discharging spores two days before side A ceased (Fig. 13). This behaviour was also observed in sporophore L-9 (Fig. 17) when two sides were compared. Observations of the hymenial surface showed that parts of the pore layer were mature when other parts had not yet matures or had already ceased discharging spores. Also, some parts of the hymenial surface showed a more rapid rate of exhaustion than others. Hymenial variation was more evident in the laboratory than the field, possibly because the sporophores used in laboratory exper-

iments had been subjected to low storage temperatures of 2 to 6C, during which time slow hymenial development was observed. In the present investigation, where the objective was to examine the general response of the fungus, it was felt that hymenial variation was compensated for by sampling large numbers of sporophores (approximately 200). Further compensation was afforded by basing conslusions on data that were consistent over at least a 2 or 3 day period for any treatment.

Stages of spore discharge: maturation, exhaustion and cessation.

Frequent observations of P. tomentosus showed that the spore discharge rate was not affected by environmental factors while the sporophore was maturing, and the rate increased gradually, but steadily, until the sporophore was completely mature. Even when optimal environmental conditions prevailed, the sporophore did not discharge spores at a maximum rate during the period of maturation. The sporophore required from 2 to 4 days from the onset of spore discharge to reach maximum spore discharge rates.

A period of only 1 to 2 days was needed to reach maximum spore discharge rates by sporophores studied in the laboratory. This was distinguished from the maturation period shown by sporophores in the field since, in the former, the sporophores were already mature when experimentation was begun. Therefore, the initial lag in spore discharge rates in sporo-

phores in the laboratory was not due to a developmental stage, but rather to an adjustment period to new environmental conditions.

A second phenomenon observed during spore discharge studies was the cyclic periods of exhaustion exhibited by most healthy sporophores. An individual sporophore did not always produce spores at the same rate under the same optimal conditions for spore discharge. The different rates could be due to temporary exhaustion of the sporophore. Differences in discharge rates by sporophores in tent B, 1968, were observed (Fig. 12), where the highest rate of spore discharge changed from day to day under identical environmental conditions. It appeared that after a day or two of rapid spore discharge, the sporophore was unable to respond as well on the third or fourth day, and it may not recover until a day or two later. The phenomenon might also partially explain why sporophores in the laboratory did not discharge spores for as long periods as those in the field. Sporophores in the laboratory were exposed to constant conditions, and when these conditions were optimal for high spore discharge rates, the sporophore might have produced spores at a high rate for several days continuously leading to a rapid and possibly permanent cessation of spore discharge. Sporophores in the field were exposed to at least one naturally fluctuating factor and they possibly did not exhaust rapidly because the fluctuating factor(s) meant that at least part of

a 24-hour period would not be suitable for spore discharge. During these periods of less suitable environmental conditions, the sporophore might 'rest'.

In the overmature stage (usually lasting 1 to 2 days) which leads to total cessation of spore discharge, the discharge rate was again uninfluenced by the environment as in the period of maturation of the sporophore. Total cessation was easily distinguished from temporary exhaustion, since in the former case, the hymenial surface became dark brown and the pores coalesced. Within 24 to 48 hours, the sporophore was infested by insects or invaded by micro-organisms; rapid decay followed.

Spore deposition patterns.

Over one hundred cardboard sheets with affixed exposed coverslips were prepared as shown in figures 25 and 26, and many examples of a changing spore deposition pattern were observed. It appears that the 18 mm. square hymenial portion being sampled in figure 25 contained a central oblique strip from which spore discharge was especially heavy under optimal conditions over a 3 to 4 day period. The changes in the deposition patterns over hourly intervals could not be correlated with environmental influences, nor was the pattern consistent from day to day at the same hours.

The gradual shift in spore deposition patterns suggests that air currents resulting from any environmental change

Fig. 25. The changing spore deposition pattern of sporophore 39 from August 24 to August 31 in darkness at high R.H. and naturally fluctuating temperature (treatment D, 1968). Spore discharge rates are shown in Fig. 14. Each coverslip represents one hour's deposit and follows in order from left to right in each row and from top to bottom.

