M.Sc. Plant Pathology

ABSTRACT

Lyall W. Small

THE EPIDEMIOLOGY OF LEAF BLIGHT DISEASE OF ONIONS INCITED BY BOTRYTIS SQUAMOSA

An explosive epidemic of the disease occurred at Ste-Clothilde-de-Chateauguay during the summer of 1969. Meteorological data, spore trapping and disease survey results indicated that the primary leaf blight symptoms were physiogenic and that subsequent symptoms were incited by Botrytis squamosa Walker. Periods of high temperature seemed to be the most important climatic factor associated with the onset of the epidemic. Fluctuations in relative humidity appear to facilitate the liberation of conidia by causing hygroscopic movements of conidiophores. In laboratory inoculations the Ste. Clothilde's isolates infected necrotic leaves as well as healthy leaves, but the latent period was shorter on the former than on the latter.

Short title.

THE EPIDEMIOLOGY OF ONION LEAF BLIGHT

THE EPIDEMIOLOGY OF LEAF BLIGHT DISEASE OF ONIONS INCITED BY *BOTRYTIS SQUAMOSA*

by

Lyall W. Small

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THE EPIDEMIOLOGY OF ONION LEAF BLIGHT

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ABSTRACT

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THE EPIDEMIOLOGY OF LEAF BLIGHT DISEASE OF ONIONS INCITED BY BOTRYTIS SQUAMOSA

An explosive epidemic of leaf blight disease of onions occurred at Ste.Clothilde de Chateauguay during the summer of 1969. Meteorological data, spores trapped on a Hirst spore trap and disease survey data indicated that the primary leaf blight symptoms were physiological and that subsequent symptoms were incited by Botrytis squamosa Walker. Periods of high temperature seemed to be the most important meteorological factor associated with the onset of the leaf blight symptoms. Fluctuations in relative humidity appear to be involved in the liberation of conidia of B.squamosa by causing hygroscopic movements of conidiophores. Laboratory inoculation experiments showed that the Ste.Clothilde B.squamosa isolates can infect physiogenic necrotic leaves as well as healthy leaves and that their latent periods were shorter on leaves which were necrotic than on leaves which were healthy at inoculation.

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1. INTRODUCTION

A major proportion of Canada's onion production is carried out in the muck soil area of the Quebec province. According to annual disease surveys carried out by the Quebec Department of Agriculture and Colonization, one of the most important diseases of onions in this area is leaf blight disease incited by species of Botrytis.

No accurate estimates of yield losses due to this disease are available and some workers are of the opinion that the disease generally occurs at a stage of growth of the onion plant when it results in only insignificant yield losses. The project described in this thesis was first envisaged mainly as a disease measurement programme to quantify the extent of yield losses caused by the Botrytis incited disease if there was such an epidemic in the summer of 1969 at Ste. Clothilde de Chateauguay.

On the 22nd July 1969, after it was thought that onion leaf blight disease would either be very late in occurrence or would not be present at all at Ste. Clothilde in 1969, lesions and dieback symptoms of the disease were observed widespread in the field with no apparent foci of infection. The disease measurement programme could therefore not be carried out along the lines of Le Clerg's paired

plant technique as had previously been planned, and the disease measurement aspect of the disease had to be subordinated to an epidemiological investigation. A few disease measurement parameters were taken however, one of these was a correlation analysis between number of spots per plant and number of spots on individual leaves. This analysis was carried out in an attempt to find a labour saving measurement for surveys of this disease which could be more fully investigated in future experiments in the field, the greenhouse and the laboratory. Correlations were also carried out between lesions per plant and yield as estimated from the weight of the bulbs at the time when samples were taken.

The epidemiological investigations had therefore assumed the greater amount of importance in this study and spore trapping studies, using a Hirst automatic volumetric spore strap formed a major part of these investigations. Taking continuous data on various environmental factors including rainfall, sunlight hours, relative humidity, etc. was also important in this phase of these studies. Dew duration was measured by means of a dew meter which was a modification of the Theis and Calpouzas type and was designed to accellerate the choice of a suitable pencil and also to give more accurate readings. Isolations of the causal fungus were also made and Koch's postulates were eventually carried out on these isolates.

Some of the Ste. Clothilde isolates were kept in a refrigerator at Macdonald College for a number of months and hope had almost gone that these cultures would sporulate when some isolates did sporulate in February 1970. Prior to this it had been thought that this thesis would have to be written on the Ste. Clothilde results alone as sporulation was necessary for identification of the causal organism as well as for carrying out inoculation experiments to determine various epidemiological parameters. The sporulation of only a few isolates at this late stage meant that only a limited number of inoculation experiments could be carried out in the laboratory to determine latent periods, etc.

Onion plants were sown in pots in the greenhouse and growth chamber and single spore isolates from the sporulating Ste. Clothilde isolates were plated and incubated at various temperatures and light regimes in the hope that in late April or May when the plants would be at a suitable stage for inoculation they would provide an adequate supply of inoculum. However, when the plants were at the correct stage for inoculation, there was no new inoculum from these sporulation experiments and the spores from some of the previously sporulating isolates had to be bulked and used for the inoculation experiments. Shortly after the inoculation experiments were set up

spores of B. squamosa were formed at low temperatures but at this stage it was too late to start new inoculation experiments.

This thesis is divided into two main sections, the Ste. Clothilde observations and the Laboratory experiments. The laboratory experiments are generally of a preliminary nature and were primary designed to determine latent, incubation, and infections periods of the fungus on onion leaves.

2. LITERATURE REVIEW

2.1 Background

This literature survey relates mainly to the leaf blight disease of onions incited by Botrytis squamosa Walker. However, various references are given which do not directly involve this disease but relates to other symptomatologically similar diseases of onions incited by other species of Botrytis and by non-parasitic agents.

Leaf blight appears to be a disease complex of commercially grown salad and bulbing onions, variously called: onion blast, sunscald, blight, tipburn, tip blight, leaf blight and leaf fleck. This complex is prevalent in many onion grown areas of the world. It has been reported by Whetzel in the USA (1904), by Ogilvie and Mulligan in England (1941), by Page in Canada (1953), by Viennot-Bourgin in France (1953), by Hennebert in Belgium (1964), and by Garibaldi in Italy (1968).

2.2 Symptoms

The disease syndrome as described by Clinton(1904), Jones(1944), Hickman and Ashworth(1943), Page(1957), Segall and Newhall(1960), Hancock and Lorbeer(1963), and Engle et al (1965),may conveniently be divided into two phases: 1) The

leaf fleck phase, in which affected onion leaves exhibit circular to elliptical greyish white, slightly depressed dessicated lesions, in size 1-10x1-4mm. The lesions are usually discrete especially during the early stages of infection. Sometimes, however, the margins are water-soaked and in the later stages contiguous spots occasionally coalesce. Older leaves are more severely affected than young ones.

2) The tip blight phase, in which there is a pendant withering of the leaf tips, particularly of the outer leaves. The tissues at the junction of the withered portion with the remaining part of the leaf are generally water soaked. The withering of the leaf tips is characteristically, but not invariably, associated with leaf flecking and usually follows 5-12 days after leaf flecking (Segall and Newhall , 1960).

A sign commonly associated with the disease complex is the profuse sporulation of various combinations of four species of Botrytis viz-: B.squamosa, B.cinerea, B.allii and B.byssoidea, on necrotic onion leaf tips. The most prevalent of these species are B.squamosa and B.cinerea. No sporulation is generally observed on the discrete lesions lower on the leaf.

2.3 Etiology

The etiology of this disease is still not very

clearly established. The first report of symptoms similar to the disease now called blast of onions was made by Whetzel in 1904. He made no mention of the leaf spotting phase and he considered the disease to be due to wet weather and poor soil drainage. Doran and Bourne (1931), and Linus Jones (1944) also concluded that the disease was of a physiological nature. Jones thought that the disease was incited by a non-parasitic agent because investigations prior to his own had failed to show the presence of a living pathogen when the plants exhibited the first signs of injury, and because the disease developed in bright sunshine and low humidity in marked contrast to the inception and spread of parasitic diseases. He concluded, in similar vein to Doran and Bourne, that blast results from too rapid a loss of water from tissue exposed to the sun after an abnormal development in subdued light, high relative humidity, and high temperatures.

Clinton in Connecticut in 1904 apparently was the first person to describe the leaf spotting phase of the disease and also to attribute causation to a Botrytis species. Munn in 1917 in New York State described Botrytis allii Munn as being pathogenic on onion leaves and to this Walker added B.squamosa in 1926. Ogilvie and Mulligan in England in 1932 described a small sclerotial Botrytis as being the causal agent of onion blast but later in 1941 Ogilvie reported that

Botrytis cinerea Pers was the causal agent. This fungus was also implicated by Yarwood in California in 1938. However Hickman and Ashworth in England in 1943 reported that the isolates of Ogilvie and Mulligan consisted of both B.squamosa and B.cinerea and concluded, on the basis of experiments with their own isolates, that B.squamosa was the predominant causal organism although B.cinerea and another unidentified Botrytis were also associated with the disease. Page in Canada in 1952, Viennot-Bourgin the same year in France, and McLean and Sleeth in Texas in 1959 reported similarly that B.squamosa was the causal agent of onion blast. In 1960, the situation was further complicated by Segall and Newhall who reported on the basis of work in New York State that four Botrytis species were causal agents viz-: B.allii, B.cinerea, B.tulipae (Lib) Lond and B.paeoniae Oud.

In 1960 then, five identified Botrytis species and possibly two other unidentified ones had been implicated as the pathogens of onion blast. In 1963 Hancock and Lorbeer reported on studies which had been carried out to clarify this situation. On the basis of data obtained with numerous isolates from onion fields in New York state, they proposed that onion blast consists of three separate diseases and that: 1) Botrytis Leaf Blight should be the recognized name of the disease incited by B.squamosa involving initial lesion

formation followed by rapid blighting, 2) Botrytis Leaf Fleck should be the name given to the disease caused by B.cinerea and characterized by superficial leaf flecks and that 3) The term Onion Blast should be retained for the non-parasitic disease first described by Whetzel in 1904. They found that B.allii and B.byssoidea, the only other Botrytis species found on diseased onions in their collection, were not appreciably pathogenic under normal conditions. Tichelaar (1967) confirmed in Holland that B.allii was non-pathogenic to onion leaves.

In 1965 after the etiology of onion "blast" had apparently been established unequivocally by Hancock and Lorbeer, some workers in Wisconsin (R.L. Engle, et al,(1965), on the basis of a) weather and disease measurement, b) anatomical studies of flecking and mechanical induction of the disease, c) ozone fumigation of onion seedlings and fully expanded onion leaves and d) correlations of ambient ozone with rainfall, provided evidence that a disease with symptoms essentially identical to onion "blast" was incited by high levels of ozone only. They showed that there was a close relationship between the presence of tipburn (the name they proposed for the ozone incited disease) in onions and high levels of ozone which generally occur after periods of heavy rainfall.

They found that high levels of ozone give rise to two types of symptoms. An immediate tipburn caused by damage to cells of the leaf tip and flecking. The flecks often extend through the entire thickness of the leaf.

These symptoms are essentially the same as those incited by B.squamosa except that in tipburn the leaf tip dieback is gradual and seldom extends to more than one third of the leaf's length.

The work of Engle et al (1965) seems to substantiate to some extent the conclusions drawn by the early workers (Whetzel, Doran and Bourne, and Jones) as to the non-parasitic nature of onion blast disease.

In summary, a disease syndrome involving leaf spotting and tip dieback phases, has been variously ascribed to a) weather conditions, when it is called onion blast, to b) pathogenic Botrytis species, when it is called Botrytis Leaf Fleck or Botrytis Leaf Blight depending on the species of Botrytis, and to c) high levels of ozone in the atmosphere when it is called Tipburn.

2.4 Biology of Botrytis Squamosa

Botrytis squamosa was first described by Walker in 1925. His description is wholly reproduced here as it is

pertinent to the section on the identification of the causal agent of the disease occurring at Ste. Clothilde:

B.squamosa N.S.

Mycelium hyaline, septate, variable in diameter, branches not ordinarily constricted at the base. Conidiophores comparatively rare at 20-22 degrees C, more abundant at cooler temperatures; seldom rising direct from the mycelium, more often in tufts from sclerotia, erect, becoming flattened and twisted with age, hyaline at first, turning dark with age; septate, slightly swollen at base; branches common and constricted at the base; growing tips branch and rebranch previous to sporulation; conidia borne on short hyaline sterigmata arising from swollen apices of branches and become detached at maturity; side branches degenerate after fructification, the walls drawing back in characteristic accordion-like folds; degenerating side branches cut off by septa laid down near the base, leaving distinct scars or knobs upon the main stalk; main stalks proliferate and sporulate repeatedly depending upon conditions. Conidia obovoid to ellipsoid, smooth, continuous, hyaline, ashen grey in mass when young becoming somewhat darker with age; sterigmata seldom remaining attached; 13-22x10-17μmostly 15-20x12-15 μ . Microconidia globose, about 3μ in diameter, borne upon short, hyaline conidiophores.

Sclerotia white at first, turning black with age; most common on dry outer scales of the host, roughly circular in outline, flat scale-like, 1/2 to 4mm. in diameter, rarely more than 1/8 mm. in thickness; often converging into large scale-like conglomerates.

Parasitic on outer scales of bulbs of Allium cepa, especially upon white varieties; distribution, Wisconsion, Illinois.

The salient characters of the grown of B.squamosa on Potato dextrose agar plates at room temperature-: The colony which enlarges more slowly than either B.allii or B.byssoidea. Abundant sclerotia appear over the entire plate after two or three weeks, first as dense whitish masses which become hard and black with age; mostly 1-2 mm. in width and thicker and more rounded on the upper surface than those which develops upon the host. There is very little sporulation at room temperature (20-22 degrees C.) but profuse production of conidiophores arising directly from the sclerotic occurs at 12-16 degrees C.

Hickman and Ashworth in 1943 next isolated the fungus in England and the characteristics of their isolates agreed closely with Walker's description, they however added that conidia germinate by one or two germ tubes and that

conidia produced in culture are generally slightly smaller than those produced on the host, these findings were verified by Hancock and Lorbeer (1963) with their New York isolates.

Many workers have noted that conidia are produced in culture almost exclusively from the surface of sclerotia at low temperatures around 12 degrees C. In April 1970, Bergquist and Lorbeer reported that they achieved abundant sporulation in Pyrex glass petri plate cultures at 20-23 degrees C. on a defined medium in 5-7 days when B. squamosa was exposed simultaneously to near ultraviolet light (340-380 mμ) and light from fluorescent tubes (425-675 mμ) for an illumination regime of 14 hours light, 10 hours dark. Sporulation did not occur in complete darkness or when only fluorescent or fluorescent-incandescent light sources were used.

The first record of the perfect stage of the fungus was made by Cronshey (1946), in England. He described the production of apothecia directly from the sclerotia of some isolates cultured on Dox's agar. The characteristics of these apothecia were consistent with those of the genus Sclerotinia.

Apothecial production by B.squamosa appears to be a relatively rare occurrence (Page 1956) and it has been reported only twice since Cronshey's work. Viennot-Bourgin (1953) renamed the fungus Botryotinia squamosa on the basis of the characteristics of the apothecia developed on Czapek's agar and the other report of the perfect stage was by Bergquist and Lorbeer (1968), who achieved apothecial production by programmed changes of temperature and illumination using first a wheat seed - Water medium and then a sterile sand medium.

Page (1956) reported that aeration and nutritional factors influenced the production of sclerotia. Light in excess of 100 ft. candles was found to inhibit the organism in culture. Periods of darkness alternating with periods of incandescent or fluorescent light influenced the development of concentric rings of sclerotia on Czapek's agar. Page also found that the fungus made adequate mycelial growth at 12°C. but its optimum temperature for mycelial growth was 20°C. Some of these conclusions were later up-dated by Stinson and Gage (1958) who concluded that the inhibitions reported by Page was due to high temperatures rather than to light and that sclerotial zonation was probably due to the effect of temperature variations as well as to light and dark alternation

2.5 Host-Parasite Relationships

The Cytological aspects of penetration and invasion of onion leaf tissue by Botrytis species, notably B.squamosa and B.cinerea, has not been thoroughly investigaged. Segall and Newhall (1960) working with B.allii found germinating conidia only on the surface of lesions. There was no penetration by the germ tubes through either cuticle or stomata and no mycelium could be found in the leaf tissue. The germ tubes on the leaf surface did not grow in any particular direction with relation to the position of the stomata, and the fungus could not be re-isolated from surface sterilized lesions. In view of these findings and also because individual lesions were larger where concentration of spores were highest, and sterile culture filtrates of the fungus on Potato Dextrose Broth gave rise to typical disease symptoms, they proposed that toxins were involved in a type of pathogenesis without parasitism and penetration.

Hancock and Lorbeer (1963) confirmed Segall and Newhall's findings that leaf spots,resulting from inoculation with B.allii,did not contain hyphae and did not yield the pathogen on surface sterilization. Tichelaar (1967) working in Holland and using more precise inoculation and staining techniques than the New York State workers adequately demons-

trated that germ tubes of B.allii, by the formation of appressoria and infection hyphae, penetrate onion leaves through the stomata. Only the guard cells, the adjacent epidermal cells and the substomatal cavity are invaded. During the green leaf stage, the fungus may either remain quiescent in the epidermis or if moisture conditions are favourable it may grow further over the leaf surface occasionally entering a stomata, however there was no evidence of spotting caused by B.allii. He found that growth through the underlying mesophyll does not occur until the leaves become senescent. In infected leaves which have wilted and become chlorotic the fungus spreads rapidly intracellularly, and under high Relative Humidity there is abundant sporulation on the leaf. Also plants of all ages were infected, older plants being somewhat more susceptible than the younger ones.

Hancock and Lorbeer (1963) found that mycelium of B.squamosa, unlike that of B.cinerea or B.allii could be observed in necrotic leaf tissue and also on the inner leaf surface, i.e. within the leaf lacunae. Hence, B.squamosa could apparently invade healthy onion leaf tissue whereas B.cinerea and B.allii could not. They postulated that the ability of B.squamosa to grow rapidly in the lacunar environment of the leaf may account in part for the withering of the leaves 5-12 days after the primary spotting symptoms appear. These explanations were rendered more likely by the fact that

Page got dieback symptoms by injecting B.squamosa inoculum directly into the leaf lacunae and also they themselves, Page (1957) Cronshey (1946) and Viennot-Bourgin (1958) were able to isolate B.squamosa from lesions incited by it.

In 1964, Hancock et al reported further investigations into the toxigenic aspect of the host-parasite relationships of the Botrytis leaf blight and leaf fleck diseases of onions. They found that B.allii, B.cinerea and B.squamosa all produced pectin methylesterase and cellulase. B.allii produced both exo- and endo-polygalacturonase in detached and intact onion leaves. B.cinerea and B.squamosa also produced exo- and endo-polygalacturonase, the exo type, however, only on detached onion leaves. In another paper (1964b) Hancock et al concluded that endo-polygalacturonase produced by germinating spores of Botrytis squamosa has a primary role in leaf blight disease and that cellulase is not a significant factor. The fact that culture filtrates and commercial pectolytic enzyme preparations induced symptoms on onion leaves similar to those caused by the pathogen suggested to them that killing in advance was taking place. They suggested also that pectolytic enzymes are secreted into the infection droplets by germinating conidia of Botrytis spp. and that the enzymes are retracted into the substomatal cavity of the onion leaf in the presence of light. The leaf

flecking phase is thus induced by enzymatic action on pectic substances of the middle lamella. They thought that the tip dieback phase developed in the case of B.squamosa and not B.cinerea because the former might theoretically be able to produce sufficient pectolytic enzymes to kill an appreciable amount of underlying leaf tissues and thus provide ready entrance for the pathogen into the lacunar areas, while the latter might not be able to produce enough of such enzymes.

Segall and Newhall (1960) made histological studies of the lesions incited by Botrytis spp. and found that in the lesions themselves the epidermal cells become separated from the palisade cells below the epidermis, with both palisade cells and the parenchyma cells becoming completely disorganised. A separation of cell walls was noted which indicated that the middle lamella had been dissolved. Engle et al (1965) described histological studies on leaf flecks incited by high levels of ozone which revealed a tissue breakdown immediately under the flecks, this breakdown involved not only the palisade cells, but often the vascular bundles also. They found also that 10 or more needle punctures randomly scattered and simulating flecks, caused symptoms similar to those occurring naturally among plants affected with tipburn under field conditions.

Page (1957) reports on histological studies on sporulation on necrotic leaf tissue where he found that the initials of conidiophores developed from hyphae in the host mesophyll or more commonly, directly through epidermal cells.

2.6 Epidemiology of B.Squamosa Leaf Diseases of Onions

2.6.1 General Aspects of Epidemiology

The epidemiology of parasitic disease may be defined as the science of increase in populations of parasitic pathogens on the population of the host. It involves the study of all host, pathogen, and environmental factors which influence the penetration, invasion and fruiting of the pathogen on the host and therefore controls the rate of disease development(r). A high rate of disease development, e.g. r = 0.45 per unit per day, is characteristic of explosive epidemics such as those of potato blight incited by Phytophora infestans. (Van der plank 1963).

The rate of disease development has not been measured for the Botrytis leaf blight disease of onions but results of spray trials by Shoemaker et al (1967 and 1968) and surveys by Simard et al (1962, 1965 a, 1965 b, 1966 and 1968) indicate that this disease may be classified as one that can cause explosive epidemics.

Among the most important pathogen factors in epidemiology are aggressivity, pathogenicity, the threshold number of propagules capable of causing infection and disease under average conditions, the reproductive capacity of the pathogen, and whether or not it is capable of survival in the soil.

The reports of various workers on Botrytis leaf diseases of onion indicate that the pathogens have a reasonably high degree of aggressivity and pathogenicity. However, Garrett (1960) has stated that although the infectivity of the average spore of B.cinerea and similar pathogens seems to be adequate for infecting damaged or debilitated tissues it is not so for infection of green vigorous tissues.

The reproductive capacity of a fungal pathogen depends on the number of spores it produces in a single generation, its latent period, its infectious period and its rate of spore production. For B.squamosa none of these statistics could be found in the literature; Segall and Newhall (1960), however, reported that B.allii spores retain their infectivity on onion leaves for about 8 days.

The capacity to survive in the soil saprophytically plays an important role in the epidemiology of many crop

pathogens. Page (1957) theorized that B.squamosa, due to its production of sclerotia on onion debris might be a successful soil saprophyte, but he was unable to obtain proof for this.

Host factors important in general epidemiology are host resistance, growth type of host, density and purity of host stand and the existence of alternate hosts.

There have been no reports on varieties of Allium cepa which are resistant to B.squamosa. Bergquist and Lorbeer (1970 a) reported that A.bouddae and A.schoenoprasum were immune, A.fistulosum was highly resistant and A.pskemense and A.galanthum were moderately resistant while A.cepa and A.vavilovii were susceptible. No alternative hosts for B.squamosa have been reported.

The most important environmental factors in epidemiology are weather and climate, temperature, moisture, light, wind, edaphic factors and biotic factors. In general it appears that the environmental effects on the pathogen are more important than on the host or on disease development.

The B.squamosa leaf disease of onions has not been thoroughly investigated in terms of the environmental conditions which favours its development. The disease has been

reported in Quebec, Western Ontario, Wisconsin, New York State, Texas, Italy, France, England and Belgium. This broad geographical range indicates that the disease can develop under rather wide weather and climatic conditions. No reports on biotic factors important in disease development has been found.

2.6.2 Course of the Disease

Botrytis leaf blight disease of onions generally occurs towards the end of the growing season, sometimes before the bulbs have enlarged appreciably and while the necks are still succulent, Walker (1926), Page (1957), Segall and Newhall (1960). In Quebec its onset on spring sown onions usually takes place from mid July, Simard et al (1966, 1968).

Page O.T., (1957) and Hickman and Ashworth (1943) imply that the disease is general and not sporadic (cf.Jones 1944) as they could find no suitable healthy control plants in diseased fields. The source of the initial inoculum has not been clearly established for spring sown onions. Page (1957) hypothesized that B.squamosa and other Botrytis species over winter as sclerotia and possibly as conidia or mycelia in the soil in southern Ontario, from the fact that

sclerotia are found on onion bulbs in the field though not on infected leaves. He, however, was unable to achieve typical infection from soil inoculum and concluded that the atypical symptoms were the result of systemic infection of seedlings and mature plants which had become serious under the extreme and abnormal conditions of the experiment.

Segall and Newhall (1960) observed abundant sporulation of Botrytis species on previously discarded cull onions. They postulated that spores from these were a source of inoculum, implying that cull onions gave rise to the initial wind disseminated inoculum while subsequent infection is due mainly to sporulation from necrotic leaf tips. They thought that in damp, humid, warm weather great showers of Botrytis spores can occur in onion fields. Lorbeer (1966) gave the only report of spore-trapping experiments on B.squamosa. He found, in contradistinction to the expectations of Segall and Newhall (1960) that maximum numbers of B.squamosa conidia occurred during daylight periods of increasing temperatures and decreasing relative humidities. Lorbeer also observed a diurnal periodicity of conidial concentration in the air, maximum members, 80% of total, being obtained between 8 a.m. to 4 p.m. There was considerable day to day, and hour to hour variation in the number of conidia. The maximum numbers for two hour periods being 260/1.2 $m.^3$ of air and for 24 hour periods being 438/14.4 $m.^3$ of air.

The reports of Segall and Newhall (1960) and Hancock and Lorbeer (1963) imply that B.squamosa conidia penetrate onion leaves of all ages by enzyme action, then, according to Hancock and Lorbeer (1963) and Hancock, Miller and Lorbeer (1964 a and b) the deep nature of the resulting lesion allows the fungal hyphae to enter the leaf lacunae in which it spreads rapidly to the leaf tip where it causes dieback symptoms. In the field, however, this sequence has not been obvious because most workers state only that spots in the field are associated with dieback - Page (1957), Hickman and Ashworth (1943).

Having initiated the dieback symptoms, B.squamosa then sporulates after an undetermined latent period on the necrotic leaf tips. The spores are presumably wind borne to healthy onion leaves where the disease cycle is repeated during favourable conditions. The disease develops at an explosive epidemic rate when environmental, host and pathogen factors are favourable.

2.6.3 Macroclimate

The macroclimatic conditions favourable for the onset and development of onion dieback and fleck disease appear to have been well studied. They seem to be very similar for both the parasitic and non-parasitic aspects of the

disease. Jones (1944) observed that onion blast injury appears suddenly and is widely extended, it is noticed first in the presence of intense sunlight and high temperatures following a period of cloudy wet weather. The nature of the appearance of the injury is general instead of sporadic and any increase in its development is on individual plants and not from plant to plant, where there may be borderline conditions of injured and uninjured plants.

Engle et al (1965) found that the environmental conditions for the onset of ozone incited tipburn were exactly the same as described above for blast.

With respect to the parasitic disease, Page O.T.1957, in inoculation experiments, achieved significant typical leaf flecking symptoms only in controlled environments with a regime of 10 hours darkness and 16 hours light, the light period was maintained at 25°C. and the dark period at 12°C., he found that continuous darkness gave rise to only a few atypical lesions. He postulated that infection probably occurs during the dark period when the change from a higher to a lower temperature resulted in the formation of a condensate on the leaves. Segall and Newhall (1960), observed that in the field the leaf flecking phase occurred when the relative humidity was close to 100% for at least 24 hours,

Botrytis spores were present, and there was strong light. They found that the leaf blighting phase occurs after the leaf spotting phase and requires temperatures above 27°C. Simard et al (1968) developed an empirical forcasting method for the date of onset of various leaf blights of vegetables including Botrytis leaf blight and leaf fleck of onions. They postulated that if the June rainfall in any year approximates the 10 year mean, (ca.9 cms) leaf blights will develop in July or August depending on the date of onset and the development of July and August rainfalls. They also suggested, in 1968, that high temperatures may act selectively for the development of B.squamosa leaf blight in preference to B.cinerea leaf fleck.

2.6.4 Microclimate

B.squamosa may be placed in the Anthracnose class of Yarwoods classification of foliage pathogens based on humidity requirements (Yarwood 1956) as:1) The results of inoculation experiments by various workers including Cronshey (1946), Page (1957), Segall and Newhall (1960) and Hancock and Lorbeer (1963) indicate that high relative humidities are essential for the leaf flecking phase. 2) Page (1957) implies that moisture on the leaves is important in the development of leaf flecking. 3) Sporulation is observed

on necrotic onion leaf tips during periods of high relative humidity (Page 1957), Segall & Newhall (1960), Hickman & Ashworth (1943), Hancock & Lorbeer (1963), and 4) Necrotic onion leaf tips from infected plants which are not observed to sporulate in the field, readily do so when placed in humid containers, Page O.T.(1960), Hancock & Lorbeer (1963).

High temperatures have been found necessary for the leaf blight phase, Segall & Newhall (1960), Simard et al (1968), but whether the action of high temperature is on the susceptibility of the plant or on the pathogen itself has not been clearly established.

Light has been found essential for the leaf spotting phase by Page O.T. (1956, 1957) and by Segall and Newhall (1960). However, in constant darkness, with alternate 12 hour periods at 13°C. and 24°C., Segall and Newhall obtained an unexplained significant number of lesions.

Near u.v. light was found essential for the rapid production of conidia of B.squamosa in axenic culture by Bergquist and Lorbeer (1970).

Hancock et al (1964b) have hypothesized that light could be important in its effect on the opening of the stomata and hence influence the retraction of the toxin contained in the infectious droplet.

2.7 Disease Control

Onion leaf fleck disease has been found amenable to chemical control measures even at the times when it was thought of as being a non-parasitic disease. Doran and Bourne (1931) were able to delay the onset of blast by spraying with Bordeaux mixture, but the resulting increased yield was insufficient to justify yearly spraying.

Segall and Newhall (1960) and Newhall and Rawlins (1952 and 1958) reported effective control by carbamate sprays. But Engle et al (1965) pointed out that these carbamates are effective antiozonants as well as fungicides, and control could have been due to the action on ozone rather than on Botrytis spp.

Page O.T. (1957) also reported control by carbamate sprays. Shoemaker et al (1967 and 1968) reported similar control by aircraft-application. Tartier (1970) reported significant increases in yield due to well-timed spraying with carbamates using Simard et al's (1968) disease forecasting method.

3. STE. CLOTHILDE FIELD OBSERVATIONS

3.1 Background

Field investigations on the epidemiology of B.squamosa leaf blight disease of onions was carried out at the Ste.Clothilde Experimental Station of the Canada Department of Agriculture situated in a muck soil area of the Province of Québec. These investigations consisted of:

a) The continuous sampling of the air for its content of conidia of B.squamosa by the daily use of a Hirst spore trap.

b) The collection of meteorological data related to the macro and microclimatic environment of the onion plants grown at Ste.Clothilde.