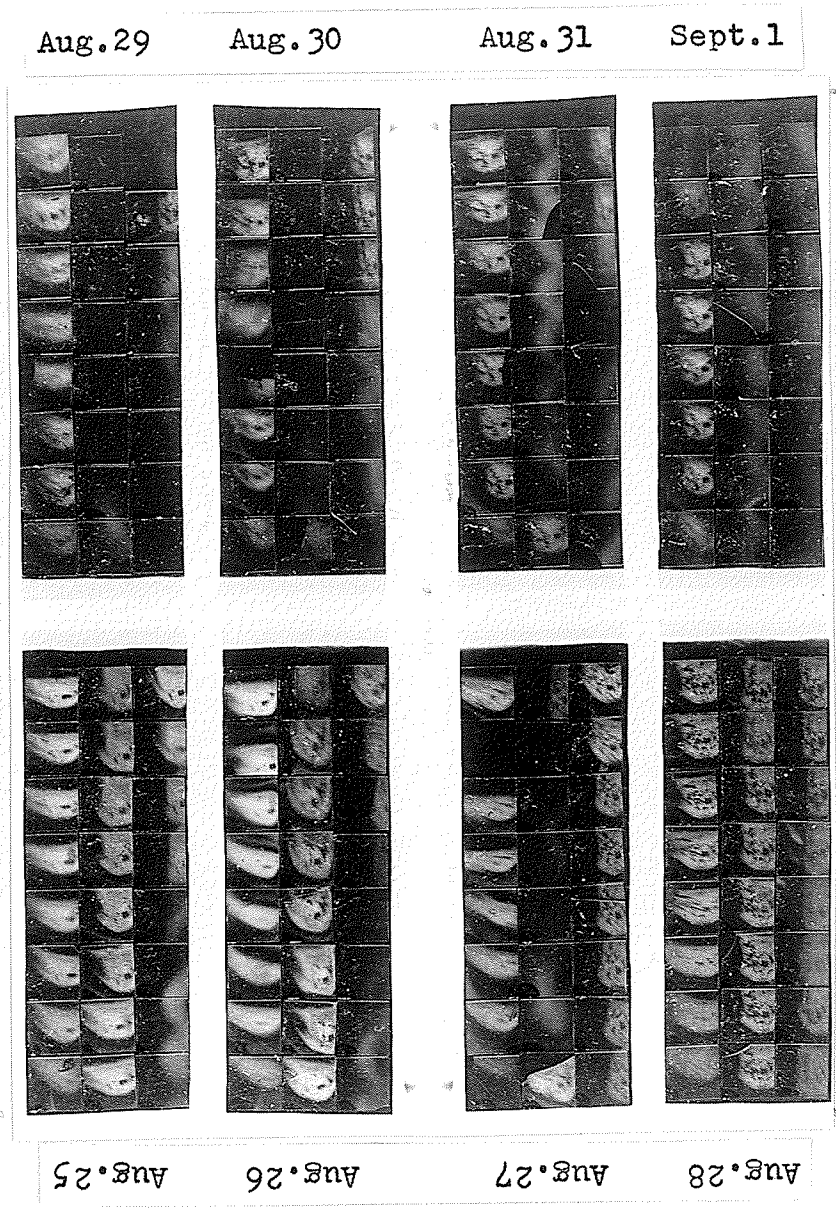


Fig. 26. The changing spore deposition pattern of sporophore 102 from August 25 to September 1 at high R.H. and naturally fluctuating temperature (treatment D, 1968). Spore discharge rates are shown in Fig. 14. Each coverslip represents one hour's deposit and follows in order from left to right in each row and from top to bottom.

were not the cause, since they would result in much more abrupt changes that would not be uniform from hour to hour. Slight air movements may have been present in some of the enclosures due to natural fluctuation in temperature and R.H. However, it was purposely attempted to eliminate air movement by building small air-tight enclosures. The changes in spore deposition patterns were not correlated with periods when there would be air circulation due to movements by the investigator during daily examinations which seldom lasted more than ten minutes nor were they correlated with the movement of the collecting disc of the spore sampler. Neither of the sporophores whose spore deposition patterns are shown in figures 25 and 26 had been placed under artificial light and therefore heat emission from instruments was not a likely cause of the variation.

Other evidence supporting a developmental change in the hymenial layer as the cause of the variation is the difference in spore discharge rates by two sides of the same sporophore during the same time interval and the phases of spore discharge rate due to the developmental stage of the sporophore as described in the previous section.

Spore discharge in relation to moisture content of the sporophore and the soil.

Spore discharge rates by sporophores of P. tomentosus in the field were significantly higher in 1968 than in 1969. Sporophore moisture content was evidently not the reason for

this difference, since there was no significant difference in the sporophore moisture contents in 1968 and 1969 (Table V). Although there was greater variation in the sporophore moisture content in 1969 between plots, the spore discharge rate was the same in all plots, further evidence that the sporophore moisture content does not determine spore discharge rates.

In figures 27 and 28, sporophore moisture content and graded spore discharge rates are plotted on a scatter diagram. The graded spore discharge values were determined from the average hourly spore discharge rates per day, and the relationship is as follows;

Grade	Average hourly number of spores per microscope field per day
1	1-50
2	51-100
3	101-200
4	201-500
5	501-1,000
6	1,001-2,000
7	2,001-3,000
8	3,001-4,000
9	4,001-5,000
10	5,000+

In 1968 and 1969, a sporophore moisture content of less than 180% was unsuitable for a high rate of spore discharge (grade 3 or better). Above 180% moisture content, spore discharge rates were not correlated with sporophore moisture content.

The range in values of soil moisture contents is shown for each plot in 1968 and 1969 in Table VI. Scatter diagrams

Table V. The average daily sporophore moisture content of *P. tomentosus* per plot in 1968 and 1969.

Year	Plot	Average Sporophore Moisture Content
1968	A	321.2
1968	B	472.4
1968	C	580.8
1968	D	381.0
Average		438.8***
1969	A	128.1
1969	B	1460.6
1969	C	1137.3
1969	D	565.7
Average		822.9

*** The averages are not significantly different (5% level) using the student's t-test (df=6).

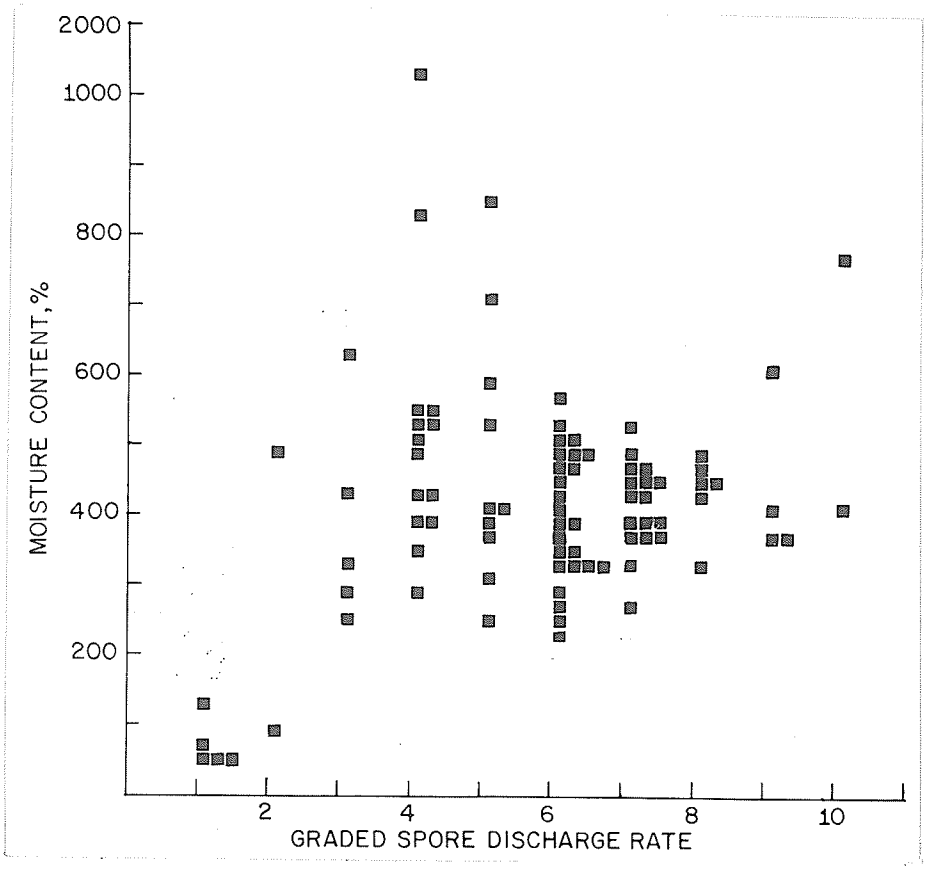


Fig. 27. The average hourly graded spore discharge rate per day versus the sporophore moisture content in the same day, 1968.

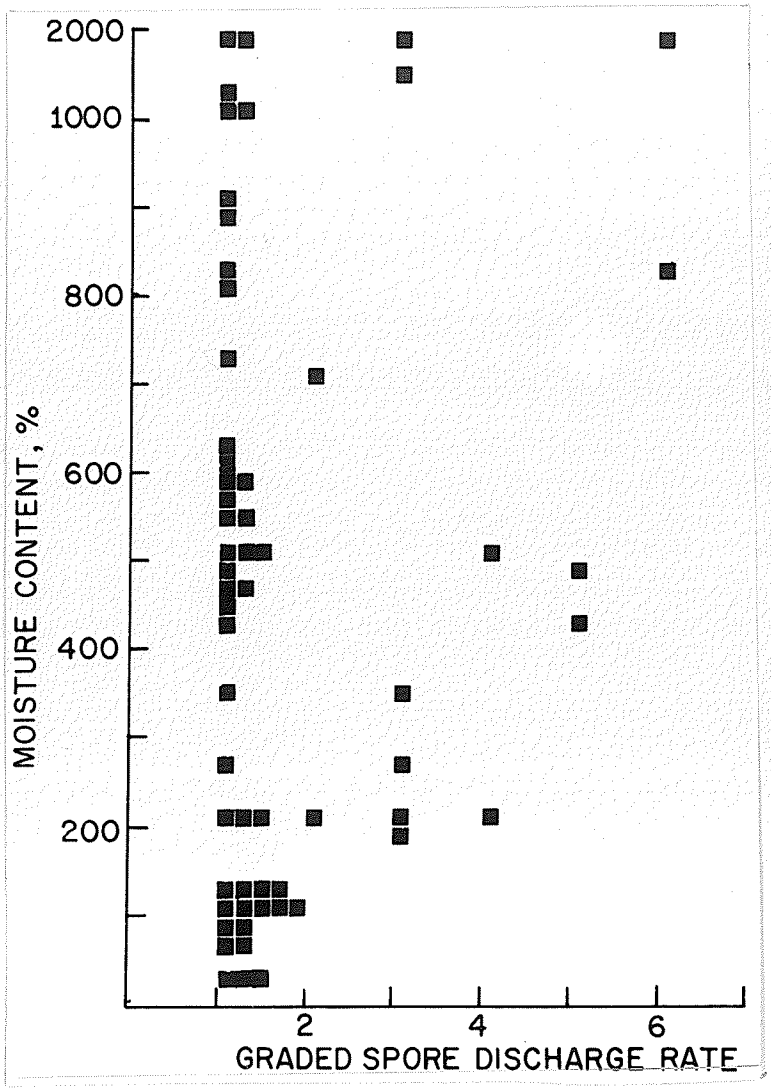


Fig. 28. The average hourly graded spore discharge rate per day versus the sporophore moisture content in the same day, 1969.

Table VI. The average daily moisture content of humus and mineral soil horizons in each plot in 1968 and 1969.

Year	Plot	Average Moisture Content		
		Humus	A horizon	B horizon
1968	A	136.3	11.8	10.4
1968	B	91.6	11.0	9.2
1968	C	132.5	11.4	8.3
1968	D	155.0	8.4	6.8
Average		128.8	10.6	8.6*
1969	A	62.4	6.4	9.1
1969	B	133.6	13.0	11.7
1969	C	143.0	17.7	13.3
1969	D	149.8	16.4	13.8
Average		122.2	13.3	11.9

* Significant at the 5% level (using student's t-test).

revealed no relationship between spore discharge rates and soil moisture contents. In 1968, plot B had the lowest humus moisture content due to lack of watering in an attempt to lower the R.H. Nevertheless, the average hourly spore discharge rates still compared favorably with other plots. There was no significant difference in the moisture content of the humus or the A horizon in 1968 and 1969. There was a significant difference in the moisture content of the B horizon in 1968 and 1969, 1969 having a higher average moisture content. This may have been due to nine days of freezing temperatures in June and the low average minimum temperatures in June and July. This might have caused the lower mineral horizon to thaw less rapidly and retain moisture longer.

Spore discharge in relation to sporophore size and growth rate.