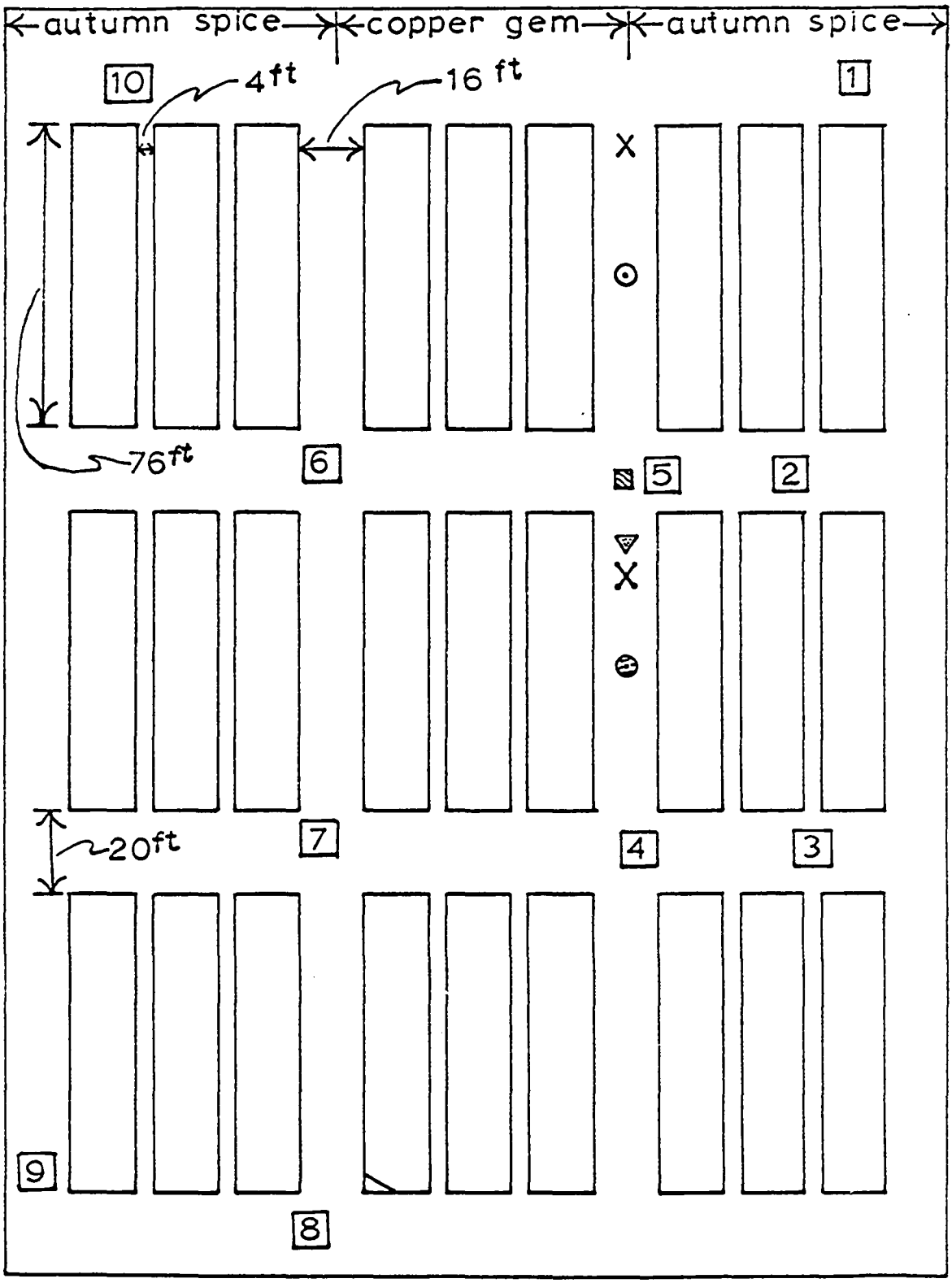
c) The isolation and identification of B.squamosa collected on necrotic onion leaves.

d) Following the disease progress by means of disease surveys.

The experimental field was set up as depicted in Figure 1. The field layout was designed primarily for a spraying trial which was carried out by M. Leon Tartier and his colleagues of the Quebec Department of Agriculture and Colonization. The epidemiological investigations were assigned the border rows for surveys, isolations, etc. The dimensions are given in Figure 1.

FIGURE 1

FIGURE 1


autumn spice
copper gem
autumn spice
10
4ft
16 ft
1
76ft
6
5
2
20ft
7
4
3
9
8

The field was seeded on May 6th, 1969. Two onion varieties were used: Autumn Spice and Copper Gem. The Autumn Spice variety was grown in the blocks of the outer latin squares while the Copper Gem variety was grown in the blocks of the middle latin square (see Figure 1). Only the Autumn Spice variety in the border rows was sampled for disease incidence, for isolations of B.squamosa and for weights of onion bulbs.

3.2 Materials and Methods

3.2.1 Meterological Instruments Used in the Field.

The instruments in the field consisted of:

a) Two dew meters: one of which was an unmodified Theis & Calpouzos meter, the other was a modified Theis and Calpouzos type that will be more fully described below.

b) A Hirst spore trap .

c) A recording hygrothermometer placed 4 feet above soil level in a Stevenson screen.

The electrically powered instruments were provided with power from an electrical outlet of 110 volts on the edge of the field by means of a long heavy duty extension cord.

The position of the instruments is illustrated in Figures 1 and 2. The latter shows the field after the border rows were harvested and the onions in the spray trial plots had fallen over just prior to harvest.

In addition to the microclimatic data taken from instruments in the experimental field, Macroclimatic data were collected from standard meteorological instruments in the weather station of the experimental farm. The weather station was about 1/4 mile distant from the experimental field, but was at a slightly higher elevation than the field.

The data collected from the weather station were:

1) Hours of sunlight per day measured on a Heleograph.
2) Wind speed, measured somewhat arbitrarily using the Beaufort scale as the Taylor anemometer was out of order.
3) Evaporation measured on a Wright evaporimeter and reported in Livingston units.
4) Rainfall, measured on a recording rain gauge.

FIGURE 2

FIGURE 2

3.2.2 Spore Trapping

3.2.2.1 The Spore Trap

A Hirst automatic volumetric spore trap was used. The flow rate was 10 litres of air per minute . The 1/2 meter legs were used so that the orifice would be as close as possible to the level of the onion leaves to ensure that the majority of the spores trapped originated from these leaves. Vaselined slides were changed everyday at approximately 8 a.m. from mid May until August 22nd.

Hirst (1952) and the Cassella instruction leaflet No. 3061/RO describe adequately various aspects of the use of the Hirst spore trap. However, in this study some modifications of Hirst's techniques of coating the slides and of counting spores were made and these are described more fully below.

3.2.2.2 Coating the Slides

It was found that very rapid and even coating of glass slides could be made by the following technique: Vaseline contained in a 200 ml beaker was melted over a hot plate and maintained at a temperature slightly higher than its melting point. Meanwhile, glass slides were measured and marked in the manner described in the Cassella instruction

leaflet and the ends to be coated were briefly warmed on the hot plate. A smooth-edged slide (Fisher BEV-L-EDGE 2.5x7.6 Cm), of the type used in making blood smears in Medical Laboratory technology, was then dipped in the hot vaseline and used to put a drop of the hot liquid on the start mark of a warm slide. Then, with a quick even motion in the manner used in making blood smears, the smooth Bev-L Edge slide was drawn over the vaseline area. The resulting uniformly thick vaseline coat was then allowed to solidify. The vaseline was later scraped from strips 3 mm wide along the edges and 5 mm wide along the short edge of each slide with a small piece of soft wood cut to these dimensions at either end.

Solvar and Gelvatol were tried as adhesives and as mountants for the vaselined slides but were generally unsatisfactory. The Gelvatol, in particular, had the major disadvantage that its stock solution quickly became contaminated with a rapidly growing fungus of undetermined taxonomic nature.

Exposed slides could also be observed under the microscope more conveniently without the addition of a cover slip and these slides kept well in dust proof slide boxes at room temperature.

3.2.2.3 Counting The Spores

In the appendix page (237) there is given a personal communication from Dr. Hirst to Professor Sackston which describes in detail a method of counting and calculating concentrations of spores deposited on the Hirst spore trap and also corrects an error made in Hirst's 1952 paper and in the Casella instruction leaflet. Before this communication was received, however, an alternative method of counting and calculating B.squamosa spore concentrations was devised as it was realized that there was an error in Hirst's original papers.

A piece of transparent plastic about 1/2 mm thick was cut to the dimensions of 2 1/2 x 6 1/2 cms and 28 very fine lines, 2 mms apart and parallel to the short edges, were ruled across it with a specially sharpened dissecting needle, starting 3 mms from a short edge. A wax pencil was then used to mark over these lines and the excess pencil deposit was then wiped off leaving thin red lines on the plastic grid. The grid was then placed under the exposed vaselined slides and the lines could clearly be seen under the spore trap field with the appropriate adjustment of the microscope condenser. The grid was attached to the slide by means of Scotch tape in such a way that one line coincided

with the start of the spore deposit for a particular day and each line thereafter demarcated the average number of spores trapped in 2 hour periods or the distance the slide moved in 1 hour. Thus one could accurately and quickly estimate the time period during which spores were deposited from the position and/or number of the nearest grid lines relative to the first grid line.

In routine daily counts using the grid all B.squamosa spores were counted in 5 longitudinal traverses each 0.39 mms in width near the centre of the slide for separate 2 mm deposits to estimate average B.squamosa spore concentrations over 24 hour periods and also to get some estimate of two hourly changes in spore concentration as well.

Hirst in his paper of 1952 and in the Cassella instruction leaflet stated that the spore deposit is not of even density across the slide and recommended the centre line of the slide as the most suitable position to choose for a longitudinal travers. He also stated that in wind tunnel tests there were 2 areas of dense deposit along the axis and that the position of these areas changed with the suction rate and external wind speed. In looking at many spore trap slides it was also observed that in all cases there were areas of denser deposit near the centre of the slide. In

view of the above facts and in the absence of an accurate anemometer, the recommended method of estimating the total number of spores trapped per slide would give an erroneously large estimate due to the non-uniformity of the spore deposit. A correction factor was therefore calculated which gave estimates nearer to the correct totals. To derive this correction factor all the B.squamosa spores on one slide was counted for each 2x14 mm area using the grid described above. Then the spores in all the areas demarcated by the grid lines were counted in 5 longitudinal traverses, each 0.39 mm wide, in the central area of the slide. A correction factor of 4.8 times the total spores counted in the five traverses gave the nearest estimate of the actual total spores counted. Table 1 gives details of this calculation. The factor 4.8 seemed to be more accurate than the factor 7.18 which is used when uniform spore deposition is assumed.

TABLE 1

HOURLY *BOTRYTIS SQUAMOSA* COUNT FOR AUGUST 1, 1969 USED TO DERIVE A CORRECTION FACTOR

Grid lines between which spores were counted	Spores in Longitudinal Traverse number					Total Spores in 5 Traverses	Calculated Total Spores in Traverses x 4.8	Actual Total Spores counted in each 2x14 mm area of slide
	1	2	3	4	5			
8 - 9	0	0	0	0	0	0	0	1
9 - 10	0	2	1	1	3	7	34	46
10 - 11	18	26	29	19	16	108	518	536
11 - 12	18	19	18	9	21	85	408	377
12 - 1 PM	5	12	6	8	10	41	197	192
1 - 2	6	6	3	2	0	17	82	178
2 - 3	1	21	1	0	1	15	72	33
3 - 4	0	7	6	2	0	15	72	50
4 - 5	12	4	7	2	4	29	139	111
5 - 6	6	1	4	6	2	19	91	81
6 - 7	4	9	2	11	10	36	173	128
7 - 8	2	2	3	6	0	13	62	138
8 - 9	2	3	6	1	0	12	58	201
9 - 10	1	0	0	0	0	1	5	28
10 - 11	0	0	0	0	1	1	5	7
11 - 12	0	0	0	0	0	0	0	5
12 - 1 AM	0	0	0	0	0	0	0	0
1 - 2	0	0	0	0	0	0	0	0
2 - 3	0	0	0	0	0	0	0	0
3 - 4	0	0	0	0	0	0	0	0
4 - 5	0	0	0	0	0	0	0	0
5 - 6	0	0	0	0	0	0	0	0
6 - 7	0	0	0	0	0	0	0	0
7 - 8	0	2	0	1	0	3	14	8
TOTALS	75	105	86	68	68	402	1930	1920

On 6 days, chosen for reasons to be discussed later (page 111) short transverse hourly traverses of 0.39 mm each were made in the manner described by Hirst. The total numbers of spores per day, i.e. in 14.4 m^3 of air from these short traverses were calculated. These totals were found to be generally higher than the total daily estimates derived by multiplying the spores counted in 5 longitudinal traverses by 4.8. Details of these calculations are given in Table 2. This discrepancy between transverse and longitudinal spore counts might be due to such characteristics of the spore trap as the erratic deposition of spores in terms of periods of deposition and non-uniform impaction on the sticky slides, both normal characteristics of the Hirst spore trap and capable of causing such discrepancies when relatively narrow traverses such as 0.39 mm are used.

It is likely that with this spore trap, especially in cases where an anemometer is not used to correct its efficiency, any correction factor applied to longitudinal traverses would give only very approximate estimates of the total daily numbers of spores trapped. However, irrespective of the correction factor used, the relative values obtained with the trap are comparable. These values can give for example good indications of fluctuations in spore loads with time. This is generally a much more important parameter in

TABLE 2

HOURLY SHORT TRAVERSE B.SQUAMOSA CONIDIAL COUNTS

Time of Estimate	Estimate of Spores Trapped per hour i.e. spores per 0.39 mm Traverse x 5.128					
	July 26	July 28	July 31	Aug. 3	Aug. 7	Aug. 16
8 - 9	0	31	15	0	82	5
9 - 10	10	51	10	15	62	97
10 - 11	0	36	15	435	205	26
11 - 12	0	36	21	103	415	26
12 - 1 PM	21	21	5	123	287	0
1 - 2	46	31	0	103	164	0
2 - 3	26	10	0	72	185	5
3 - 4	5	5	0	46	241	21
4 - 5	41	0	15	26	77	62
5 - 6	0	0	0	26	149	118
6 - 7	0	5	0	15	87	77
7 - 8	10	0	10	36	0	51
8 - 9	5	0	0	10	67	5
9 - 10	5	0	0	0	108	10
10 - 11	0	0	0	0	374	31
11 - 12	0	15	0	0	385	205
12 - 1 AM	0	10	0	0	179	251
1 - 2	0	0	5	5	256	41
2 - 3	0	0	0	0	395	10
3 - 4	0	0	0	0	138	67
4 - 5	0	0	0	10	21	62
5 - 6	0	21	5	0	21	31
6 - 7	0	0	0	0	108	21
7 - 8	0	0	0	0	21	0
Total per Day	169	272	103	1026	4025	1220
Estimated Total from Longitudinal Traverses	106	305	78	951	3001	980

epidemiology than the absolute numbers of spores trapped.

All of the longitudinal traverses in this study were made in the central area of the slides and a correction factor of 4.8 was used in all cases to give an estimate of the number of spores in 14.4 cu.metres of air. Perhaps the best method of making estimates of absolute daily totals of spores trapped from counts derived from longitudinal traverses would be by making 5 random traverses which are not concentrated in the central area as suggested by Hirst, and then use a correction factor of 7.18 to compute daily totals.

3.2.2.4 Identification of B.squamosa Spores on Vaselined Slides

B.squamosa spores were relatively easy to identify after they had been pointed out by Mr.Leon Tartier. The conidia are hyaline and characteristically distorted, apparently as a result of dehydration (Figure 3). All the Botrytis type spores caught on the vaselined slides had the typical dimension of B.squamosa, i.e. 16-24 µ x 9-16 µ. They were thus generally larger than conidia of the other Botrytis species commonly associated with onions. The shape of the conidia was characteristic of that of B.squamosa. From spore size it is concluded that most, if not all, of the conidia trapped during this season at Ste. Clothilde were conidia of B.squamosa.

To see whether identified B.squamosa conidia from necrotic onion tips would become distorted and assume the same characteristics in Vaseline, spores from necrotic onion leaves were dusted onto vaseline coated slides and left for a few days before examination. When looked at under the microscope a few distorted Alternaria type spores and numerous Botrytis type spores, undistinguishable from those trapped normally on vaselined slides, were seen. This was taken as further evidence that the hyaline distorted Botrytis spores of the dimensions given above were conidia of B.squamosa and that distortion was due to action of the

FIGURE 3

FIGURE 3

3.2.3 Isolation of Pathogen from Diseased Leaves

Leaf blight disease symptoms were first noticed on July 22nd and one week later isolations were made from three types of leaf pieces:

1) Lesions, and a small portion of the surrounding healthy leaf tissue.

2) Necrotic leaf pieces on which the fungus was sporulating.

3) Apparently healthy leaf tissue from plants which generally had lesions on other leaves as very few plants were free of lesions at the stage when isolations were made.

In the isolation procedure no surface sterilants were used as they generally kill the pathogen. Leaf pieces, about 5 mm^2, were washed in 4 changes of sterile distilled water contained in 12 ml specimen bottles. They were then placed aseptically on PDA slants contained in screw capped test tubes 150 x 22 mm. 152 such isolates were made, these isolates were examined for growth of micro-organisms and tissue transplant transfers were made of the resulting white aerial mycelial cultures which produced sclerotia after about 10 days growth. Transfers were made until the cultures were visually pure.

The B.squamosa like isolates were brought to the Plant Pathology Department of Macdonald College on the 1st September 1969, some were placed in a refrigerator at 4°C, and the others were placed in a cupboard with a glass door in the laboratory where the temperature ranged from 16 to 29°C.

When sporulation occurred, single spore isolates were made by dusting spores onto partially dried PDA media in petri dishes and selecting individual spores by means of a specially sharpened dissecting needle. The single spores were then transferred, one per tube, to PDA media in screw capped test tubes 150x22 mm.

3.2.4 Measurement of Dew Periods

3.2.4.1 Background

Many instruments are used to record the duration of dew or moisture deposits on plant surfaces. Wallin (1967) lists several of these. The seven-day Taylor Dew Meter modified by Theis and Calpouzos (1957) was chosen for this project because the duration of the moisture deposit it measures is comparable to that on plant surfaces of similar orientation. The instrument is easily available, inexpensive and easy to set up and operate.

Basically, the Dew Meter consists of a pencil connected to a hygrothermograph clock mechanism which moves the pencil at the rate of approximately one revolution per week over a circular ground glass plate. The pencil leaves a mark whenever the ground glass surface is wet.

Many workers have found that the choice of a pencil is a major problem with this type of Dew Meter. The properties of any given type of pencil often vary from batch to batch and even within one pack the properties of individual pencils vary. A type of pencil suitable for one region may not be so for another region, experiments therefore have to be carried out to find a suitable pencil for any particular region. Experiments for this purpose are described below.

3.2.4.2 Determination of a Suitable Type of Pencil for the Dew Meter

A suitable pencil must have the following properties

1) A lead which is evenly and relatively slowly dissolved in dew as well as in rain.

2) A firm point which will last for at least a week.

3) A lead which leaves a trace that is not removed by subsequent rain.

The Theis and Calpouzos modification of the Taylor Dew Meter involves the use of a compass in which only one pencil is held at a time. Instead of this, a holder from which 5 pencils could be suspended was used to permit the testing of 5 pencils at a time and thus accelerate the choice of suitable pencils.

Another modification to the Dew Meter was made to allow an easier and more precise evaluation of the length of the dew trace. In the apparatus described by Taylor (1956) the ground glass plate is taken from the instrument and placed over a ruled chart for evaluation of the duration of the dew period. On the apparatus used radial lines were ruled in India ink (Reeves Waterproof) on the ground glass plates at arcs corresponding to hourly intervals. These lines remained distinct on the plates for over a month and gave a much more accurate estimate of the duration of the moisture deposit. Moreover, daily readings could be easily made in situ in the field.

The following pencils were used in preliminary tests:

Venus	Super Color	6200	RED
	"	"	BLUE
	"	"	PURPLE
	"	"	BROWN
	"	"	HELIOTROPE
	"	"	GREEN
	"	"	LIGHT GREEN
	"	"	ORANGE
	"	"	LIGHT BLUE
	"	"	SCARLET
	"	"	YELLOW
	"	"	BLACK
	Laurentian	1	DEEP YELLOW
	"	2	SARASOTA ORANGE
	"	3	POPPY RED
	"	5	ORCHID PURPLE
	"	6	NAVY BLUE
	"	9	DEEP CHROME GREEN
	"	11	CHESTNUT BROWN
	"	12	MIDNIGHT BLACK
Eberhard Faber	Captor Copying		712 EXTRA HARD
	"		712 MEDIUM
	"		122 HARD
	Weatherproof		6639
	Blue-Black Ink		
		Pencil	740 HARD
	"	"	740 MEDIUM
		Van Dyke	716 MEDIUM
	Colorbrite		2114 PURPLE
	Microtomic		H
	Mongol		863
	"		844
	"		865
	"		866
	"		944
Eagle	Chemi-sealed Verithin		741 INDIGO BLUE
	Turquoise		411
	Alpha		

Medium, Indelible copying, Government of Canada.

Paradise 114, cited as suitable types by Theis and Calgouzos, was not available.

Preliminary screening of pencils was done by performing the following test: A marked portion of ground glass was moistened and the other portion left dry. Each pencil was drawn slowly and with even pressure across first the dry and then the wet portion. The latter was then allowed to dry. The pencil traces on the dry and the previously wet areas of the ground glass were then compared.

For some pencils there was no apparent difference between the mark left on the wet and on the dry surfaces. Others, generally the indelible type of pencil, gave indistinct traces on the dry area and very broad diffuse ones on the wet area. Finally, others gave distinctly heavier but not very diffuse traces on the wet area as compared with the dry area. The pencils in this last category were further tested under field conditions on the dew recorder. Results of these tests are presented in Table 3.

Venus Laurentian Poppy Red 3 gave the best results followed by Eagle Verithin 741. The latter gave a more diffuse trace in the rain and its point did not last as long as the former. Weatherproof #6639,which was found by Mederick (1968) to be a suitable dew meter pencil under Macdonald College conditions did not differentiate well between wet and dry periods, although all its other characteristics were good. The Mongol indelible pencils recommended by Taylor (1956)

TABLE 3

CHARACTERISTICS OF PENCILS TESTED IN THE DEW METER

Pencil Type	Differential Mark	Diffusion of Pencil Trace in Rain	Pencil Point Lasting Ability	Remarks
Venus 6200 Black	Good	Very soluble great diffusion	Poor	
Venus Laurentian 3	Very Good	Very little diffusion	Good	Best tested
Venus Laurentian 5	Good	Medium diffusion	Medium	
Venus Laurentian 6	Good	Medium diffusion	Medium	
Venus Laurentian 11	Good	Medium diffusion	Medium	
Eberhard Faber Weatherproof #6639	Little difference	No diffusion	Very Good	Difficult to determine start of dew period.
Eberhard Faber Van Dyke 716	Good	Very soluble, great diffusion	Medium	
Eberhard Faber Colorbrite 2114	Medium	Medium diffusion	Poor	
Eberhard Faber Mongol 844	Good	Very soluble, great diffusion	Poor	
Eberhard Faber Mongol 944	Good	Very soluble, great diffusion	Poor	
Eagle Verithin 741 (indigo blue)	Very Good	Medium diffusion	Medium	Second best

gave too diffuse a trace in both heavy dew and rain to be suitable under Ste.Clothilde conditions.

Even though the Venus Laurentian Poppy Red 3 pencil was the best of the pencils tested, it made a trace in dew that was little different from that made in rain and pencils within one pack varied considerably with respect to the desired properties for the dew meter.

3.2.4.3 Duration of Dew on Meter Plate and on Leaves at Different Locations

In addition to the testing of pencils, a few observations were made on dew duration in the three different locations mentioned before. These locations were: 1) in low pasture, 2) in medium pasture under an apple tree, 3) in high pasture.

In medium and high pasture it was noticed on the nights during which dew was formed that the onset of Dew formation on horizontal exposed leaves generally approximated the onset of dew formation on the dew meter plate. In low pasture the onset of dew formation on horizontal exposed leaves varied from about the same time to one hour earlier than its onset on the dew meter plate. Cessation of dew on leaves of similar orientation and exposure as the dew meter

plate, and the dew meter plate itself was approximately the same for both medium and high pasture. However, here it was observed that dew generally remained longer on horizontal leaves than on vertical leaves, the additional length of time probably depending mainly on evaporation influencing factors such as wind speed, relative humidity and temperature. For location #1 dew cessation on the dew meter approximated dew cessation on nearby leaves.

After a suitable pencil was found the dew meter was set up in the onion field as shown on the map, (page 33). The plate was changed at weekly intervals and on a few occasions the duration of dew deposits on the dew meter was compared with dew duration on representative onion plant leaves. In the late stages of growth, when the onion plants were larger and ground cover was better, the dew duration as recorded by the dew meter approximated the visual duration of dew deposits on onion leaves in general, while earlier in the growing season the dew duration as recorded by the dew meter tended to be slightly less than the actual dew duration on onion leaves as determined by visual inspection. Figures 4 and 5 illustrate the design of the dew meter.

FIGURE 4 and FIGURE 5

FIGURE 4

Details of plate and pencil-bearing mechanism of Dew meter.

FIGURE 5

The Dew meter in operation.

FIGURE 4

FIGURE 5

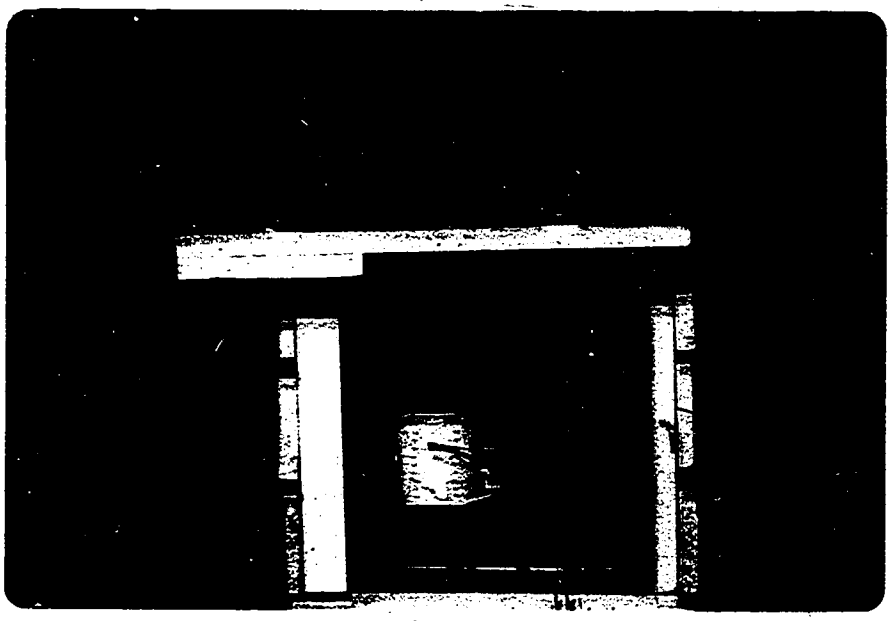


FIGURE 4

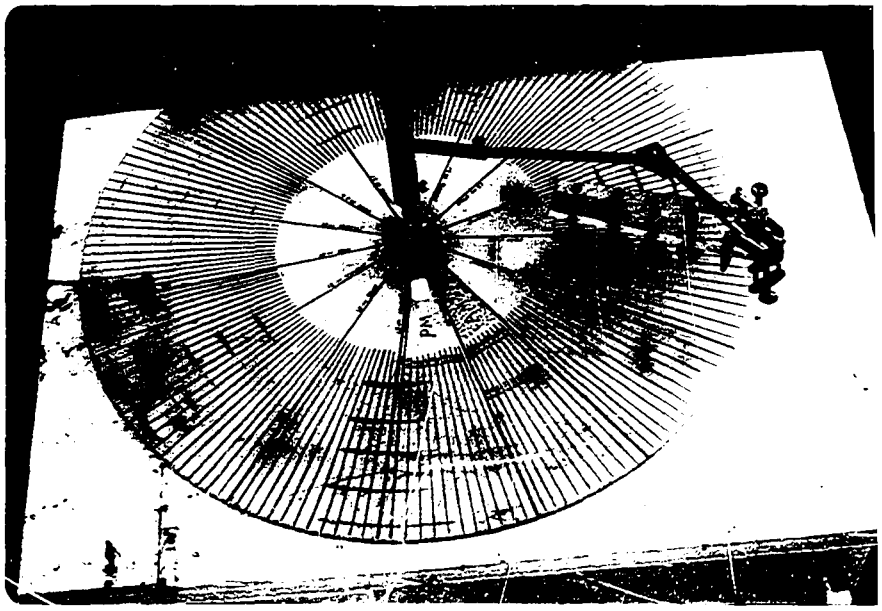
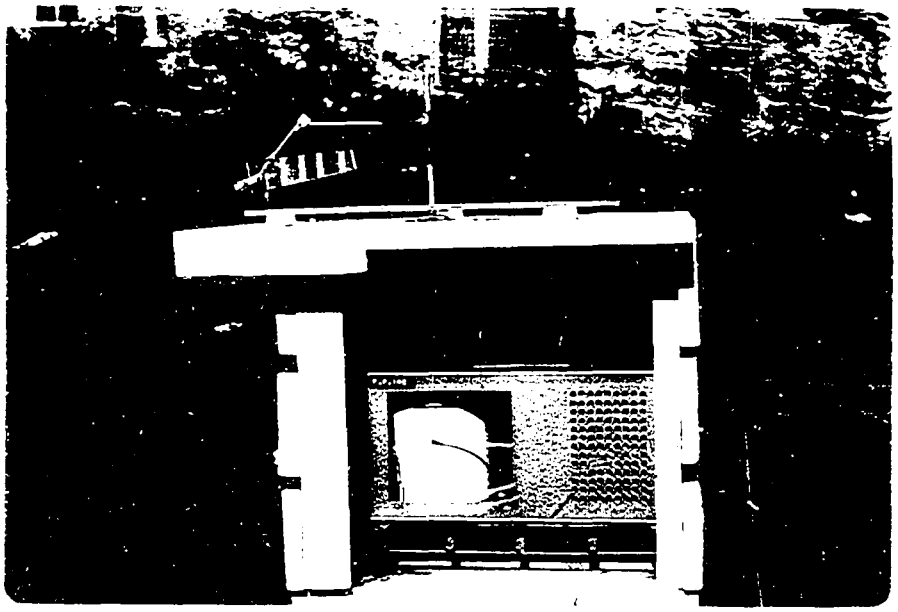


FIGURE 5



3.2.5 Disease Surveys

Some spots and tip dieback symptoms on the older leaves of plants in the field were noticed for the first time on 22nd July 1969. There was no apparent foci of infection as the symptoms appeared widespread on the majority of the onion plants. One week afterwards, on July 30th, the first leaf blight survey was made when it was confirmed that the lesions were most likely incited by a species of Botrytis because by then the pathogen was sporulating profusely on the necrotic leaf tips. Subsequent surveys were made on the 4th and 12th August.

The disease intensity was measured by counting the number of spots per leaf and per plant. Van der Plank (1963) gives an example for stem rust of wheat which affords a rational basis for doing this; also a group at Cornell University have been measuring disease intensity in terms of spots per plant as reported by Shoemaker et al (1967 a & b and 1968). Spots were counted on 10 samples of 5 plants each. Randomization of sampling was achieved by taking samples wherever a stick thrown in the general direction of the experimental plots had landed.

3.3 General Observations at Ste. Clothilde

3.3.1 Identification of *Botrytis* sp. Collected at Ste. Clothilde

Lesions, presumably incited by a Botrytis species, were first observed widespread on onion leaves in the field on the 22nd of July 1969. A species of Botrytis with large conidia was observed sporulating on these leaves at about the same time. The average size of the conidia was 20 x 14 μ which indicated that this fungus was probably *B.squamosa*. However, none of the Botrytis conidiophores examined showed the accordion like degeneration of the sporulating branches which is diagnostic of *B.squamosa*. It was realized later that this was probably because the conidiophores examined were not at the right stage to show this degeneration.

The symptoms of the disease observed in the field at Ste.Clothilde was essentially the same as those of onion blast described by various workers and noted in the Literature Review. Figure 6 shows the leaves of a three-month old onion plant at Ste.Clothilde with the typical dieback and lesion symptoms of onion leaf blight disease.