Spore discharge rates were significantly different in 1968 and 1969, sporophores in 1969 discharging spores at a consistently lower rate (Table VII). The final average sporophore size per plot was not significantly different in 1968 and 1969, nor was there a significant difference between these years when the total growth rates and growth rates during collection only were compared (Table VII). The growth rate during collection was consistently lower than the total growth rate indicating that the sporophore does not expand rapidly once it has matured and is discharging spores. Apparently however, the differences in spore discharge rates be-

Table VII. The size, growth rate, growth rate during collection, and the graded spore discharge rate of sporophores of P. tomentosus in 1968 and 1969.

Year	Plot	Sporophore	Mature Size Diam. (cm.)	Average Growth Rate (mm.)/day	Growth Rate During Collection (mm.)/day	Average Graded Spore Discharge Rate/day
1968	A	24	10.2	1.6	0.4	4.2
		25	6.9	1.1	0.8	4.2
		26	6.8	1.3	0.4	5.8
		86	6.0	0.8	1.7	2.6
		88	7.1	0.9	0.06	5.4
1968	B	27	8.0	1.8	1.9	6.7
		30	10.2	1.9	1.2	6.5
1968	C	32	8.7	1.7	1.1	6.6
		60	8.4	2.1	1.7	3.9
		59	8.6	1.8	1.5	7.0
		190	8.6	2.2	0.4	5.0
1968	D	39	7.1	1.5	0.9	6.4
Average -1968			7.8	1.5	0.9	5.4**
1969	A	166	4.6	2.2	0.06	1.0
		73	4.8	1.0	0.0	1.2
		167	6.6	1.9	0.1	2.0
1969	B	76	13.0	3.8	5.2	1.9
		107	4.6	1.2	0.0	3.1
		16	8.6	1.9	2.7	2.7
1969	C	29	5.6	1.4	0.5	1.4
		30	5.0	1.2	0.3	1.0
		7	9.1	2.0	0.1	1.0
1969	D	19	3.8	0.9	0.0	1.0
		117	8.0	2.5	2.0	1.0
		28	9.3	1.4	0.8	2.6
Average -1969			6.5	1.7	0.9	1.6

**Significant at the 1% level (using the student's t-test).

tween years were not due to either sporophore growth rates or mature sporophore size.

Numbers of sporophores in 1963 to 1969, and relation to temperature and rainfall.

Information on the numbers of sporophores appearing in the summers of 1963 to 1966 was supplied by R.D. Whitney. Sporophores were observed on approximately July 15 at the earliest every season and approximately October 1 at the latest. Few searches were made after this date, although sporophores appeared in such low numbers during September, that it may be assumed that the numbers presented adequately represent the total crop in the area studied. Data on rainfall and temperature were obtained from the monthly records of the Meteorological Branch of the Department of Transport and the readings were taken at Prince Albert, Saskatchewan, which is approximately 50 miles southwest of the study area. The data described above are shown in Tables VIII and IX.

The number of sporophores appearing each year appears to be partially dependent on the amount of rainfall both during and preceding the period when the sporophores are developing (Table VIII). Although 1969 produced a large number of sporophores, the quality of these sporophores was poor, and this was probably partially due to the low total amount of rainfall. The number of days of freezing also appears to have an influence on the number of sporophores which

Table VIII. 1963-1969. Total number of sporophores of P. tomentosus per year and monthly rainfall.

Year	No. of Sporophores	Mar.	April	May	June	July	Aug.	Total
1963	363	0.77	1.24	0.78	4.45	3.95	1.51	12.70
1964	56	0.30	0.11	0.57	0.16	1.55	2.69	4.38
1965	270	0.19	0.38	1.13	3.22	2.01	3.12	10.05
1966	349	1.05	0.38	0.16	4.37	3.46	3.03	12.45
1967	100	0.70	0.36	0.10	1.02	2.77	1.23	6.18
1968	353	0.55	0.68	3.66	1.77	3.64	3.44	13.74
1969	245	0.28	0.38	0.64	0.73	3.40	0.87	6.30

Table IX. Total number of sporophores of P. tomentosus per year and average daily maximum and minimum temperatures (F), 1963-1969.

Year	No. of Sporo-phores	MARCH Min.	MARCH Max.	MARCH Days below 32F	APRIL Min.	APRIL Max.	APRIL Days below 32F	MAY Min.	MAY Max.	MAY Days below 32F
1963	363	7.3	29.7	31	25.7	49.4	27	33.6	62.4	13
1964	56	-9.3	17.1	31	26.4	50.2	26	42.7	74.1	7
1965	270	-6.7	19.9	31	26.1	47.0	22	35.3	61.4	11
1966	349	5.9	29.2	30	22.0	39.8	28	37.1	63.3	10
1967	100	-3.4	32.7	31	17.4	38.1	29	34.0	62.1	16
1968	353	15.9	36.5	30	25.8	49.7	21	35.4	63.1	12
1969	245	-1.6	26.2	31	28.0	54.2	21	36.3	62.3	8

Year	No. of Sporo-phores	JUNE Min.	JUNE Max.	JUNE Days below 32F	JULY Min.	JULY Max.	JULY Days below 32F	AUGUST Min.	AUGUST Max.	AUGUST Days below 32F
1963	363	37.9	69.2	0	54.2	76.9	0	51.0	76.4	0
1964	56	42.7	74.1	1	54.6	79.4	0	46.5	69.8	2
1965	270	47.5	70.4	1	51.5	76.9	0	49.2	73.5	0
1966	349	45.8	68.4	2	51.6	74.4	0	46.8	72.8	0
1967	100	42.9	69.5	3	50.6	76.9	0	49.1	77.0	0
1968	353	44.7	70.5	1	48.3	73.2	0	45.5	67.6	0
1969	245	38.1	69.8	9	47.7	74.4	0	49.1	77.9	0

develop (Table IX). It has been observed in the fall that frequent days of freezing temperatures lead to faster deterioration of the sporophores. The temperature conditions are probably most important in June, July and August. In 1969, a year of unusually poor spore discharge even though a large sporophore crop was produced, there were nine days of freezing temperatures in June and the average minimum temperature in June and July were the lowest recorded for any year. The occurrence of a great amount (3.4 inches) of rain in August, which in other years had been associated with a large sporophore crop, was probably the reason that so many sporophores appeared in 1969.