Isolations were made as described in Materials and Methods. Micro-organisms which grew out of the explants besides Botrytis were species of Alternaria, Stemphylium,

FIGURE 6

FIGURE 6

Onion leaves from Ste. Clothilde showing tip dieback and flecking symptoms.

FIGURE 6

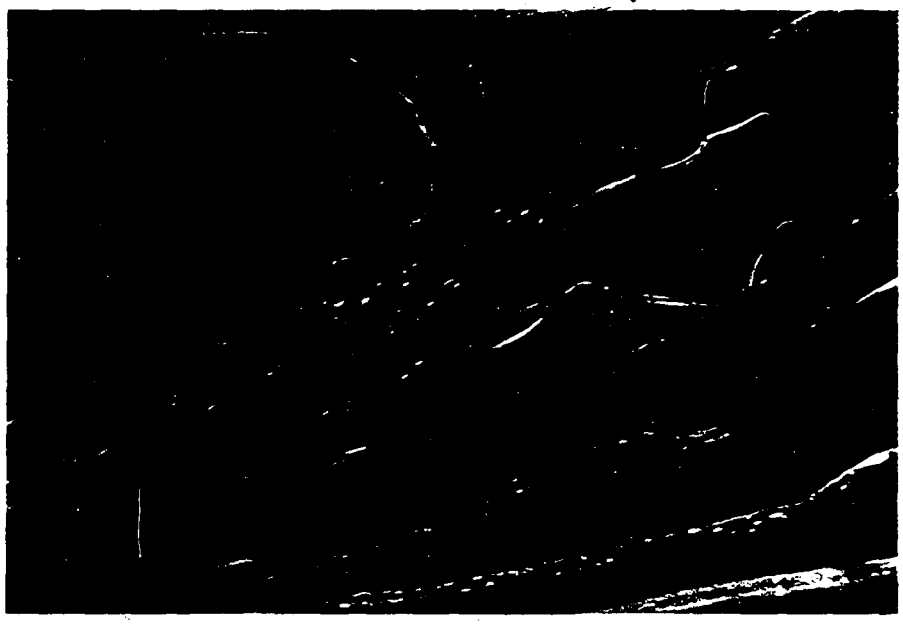
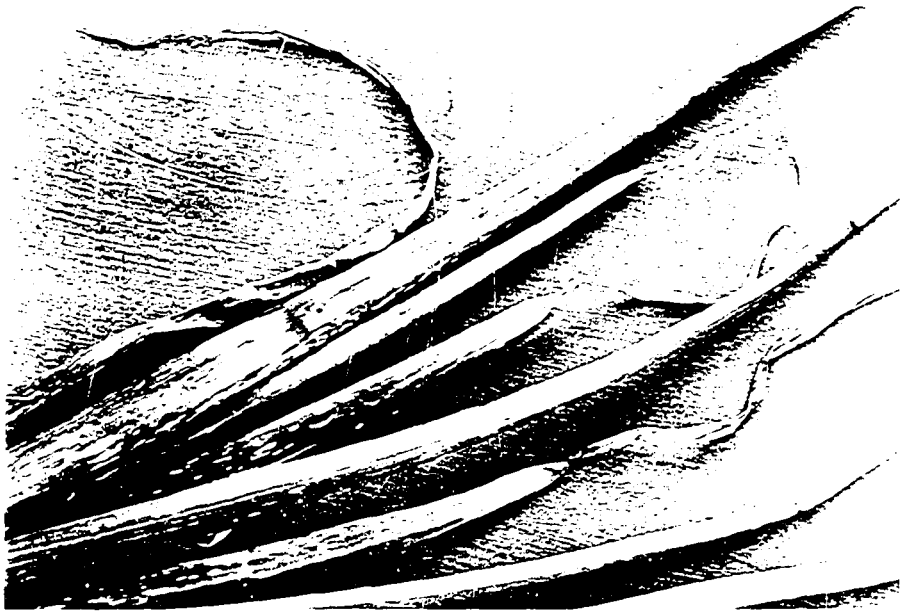


FIGURE 5



Fusarium, Penicillium and various unidentified bacteria. A fungus with white aerial mycelium was present in a few cultures but it did not form sclerotia or spores by which it could be identified. A Rhyzopus sp. was also present in one culture.

About 70% of the isolates obtained from lesions and 78% of those obtained from necrotic tissue had the mycelial and sclerotial characteristics of B.squamosa. However, only one of these was obtained from healthy leaf tissues.

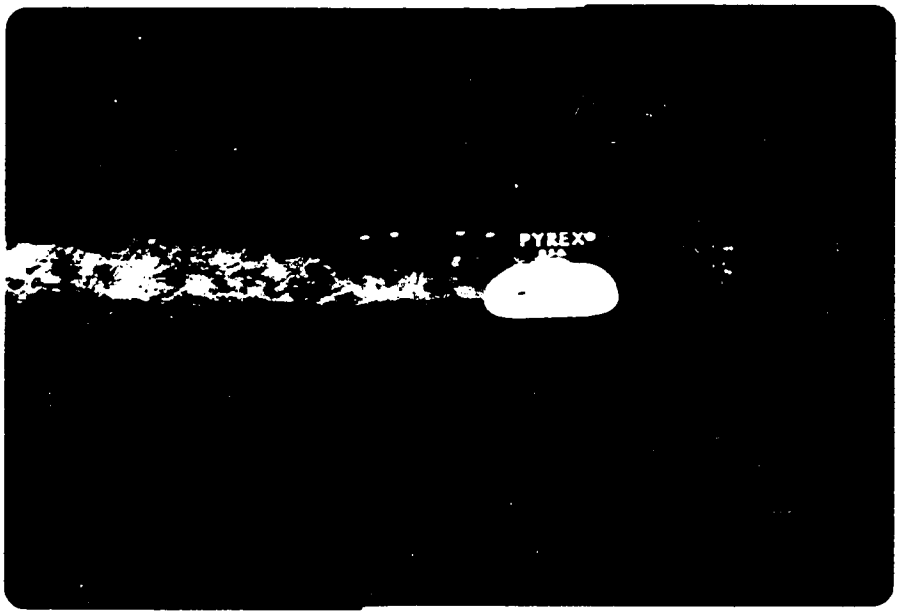
The isolates placed in the refrigerator at Macdonald College as described in Materials and Methods were examined periodically for conidial production until the end of December 1969 up to which time no sporulation was observed. When examined again on the 12th of February 1970,there was profuse sporulation on several isolates arising directly and exclusively from the surface of sclerotia. The isolates at room temperature produced no conidia. However,in 3 tubes (isolates No.120, No.122 & No.123) structures resembling apothecia were seen arising from some of the sclerotia (see Figure 7). Numerous microconidia were seen on the apothecia and mycelium of these isolates. However no asci or ascospores were seen. It is possible that these spores might have been shed at the stage when the apothecia were discovered. Isolates No.120,No.122,and No.123 and some others of the group of isolates

FIGURE 7

FIGURE 7

Apothecia arising from sclerotia in test tube culture.

FIGURE 7



kept in the laboratory at room temperature were then placed in the refrigerator at 4°C. and after one month many of these isolates had formed conidia, one of the isolates with apothecia producing typical B.squamosa conidiophores bearing conidia both from sclerotia and from the neck of an apothecium. The isolates forming conidia after 4 weeks incubation at 4°C. were isolates No.'s 120, 122, 123, 139, 148, 130, 141, 137, 129, 125 and 140;none of the other isolates with typical B.squamosa mycelial and sclerotial characteristics which had been kept at room temperature from September 1st formed conidia.

The conidia and conidiophores of the sporulating isolates were measured by means of a Reichert Visopan projection microscope.

The characteristics of all of the Botrytis isolates collected at Ste.Clothilde and finally brought to sporulation on PDA slants at 4°C. agrees in all important respects with the description of B.squamosa given by Walker.

The dimensions of conidia sampled from 5 isolates were:- 10 - 14 µ x 13 - 29 µ

Most conidia were in the range of 10-12 µ x 15-20 µ

The average for the 5 isolates being 12 µ x 19 µ

Hyphal threads averaged 2.5 μ diameter. Conidiophores had an average diameter of 12 μ at the segments of nodes while the central portions averaged 18 μ .

Microconidia were 1.7 - 2.1 μ in diameter, the average size being 2 μ . The average diameter of microconidial clusters was 36 μ .

Figures 8, 9, 10 and 11 illustrate various characteristics of the isolates examined.

3.3.2 Disease Surveys

The average number of lesions per plant on the survey dates are given in Figure 19, page 95.

About 550 spots per plant corresponded to 100% disease intensity. This conclusion was reached from a consideration of: 1) the number of spots per plant observed in these surveys, 2) disease progress curves drawn from data gathered by M. Tartier on the same epidemic at Ste. Clothilde and in which the disease intensity was measured according to the Horsfall and Barratt's system, (Horsfall and Barratt 1945), and 3) data taken from Shoemaker et al's reports of 1967 and 1968. Using 550 lesions per plant as 100% disease, disease progress curves were drawn.

FIGURE 8

FIGURE 8

Typical degenerating accordion-like side branches of a conidiophore of *B. squamosa*.

FIGURE 8

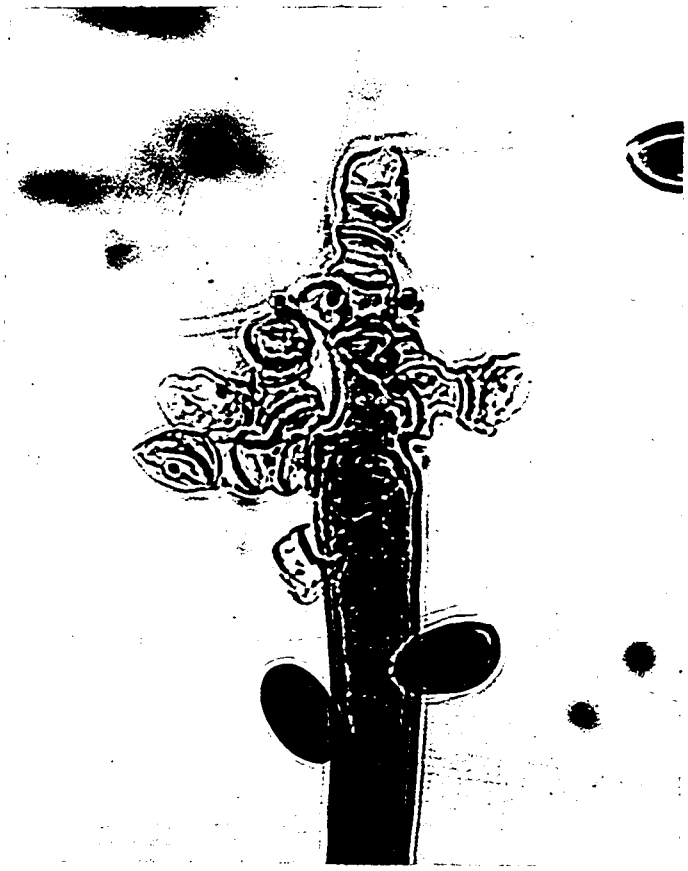


FIGURE 8

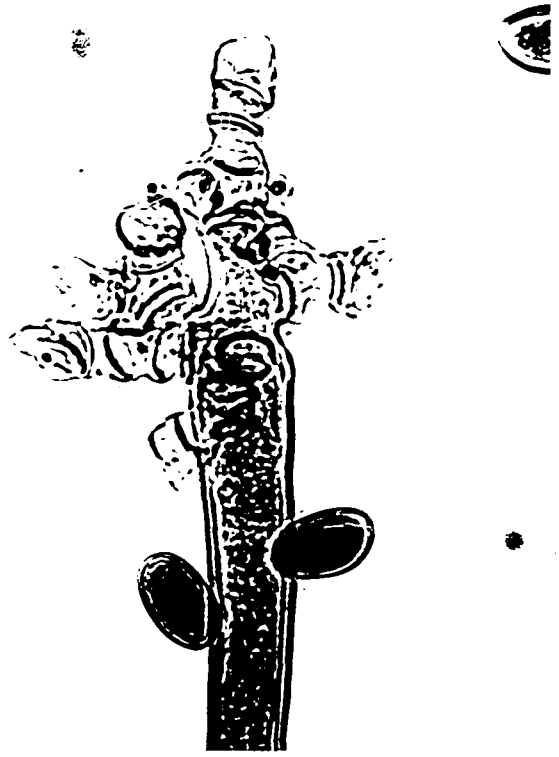


FIGURE 9 and FIGURE 10

FIGURE 9

Young conidiophore of B. squamosa with attached conidia.

FIGURE 10

Conidiophore of B. squamosa with sterigmata still attached.

FIGURE 9

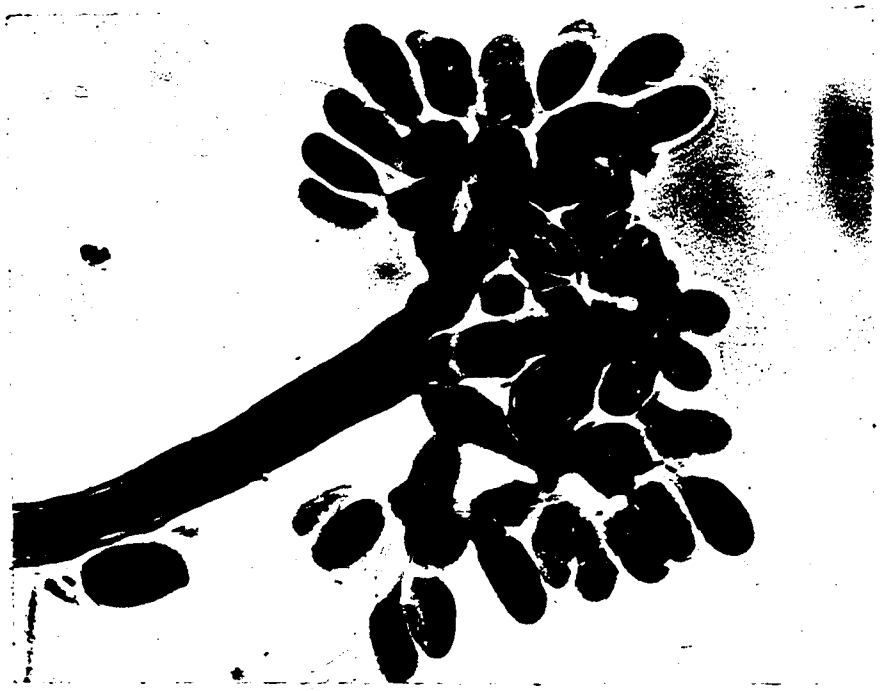


FIGURE 10

FIGURE 9

FIGURE 10

FIGURE 11

FIGURE 11

Petri dish cultures of 3 B.squamosa isolates showing sclerotia.

FIGURE 11

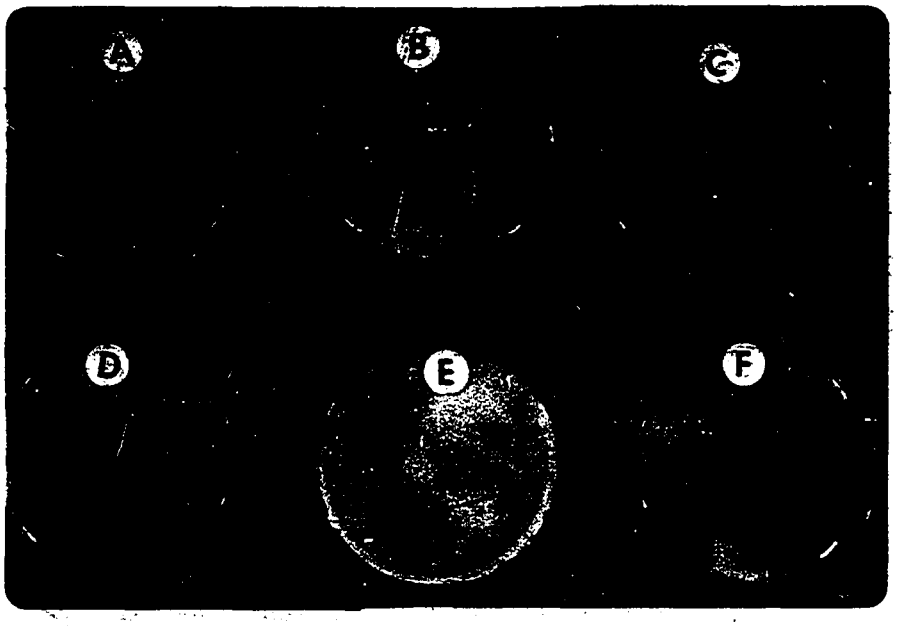


FIGURE 11

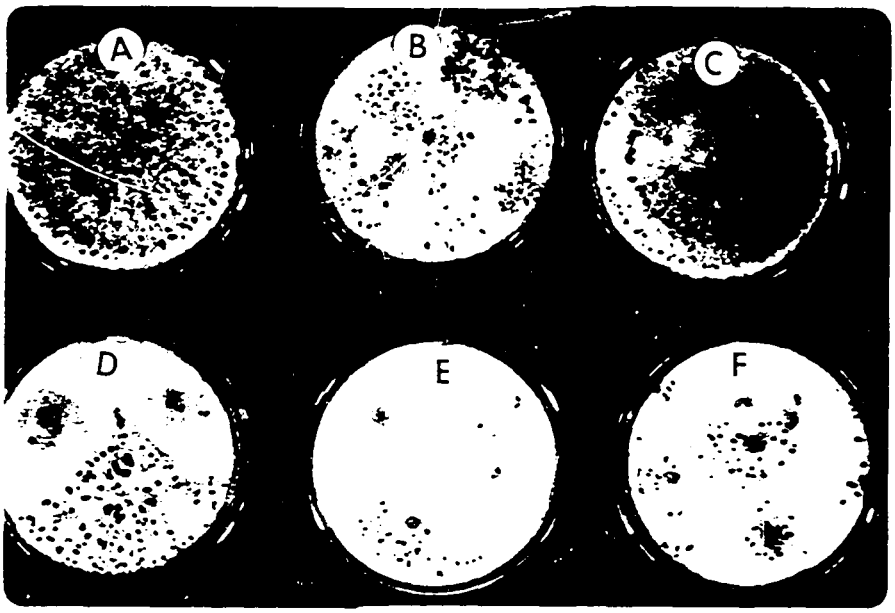


Figure 12, Curve A shows that the epidemic progressed at a very rapid rate until August 12th. One would expect that it would decrease subsequently but no survey was made after that date. Curve B represents the same data after Van der Plank's(1963) transformation was applied to them. The apparent rate of infection is typical of an explosive epidemic being 0.31 lesions per unit per day for the period between the first and last survey.

Figure 13 shows the rate of involvement of leaves in general with the disease. The curves in Figure 13A show that the percentage of leaves with tip dieback symptoms were generally lower than those of leaves with spots. However, as the epidemic progressed, these percentages approached one another as the rate of dieback development increased and that of leaf spotting decreased. The curve in Figure 13B shows the progressive decrease in the percentage of healthy leaves as the disease developed.

The histograms of Figure 14 give the percentage of plants with dieback and that of plants with spots. Results are given for each leaf of the plants on 3 dates, leaf No. 1 was the oldest leaf.

FIGURE 12

FIGURE 12

Disease progress curves of Ste. Clothilde epidemic.

FIGURE 12

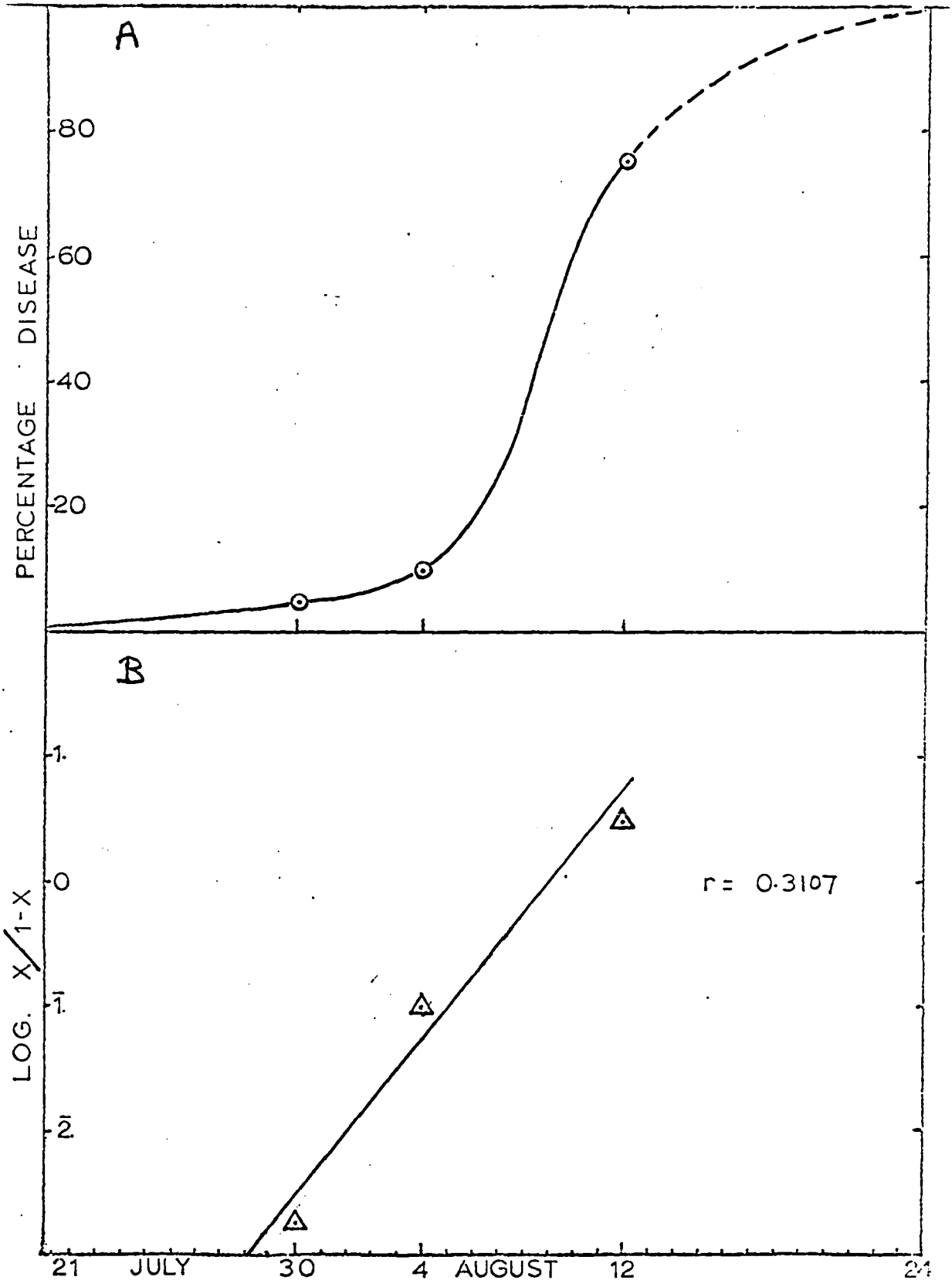

A
80
60
40
20
PERCENTAGE DISEASE
B
1.
0
1̄.
2̄.
LOG. X/1-X
r = 0.3107
21 JULY
30
4 AUGUST
12
24

FIGURE 13

FIGURE 13

Percentages of leaves with spots and dieback on survey dates.

FIGURE 13

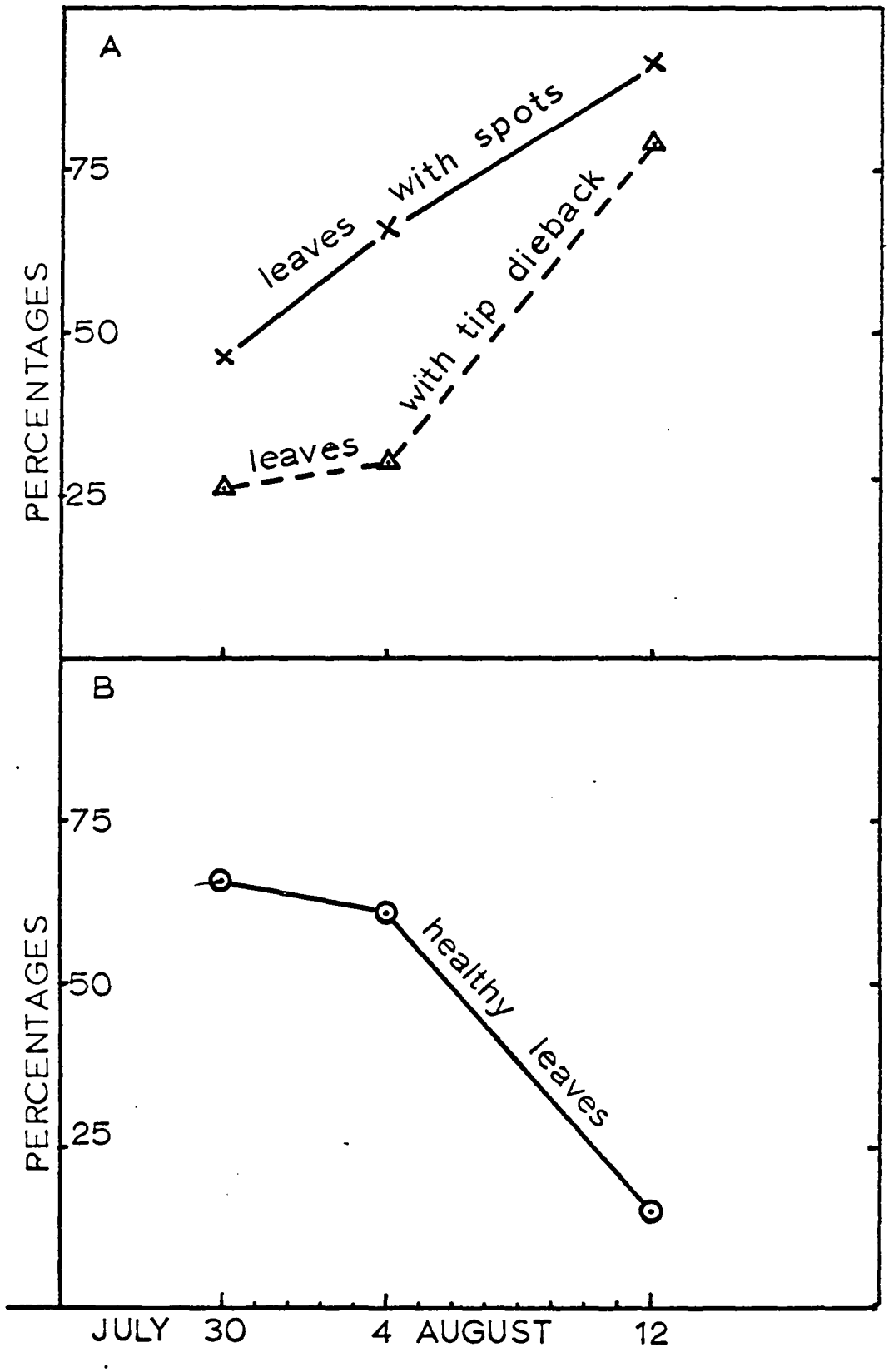
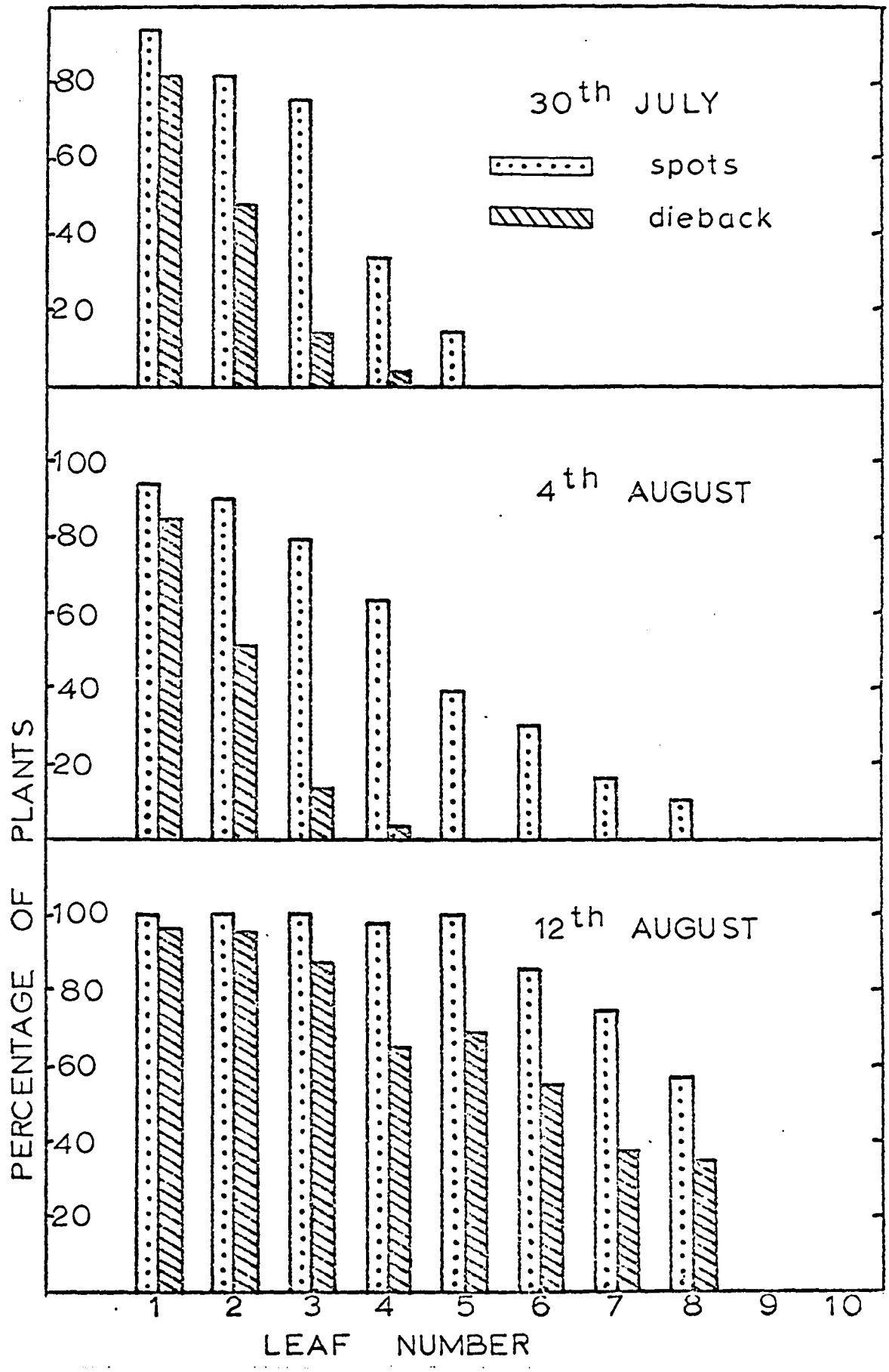

A
75
50
25
PERCENTAGES
leaves with spots
leaves with tip dieback
B
75
50
25
PERCENTAGES
healthy leaves
JULY 30
4 AUGUST
12

FIGURE 14

FIGURE 14

Percentages of plants with individual leaves having spot and dieback symptoms.
Leaf No. 1 is the oldest leaf. There were 8 leaves per plant in survey 1, 9 in survey 2 and 10 in survey 3.

FIGURE 14


30th JULY
spots
dieback
4th AUGUST
12th AUGUST
PERCENTAGE OF PLANTS
20
40
60
80
100
1
2
3
4
5
6
7
8
9
10
LEAF NUMBER

It appears that:

1) On younger leaves spots developed prior to dieback.

2) Generally, a greater percentage of older leaves had spots than that of younger leaves.

3) The proportion of leaves affected with dieback was smaller than that with spots.

4) The rate of dieback development on younger leaves increased greatly in the latter stages of the epidemic.

The average number of lesions on each of the different leaves of the plant is given in Figure 15.

In general, older leaves had more lesions than younger leaves.

A multiple correlation analysis was set up to determine mainly if there was any significant relationship between dieback and number of spots/leaf, dieback was the dependent variable y, and the number of spots/leaf and the leaf number were the independent variables x_1 and x_2 respectively, (for the notations used see Steel and Torrie 1960).

The first four leaves for each sample were analysed, the oldest leaf being numbered '1', the second oldest '2', etc. The average number of lesions per sample for each of the first four leaves were taken giving a total of 40 leaves sampled per survey. Tip dieback was classified into four

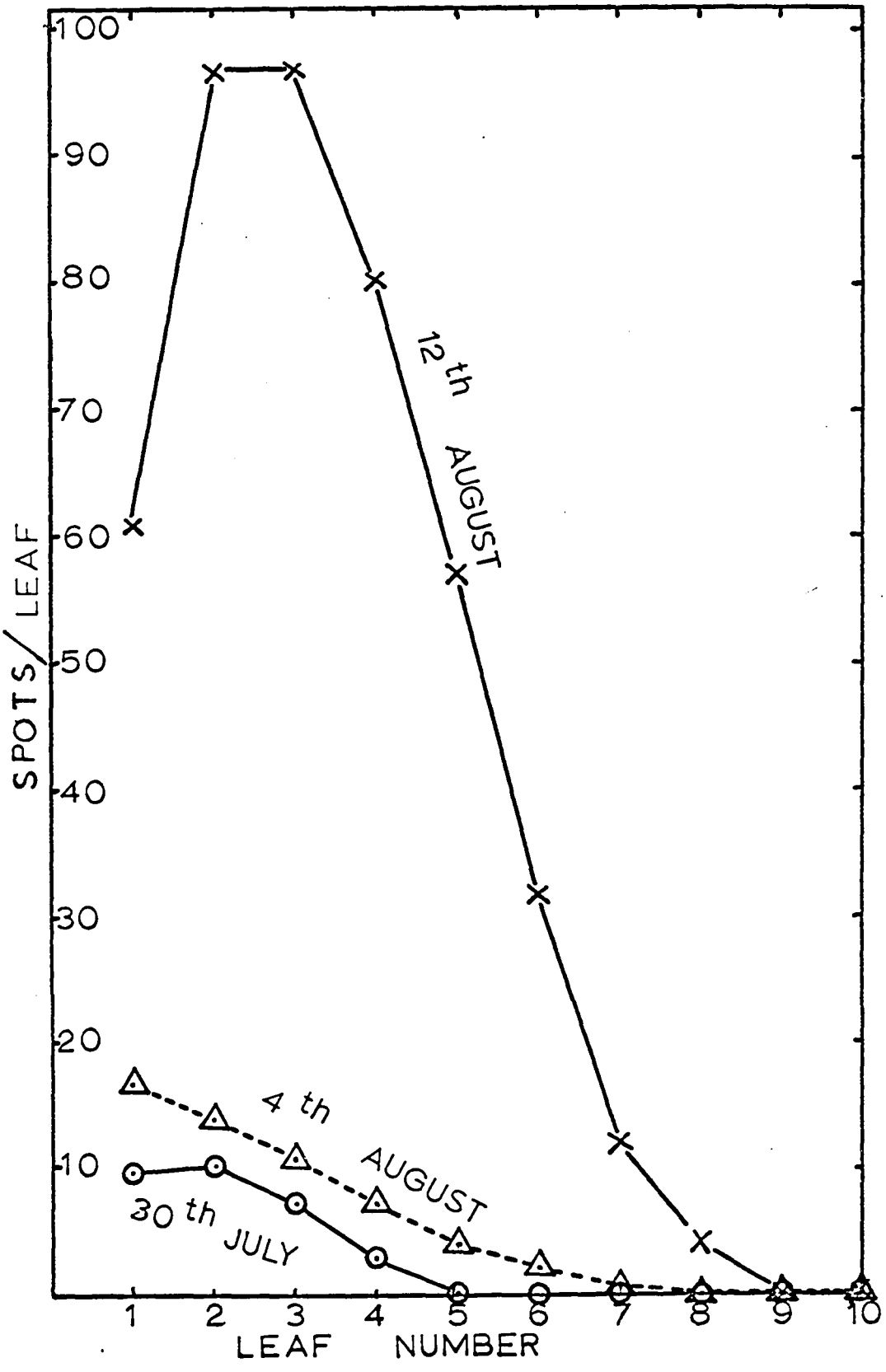
FIGURE 15

FIGURE 15

Relative mean numbers of spots on different leaves on 3 survey dates.

Leaf No. 1 is the oldest leaf.

FIGURE 15


100
90
80
70
60
50
40
30
20
10
SPOTS/LEAF
12th AUGUST
4 th AUGUST
30 th JULY
1
2
3
4
5
6
7
8
9
10
LEAF NUMBER

categories or scores: 1 - Tip necrosis one inch or less, 2 - Tip necrosis one to two inches, 3 - Tip necrosis about half of leaf, 4 - Tip necrosis over half of leaf. There were 5 leaves per sample hence the maximum score for tip dieback per leaf was 20. There were a number of zero scores for tip dieback in the first two surveys and so the transformation tip dieback + ½ was used in the correlation analysis of these surveys.

Appendix Table 3 summarizes the results of this analysis.

There was no correlation with fixed leaf number between lesions/leaf and dieback in any of the surveys, survey #2 however was near the .05 level of significance.

In survey #1 there was a significant correlation between lesions and leaf no. but in the other two surveys there was no such correlation. This result suggests that in the early stages of the epidemic some leaves were more susceptible than others and as inoculum and environmental conditions became more favourable for the pathogen there was no significant difference in susceptibility of leaves to lesion development.

In all three surveys there were very significant correlations between the leaf no. and dieback which implies that older leaves were more susceptible to dieback than

younger leaves (Figures 13 and 14). These very significant correlations also explain the apparent discrepancy between the results of the partial correlation and the sample correlation analysis for correlations between lesions and leaf number, as it is due to this high correlation between leaf number and dieback that there were significant simple correlations ($P < .05$) between lesions and tip dieback in the first two surveys.

3.3.3 An Attempt to Derive a Regression Factor for Converting number of Spots on a Particular Leaf to Number of Spots per Plant.

Large (1967) has pointed out that in a disease measurement programme it is always useful to develop a measure of disease intensity which is easy to compile and gives a good correlation with actual disease intensity derived from assessments on whole plants. K. Starr Chester (1959) also stated that it would be very helpful if 2 or more expressions of disease were correlated with each other. It was therefore decided to set up regression analyses of the number of spots/plant vs. the number of spots on other specified leaves for each survey. In this analysis, for each variable, the average per sample was taken.

Appendix Table 1 is a summary of the results of these simple regressions. It indicates that for each survey

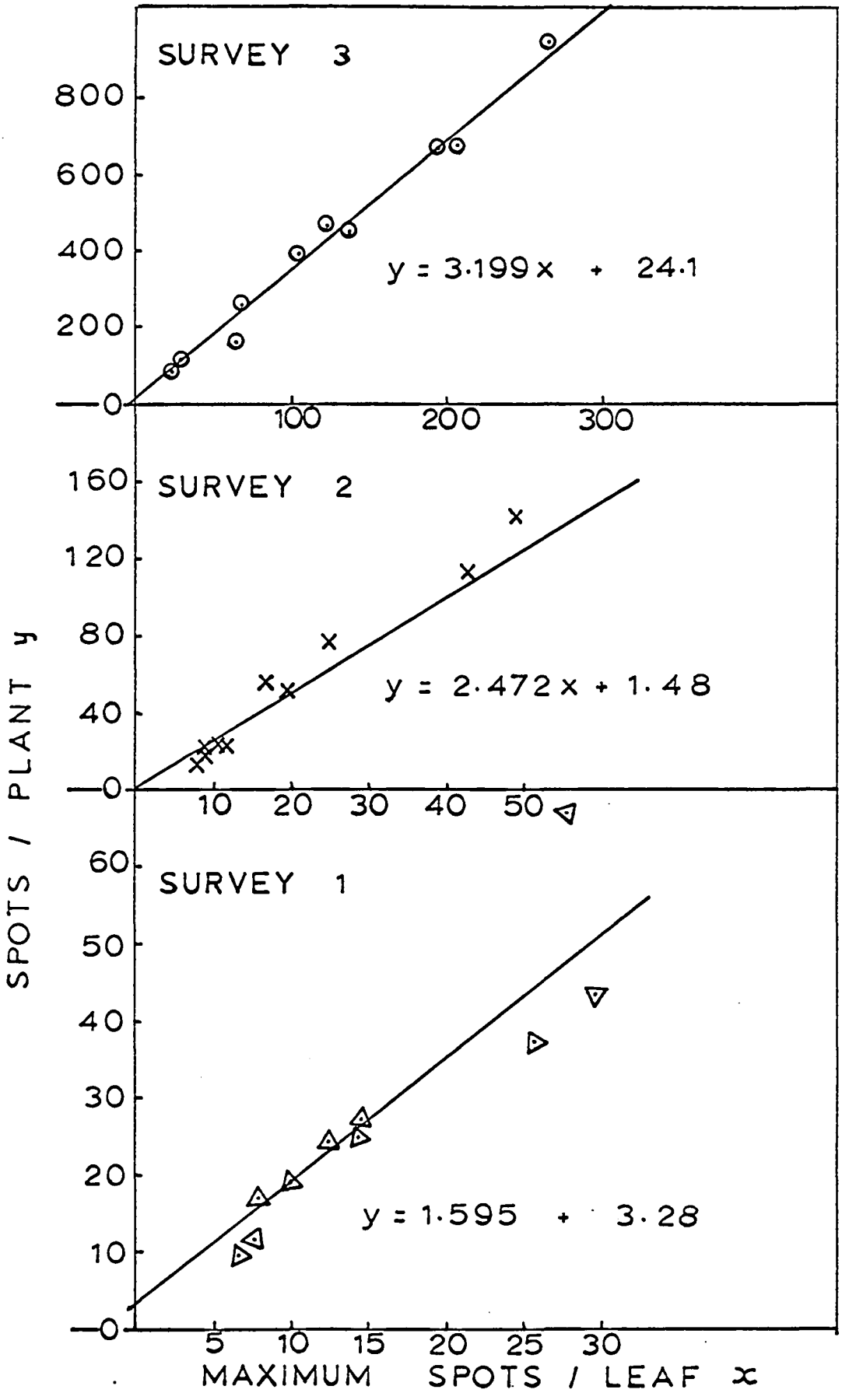
the number of spots on the leaf which had the maximum number of spots was highly and positively correlated with the total number of spots per plant. The number of spots on the 4th youngest leaf was the next best variable in this respect. A multiple correlation analysis was then set up using the three variables: y - total number of spots/plant, x_1 = number of spots on the 4th youngest leaf, and x_2 = number of spots on the leaf which had the most spots/plant. Appendix Table 2 gives a summary of the results of this analysis. In all surveys the number of spots on the leaf with the most spots/plant was highly correlated with the total number of spots/plant while the same result was found for the 4th youngest leaf only in surveys #2 and #3. Figure 16 comprises graphs in which the number of spots/plant is plotted against the number of spots on the leaf with the most spots. The results of these correlation analyses indicate that it is possible to obtain a good estimate of the total number of spots per plant by merely counting the number of spots on the leaf with the highest number of spots at most stages of the epidemic. Counting the number of spots on the 4th youngest leaf would only give a good estimate of the total number of spots per plant in the latter stages of the epidemic. If these correlations bear up under future investigations where more samples are taken the presently described method would be a quicker and more accurate method of disease measurement

FIGURE 16

FIGURE 16

Relationship between number of spots per plant and number of spots on the leaf with the most spots for the three survey dates.

FIGURE 16


SURVEY 3
y = 3.199x + 24.1
SURVEY 2
y = 2.472x + 1.48
SURVEY 1
y = 1.595 + 3.28
SPOTS / PLANT y
MAXIMUM SPOTS / LEAF x

for onion blight disease on a field scale than the presently used Horsfall and Barratt system.

3.3.4 General Spore Trapping Results

B.squamosa spores were first observed on the 19th of July and, with the exception of the 20th and 21st of July, spores were noticed every day afterwards until the 22nd of August when this phase of the investigation was terminated.

The number of spores trapped per day varied greatly from day to day as can be seen in Figure 17. There were apparently regular peaks and troughs in daily spore concentrations. The maximum daily concentration was $30x10^2$ spores per 14.4 M^3 of air.

Diurnal variation of the B.squamosa conidia trapped is clearly seen in Figure 18. The maximum numbers of conidia occurred during the day and minimum numbers at night. There were atypicalconidial deposits on the 7th and 16th of August (see Figure 26, page 117); these deposits were atypical in that considerable numbers of spores were trapped during night-time hours. Similar atypical deposits (Appendix Table 3) were observed on the 8th,9th,and 10th of August, but in these cases the night-time spore deposits were not as high relative to the daytime deposits as were those on the 7th and 16th of August. The maximum number of conidia trapped per hour was 435 per o.6 cubic metres of air.

FIGURE 17

FIGURE 17

Spores trapped per day and weekly averages during spore trapping period.

FIGURE 17

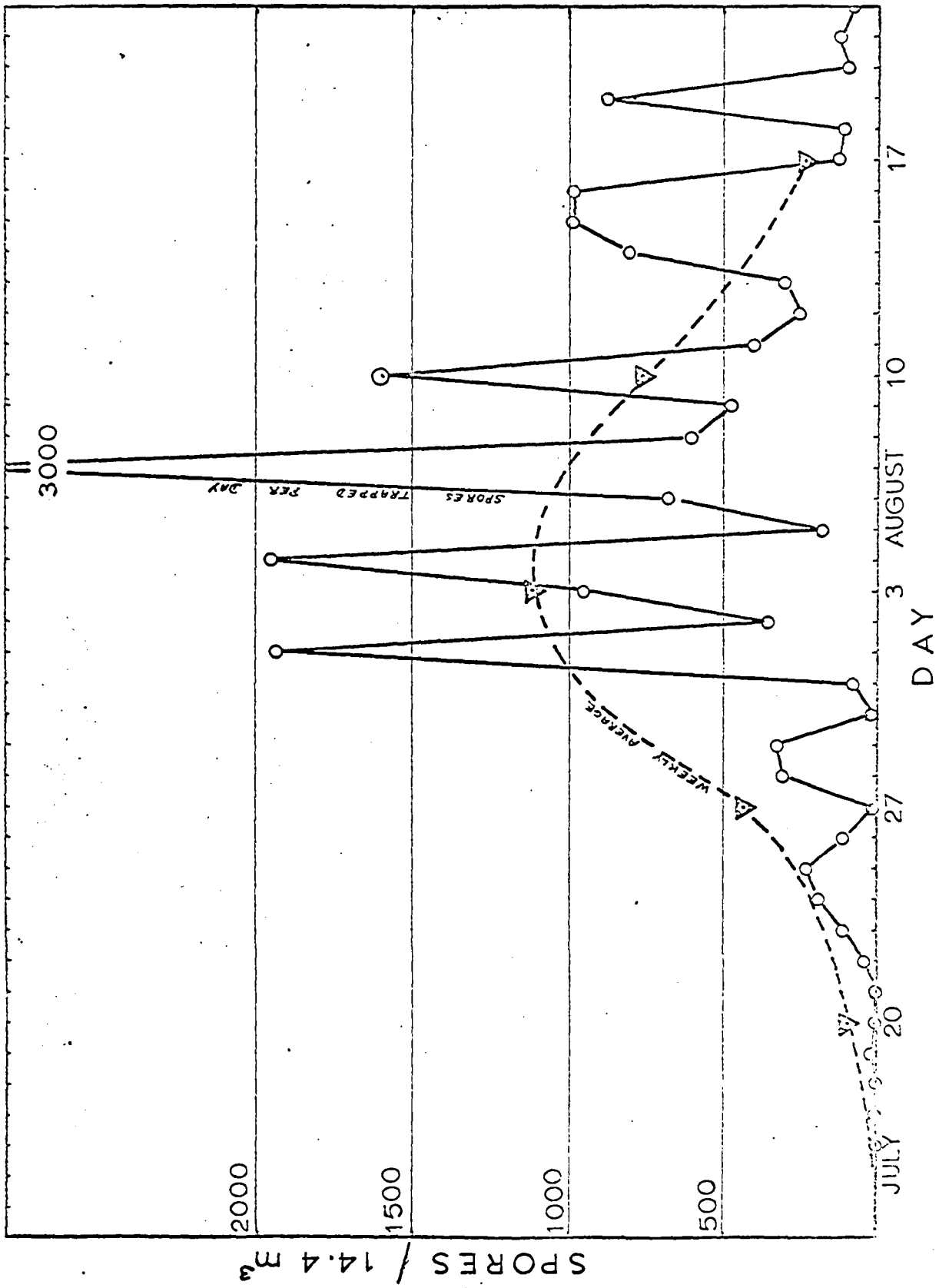
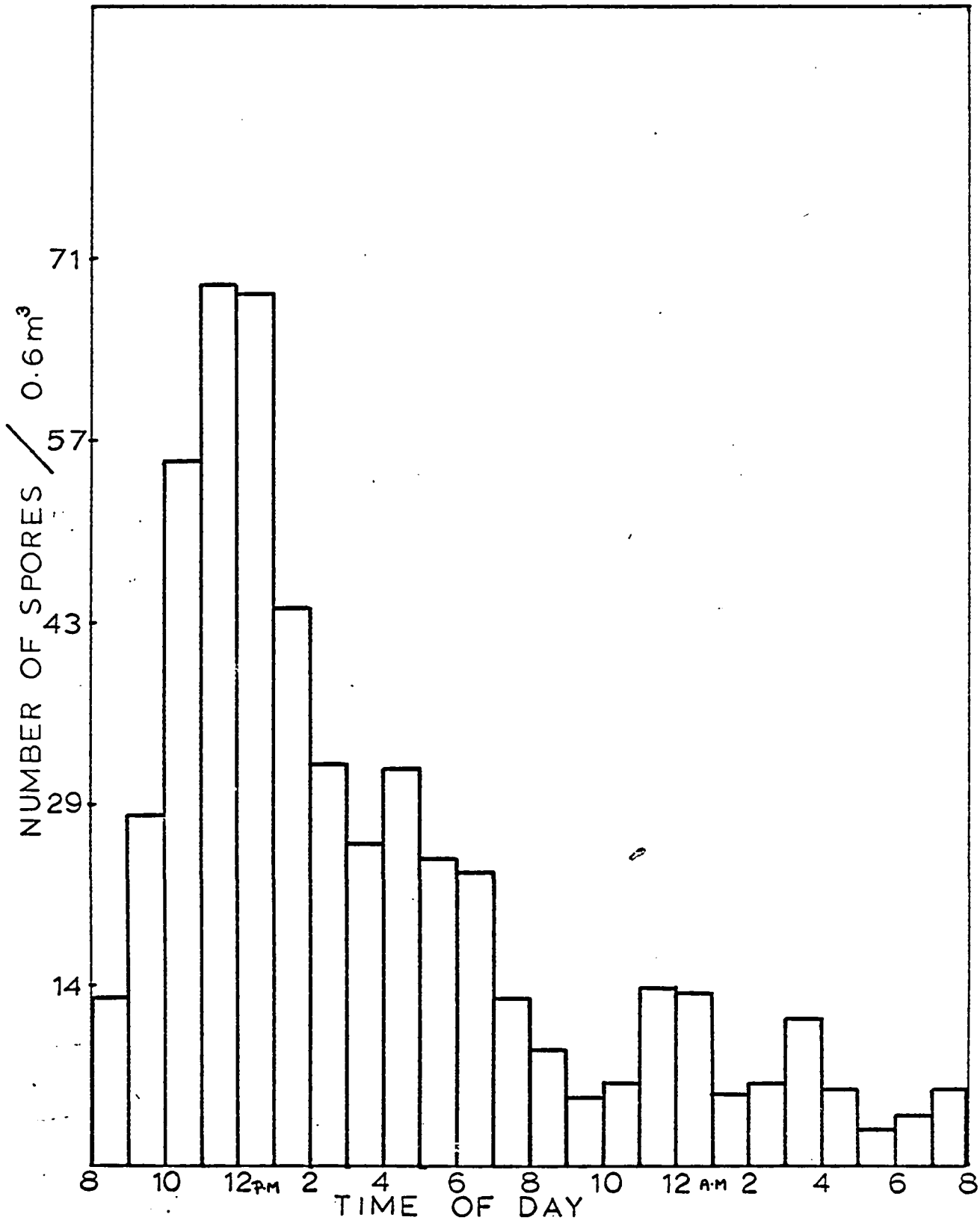

SPORES / 14.4 m³
2000
1500
1000
500
3000
SPORES TRAPPED PER DAY
WEEKLY AVERAGE
JULY
20
27
3 AUGUST
10
17
DAY

FIGURE 18

FIGURE 18

Average hourly spore trap counts for longitudinal traverses for spore trapping period.

FIGURE 18


NUMBER OF SPORES / 0.6 m³
71
57
43
29
14
8
10
12 P.M
2
4
6
8
10
12 A.M
2
4
6
8
TIME OF DAY

3.3.5 Effect of Spore Concentration on Disease Intensity

In a natural or artifical plant disease epidemic there is generally some relationship between inoculum concentration and the resultant disease intensity on infected plants. Coulson (1968) gives an excellent discussion of many aspects of this. In this study it was thought that spore trap data could give some indication of the amount of inoculum present in the atmosphere at various times and that this could be compared with disease survey data to determine if there was any relationship between numbers of spores trapped and disease intensities. Since a one-day incubation period for symptom development was indicated by laboratory experiments (page166) cumulative spores trapped one day before each survey were totalled and compared with the disease intensities on the survey dates in three types of dosage-response analyses.

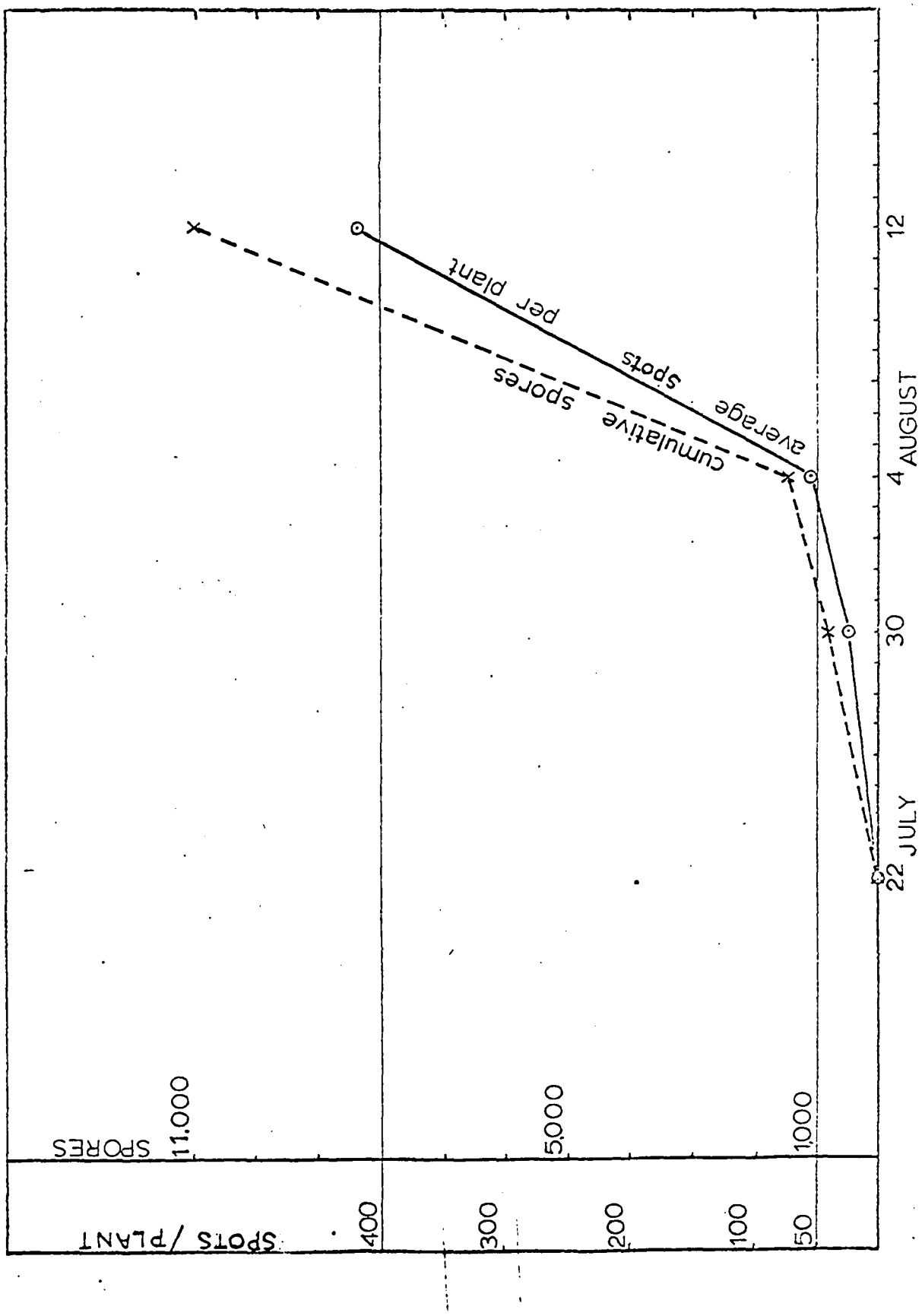
Figure 19 shows the average number of spots per plant at the three survey dates. Superimposed on this is the cumulative number of spores trapped one day prior to the days on which the surveys were carried out. The graph shows that these two variables appear to be closely related to each other for the first two surveys but diverge from each other on the final survey. This suggested the possibility that at the last survey the large number of spores were competing for infection

FIGURE 19

FIGURE 19

Average spots/plant and cumulative spores trapped on survey dates.

FIGURE 19


SPOTS / PLANT
400
300
200
100
50
SPORES
11,000
5,000
1,000
average spots per plant
Cumulative spores
22 JULY
30
4 AUGUST
12

sites on the onion leaves and hence their infection efficiency was reduced.

Garrett (1960) recommended plotting log dose: probit response curves for studying the relationship between inoculum density and resulting disease. Figure 20 is a log dose: probit response curve for the data given in Table 4. A regression analysis was carried out for this data and was found to be non-significant. There was therefore no significant relationship between log dose and probit response.

Van der Plank(1963) suggested the use of the logit transformation $\log_{10} \frac{x}{1-x}$ to correct for disease intensities over 0.1% in which the amount of available host tissues becomes limiting. As Figure 20 suggested such a situation, this transformation was tried. The log of cumulative spores trapped one day before surveys was plotted against the logit of the percentage disease in Figure 21. This transformation of the data gives a well fitted straight line and indicates that there might indeed be some competition between propagules; however, as Coulson(1968) points out, the factor 1-x corrects only for the influence of x and not for a number of other factors such as loss of tissue bearing fruiting of the pathogen, production of new tissue by the host, the latent period, etc.

TABLE 4

NUMBER OF SPORES TRAPPED ONE DAY BEFORE SURVEYS, NUMBER OF SPOTS COUNTED DURING SURVEYS AND TRANSFORMATIONS OF THESE VARIABLES

	Dosage x		Response y			
Date	Cumulative spores trapped one day before survey date	Log of cumulative spores trapped	Average Number of spots/ plant	Percen-tage Disease	$\log_{10} \frac{y}{1-y}$	Profit of y
30.7.69	1,316	3.1206	28	5.1%	$\bar{2}.730$	3.355
4.8.69	4,645	3.6675	52	9.5%	$\bar{1}.021$	3.718
12.8.69	13,513	4.1303	413	75.1%	0.477	5.665

FIGURE 20

FIGURE 20

Log of cumulative spores on survey dates vs probit of number of spots/plant.

FIGURE 20

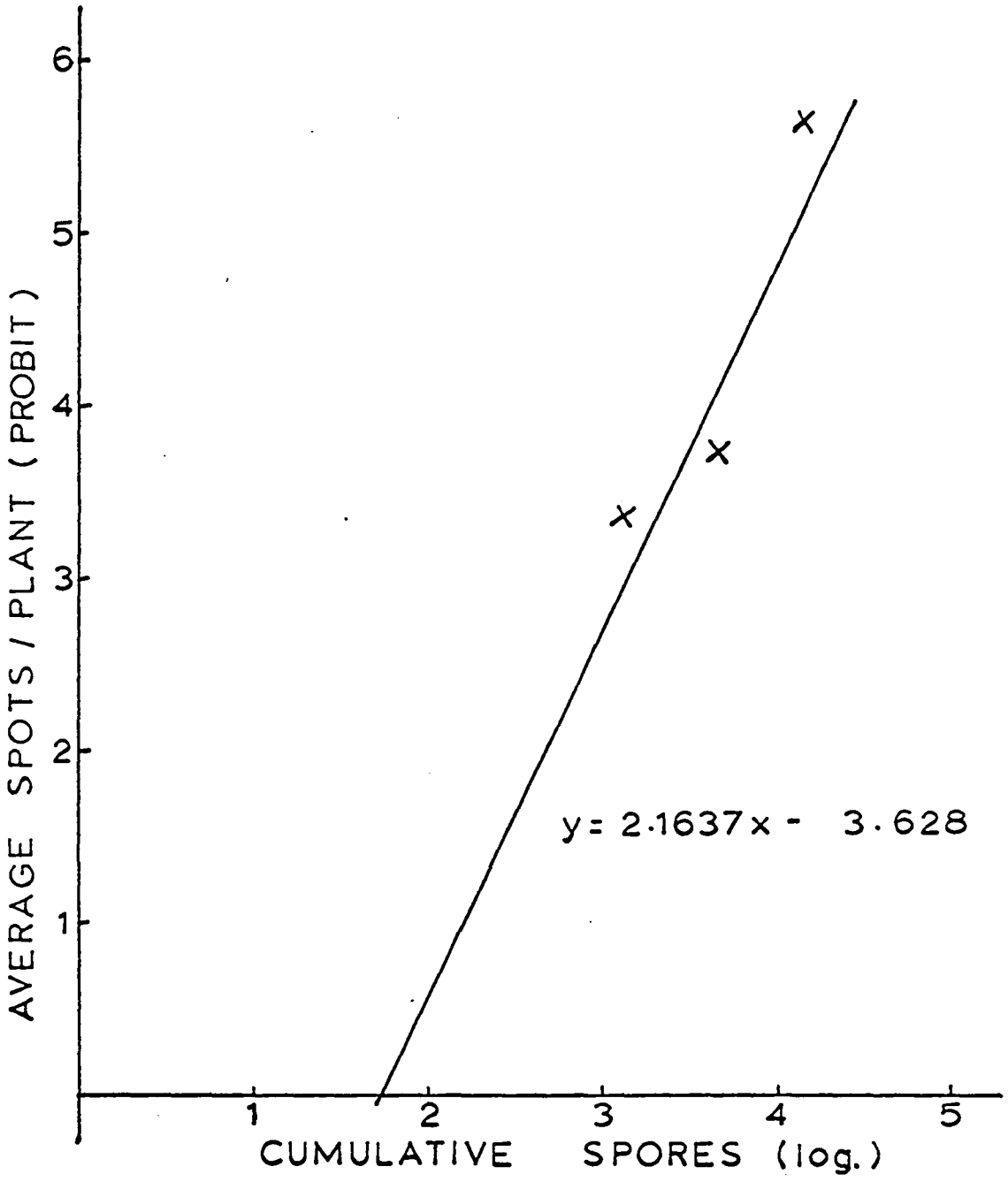

AVERAGE SPOTS / PLANT (PROBIT)
6
5
4
3
2
1
y = 2.1637x - 3.628
1
2
3
4
5
CUMULATIVE SPORES (log.)