It is suspected that although low temperatures did not inhibit the numbers of sporophores produced in July and August in 1969, they seem related to the cause for about one third of the sporophore crop having abnormal hymenial layers which produced few spores. Some undersurfaces of sporophores did not even develop a pore layer and remained the same color and texture as the upper surface. This phenomenon was not observed in 1967 or 1968, although Whitney[1] reports its occurrence in earlier years. In 1964, in which the poorest sporophore crop was produced, two days of freezing temperatures were recorded in August. Low temperatures might have destroyed some of the existing sporophores and inhibited others from developing.

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[1]Personal communication.

A more detailed view of the date of appearance of sporophores and of rainfall is shown in figures 29 and 30. In figure 29, although numbers of sporophores are shown only for every second day, some of the sporophores may have been located on the previous day. Searches were not made every day which accounts for the large values shown in 1969 on August 6, 22, and September 1. The value shown on each date for a particular year represents the total number of sporophores located since the last date on which a value is shown and the sporophores did not necessarily all appear on the same day. The low level of rainfall in 1967 appears to be associated with low numbers of sporophores.

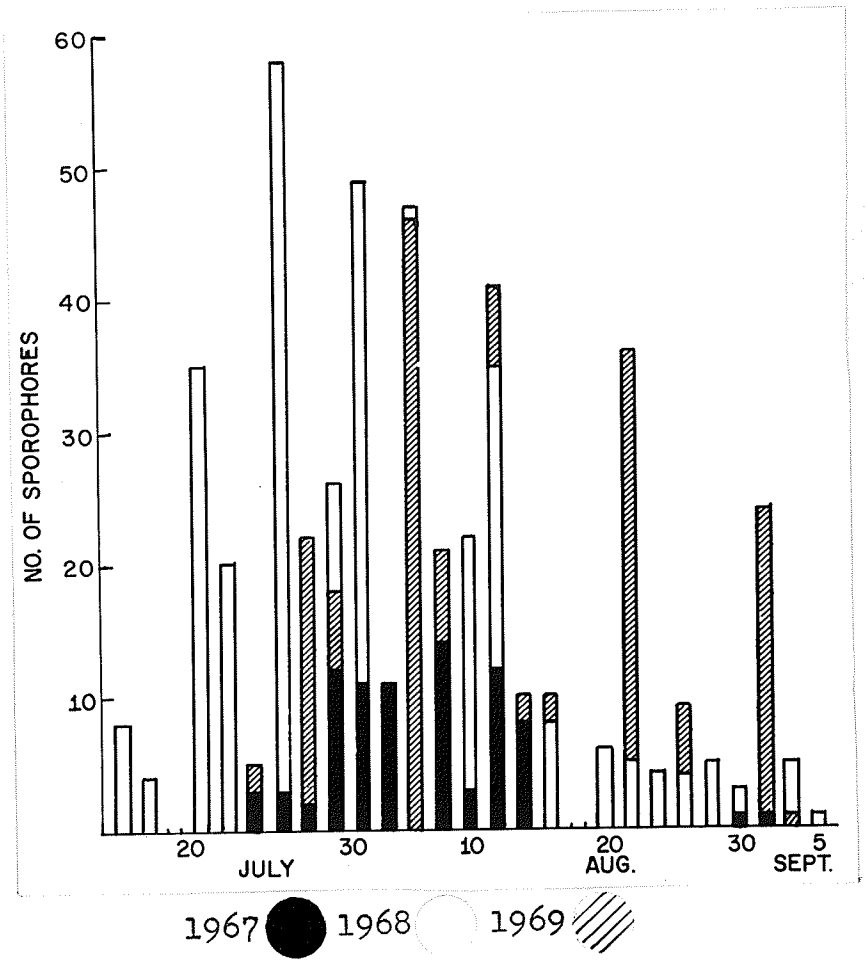

Fig. 29. The date of appearance of sporophores of P. tomentosus in July, August, and part of September in 1967, 1968, and 1969.