FIGURE 21

FIGURE 21

Log of cumulative spores vs. logit of number of spots/plant on survey dates.

FIGURE 21

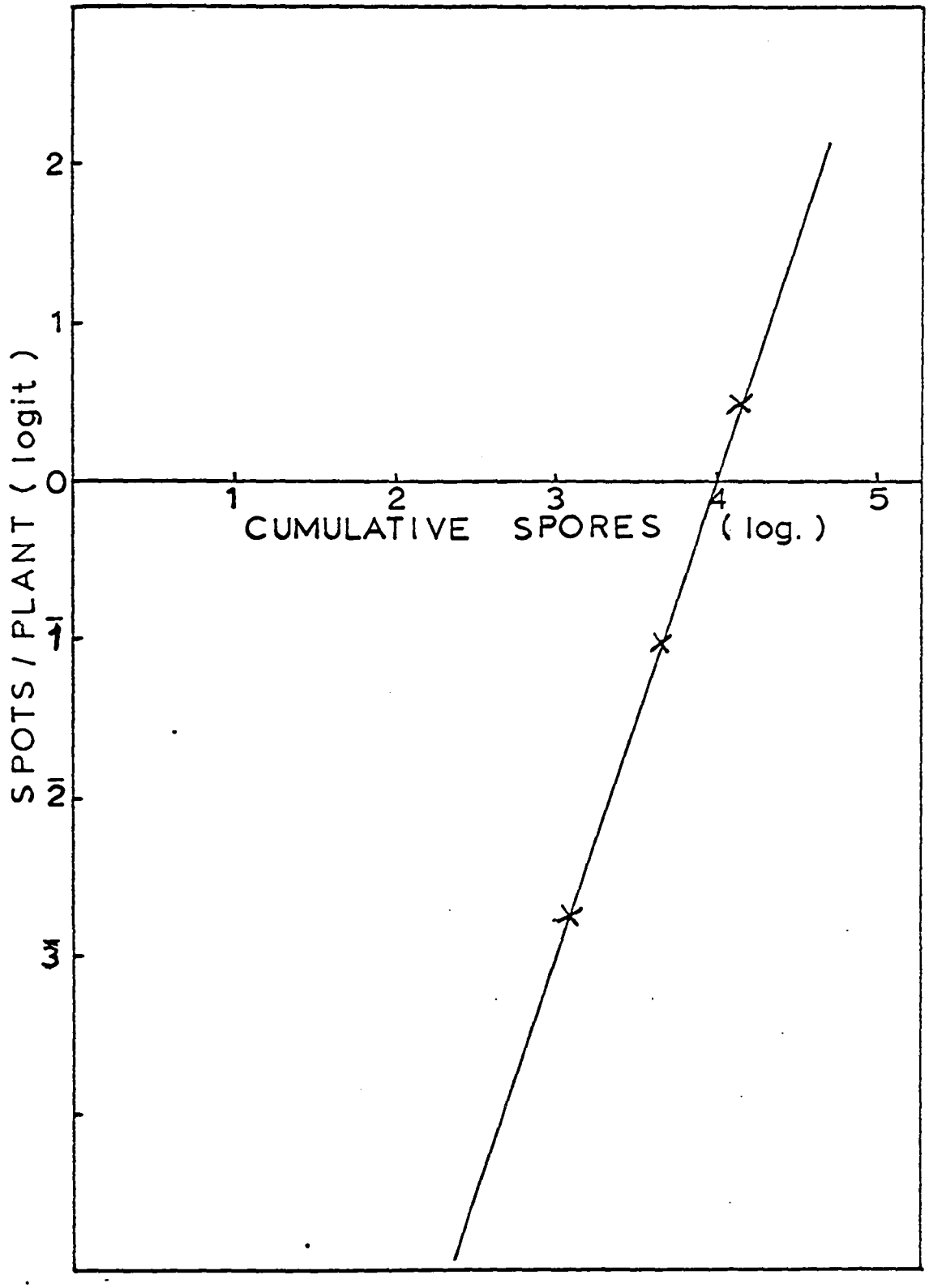


Figure 22 is a dosage response curve in which the untransformed data of Table 4 is presented graphically. There was no significant correlation between dosage and response. The graph also implies that there is a threshold value of ≏ 1,700 cumulative spores needed before appreciable disease results.

3.3.6 Observations on the Influence of Various Meteorological Factors on B.squamosa Spore Fluctuations and on the Onset of Leaf Blight Disease.

3.3.6.1 Background

This section deals mainly with an attempt to characterize the meteorological conditions which were associated with the onset of the onion leaf blight epithytotic at Ste. Clothilde and compare these conditions with the meteorological conditions reported in the literature as favouring onion blast disease. The other objective of this phase of the investigations was to study the environmental conditions which influenced the development of the disease at Ste.Clothilde as manifested in the number of spores trapped per day and the progress curve of the epiphytotic.

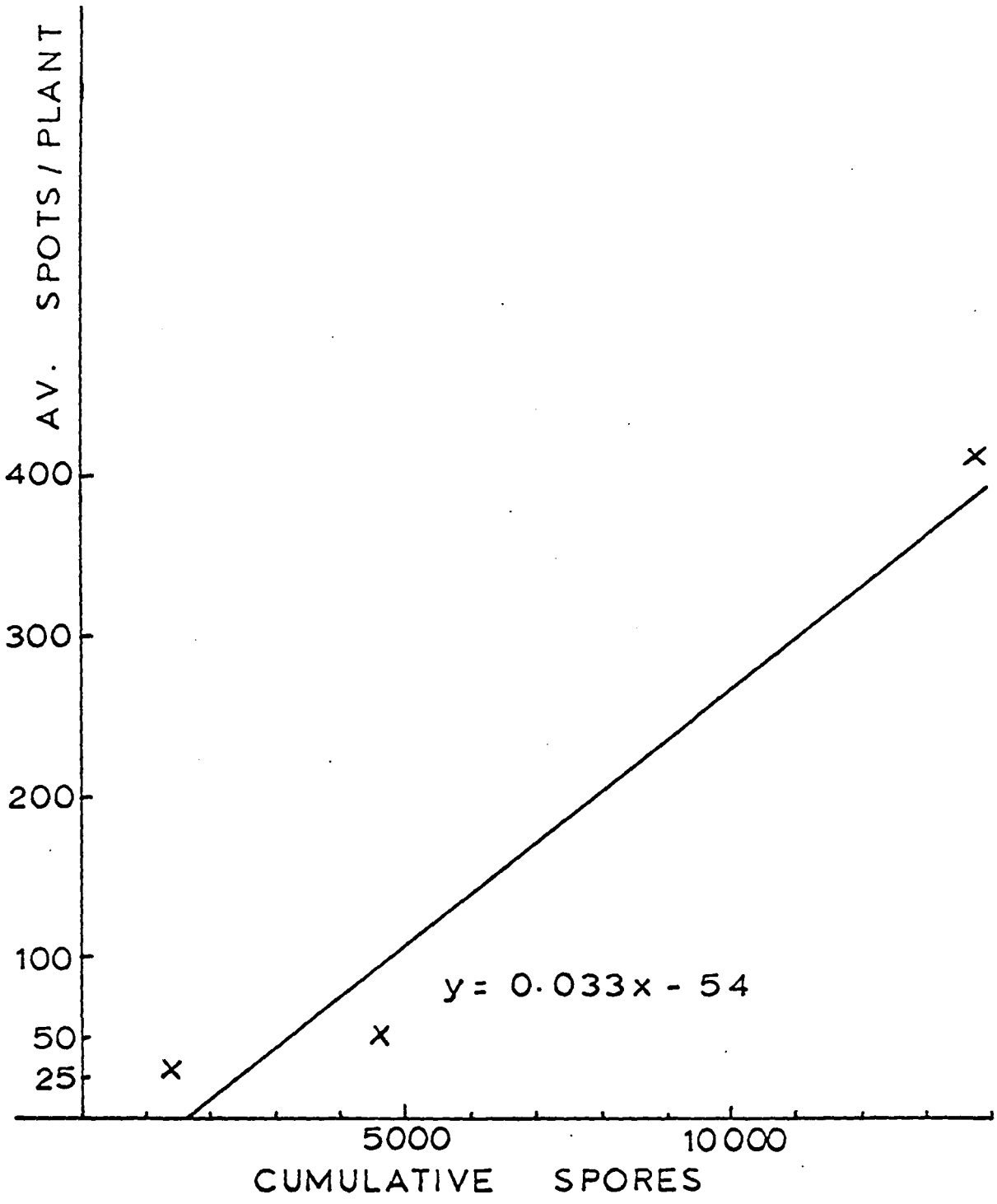
This means whereby this problem was tackled was mainly graphical. Various daily, and in some cases hourly,

FIGURE 22

FIGURE 22

Cumulative spores vs. number of spots/plant on survey dates.

FIGURE 22


AV. SPOTS / PLANT
400
300
200
100
50
25
y = 0·033x - 54
5000
10000
CUMULATIVE SPORES

environmental data were collected from the Ste.Clothilde meteorological instruments. Individual environmental factors were then compared in graph form with spores trapped and/or the disease progress curve.

This type of analysis would necessarily not be able to show interrelationships between various individual environmental factors and also cannot show the influence of composite environmental factors on the epiphytotic. Nevertheless, it is valuable in that it can segregate single environmental factors which have been proven to be of significant epidemiological importance in this and other plant diseases. This type of analysis also is the main type reported in the literature for numerous plant diseases. A much better analytic tool would perhaps involve the collection of much more extensive data on a continuous basis for analysis by a computer.

This section is divided into 7 subsections in each of which one environmental factor is considered and its influence on the onset of disease and disease development is briefly discussed and illustrated in graph form. The environmental factors considered are: 1) rainfall, 2) relative humidity, 3) dew duration, 4) temperature, 5) hours of sunlight, 6) evaporation, and 7) wind speed.

3.3.6.2 Rainfall

The rainfall conditions for the leaf blight disease of onions and onion blast as determined by various workers are detailed in the Literature Review. Most relevant is the hypothesis of Simard et al (1968) who stated that if June rainfall in any year approximates its 31 year average there is a consequent early development of onion leaf blight in that year depending on the onset and amount of July rainfall. Rainfall (in inches) for the period June to August, the logarithms of the number of spores trapped per day and a portion of the disease progress curve are plotted in Figure 23. The June rainfall totalled 5.97 inches and so surpassed the 31 year average of 3.40 inches. However, July rainfall was relatively low and the first heavy rainfall in July was on the 23rd. B.squamosa spores were seen on the spore trap slides 4 days before this heavy rainfall, and also an appreciable amount of disease was noticed 2 days before the rains came. Rather heavy rains fell on the 25th, 26th, and 27th of July and this was accompanied by a rapidly increasing rate of disease development.

The rainfall histogram for the period 23rd to 27th of July seems to fluctuate as the log of the number of spores

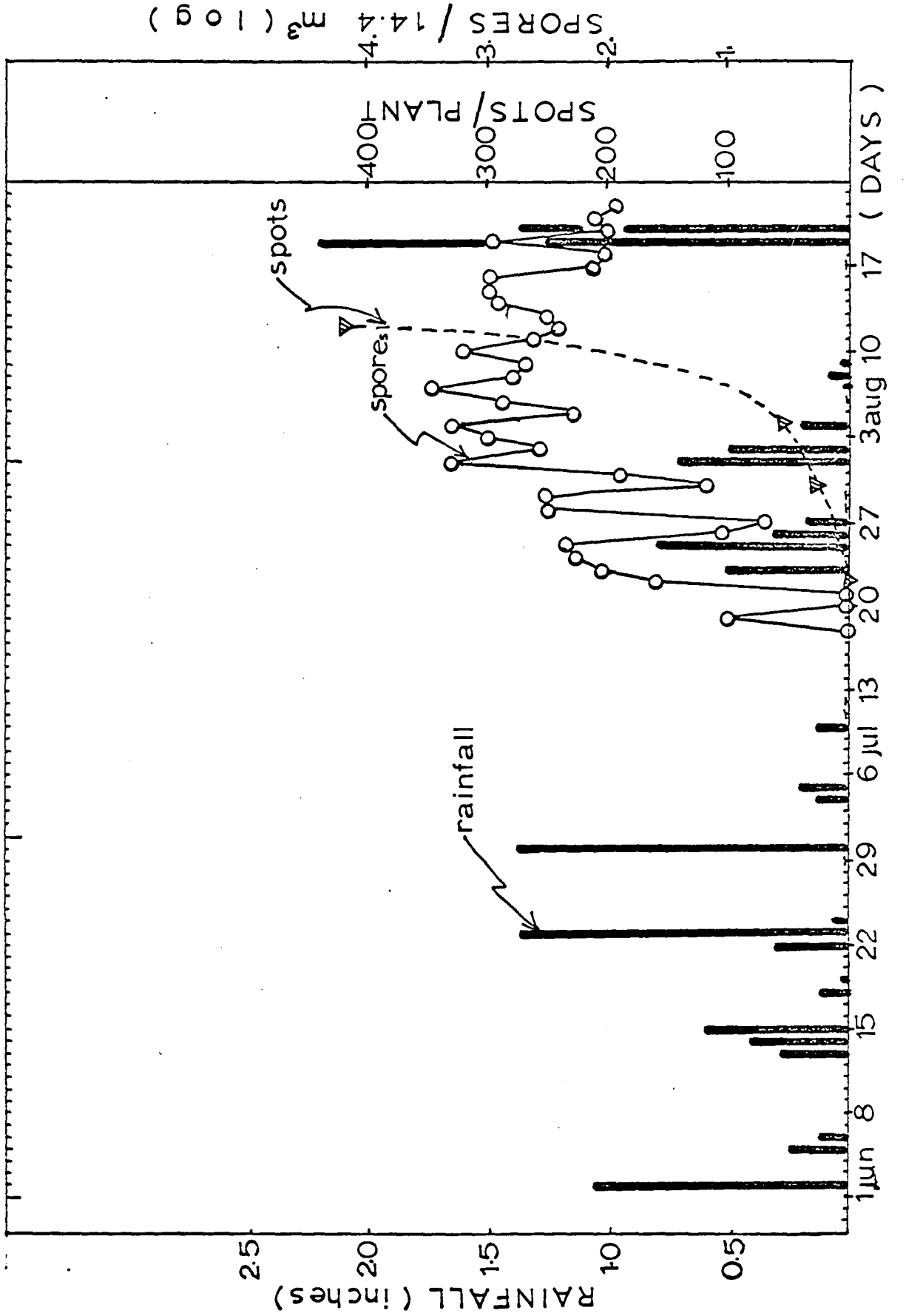
FIGURE 23

FIGURE 23

Rainfall, log of spores trapped and average spots/ plant for the period May to August.

The onset of disease symptoms was on July 22nd.

FIGURE 23


SPORES / 14.4 m³ (log)
4.
3.
2.
1.
SPOTS / PLANT
400
300
200
100
spots
spores
rainfall
RAINFALL (inches)
2.5
2.0
1.5
1.0
0.5
1 Jun
8
15
22
29
6 Jul
13
20
27
3 Aug
10
17
(DAYS)

trapped per day.

In general, therefore, the observations shown in Figure 23 seem to substantiate Simard et al's hypothesis.

The rainfall conditions accompanying physiogenic onion blast disease, as elucidated by Jones(1944), involves a period of low relative humidity conditions following a period of cloudy wet weather. These conditions were met to a great extent at Ste.Clothilde; June and early July were characterized by cloudy rainy weather and there were dry conditions from the 13th to the 22nd of July when leaf spots were first observed.

The rainfall conditions theoretically were not favourable to tipburn incited by ozone as it was only after the 23rd of July when symptoms had already been noticed that thunderstorms, which were possibly capable of liberating ozone, occurred.

3.3.6.3 Relative Humidity

Relative humidity is known to be an important factor in the epidemiology of many plant diseases (Yarwood 1956 and 1959). With specific reference to B.squamosa,the studies of most workers indicate that it is one of the category of foliage

pathogens which requires high relative humidity for inoculation, incubation and sporulation.

Jarvis (1962b) working with B.cinerea found that abundant dispersal of conidia was correlated with a rapid rise or fall of 2-3% in relative humidity especially in the range of 65-85%. The changes in relative humidity caused hygroscopic movements of conidiophores which dislodged spores that were then transported by air movements.

Hygroscopic movement of conidiophores of B.squamosa was noticed during the microscopic examination of pieces of necrotic leaves incubated in moist petri dishes. It thus appeared possible that spore liberation by B.squamosa is affected by relative humidity as is B.cinerea. It was thought that possible evidence for this hypothesis could come from a similarity in the pattern of the hourly relative humidity fluctuations and that of the spore numbers in the air on days with remarkable fluctuations in relative humidity. Hence, relative humidity charts for every day from July 16th to August 20th were examined and the rate of change of the relative humidity from 85% to 65% and from 65% to 85% was noted for each day. Six days, 5 of which had more than the normal degree of fluctuations in relative humidity were then chosen and for each of these days curves were drawn for the

hourly number of spores trapped using short traverse counts and for the hourly changes in relative humidity - Figures 24, 25, and 26. (Also plotted in these figures were dew duration, sunlight and rainfall which will be referred to later.)

From visual inspection of the curves one can detect a synchronization between relative humidity and number of spores trapped. It therefore appears that the mechanism of spore discharge in B.squamosa, like that for B.cinerea, is by hygroscopic movements of the conidiophores.

Figure 27 shows the hours of relative humidity above 90% for the period under study.

The onset of the disease on the 22nd of July was not marked by any noticeable irregularities in the hours of relative humidity over 90%. From the 14th of July to the 17th of July maximum relative humidities dropped appreciably and remained at a lower than normal level until the 21st of July. These observations are reminiscent of the conditions postulated by Jones for the onset of onion blast disease. Minimum R.H.'s were also generally lower than normal in the week preceding the onset of onion leaf blight disease symptoms.

FIGURE 24

FIGURE 24

Hourly fluctuations in spores trapped, R.H., Rainfall, Dew Duration and Sunlight hours for the 26th and the 28th of July.

FIGURE 24

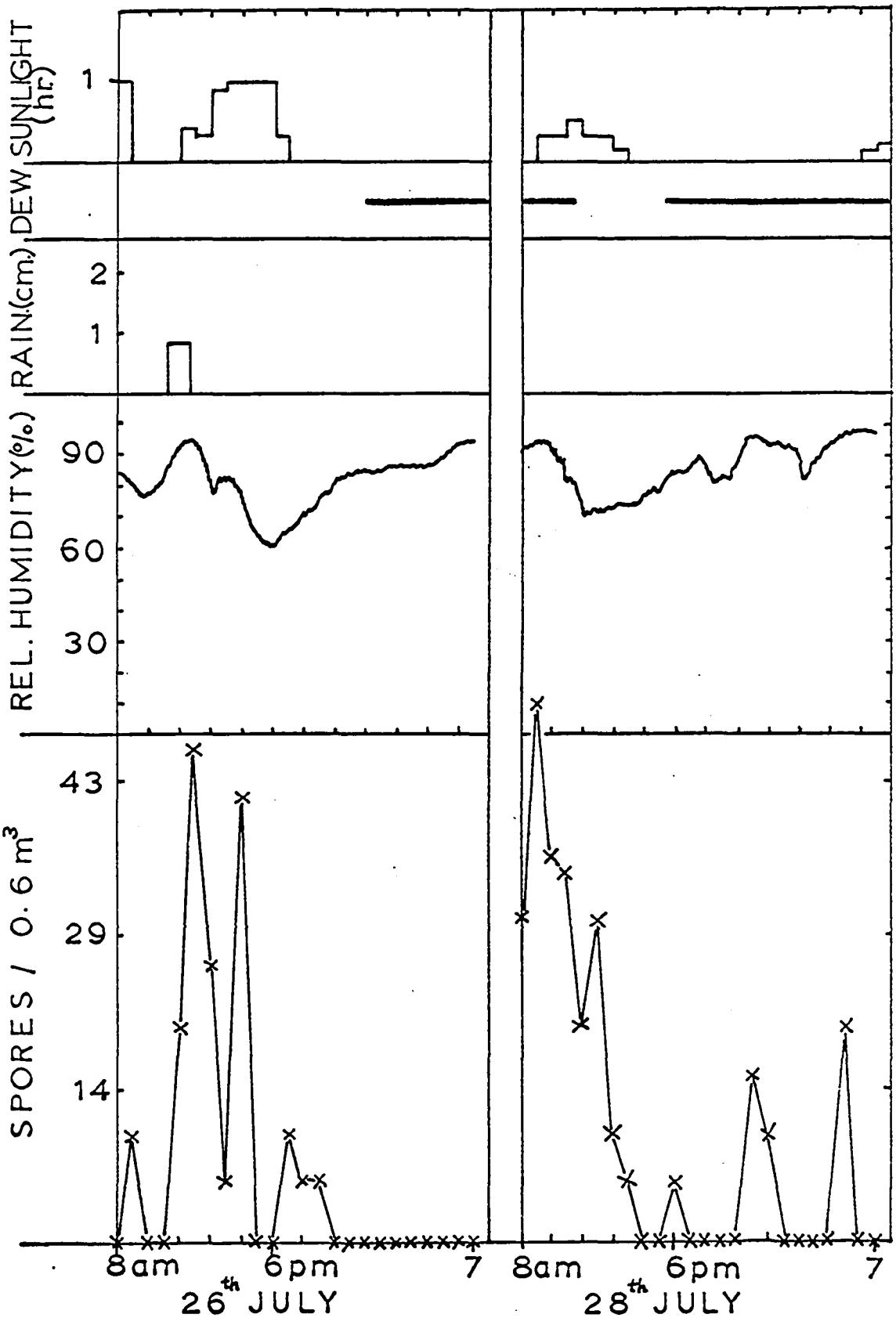

SUNLIGHT (hr)
1
DEW
RAIN (cm)
2
1
REL. HUMIDITY (%)
90
60
30
SPORES / 0.6 m³
43
29
14
8am
6pm
7
26th JULY
8am
6pm
7
28th JULY

FIGURE 25

FIGURE 25

Hourly fluctuations in spores trapped, R.H., Rainfall, Dew duration and sunlight hours for the 3rd of August and the 31st of July.

FIGURE 25

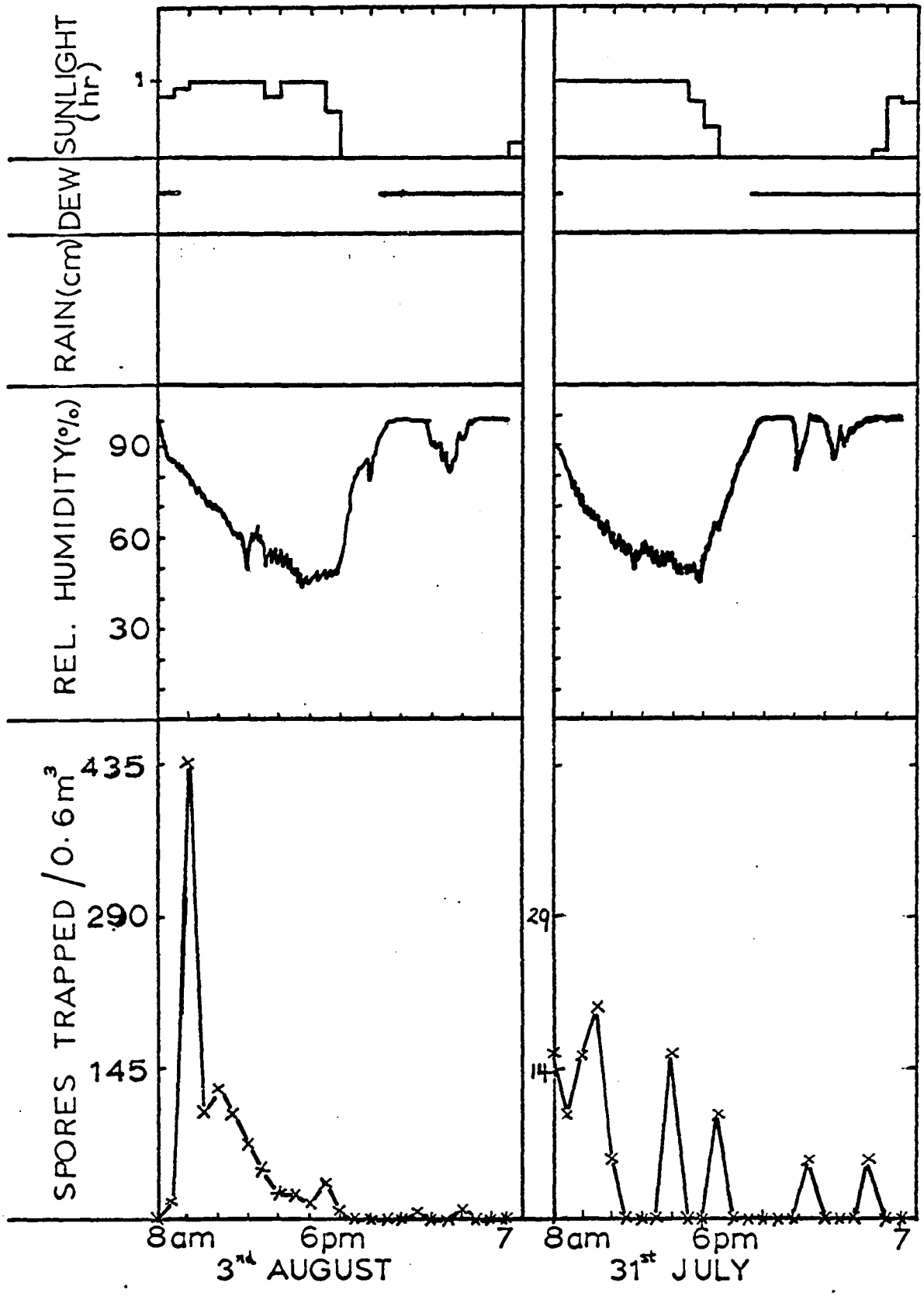

SUNLIGHT (hr)
1
DEW
RAIN(cm)
REL. HUMIDITY(%)
90
60
30
SPORES TRAPPED /0.6m³
435
290
145
29
14
8am
6pm
7
3rd AUGUST
8am
6pm
7
31st JULY

FIGURE 26

FIGURE 26

Hourly fluctuations in spores trapped, R.H., Rainfall, Dew duration and sunlight hours for the 16th and the 7th of August.

FIGURE 26

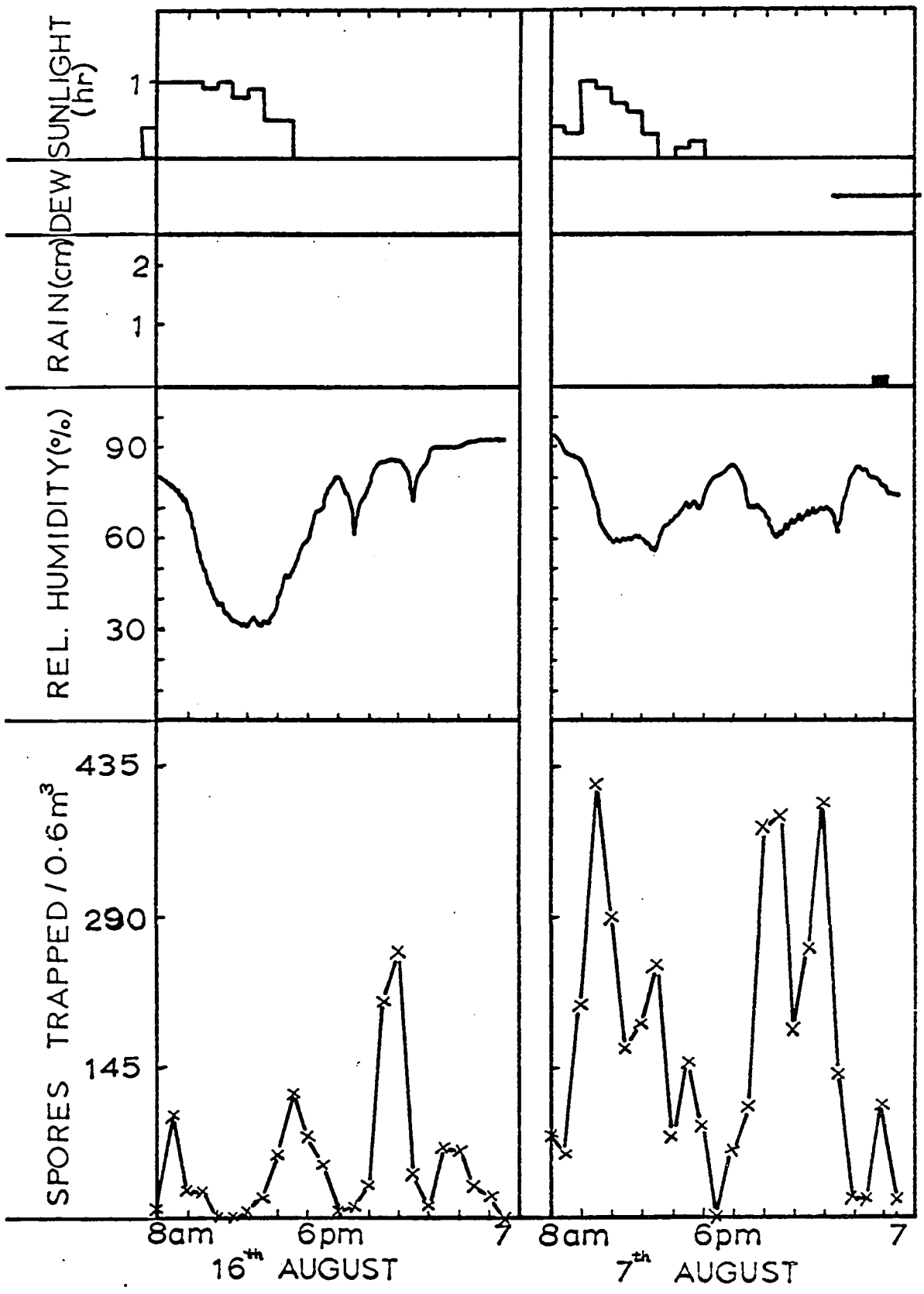

SUNLIGHT (hr)
1
DEW
RAIN(cm)
2
1
REL. HUMIDITY(%)
90
60
30
SPORES TRAPPED/0.6m³
435
290
145
8am
6pm
7
16th AUGUST
8am
6pm
7
7th AUGUST

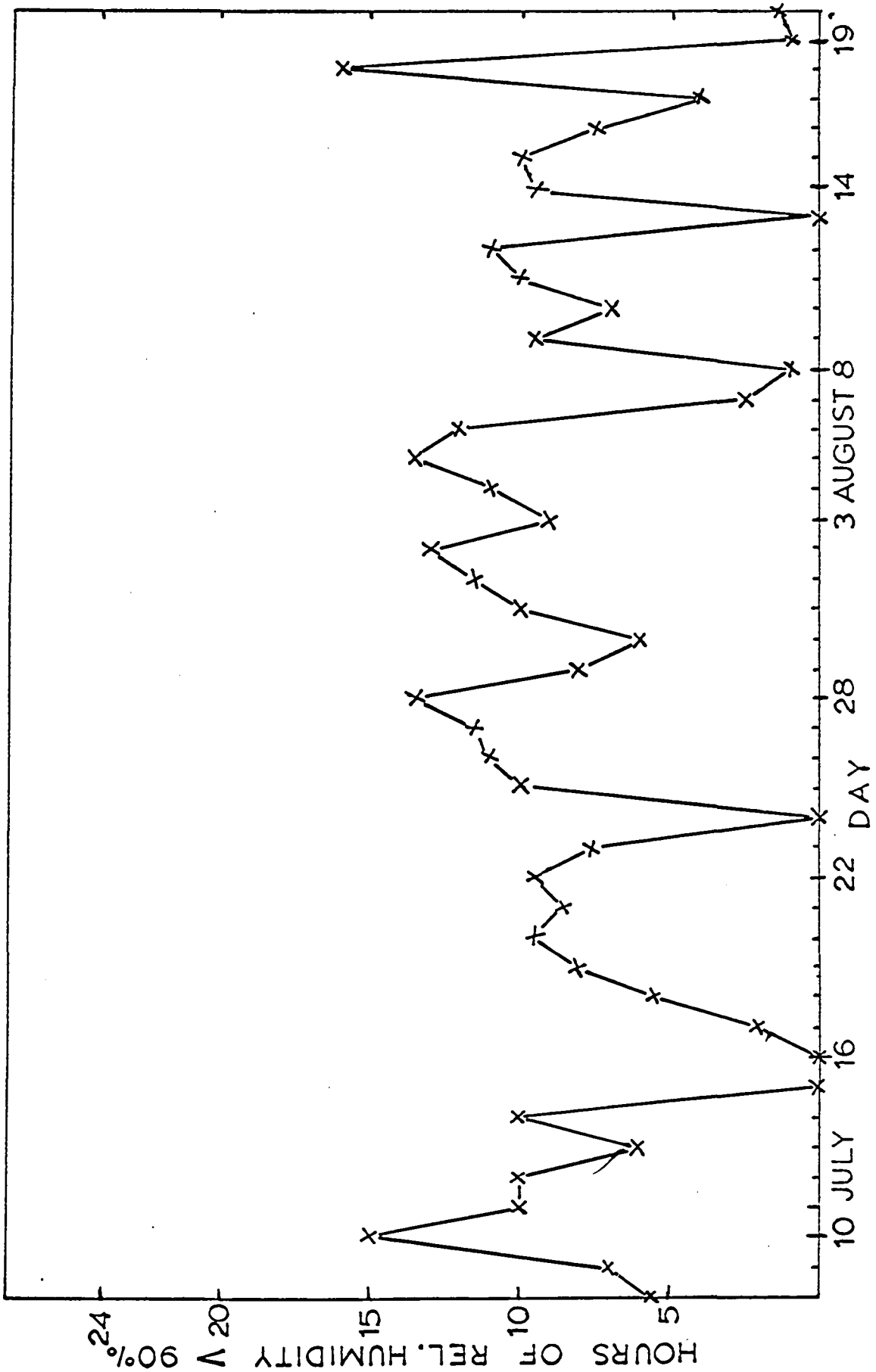
FIGURE 27

FIGURE 27

Hours of Relative Humidity above 90% for July and August.