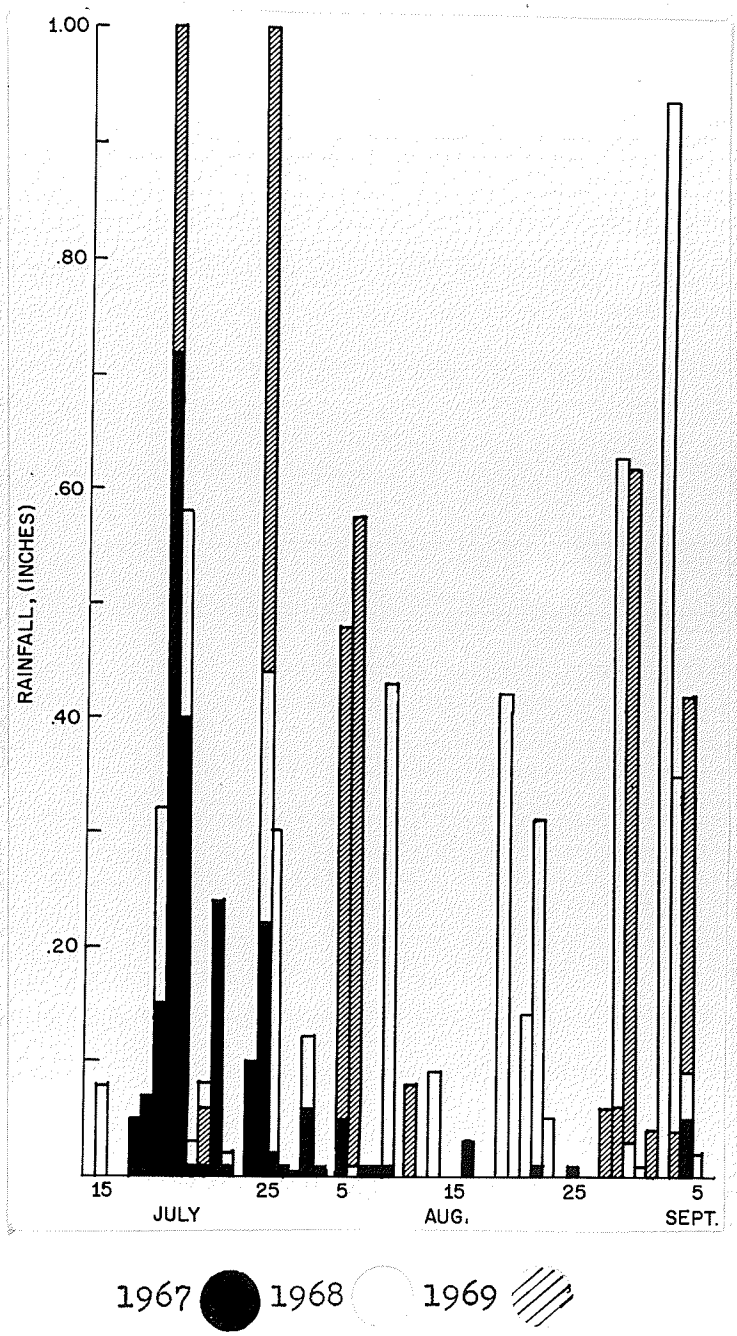

Fig. 30. Rainfall on the study site in July, August, and part of September in 1967, 1968, and 1969.

DISCUSSION AND CONCLUSIONS

Sporophores of P. tomentosus with moisture contents above 180% discharged basidiospores at highest rates under conditions of high R.H. (85 to 100%) when temperature was held at a favorable constant level (60 to 70F) in darkness. Reductions in R.H. from saturation to 70% or lower under the same conditions of constant temperature and darkness resulted in immediate abrupt reductions in discharge rates which again increased when R.H. was restored to saturation. Reductions to 80% R.H. resulted in no decrease in spore discharge and the threshold for an effective reduction in spore discharge rates appeared to be between 70 and 80% R.H. at constant temperature in darkness. Most other members of the genus Polyporus which have been studied produced spores in greatest numbers at night (which suggests that high R.H. is favorable) or under conditions of high R.H. (e.g. Zoberi, 1964; Moser, 1970; and McCracken, 1970). Other studies of the Polyporaceae which have been discussed in the literature review revealed that high R.H. was favorable for spore discharge. Results of these studies including the present one do not agree with Buller's (1909) theory that R.H. has no effect on spore discharge in leathery Basidiomycetes as long as the sporophore moisture content is high.

Sporophores of P. tomentosus required at least 180% moisture content to discharge spores at a high rate. Above

the threshold of 180%, there was no correlation between sporophore moisture contents and spore discharge rates, so that it is unlikely that R.H. fluctuations would have affected spore discharge rates by changing the moisture content of the sporophore. This, and the fact that the present studies involved short periods of low humidity in controlled experiments, lead one to conclude that the changes in spore discharge rate were indeed due to R.H. changes rather than sporophore moisture content. Zoberi (1964) suggested that studies of R.H. fluctuations should be of short duration so that the moisture content of the sporophore is unaffected. Myren (1969) observed that basidiospores of P. tomentosus were discharged for 72 hours at 0% R.H., and he suggested that this was probably the time required, under test conditions, to lower the moisture level of the sporophore below that which was needed to allow spore production and liberation.

Under field conditions which allowed natural fluctuations of temperature, spore discharge in P. tomentosus was directly related to temperature in the range of 45 to 75F when sporophores were maintained in darkness with continuously high R.H. Whitney[1] and Myren (1969) noted that temperatures above 27 to 30C caused a halt in spore discharge in P. tomentosus. No investigations of specific temperatures were attempted in this study. Temperatures above 78F occurred infrequently and were of short duration

[1]Unpublished data of R.D. Whitney.

in the field, and they did not appear to inhibit spore discharge. Myren (1969) observed a permanent cessation of spore discharge in P. tomentosus at 36C. He did not mention how long the sporophores must be maintained at this temperature for permanent cessation to occur, but it is unlikely that it would occur under natural conditions unless these temperatures were maintained for several hours or reccurred on several days in succession. Other examples cited in the literature review show that increasing temperature appears to be a favorable influence among other leathery fungi as well as P. tomentosus, until it reaches an upper limit whereafter the discharge rate is lowered and may cease.

Myren (1969) reported that spores were discharged in P. tomentosus at the low temperature of 4C during test periods of 48 hours. Whitney and Bohaychuk (1969) stored sporophores at 1C, at which temperature they did not produce spores when tested two weeks after storage. Since no tests were made immediately after storage, spores may have been produced undetected in low numbers for two to three days until the sporophore ceased discharging spores altogether. It is suspected that Myren's sporophores would not have continued to discharge spores much beyond 48 hours at 4C. Temperatures down to 1C in field studies presented in this report did not cause cessation of spore discharge. Temperatures below freezing levels appeared to cause physical damage to the sporophores from which they did not always recover. However, low temp-

eratures just above the freezing point in August and September would probably only cause a temporary lowering of the spore discharge rate.

Light of an intensity above 190 ft-c was found to inhibit spore discharge in P. tomentosus. Studies conducted with artificial light in the laboratory and with both artificial and natural light in the field showed definite reductions in discharge rates at intensities from 190 to 940 ft-c. Spore discharge rates also appeared to be directly related to light intensity since 940 ft-c inhibited spore discharge more than 190 ft-c. Although light effects were difficult to separate from the effects of R.H. due to simultaneous reductions in the latter factor when light was applied, the fall in R.H. was judged (on the basis of results in total darkness) to be insufficient to have caused the reduction in spore discharge rates. Field studies conducted under constantly high R.H. in plastic enclosures showed that the application of light during temperature increase resulted in reduced discharge rates when these rates would have increased during temperature increases in darkness. With natural light conditions, spore discharge peaks occurred 4 to 8 hours after the peak in daily temperature, while in darkness the peaks coincided. These results indicate that light inhibits spore discharge in P. tomentosus.

Ingold (1952) stated that light exerts little direct influence on spore discharge in Basidiomycetes, but this

statement hardly seems valid since unlike non- Basidiomycetes, Basidiomycetes have not been extensively studied for a possible relation between spore discharge and light. Nevertheless, light may have only an indirect influence on spore discharge rates by members of the Polyporaceae, since the pores of this family are directed downwards and are on the undersurface of the sporophore so that mainly reflected light would reach the hymenial layer and direct light would only reach the upper surface of the sporophore. In the present studies, the sampling equipment involved a foil plate on the collecting disc beneath the sporophore, which would have caused greater light reflection than would occur naturally. In the field, depending upon the location of the sporophores, direct sunlight would be needed for the intensity required to inhibit spore discharge. Light may also act as a heating influence which would lower the R.H. in the pores so that light and R.H. would have an additive effect in reducing spore discharge. To investigate this, it would be desirable to eliminate light from the undersurface of the sporophore and to measure R.H. changes within the tubes. In this study, there were cases of lowered discharge rates occurring when little reflected light reached the undersurface of the sporophore and strong light was directed only to the upper surface. This suggests the possibility of a photoreceptor within the pileus which influences the liberation of spores. Ingold's view (1962) is that the photoreceptor in light-de-

pendent processes in fungi is a carotene or a flavin which may be associated with a protein. Carlile (1965) reviewed the literature on the photobiology of fungi and found that although several theories to explain the light-sensitivity of fungi have been suggested, there is little supporting evidence for any of the theories, so that the problem is largely unsolved.

When two or more environmental factors were allowed to fluctuate naturally, the changes in spore discharge rates in P. tomentosus did not coincide with the changes in any one of the factors of light, temperature, or R.H. For example, while discharge was directly related to temperature at high R.H. in continuous darkness, the relationship was disrupted when R.H. and light varied according to natural circadian cycles. The interaction of all three factors appears to be most important in the determination of the periodicity of spore discharge rates and trends in combinations of light, temperature or R.H. changes seem to be more important than the absolute value of a single factor in a particular time interval. Under natural conditions, the peak of spore discharge occurred in the late afternoon or early evening. By consideration of the influences of light, temperature, or R.H. singly, and of the combination of two or three of these factors, the following pattern of spore discharge rates in P. tomentosus might be formulated for a cloudless day.

a. Spore discharge is lowest at approximately 1200

hours when there is strong sunlight, the R.H. is decreasing, and the temperature is increasing.

b. Spore discharge increases in the afternoon or early evening when the temperature reaches a peak. The R.H. is increasing and the light intensity is slightly decreasing.

c. Spore discharge reaches a peak when light intensity is low, the R.H. is high, and temperature is favorably high, although decreasing.

d. Spore discharge decreases in the morning mainly in response to temperature being at its lowest as well as the appearance of sunlight, even though R.H. is high.

There was evidence that cloud cover and rainfall disrupted the pattern of spore discharge, since on these days neither temperature nor R.H. fluctuated as on clear days and the cloud cover prevented much natural light fluctuations.

The spore discharge rate in P. tomentosus under natural conditions was influenced by the interaction of light, temperature and R.H., and exhibited a periodicity which did not coincide with the natural periodicities of any of the three factors. The majority of studies considered in the literature review indicated that Basidiomycetes have a nocturnal habit of spore discharge. Basidiospores of P. tomentosus were discharged at highest rates in the evening hours and the rate usually fell in the early morning hours. These facts would place P. tomentosus in the category of nocturnal sporulators. The periodicity in spore discharge rates in P. tomentosus

under natural conditions was perhaps most similar to the periodicity of the light regime in that highest rates of discharge occurred when light intensity was low and lowest rates occurred when light intensity was at its peak. It is believed that this might be the case with many other Basidiomycetes if the methods of collection and interpretation of the data were reconsidered as discussed in the literature. If this is the case, it is surprising that so few studies of the influence of light on discharge by the Basidiomycetes, and particularly the Polyporaceae, have been completed.

The high rate of spore discharge in P. tomentosus observed in the early morning hours was not maintained throughout the night as the decreasing temperature exerted its influence on the spore discharge rate. The interaction of the three factors makes it difficult to assign an order of priority to any one factor. The moisture relationships, including R.H., rainfall, and sporophore moisture content are probably most important for the development of a mature sporophore which discharges spores at a high rate under appropriate conditions, whereafter interaction of the three factors, light, temperature, and R.H. is most important in determining the periodicity of the spore discharge rate.