The first leaf blight symptoms were seen on July 22nd.

FIGURE 27


HOURS OF REL. HUMIDITY ≥ 90%
24
20
15
10
5
10 JULY
16
22
28
3 AUGUST
8
14
19
DAY

Segall and Newhall (1960) reported that the leaf spotting phase was characterized by relative humidities close to 100% for over 24 hours. This was not seen at Ste. Clothilde during the period near the onset of leaf spotting.

Total spores caught per day did not seem to be appreciably influenced by either the duration of periods of high relative humidities or by maximum or minimum relative humidities. However, when periods of high relative humidities was shorter than 5 hours there was generally low sporulation on the following day. There were some exceptions to this observation which could possibly be explained on the basis of spores deposited from other onion fields by prevailing winds.

3.3.6.4 Dew Duration

The duration of moisture on plant surfaces has been shown to be essential to the infection processes of many plant pathogenic fungi (Wallin 1967). To investigate the influence of dew duration on the fluctuations of spores trapped and the onset of onion leaf blight disease the readings obtained from the dew recorder were compared graphically with daily and hourly numbers of spores of B.squamosa trapped.

Figure 28 is a graph which shows the dew duration per day for the whole period which was studied. This graph shows that on 4 days prior to the first spores being trapped, i.e. on the 15th, 16th, 17th, and 18th of July, there was less than 7 hours of dew recorded per day. It also shows that on only one day (the 20th of July) of the week preceding the onset of the disease did the dew duration exceed 10 hours. These conditions are consistent with the conditions postulated by Jones as suitable for the physiological onion blast disease.

It was noticed that there were peaks of high spore numbers of B.squamosa following roughly 3 days after one another; in view of this observation, graphs were drawn comparing spores trapped with dew periods 3 and 4 days earlier. Also, since dew on a particular night might have some influence on the numbers of spores trapped on the following day, a graph of dew duration compared with spores trapped one day later was also drawn (Figure 29). These graphs show there was no apparent relationship between dew periods and numbers of spores trapped. However, Figure 29 shows that generally dew periods do not seem to be correlated with the numbers of spores trapped on the following day, but whenever the dew period was 8 hours or less there were generally few spores trapped on the following day. It therefore seems

FIGURE 28

FIGURE 28

Dew periods and log of spores trapped per day for the period June to August.

First symptoms were noticed on ~~August~~ July 22nd.

FIGURE 28

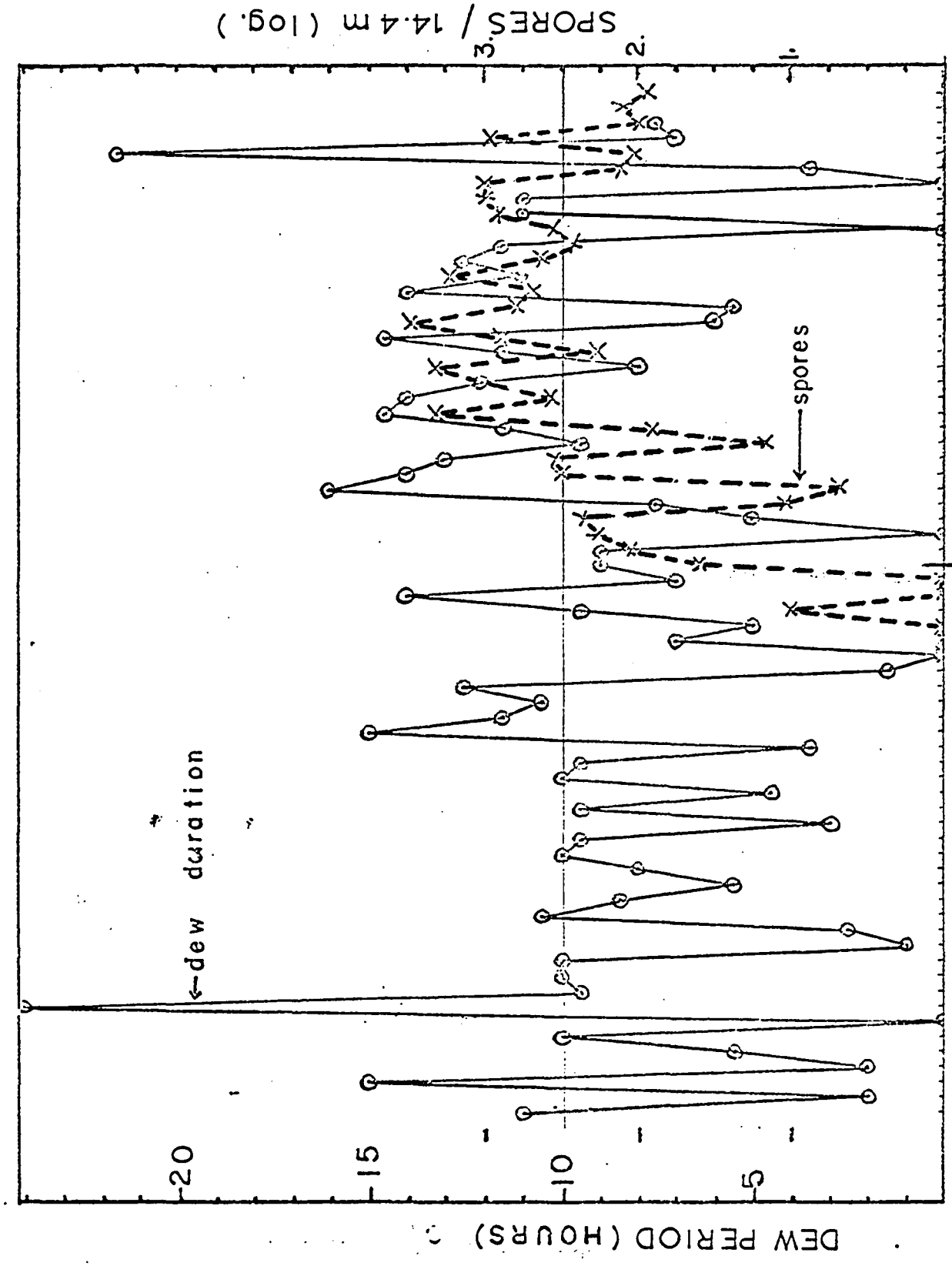

SPORES / 14.4 m (log.)
3.
2.
1.
spores
dew duration
16JUNE 24
1JULY 8
15
22*
29
5AUG. 12
19
DEW PERIOD (HOURS)
20
15
10
5

FIGURE 29

FIGURE 29

Dew periods and log of spores trapped on subsequent days for July and August.

First symptoms were noticed on July 22nd.

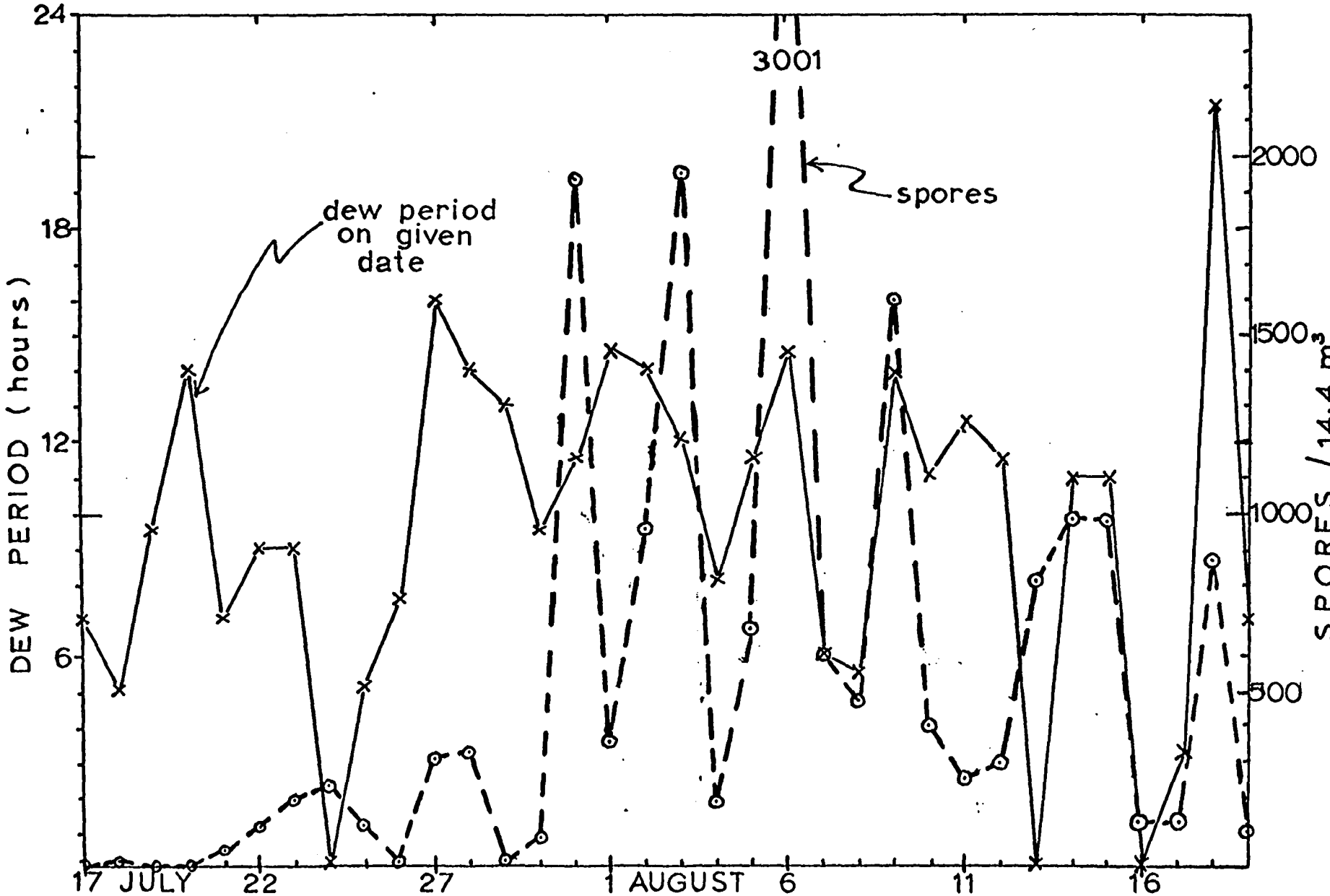

24
18
12
6
DEW PERIOD (hours)
dew period on given date
3001
spores
2000
1500
1000
500
SPORES / 14.4 m³
17 JULY
22
27
1 AUGUST
6
11
16

FIGURE 29

that a threshold period of 8 hours is necessary for adequate sporulation and longer periods of dew may not necessarily lead to greater production of spores.

Figure 30, which is typical of most days during the epidemic, shows that the onset of the dew period generally coincides with a noticeable decrease in the number of spores trapped and that the cessation of the dew period has the converse effect.

Figure 31 is a graph of daily dry periods compared with spores trapped on the same day. This graph was drawn as it was noticed that spores were generally caught during dry periods and also that on some of the days on which the highest numbers of spores were trapped, e.g. the 7th of August, the dry periods were generally longer than normal. Figure 31 shows that there is no apparent consistent relationship between number of spores trapped and length of dry periods on the days during which the spores were trapped.

FIGURE 30

FIGURE 30

Hourly fluctuations in spores trapped, Rainfall, Dew duration and sunlight hours for the period 13th to the 16th of August.

FIGURE 30

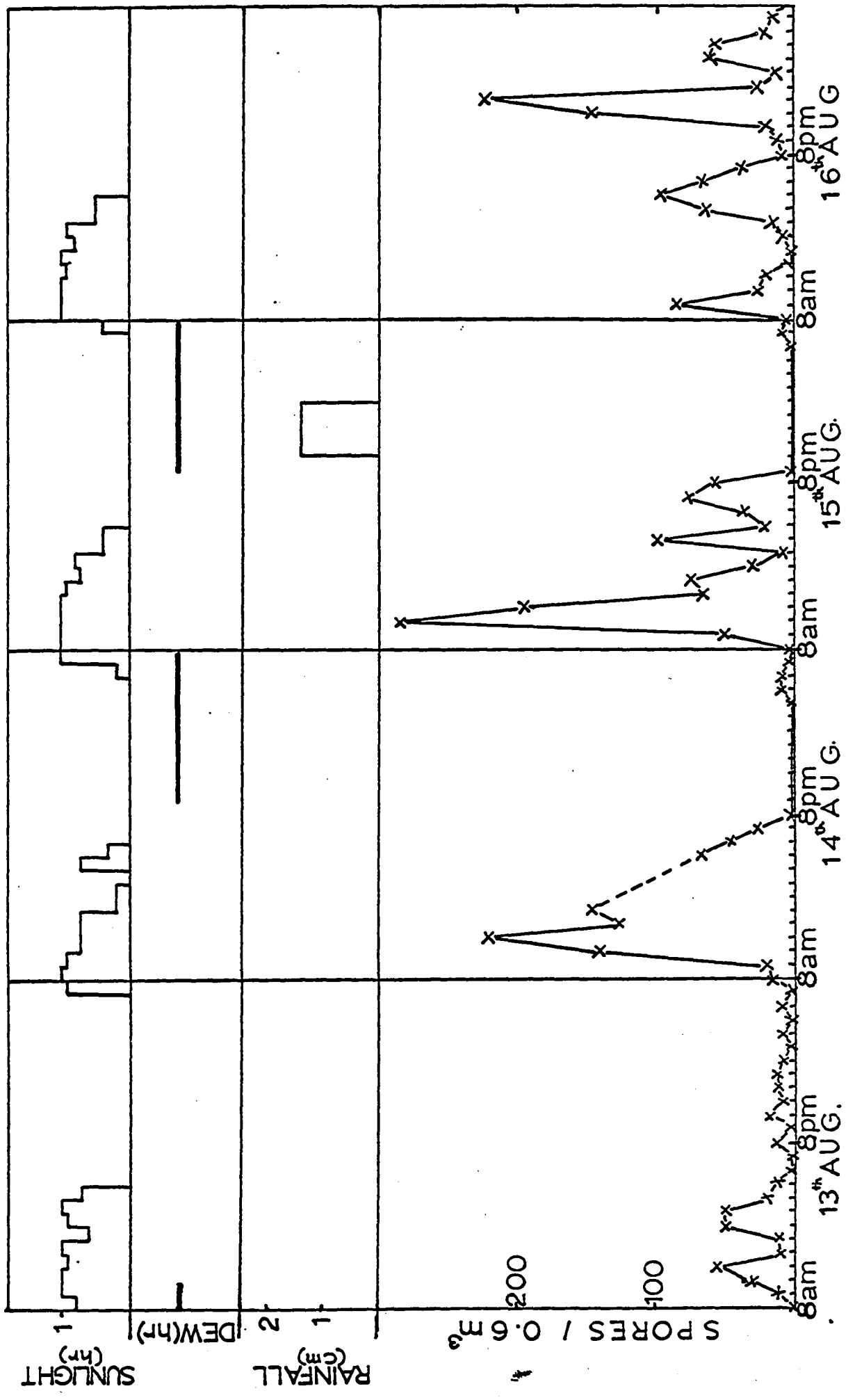

SUNLIGHT (hr)
1
DEW(hr)
RAINFALL (cm)
2
1
SPORES / 0.6 m³
200
100
8am
8pm
13th AUG.
8am
8pm
14th AUG.
8am
8pm
15th AUG.
8am
8pm
16th AUG

FIGURE 31

FIGURE 31

Dry periods and log of spores trapped on same day for July and August.

First symptoms were seen July 22nd.

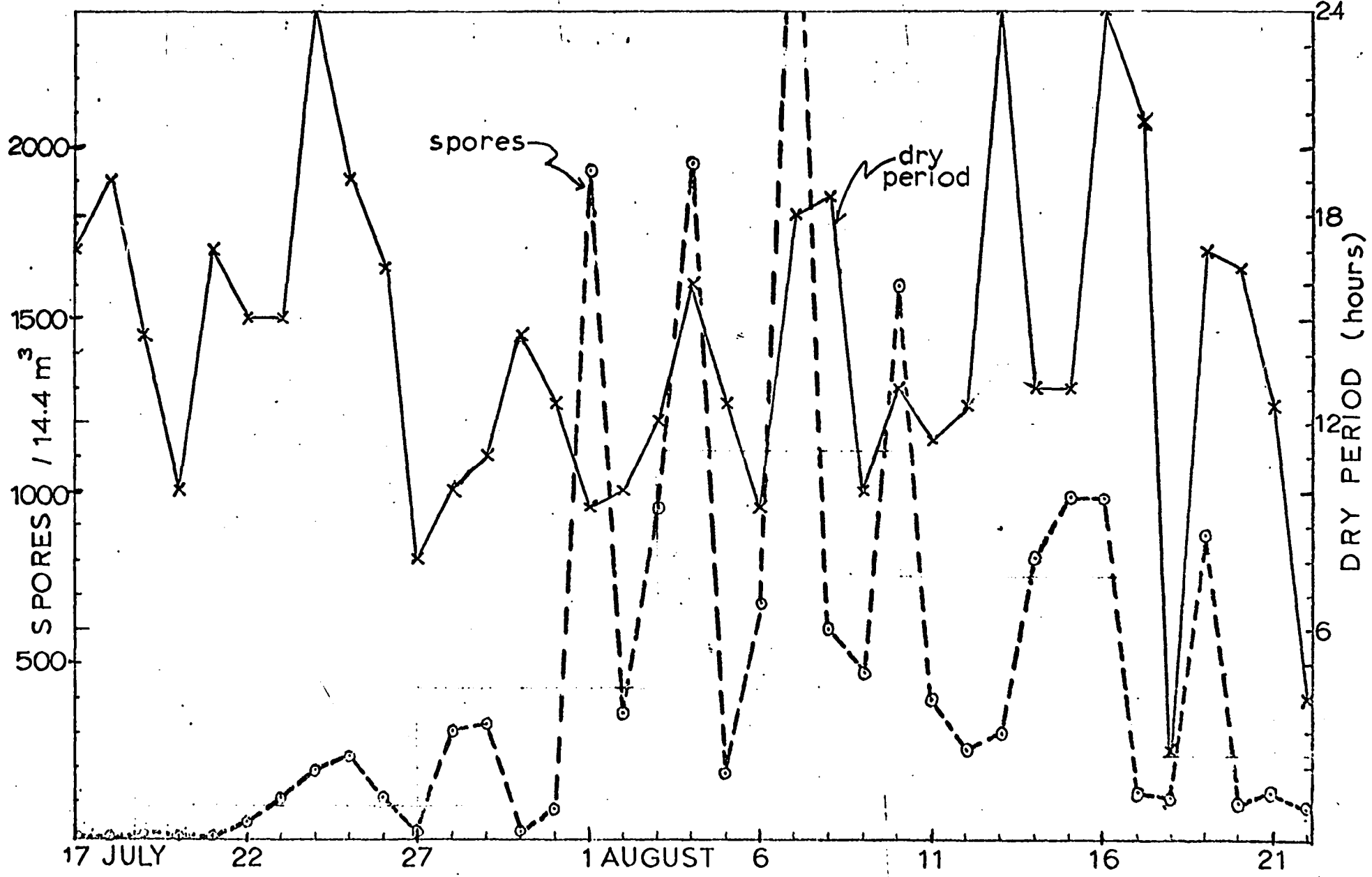

spores
dry period
2000
1500
1000
500
SPORES / 14.4 m³
24
18
12
6
DRY PERIOD (hours)
17 JULY
22
27
1 AUGUST
6
11
16
21

FIGURE 31

3.3.6.5 Temperature

Simard et al (1968) stated that high temperatures favour the development of B.squamosa leaf blight, also Segall and Newhall (1961) reported that temperatures over 26.7°C. are essential for the leaf blighting phase of the disease. Jones (1944) found high temperatures to be one of the requirements for the physiogenic onion blast disease. However, Jarvis (1962), working with B.cinerea found that very few spores are dispersed if night temperatures are less than 13°C. in spite of otherwise suitable conditions during the day.

Graphs relating temperature to spores trapped are presented in Figures 32, 33, 34, and 35.

In Figure 32 it can be clearly seen that the only extended period in July with long hours of temperature over 26.7°C. was from the 15th to the 18th of July. This period of high temperatures preceded the trapping of spores of B.squamosa and the onset of disease symptoms, and therefore this graph of conditions at Ste.Clothilde supports the conclusions of workers, both on the pathologic and physiogenic diseases, as to the temperature conditions favourable to the onset of the disease.

The hours of temperature over 21.1°C. and 26.7°C. does not seem to bear any straight forward relationship to the development of the disease except in that the fluctuations in hours of temperature over 26.7°C seem to be generally in phase with the fluctuations in spores trapped.

In view of Jarvis' findings with B.cinerea that low temperatures inhibited spore liberation, Figure 33 was drawn. In this graph minimum temperatures are plotted with spores trapped on the same day; this graph shows mainly that these two variables were in phase with each other for the first two weeks of August. However, when the minimum temperatures were compared with spores trapped on the following days, there was no such pattern. During the first two weeks of August there were regular peaks in spores trapped following 3 days after one another. This observation indicated that the latent period for spore production at this time might also have been 3 days. Therefore a graph was drawn which related minimum night temperatures to spores trapped 3 days afterwards. This graph is shown in Figure 34. Here it can be seen that minimum temperatures and spores trapped are in phase with each other for the first 12 days of August. This observation suggests an hypothesis that the latent period for spore production was three days during this stage of the epidemic

FIGURE 32

FIGURE 32

Hours of temperature above 21.1°C. and above 26.7°C. and log of spores trapped during July and August.

The first leaf blight symptoms were noticed on July 22nd.

FIGURE 32

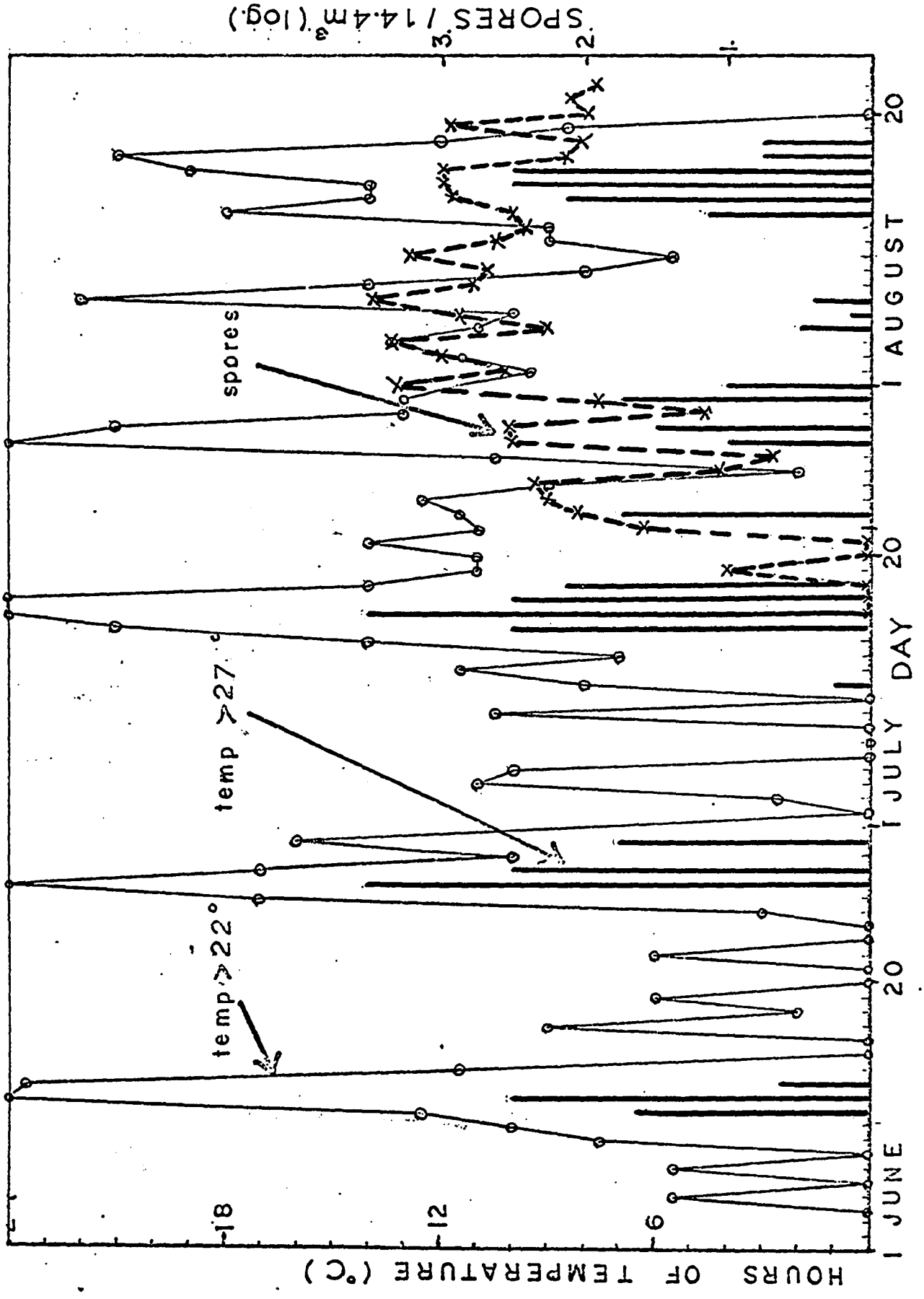

SPORES / 14.4m³ (log.)
1
2
3
spores
temp >27°
temp >22°
HOURS OF TEMPERATURE (°C)
6
12
18
1 JUNE
20
1 JULY
DAY
20
1 AUGUST
20

FIGURE 33

FIGURE 33

Minimum temperatures and log of spores trapped during July and August.

The first leaf blight symptoms were noticed on July 22nd.

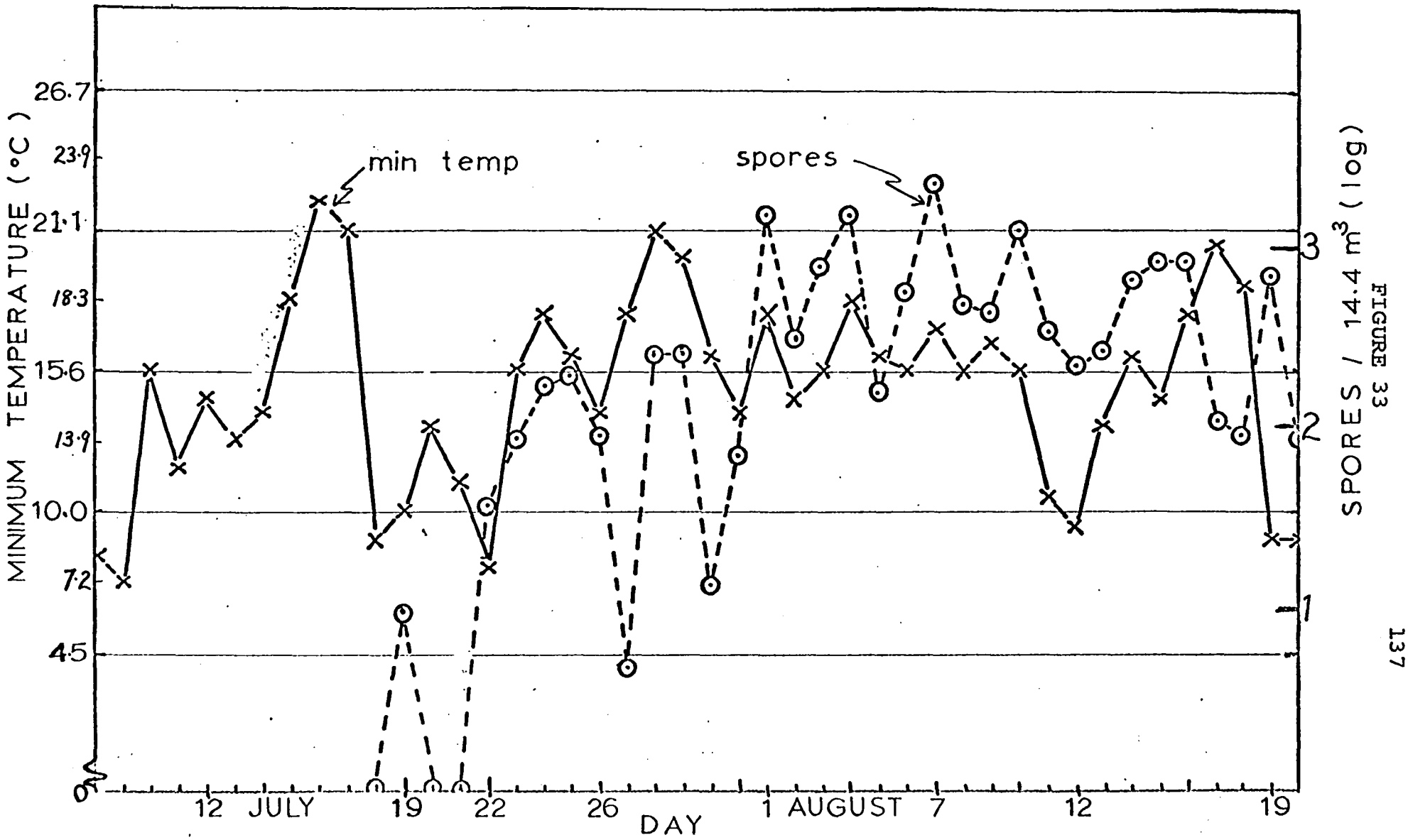

MINIMUM TEMPERATURE (°C)
26.7
23.9
21.1
18.3
15.6
13.9
10.0
7.2
4.5
0
min temp
spores
SPORES / 14.4 m³ (log)
3
2
1
12 JULY
19
22
26
DAY
1 AUGUST
7
12
19

FIGURE 33

FIGURE 34

FIGURE 34

Log of spores trapped and minimum temperatures 3 nights previously during July and August. The first leaf blight symptoms were noticed on July 22nd.