In studies of P. tomentosus, only one example of an apparently endogenous periodicity which persisted when the conditioning factors were removed was observed. According to Austin (1968b) this was not an endogenous rhythm be-

cause of the preconditioning which occurred before the periodicity in question was observed. In all other studies, the periodicity in spore discharge rates in P. tomentosus was dependent on environmental fluctuations except during developmental phases of the sporophores, such as maturation and commencement of spore discharge, and cessation of spore discharge. The lack of an endogenous rhythm in P. tomentosus agrees with the finding of few such rhythms in Basidiomycetes generally.

The interpretation of spore discharge trends in P. tomentosus demanded knowledge of certain phenomena which were observed during the study and which were not due to technical difficulties. Some sporophores of P. tomentosus showed variability in their spore discharge rates which was apparently unrelated to any other factors examined. This phenomenon occurred with a minority of sporophores; however, it was important to recognize the inherent variability in spore discharge rates displayed by certain sporophores and to make appropriate adjustments for this occurrence. Possibly this was due to environmental influences during the development of the sporophore or it may be a property of certain strains of the fungus. Insufficient observations were made to explain this behaviour.

A second more important phenomenon affecting the interpretation of spore discharge trends in P. tomentosus was the occurrence of lags in spore discharge rates during maturation

of the hymenial layer and commencement of spore discharge, and during the overmature stage of the sporophore leading to permanent cessation of spore discharge. During the spore discharge period of the mature sporophore, cycles of exhaustion caused further irregularities in spore discharge rates. This phenomenon and the fact that all areas of the hymenium did not develop uniformly may explain the occurrence of minor peaks of spore discharge rates which occurred every 3 to 4 hours and were evident in all investigations in this report. DeGroot (1960) noted this occurrence with F. igniarius and suggested that it represented variations in mechanical factors involved in spore release. He also noted that all sporophores had brief periods of inactivity.

Basidiospores of P. tomentosus from different areas of the same hymenial surface were discharged at different rates suggesting that development of the hymenium is not uniform over the whole sporophore. This phenomenon was supported by two observations. In the first case, spore collections from two sides of the same sporophore showed that the two sides did not always discharge spores at the same rate. The second observation was the changing spore deposition pattern which could not be related to environmental or other factors. Gay, Hutchinson, and Taggart (1959) noted a changing spore deposition pattern in their studies of Trametes gibbosa . They first attempted to explain this occurrence on the basis of the developmental changes in the hymenial layer and carefully inves-

tigated other possible causal factors such as an irregularity in the collecting apparatus, fluctuating air currents, or a physical condition of the apparatus. None of the latter factors were felt to be operating at the time and they concluded that the patterns of deposition were the result of rhythmic variation in the rate of spore discharge from different parts of the hymenium. Later, Taggart, Hutchinson, and Swinbank (1964) decided in an addendum to their previous study, that the pattern was after all related to the operation of the heater in the incubator in which experiments were carried out. The evidence from this study appears to support the former theory of Gay et al (1959). DeGroot (1960) also noted that portions of the hymenium of F. igniarius did not discharge spores uniformly. Perhaps a cause of the variation in P. tomentosus is that the supply of nutrients and water from the stipeor pileus was not at a uniform rate. Spore discharge in response to environment might be due more to the behaviour of groups of pores or even single pores rather than the total sporophore.

The average daily spore discharge rate by sporophores of P. tomentosus was significantly lower in 1969 than in 1968. However, there was no significant difference in the average sporophore size and growth rate or the moisture and nutrient content of the soil in 1968 and 1969 (except that the mineral A horizon had a significantly higher moisture content in 1969 than in 1968 due to climatic conditions before the spore

discharge period). Therefore, none of the latter factors appeared to cause differences in spore discharge rates, provided they were suitable for spore discharge to occur. Many sporophores in 1969 had abnormally developed hymenial layers and rarely showed a periodic response to fluctuating environmental factors. The abnormal sporophores probably developed because of the climatic conditions in 1969 preceding sporophore development.

The average spore discharge rate by sporophores of P. tomentosus was related to the total number and quality of sporophores produced in a season. The difference in the number of sporophores which appeared at Candle Lake from 1963 to 1969 was apparently determined by the climatic conditions preceding and during the growth of the sporophores. It has been observed by Whitney[1] and the author that in those years with low numbers of sporophores, the crop was of poor quality and did not discharge spores at high rates; so that the same conditions which determined the total number and development of sporophores probably determined their quality and spore producing ability.

[1]Unpublished data of R.D. Whitney.

SUMMARY

1. Basidiospore discharge rates by P. tomentosus were positively correlated with R.H. in darkness at constant temperature.

2. Spore discharge rates were positively correlated with naturally fluctuating temperature in darkness at high R.H.

3. Spore discharge rates were negatively correlated with light intensity above 190 ft-c at constant temperature and high R.H.

4. Under natural conditions, spore discharge rates showed a periodicity which was not associated with any one of light, temperature, or R.H. alone, but which was modified by the interaction of all three.

5. The periodicity of spore discharge rates was exogenous, being totally dependent on environmental influences.

6. Spore discharge rates were not influenced by environmental factors during two developmental stages; as the hymenial surface was maturing, and in the overmature stage leading to cessation of spore discharge. Between these stages, most sporophores exhibited cycles of exhaustion during which they would not produce spores at optimum levels if favorable conditions persisted for longer than 2 to 3 days.

7. Spores from two areas of the same sporophore were discharged at different rates and spores from one area of the hymenium were deposited in different patterns from hour to hour. Neither of these occurrences were related to environ-

mental factors and it appears that the hymenial surface does not develop uniformly.

8. Spore discharge rates were not correlated with sporophore size, growth rate or moisture content, nor soil moisture content, although a minimum level (about 180%) of sporophore moisture content was found to be necessary for high spore discharge rates.

9. The numbers of basidiospores discharged in a season was related to the number and quality of sporophores produced in the same season, which in turn was related to the climatic conditions preceeding and during sporophore development.

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