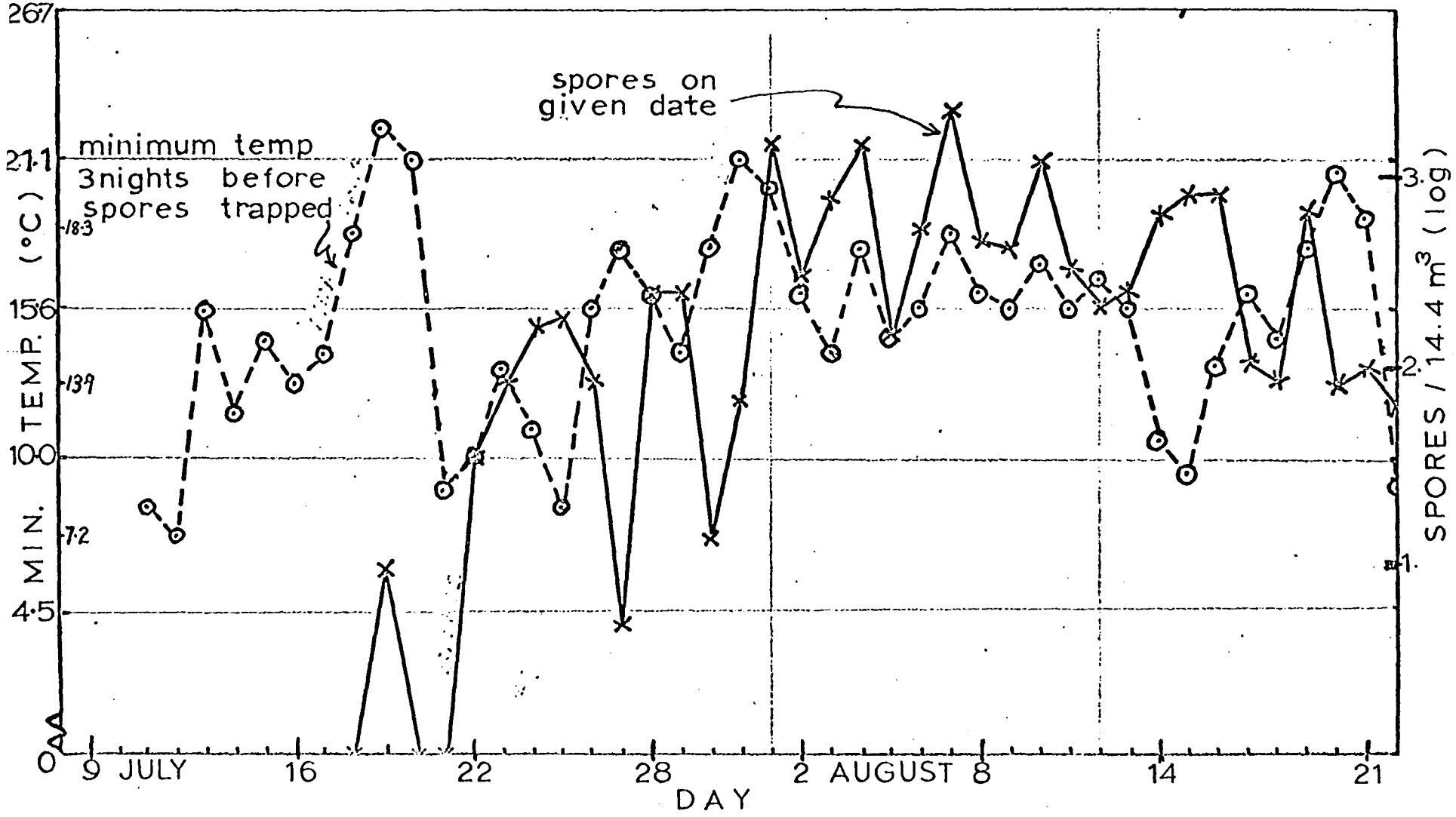

26·7
21·1
15·6
10·0
4·5
0
MIN. TEMP. (°C)
18·3
13·9
7·2
minimum temp
3nights before
spores trapped
spores on
given date
3·
2·
1·
SPORES / 14.4 m³ (log)
9 JULY
16
22
28
2 AUGUST
8
14
21
DAY

FIGURE 34

FIGURE 35

FIGURE 35

Log of spores trapped and maximum temperatures of previous night during July and August.
The first leaf blight symptoms were noticed on July 22nd.

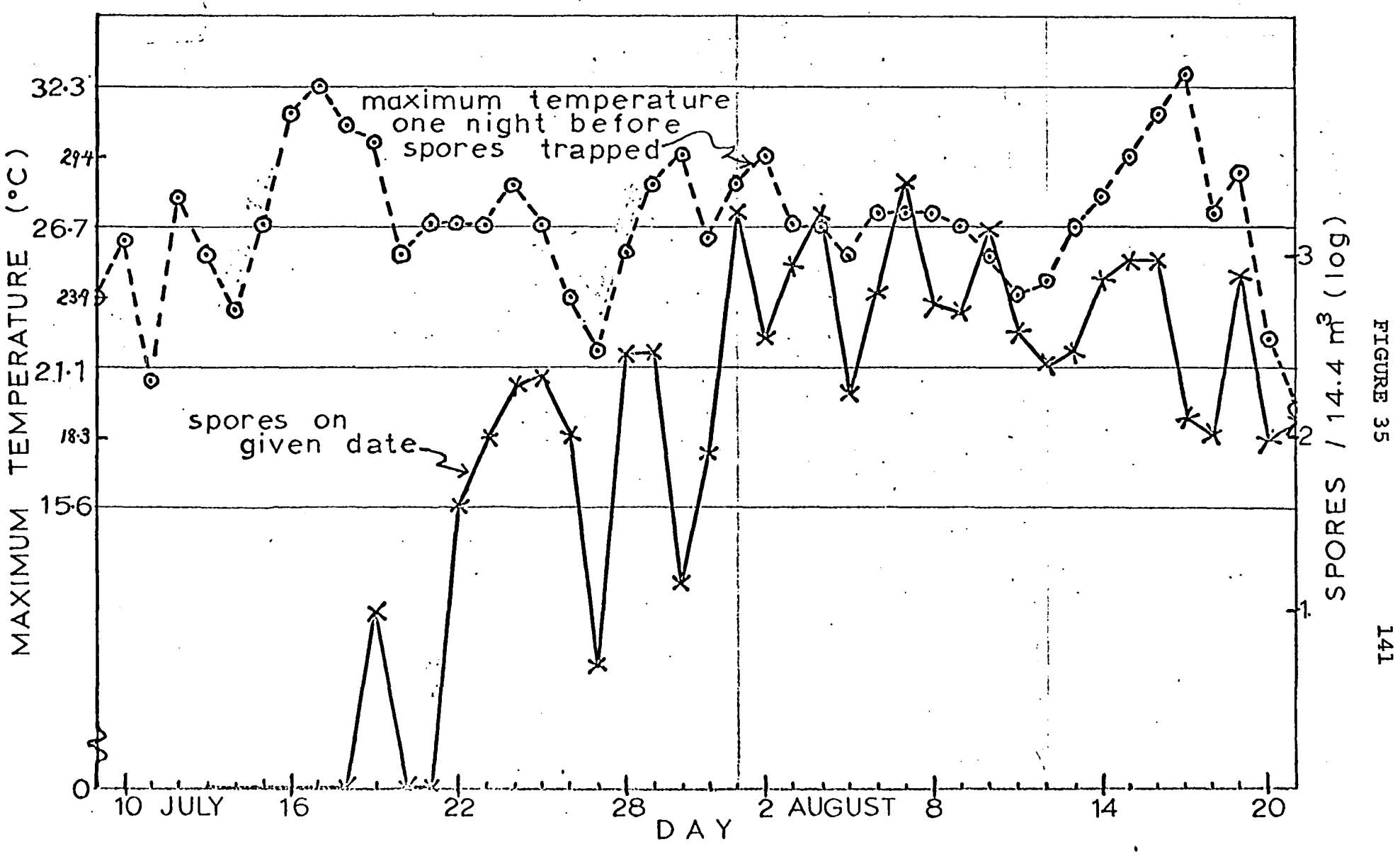

32·3
29·4
26·7
23·9
21·1
18·3
15·6
0
MAXIMUM TEMPERATURE (°C)
maximum temperature one night before spores trapped
spores on given date
10 JULY
16
22
28
2 AUGUST
8
14
20
DAY
3
2
1
SPORES / 14.4 m³ (log)

FIGURE 35

and that in other stages of the epidemic either latent periods changed due to changing environmental conditions or complex unfavourable environmental, host, or pathogen factors obscured a regular pattern of peaks and troughs in spore production.

Figure 35 is a graph of maximum temperatures compared with spores trapped on the following day. This graph does not show any straightforward relationship between these two variables.

3.3.6.6 Hours of Sunlight

Jones (1944) has stated that part of the conditions favouring the physiological onion blast disease involves periods of bright sunshine following a period of cloudy wet weather. Page (1957) and Segall and Newhall (1960) also found that light was important in some aspects of the development of leaf blight disease of onions.

In Figure 36 the hours of sunlight per day and the number of spores trapped, are plotted together against days. It shows that the period comprising the 10th, 11th, 12th and 13th of July was the longest consecutive period after the end of May during which sunlight duration was less than 5 hours/day. Also there were only 8 days with sunlight dura-

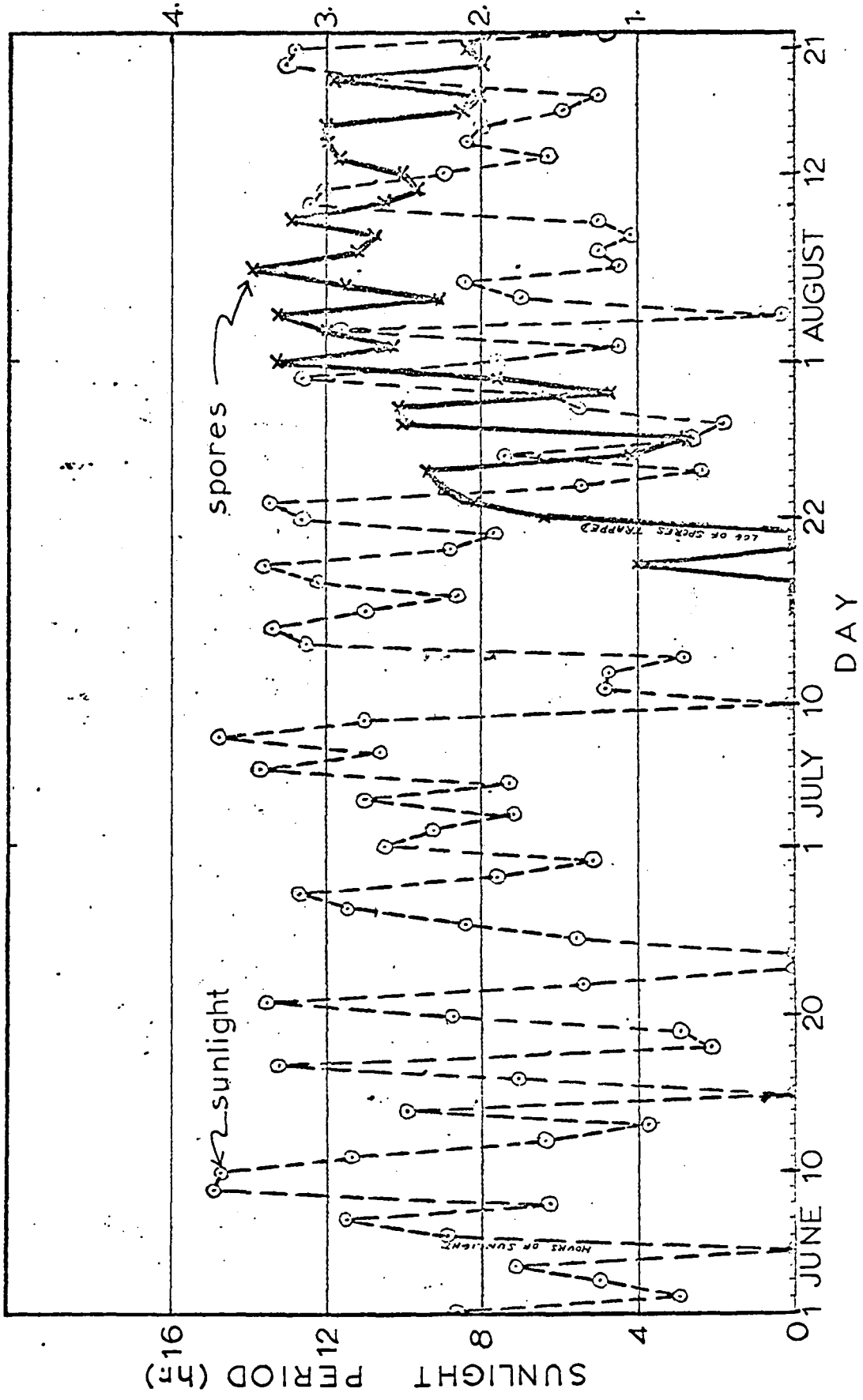
FIGURE 36

FIGURE 36

Sunlight periods and log of spores trapped during June, July and August.

The first leaf blight symptoms were noticed on July 22nd.

FIGURE 36


SPORES / 14.4 m³ (log)
4
3
2
1
spores
sunlight
SUNLIGHT PERIOD (hr)
16
12
8
4
0
1 JUNE
10
20
1 JULY
10
22
1 AUGUST
12
21
DAY

tion in excess of 12 hours from the 26th of May to the 10th of July. In contrast to this, between the 14th and the 22nd of July, when the first blight symptoms were observed, there were 5 days with sunlight duration over 12 hours and the remaining days in that period had sunlight lasting for 8 hours or more. These observations on sunlight conditions are consistent with the Ste.Clothilde epidemic being either onion blast or onion leaf blight disease.

The number of spores trapped per day seems to be independent of sunlight duration as the two variables seem to be either unrelated or out of phase with each other as seen in Figure 36. However, in the first half of August the hours of sunlight show similar regular sequence of peaks to that observed with temperature and spores trapped and hence perhaps there may be some relationship between sunlight and number of spores trapped which is not readily apparent with this type of analysis.

Figures 24, 25, 26, and 30 show that spores are liberated during daylight hours, both in the absence and presence of sunlight. However, there are indications that generally more spores are liberated during periods of short and intermittent sunlight than during long periods of sunlight.

3.3.6.7 Evaporation

Robertson (1953) states that the plant pathologist should be concerned with this factor from the point of view of drying plant surfaces. Figure 37 compares evaporation, measured in Livingston units, with spores trapped. It shows that for a week before the onset of the disease at Ste.Clothilde evaporation was relatively high. This observation supports the theory of the physiological causation of the initial stages of the onion leaf blight epithytotic at Ste. Clothilde as this high evaporation rate could perhaps be involved in a physiological stress which gives rise to blast symptoms.

Evaporation, as seen in Figure 37, is similar to temperature and sunlight hours in its relationship to the daily fluctuations of B.squamosa spores, for it shows similar regular peaks and troughs to the sunlight hour and temperature graphs of Figures 34 and 36. These observations on evaporation are not surprising as evaporation is a resultant of (1) solar and sky radiation, (2) an atmosphere in which the vapour pressure is less than the saturated air temperature of the surface, (3) wind, and (4) air temperature, Robertson (1953).

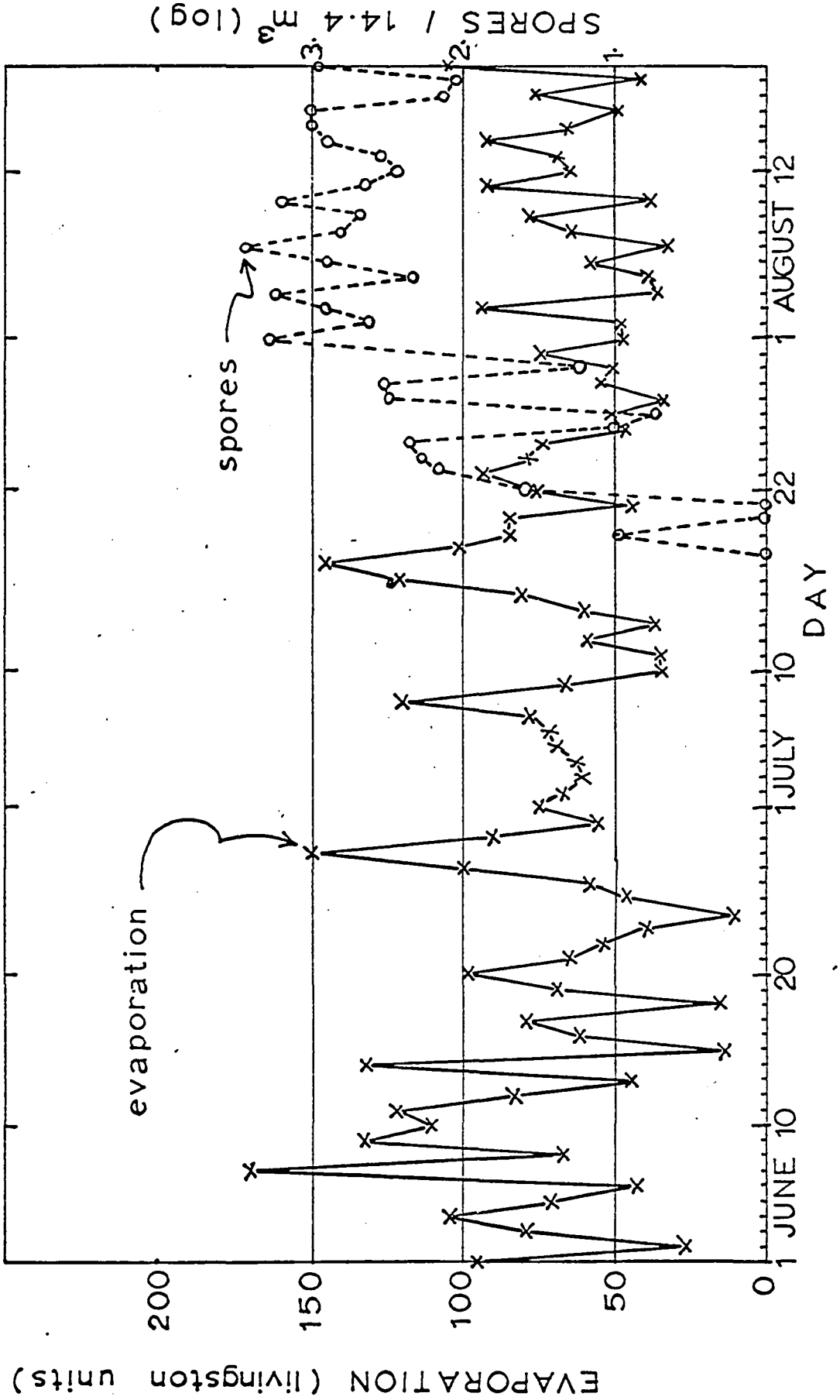
FIGURE 37

FIGURE 37

Evaporation and log of spores trapped during June, July and August.

The first leaf blight symptoms were noticed on July 22nd.

FIGURE 37 .


SPORES / 14.4 m³ (log)
3
2
1
spores
evaporation
1 JUNE 10 20 1 JULY 10 22 1 AUGUST 12
DAY
EVAPORATION (Livingston units)
200
150
100
50
0

3.3.6.8 Wind Speed

Jarvis (1962a), working with B.cinerea, found that the slightest of winds can disperse the spores of this fungus and that considerably stronger winds do not appreciably increase the spore concentrations in the air. He also found that air movements dispersed spores of B.cinerea only after they had been released by hygroscopic movements. Rotem(1969) found that the average wind velocity was the chief factor influencing the amount of alternaria spores dispersed each day. Rotem also found that high winds for a number of days diminished the number of air-blown spores. However, no report has been found on the influence of wind velocity on any aspect of B.squamosa leaf blight disease of onions.

The maximum wind speeds on the Beaufort scale can be compared to the airborne spore concentration Figure 38. There is little apparent difference between the daily fluctuations in wind speed, both before and after the onset of dieback symptoms on the 22nd of July, hence it is likely that wind speed played only a minor role in determining the onset ofthe disease. However, the 5th and 17th of July were very noticeably atypical days with respect to wind speed and the possibility that the high winds on these days might have transported the B.squamosa spores which started the epiphytotic from other regions should not be discounted. No con-

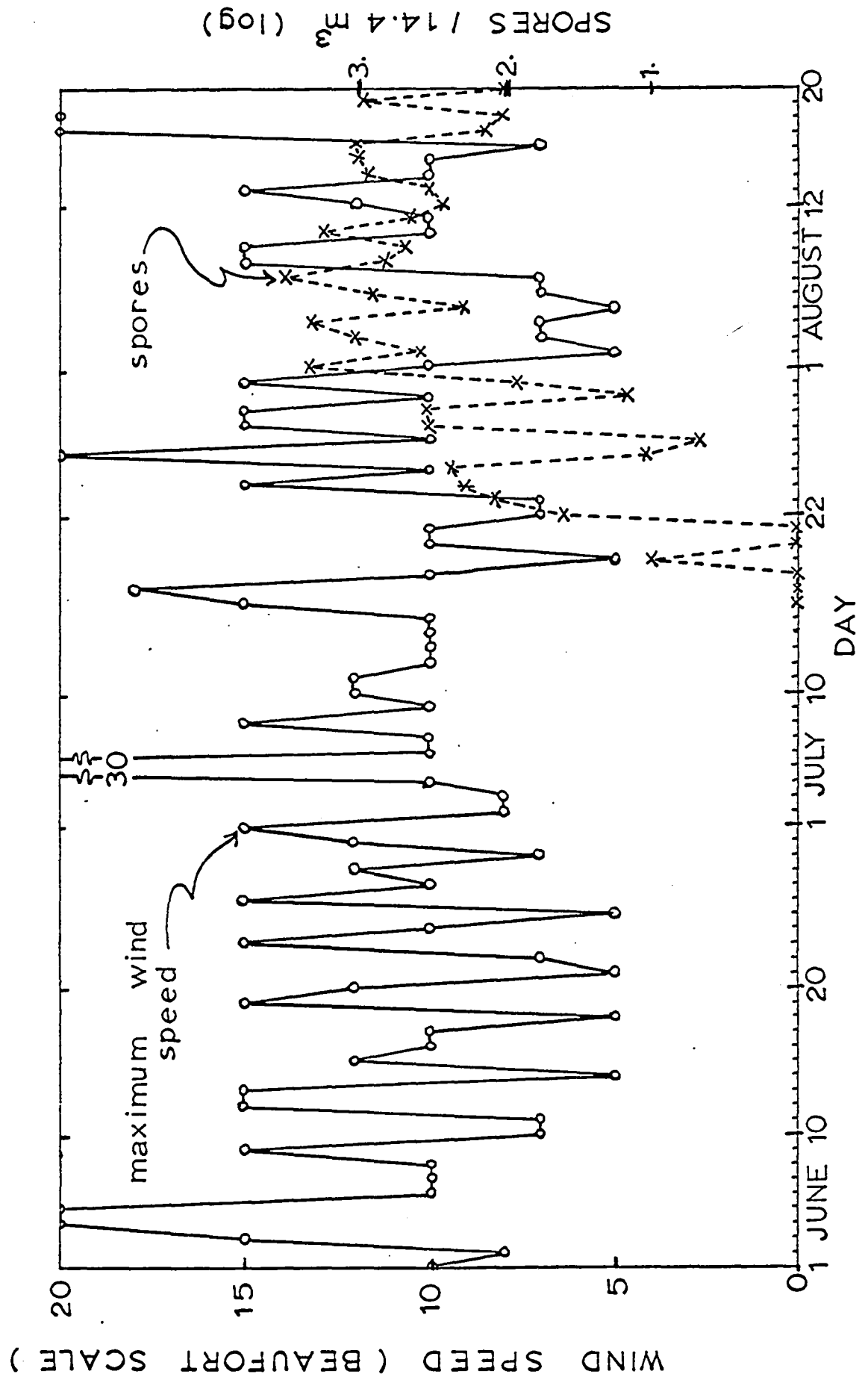
FIGURE 38

FIGURE 38

Maximum wind speed and log of spores trapped during June, July and August.

The first leaf blight symptoms were noticed on July 22nd.

FIGURE 38


SPORES / 14.4 m³ (log)
3.
2.
1.
spores
maximum wind speed
30
1 JUNE 10 20 1 JULY 10 22 1 AUGUST 12 20
DAY
0 5 10 15 20
WIND SPEED (BEAUFORT SCALE)

clusions can be drawn on the influence of wind speed on daily fluctuations of spores trapped due to the relative inaccuracy of the wind speed estimates which were made on the Beaufort scale.

3.3.6.9 Relation of the Cyclic Fluctuations in Airborne Spore Concentration to the Latent Period.

Diseases have a latent period which is the time needed for one generation of the pathogen. Theoretically, therefore, the number of spores trapped in any particular day should be related to the number of spores trapped on a subsequent day one latent period later. If conditions favourable to disease development are assumed, airborne spore concentration should form a series of increasingly higher peaks when plotted against time. A constant latent period would produce a constant frequency of peak concentrations of spores. If, on the other hand, conditions are unfavourable the duration of the latent period would differ, for the duration of the latent period is significantly affected by environmental, host and pathogen factors. The regular sequence of peaks would then be obscured.

An examination of the data on spore concentration (Figure 17, page 90) revealed a 3-day interval between peaks

for most of August. In order to make evident any relation between immediately succeeding peaks, the data were plotted as follows: Spore concentration at the different dates were plotted (Figure 39) then at each of these dates the spore concentration for 3 days before was added. The two curves are in phase for the majority of the period under study, especially for the period extending from July 30th to August 11th. However, on the 4th and the 10th of August, the spore concentrations were not higher than they were three days before, in fact the spore concentration for August 10th was even lower than on the 7th of August.

It is concluded that there was a latent period of 3 days, the duration of which was not much affected by environmental conditions in the first week and a half of August. However, it seems that unknown environmental conditions prevented the increase in amplitude of the spore concentration peaks on the 4th and the 10th of August. In an attempt to characterize the environmental factors which were involved, Table 5 was set up to determine what similarities there were between the environmental factors obtaining 3 days before each peak date and also on the preceding nights when sporulation presumably took place.

FIGURE 39

FIGURE 39

B. squamosa spores trapped on given date and spores trapped 3 days earlier during July and August. The first leaf blight symptoms were noticed on July 22nd.

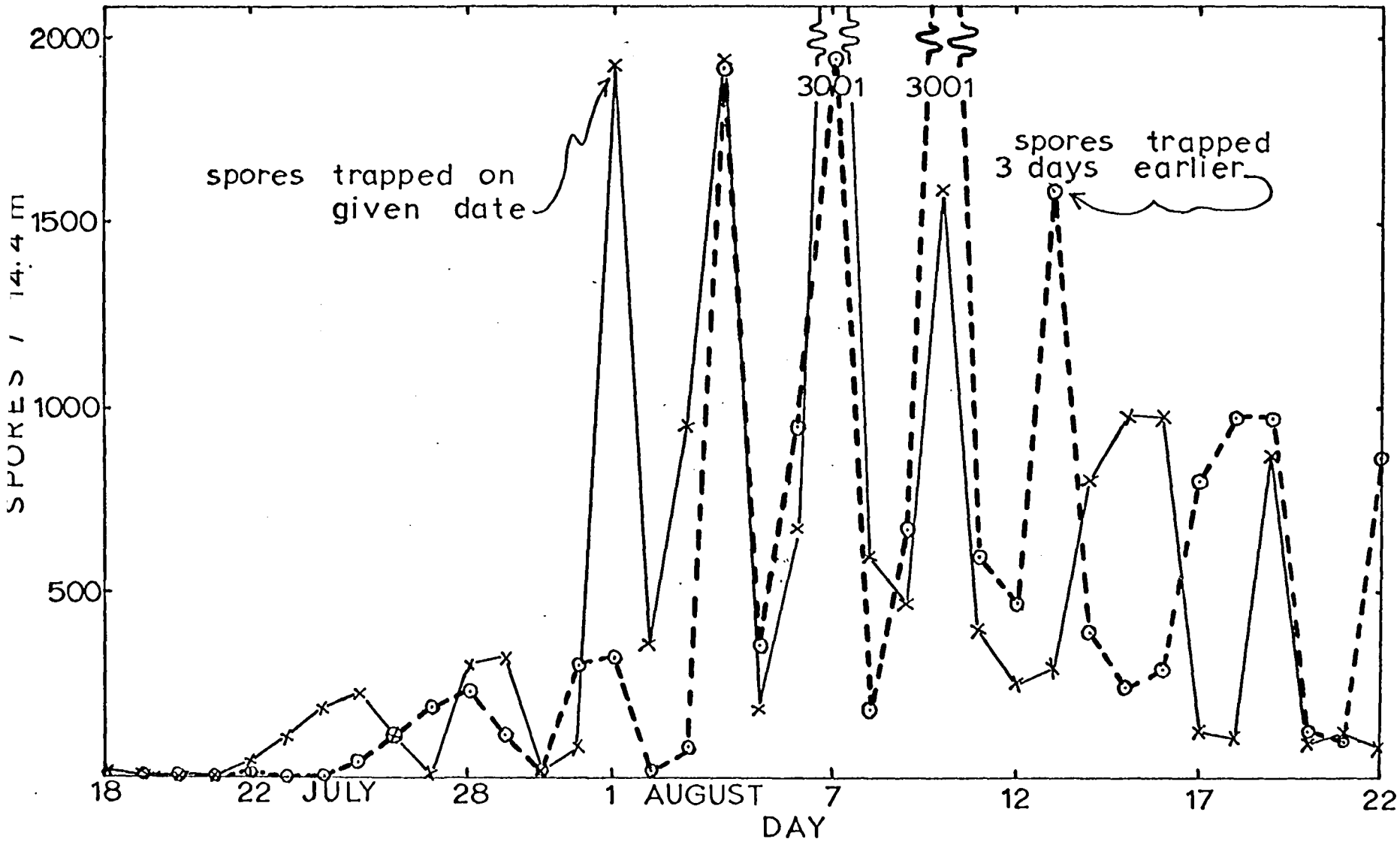

2000
1500
1000
500
SPORES / 14.4 m
3001
3001
spores trapped on given date
spores trapped 3 days earlier
18
22 JULY
28
1 AUGUST
7
12
17
22
DAY

FIGURE 39

TABLE 5

A COMPARISON OF METEOROLOGICAL DATA FOR DAYS ON WHICH THERE WERE PEAK NUMBERS OF SPORES TRAPPED

DATE	Temp. (hours 80°F)			Max. Temp. (°C.)			Min. Temp. (°C.)			RH (Hours) 90%			Wind Speed		
	On Same Date	3 days earlier	1 day earlier	On Same Date	3 days earlier	1 day earlier	On Same Date	3 days earlier	1 day earlier	On Same Date	3 days earlier	1 day earlier	On Same Date	3 days earlier	1 day earlier
Aug. 1	4	6	7	29.4	29.4	28.0	17.7	20.0	14.0	11½	8	10	10	15	15
Aug. 4	0	4	0	28.3	29.4	26.7	18.3	17.7	15.5	9	11½	9	22	10	7
Aug. 7	1½	0	1½	29.0	28.3	27.2	17.2	18.3	15.5	2½	9	12	7	22	7
Aug.10	0	1½	0	24.0	29.0	28.3	15.5	17.2	16.7	7	2½	9½	10	7	15
Mean	1.4	2.9	2.05	27.0	28.0	27.0	17.2	18.3	15.4	7.5	7.8	10.1	12	14	11

	Sunlight (hours)			Rainfall (Inches)			Dew (Hours)			Dryness (Hours)			Evaporation		
	On Same Day	3 days earlier	1 day earlier	On Same Day	3 days earlier	1 day earlier	On Same Day	3 days earlier	1 day earlier	On Same Day	3 days earlier	1 day earlier	On Same Day	3 days earlier	1 day earlier
Aug. 1	7.6	5.5	12.6	0.71	Trace	0	14½	13	11½	9½	11	12½	48	55	76
Aug. 4	7.0	7.6	11.8	0.3	0.71	0	8	14½	12	16	9½	12	37	48	94
Aug. 7	4.5	7.0	8.4	0.2	0.3	Trace	6	8	14½	18	16	9½	33	37	60
Aug. 10	5.0	4.5	4.2	0	0.2	.03	11	6	14	13	18	10	37	33	78
Mean	6.02	6.15	9.25	0.30	0.31	.007	9.9	10.4	13	14.06	13.6	11	41	43	77

The only factors in which the atypical days differed appreciably from the other days of peak production of spores were:

(1) hours of temperature over 27°C., there being no periods above 27°C. for the atypical days,

(2) rainfall, for the 10th of August, .03 inches of rain fell on the night before the 10th of August. There was no rainfall on the other days of peak sporulation.

It thus appears that the most influential factor in depressing the number of airborne spores is the absence of periods of high temperatures. The occurrence of rainfall the night before spores are liberated may also be of importance.

3.3.6.10 Conclusions

Symptoms of onion leaf blight disease were first observed at Ste.Clothilde on the 22nd of July 1969. Around this time there was no rain, relative humidities were relatively low, hours of dew duration were low, temperatures were high, there were relatively long hours of sunlight duration and evaporation was high. Prior to this period, in late June and early July, rainfall was relatively high, humidities were high, there were reasonably long hours of dew duration, temperatures were relatively low, sunlight duration

After the 22nd of July and the onset of heavy rains the epidemic developed rapidly. The environmental factors most influential in the development of the disease were:

1) Dew - this factor seemed to be important in that periods of dew of under 6 hours duration generally resulted in relatively low numbers of spores trapped on the following day.

2) Temperature - relatively long periods of temperature over 27°C. seemed to be an important factor in the disease, infection seemed to be affected unfavourably by minimum temperatures of 16°C. or lower.

3) Relative Humidity - this factor was important in that its fluctuations seemed to lead to spore release by causing hygroscopic movements of the conidiophores of B.squamosa. Hours of relative humidities above 90% did not seem to be as important as dew duration in the disease because even when there were no periods of relative humidity of over 90%, and dew was formed, spores were trapped in abundance on the following days.

4) Sunlight - this factor seemed to be important in that generally more spores seemed to be liberated when sunlight was intermittent and of short duration.

5) Rainfall - the importance of this factor was not readily apparent. However, it appears that on the days most favourable for infection there was rainfall of low intensities.

3.3.7 Effect of Disease Intensity on Yield

An important aspect of most plant disease measurement surveys is the ultimate correlation of disease intensity with the yield of diseased plants. The paired plant technique of correlating disease intensity with yield was not possible here and so in the last two disease surveys carried out at Ste.Clothilde, in addition to the normal counting of lesions per leaf, the weights of the bulbs of each plant sampled were also taken and a multiple correlation technique was used to analyse the results. There were three variables: y, the dependent variable, was the weight of the onion bulbs per plant; X_1 was lesions per plant and X_2 was leaves per plant.

The results of this analysis showed that there was no correlation between any of these variables. This result might be explained by the following factors:

1) The weight of onion bulbs taken 3 weeks before harvest might not have been correlated with the weight of onion bulbs at normal harvesting after the bulbs had been allowed to bulk up in the fields.

2) A high disease intensity at the late stage of growth of the onion plants observed at Ste. Clothilde might not have affected the bulking up of food reserves sufficiently to give any significant decreases in yield. Baker and Wilcox (1961)

found that the yields of yellow globe onions were reduced most by defoliation during the period from the 6 leaf stage to maximum leaf development and early bulb development. Defoliation earlier or later in the season had less effect on yields and often these results were not statistically significant. At the period during which there was a high percentage of disease at Ste.Clothilde the onions were quite near to harvesting and were at a stage at which mechanical defoliation would cause only non-significant reductions in yield. However, mechanical injury is not generally correlated with disease intensity (Chester 1950, Grainger 1967).

The results of this experiment are in the main inconclusive and hence further experiments will have to be carried out to determine the extent of the influence of disease intensity on yield loss.

4. LABORATORY EXPERIMENTS

4.1 Introduction

In the preceding section, field observations on a B.squamosa leaf blight disease epiphytotic were described. These observations provided facts on which the environmental factors influencing the epidemic were evaluated. However, they provided little information on pathogen or host factors which might have been important in the epidemic. Laboratory experiments were therefore carried out using B.squamosa isolates from Ste. Clothilde to determine epidemiologically important pathogen factors such as incubation periods, latent periods, infectious periods, etc. Two soil inoculation experiments were also set up to investigate the problem of the origin of the initial inoculum for the Ste. Clothilde epiphytotic and histological studies on infected onion leaves as well as an onion leaf injury experiment were also carried out.

4.2 Plant Materials Used

The varieties Spanish-G-Valencia and Nugget were used. Seed was sown in a mixture composed of a 2:2:10 v/v/v mixture of peat, sand and loam in five inch pots. Three

plants were allowed to develop per pot. Some of the plants were grown in a greenhouse but these suffered from insect attack and high temperatures in early May. Greenhouse conditions were: temperature 23-35°C., light 16 hours, dark 8 hours. The other plants were grown in a controlled environment cabinet under an illumination regime of 14 hours light and 10 hours dark with a temperature of 24°C and 13°C., respectively. The average light intensity was about 1200 foot candles provided by cool-white fluorescent tubes.

The plants grown in the growth cabinet were very healthy except for a short period when there was a physiogenic chlorosis and necrosis of older leaves. These symptoms appeared after a rapid rise in temperature to 38°C. due to a power failure.

To obtain rapidly growing leaves for some of the experiments in which leaf pieces were used, mature yellow onion bulbs of an undetermined variety were obtained from a grocery and then allowed to sprout and grow under the same conditions as described above.

4.3 Inoculation Experiments

4.3.1 Determination of the Latent, Incubation, and Infectious Periods of B.Squamosa on Leaves of Onion Plants Kept Under 3 Light and Temperature Regimes.

Introduction

The determination of latent and incubation periods of B.squamosa was the major objective of this experiment. However, the field observations, as well as the results of previous workers, indicate that temperature as well as light conditions are important in the infection process. Therefore intact onion leaves were inoculated with B.squamosa spores under 3 different temperature and light regimes to increase the probability of achieving infection and also to determine if there are changes in latent periods, etc. under these different conditions.

Materials and Methods

The leaves of 9 plants, 3 in each of 3 pots, of the onion variety Spanish-G.Valencia were sprayed with a conidial suspension of the combined Ste.Clothilde isolates no.'s 68,102, 120, 130 and 140. Control plants were similarly sprayed with sterile distilled water. After inoculation the plants were quickly covered with plastic bags to maintain a humid environment.

One inoculated and one control pot was placed in each of the following environmental conditions:

a) In an incubator in continuous darkness at 12°C.

b) In a greenhouse 23-35°C., 16 hours light, 8 hours dark.

c) In a growth cabinet 14 hours light 24°C., 10 hours dark, 13°C.

Inoculation was carried out about 4 hours before the onset of the dark period in the greenhouse and growth chambers.

A haemacytometer count of the inoculum suspension indicated that the number of conidia/ml of suspension used was about 240,000. All plants were examined at regular intervals and the date of appearance of the first lesions, conidiophores and conidia were noted for leaves which were necrotic prior to inoculation as well as for apparently healthy leaves prior to inoculation. The duration of infectious periods was also noted for necrotic tissue incited by B.squamosa as well as for previously necrotic leaf tissue which supported the sporulation of the fungus. In determining the infectious period, conidia were washed off the leaves of plants incubated in the greenhouse and off the plants later transferred from the incubator into the greenhouse. Also, the transparent plastic covers were removed during the light period; this was begun 3 days after

sporulation had started and was carried out every day for three weeks.

Results

The duration of the incubation period, infectious periods and the latent periods on infected leaves which were necrotic and also on leaves which were healthy before inoculation are presented in Table 6.

The incubation period for both the greenhouse and the growth cabinet kept plants was 24 hours. The inoculated plants kept in the low temperature incubator were observed to have numerous white superficial spots twenty four hours after inoculation, these spots subsequently disappeared.

In the greenhouse the latent period on leaves which were necrotic at the time of inoculation was 3 days while on leaves that were apparently healthy at inoculation it was 7-5 days. No sporulation took place in the 12°C. incubator. The older leaf tips of all the plants kept there became somewhat shrivelled 5 days after inoculation and developed a darker green colour than leaves of uninoculated plants and the younger leaves of the same inoculated plants. Twelve days after inoculation plants from the incubator were transferred to the greenhouse where abundant sporulation took place one day later on the green shrivelled older leaves

TABLE 6

INCUBATION AND LATENT PERIODS OF B.SQUAMOSA ON NECROTIC AND LIVING LEAVES OF ONION PLANTS WHEN INOCULATED. THE PLANTS WERE KEPT UNDER THREE DIFFERENT TEMPERATURES AND PHOTOPERIOD REGIMES.

Incubation Location and Conditions	Replicate	Latent Period on Necrotic Leaves (days)	Latent Period on Healthy Leaves at Inoculation (days)	Incubation Period (days)	Time between Incubation Period and Onset of Die-back symptoms (days)
Incubator 12°C. Continuous darkness	1	-	-	-	-
	2	-	-	-	-
	3	-	-	-	-
Greenhouse 23-25°C. 16 hrs. light, 8 hrs. dark	1	3	7.5	1	6
	2	3	8	1	6
	3	3	7	1	6
Growth Cabinet 14 hrs. light, 24°C. 10 hrs. dark, 13°C.	1	-	-	1	5
	2	-	-	1	5
	3	-	-	1	5

All control plants were healthy. A dash indicates no symptoms or sporulation occurred.

and on previously necrotic leaf tips. Plants in the growth cabinet did not support sporulation while in the cabinet nor after they were transferred to the greenhouse 2 weeks after inoculation.

The infectious period of necrotic tissue bearing conidia lasted as long as the experiment was carried out, i.e. 3 weeks. A new crop of conidia was observed each morning when the plastic bags were removed, hence the infectious period under the conditions prevailing in the greenhouse was over three weeks. The average numbers of lesions/leaf of the greenhouse and growth cabinet kept plants are presented in Table 7.

The period between lesion formation and the onset of dieback symptoms was 5-6 days for the greenhouse and growth cabinet incubated plants. However, only a few of the leaves exhibited the typical dieback symptoms. Most of the leaves in this experiment did not progressively wither from the tips but a similar dieback symptom was caused by the coalescing of the chlorotic halo around spots which, in most cases, were concentrated near the tips of older leaves. The chlorotic areas eventually became necrotic and sporulation took place on the necrotic areas. Sporulation was mainly localized at the margins of the spots.

TABLE 7

NUMBER OF LESIONS/LEAF ON THE DIFFERENT LEAVES OF PLANTS KEPT IN MOIST CHAMBERS IN A GREENHOUSE AND IN A GROWTH CABINET

(1) Leaf No.	(2) Greenhouse	(3) Growth Chamber
1	20	24
2	14	29
3	8	5
4	0	1
5	0	0

(1) Leaf No. 1 is the oldest leaf which was still green over most of its length at the time of inoculation.

(2) 23-35°C., 16 hours light, 8 hours darkness

(3) 14 hours light at 24°C., 10 hours darkness at 13°C.

Figure 40 shows leaf pieces that were removed from one of the greenhouse grown plants and placed in a moist petri dish to observe more carefully the development of conidiophores conidia, etc. Figure 41 shows spots and dieback symptoms on another of the greenhouse grown plants.

Sclerotia were formed 14 days after inoculation on pendant necrotic leaves of plants kept in the greenhouse.

Control plants at each location appeared quite healthy throughout the experiement.

Conclusions

This experiment was successful in that infection was achieved and that latent, incubation and infectious periods were determined for the greenhouse kept plants. However, no conclusion can be drawn on the effect of light or temperature on latent and incubation periods. The most significant result of this experiment is that latent periods for leaves which were necrotic at inoculation were shorter than latent periods for leaves which were healthy at inoculation.

FIGURE 40

FIGURE 40

Leaves removed from an infected plant showing dieback and leaf spotting symptoms.

(a) is the oldest leaf which was completely necrotic prior to inoculation,

(b) is the youngest leaf shown.

FIGURE 40

FIGURE 41

FIGURE 41

Tip dieback and lesion symptoms on an artificially infected plant.

FIGURE 41

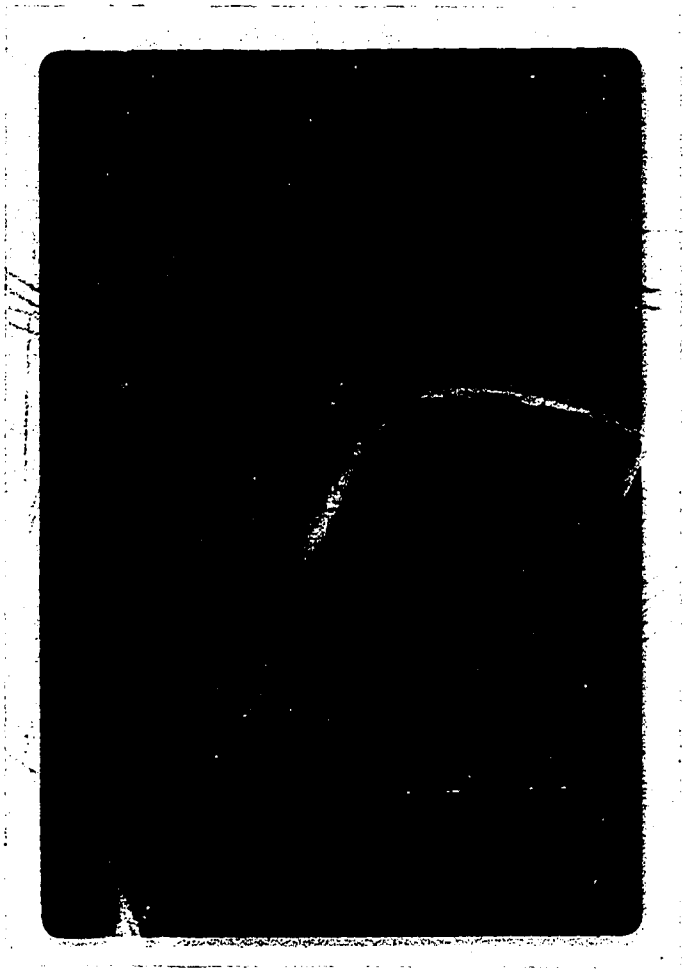


FIGURE 41

4.3.2 The effect of necrotic leaf piece and living leaf piece substrates on the latent period and reproductive capacity of B.squamosa.

Introduction

In the preceding experiment it was found that necrotic leaves on onion plants were colonized by B.squamosa and that this fungus gave rise to conidia after a shorter latent period on necrotic leaves than on leaves which were healthy at inoculation. This result was thought to be of great importance in the epidemiology of the disease and so the present experiment was set up to determine if B.squamosa on detached totally necrotic leaves in different environmental conditions of light and temperature would have different latent periods to that on healthy living leaf pieces. This experiment was also used to get some indication of the reproductive capacity of the fungus.

Materials and Methods

Twenty-five necrotic leaf pieces, each about 6 cms long, were obtained from the older leaves of Nugget plants grown in the growth cabinet. These leaves had died apparently because of normal senescense or due to a period of high temperatures caused by an electrical breakdown. The leaf pieces were examined under a stereoscopic microscope for signs of a pathogen and five pieces were placed on V-8

Agar 248 (Tuite 1968) to determine what organisms were present. The leaf pieces were washed with sterile water and sprayed with a conidial suspension of B.squamosa. (Ste.Clothilde isolate No. 62). The approximate conidial concentration of the suspension used, as determined with a haemocytometer, was 70,000 spores/ml. Five inoculated necrotic leaf pieces were placed on 2 glass slides in each of 4 autoclaved petri dishes (9 cm.diam.);on the bottom of these dishes there was a filter paper moistened with 5 mls. of a 2.5% aequous copper sulphate solution to maintain a humid atmosphere and to prevent microbial growth. The glass slides were supported above the moist filter paper by a V-shaped piece of glass tubing. One petri dish of the inoculated necrotic leaf pieces and one containing five necrotic leaves which had been atomized only with sterile water as an uninoculated control was set up in each of the locations below:

- in continuous darkness in an incubator at 12°C.
- in a controlled environment growth cabinet with a light period of 14 hours and a dark period of 10 hours, temperature 23-24°C., intensity of light 1200 ft. candles.
- in an incubator in continuous darkness at 24°C. and
- on a bench in continuous light at 24-27°C., 200 ft. candles intensity.

Living leaf pieces were obtained from the second oldest leaf of 8 onion plants of undetermined varieties which were grown from bulbs in the growth cabinet. Each leaf was cut into 4 pieces about 6 cms long. The subsequent treatment of these living leaf pieces were generally as described for the necrotic leaf pieces above. They were placed in large petri dishes (14 cm. diameter) in each of which there were 4 glass slides. Each slide supported one of the 4 pieces of one leaf. Ten mls. of copper sulphate solution was used to moisten the filter paper in these dishes.

The leaf pieces were examined every day under the stereoscopic microscope for the presence of conidiophores, conidia, sclerotia, etc. The number of conidiophores/ mm^2 was determined on a representative leaf piece for each location by counting the conidiophores in 5 random areas of 2 mm^2 each in the sporulating region and dividing by 20. Counting was done on two occasions, these were 3 days after conidiophores were first observed and 14 days after inoculation.

This experiment was repeated using the same number of necrotic leaf pieces. However, because of an insufficient number of healthy leaves of similar age and size, only 2 living leaf pieces per treatment were used.

Results

Three of the five plated necrotic leaf pieces were sterile, from the other two pieces Mucor sp., Aspergillus sp., Stemphylium sp. and an unidentified bacterium was isolated. There was no growth on the uninoculated control treatments. Some general characteristics of the disease development on healthy leaf pieces are given below for the four different conditions.

Symptom Development

Continuous Light 24-27°C.

Three days after inoculation, lesions developed on most of the inoculated leaf pieces. There was dying back from the cut ends of both the inoculated and the uninoculated control treatments. After 4 days, mycelial growth developed at the cut ends and grew towards the middle of the living leaf pieces and green islands were distinct around the lesions. The rate of mycelial growth was about 2 mm/day. Conidiophores developed after 5 days but only a few of these produced conidia at that time. All leaf pieces gave rise to sclerotia after 8 days.

Alternating Light and Darkness 23-24°C.

The characteristics of disease development in this location were essentially similar to those in continuous light except in the details which are summarized

in Tables 9a and 9b, pages 185 and 186.

Continuous Darkness 25°C.

Two days after inoculation mycelium was seen profusely growing at the cut ends of most of the leaves, the mycelial growth rate was about 5 mm/day and developed from both cut ends. After 6 days the mycelium had covered the whole external leaf surface of most leaf pieces. Numerous lesions were formed and developed green island effects after three days. Inoculated tissue remained green for a longer time then the uninoculated control leaves which became chlorotic after 2 days. Numerous sclerotia developed on the glass slides after 8 days and on one leaf piece one sclerotia was seen after nine days.

Incubator, Continuous Darkness 12°C.

One and a half days after inoculation numerous lesions were seen on most leaf pieces although the tissues was still predominantly green at this time, fast mycelial growth, comparable to the continuous darkness treatment, was observed from the cut ends after 4 days. Mycelium was also observed growing on lesions. Conidiophore and conidial production was very profuse especially on the leaf tip pieces and developed from the cut ends generally, however a few conidiophores originated from lesioned areas as well as

from non-lesioned areas of the leaves, in the later stages of disease development, the most abundant production of conidiophores was from water soaked areas near the lesions and from the borders of lesions themselves. Sclerotia developed on the glass slides after 8 days.

Figures 42 and 43 show typical inoculated and control leaf pieces on glass slides.

Latent Periods and Reproductive Capacity of *B.squamosa* on Necrotic and Living Leaf Pieces

The average periods between inoculation and conidiophore and conidial formation for necrotic and living leaf pieces under the conditions of the experiment are given in Table 8 (see also Appendix Table 6). In alternating light and darkness (23-24°C.) there was a difference of one day between latent periods on necrotic and living leaf pieces. In continuous darkness (25°C.) there was 3-5 days difference. However, there was no difference in continuous darkness (12°C.) and in continuous light (24-27°C). For necrotic leaf pieces in continuous light (24-27°C.) an average period of 1-6 days elapsed between conidiophore formation and the production of conidia.

Table 9 shows the number of conidiophores/mm^2 and on a leaf piece surface (600 sq.mm.) 3 days after sporu-

FIGURE 42

FIGURE 42

Typical living leaf pieces one week after inoculation with B.squamosa, incubated under the following conditions:

a) continuous darkness 25°C. inoculated,

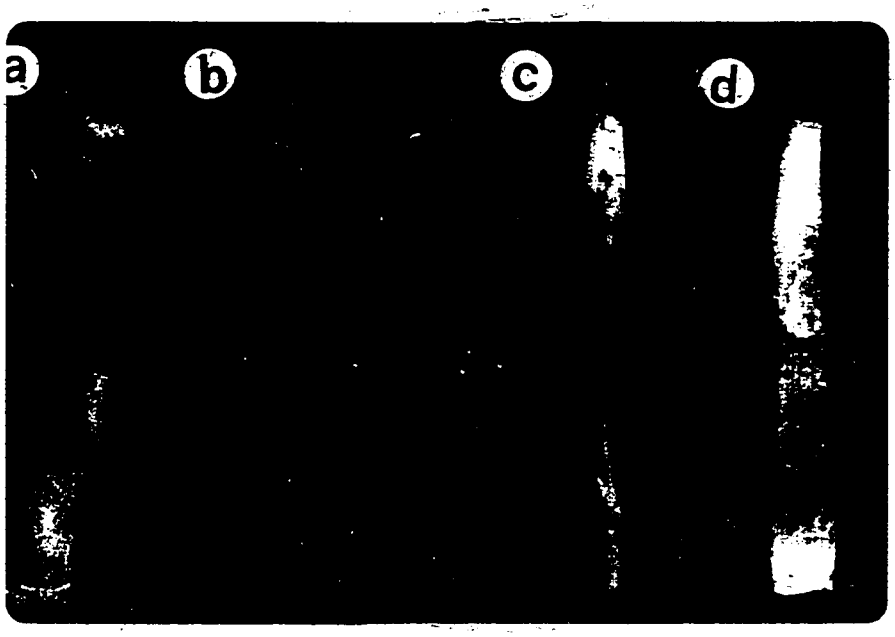
b) uninoculated control in continuous darkness 25°C.,

c) continuous light 24-27°C. inoculated,

d) uninoculated control in continuous light 24-27°C.

Mycelium is growing from all the inoculated treatments but more noticeably from (a)

FIGURE 42



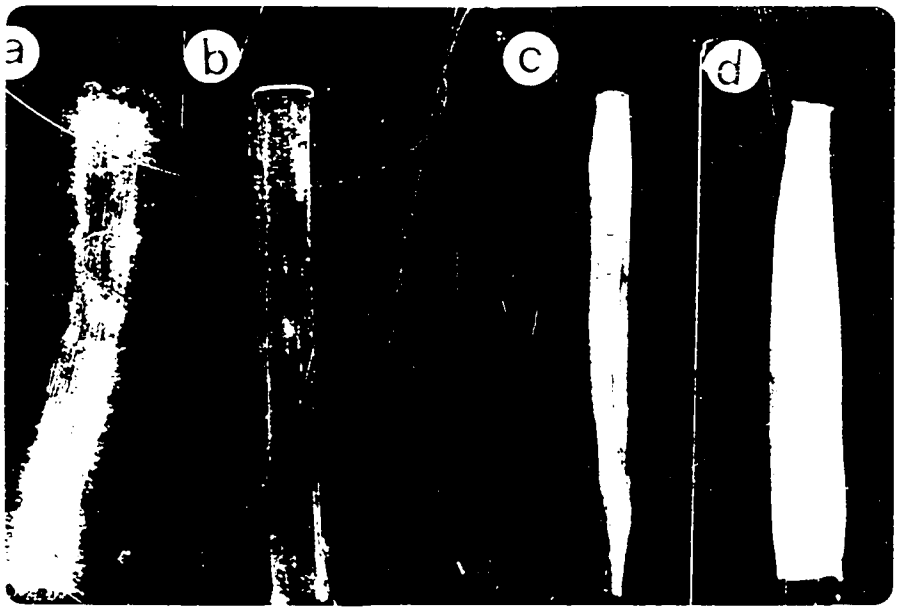

a
b
c
d

FIGURE 43

FIGURE 43

Typical living leaf pieces inoculated with B. squamosa and incubated in continuous darkness (12°C.). Conidiophores and conidia are at cut ends of inoculated treatment (a).

(b) is the uninoculated control.

FIGURE 43

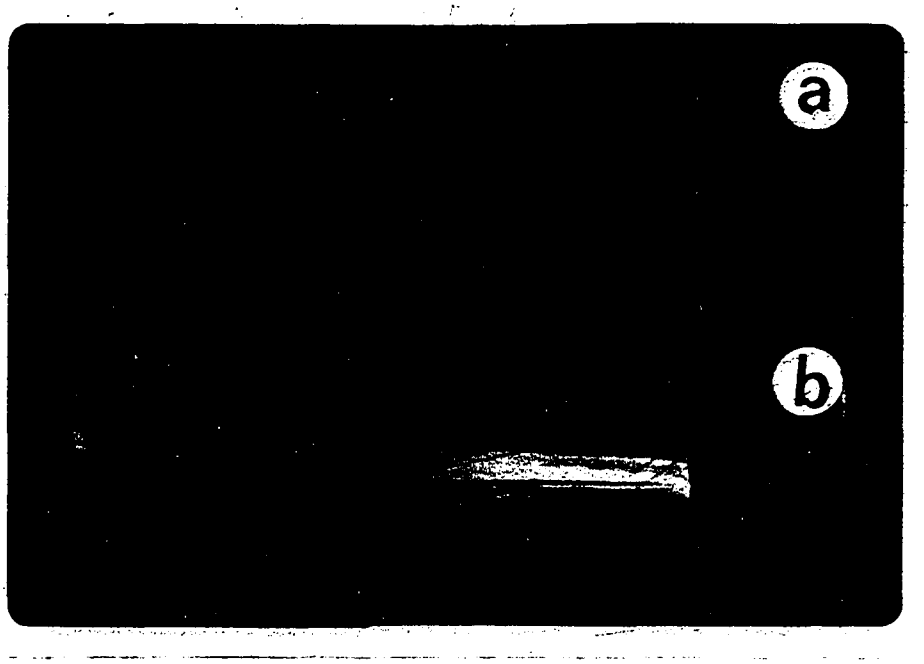


FIGURE 43

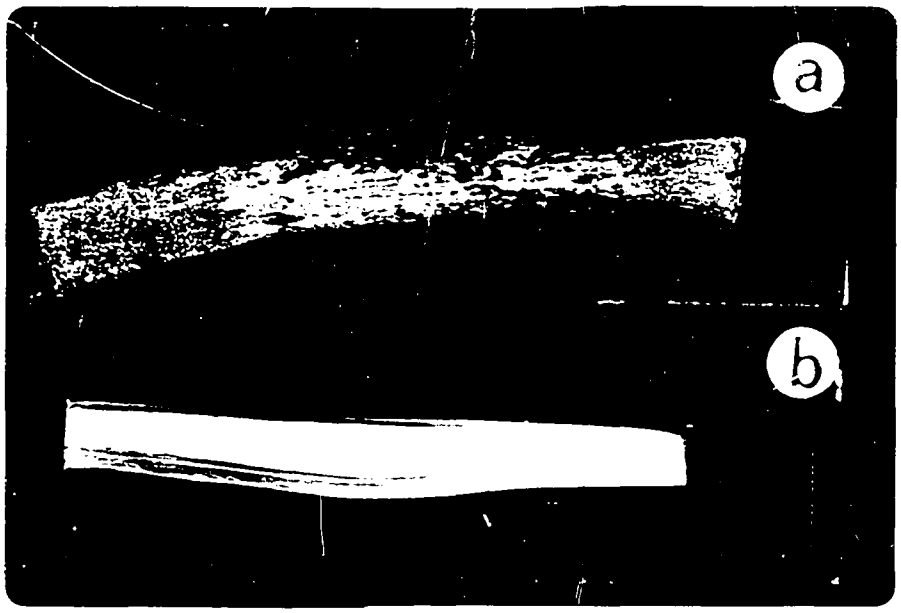


TABLE 8 a

NUMBERS OF CONIDIOPHORES PRODUCED ON NECROTIC AND LIVING LEAF PIECES UNDER DIFFERENT INCUBATION CONDITIONS ON TWO OCCASIONS AFTER SPORULATION STARTED.

Numbers of conidiophores 3 days after first conidiophores formed and 14 days after inoculation.	Continuous Darkness 12°C.		Alternating light and darkness 23-24°C.		Continuous Darkness 25°C.		Continuous Light 24-27°C.	
	Necrotic Leaf Piece	Living Leaf Piece	Necrotic Leaf Piece	Living Leaf Piece	Necrotic Leaf Piece	Living Leaf Liece	Necrotic Leaf Piece	Living Leaf Piece
Number of conidiophores/mm^2 3 days after first conidiophores formed (a)	15	15	17	21	2	0.2	25	29
Calculated number of conidiophores on one leaf piece surface (600 sq.mm) 3 days after first conidiophores formed (b)	9000	4500	10000	2500	1200	60	15000	3500
Number of conidiophores/mm^2 14 days after inoculation (a)	29	29	21	20	4	0.6	37	30
Calculated number of conidiophores on one leaf piece surface 600 mm^2 14 days after inoculation (b)	17500	17500	12500	12000	2400	360	22000	18000

(a) For sporulating region on leaf surface only.
(b) Calculation on basis of area of sporulating region, and number of conidiophores/mm^2

TABLE 8 b

NUMBERS OF CONIDIOPHORES PRODUCED ON NECROTIC AND LIVING LEAF PIECES UNDER DIFFERENT INCUBATION CONDITIONS ON TWO OCCASIONS AFTER SPORULATION STARTED.

Numbers of conidiophores with conidia 3 days after first conidiophores formed and 14 days after inoculation.	Continuous Darkness 12°C.		Alternating light and darkness 23-24°C.		Continuous Darkness 25°C.		Continuous Light 24-27°C.	
	Necrotic Leaf Piece	Living Leaf Piece	Necrotic Leaf Piece	Living Leaf Piece	Necrotic Leaf Piece	Living Leaf Piece	Necrotic Leaf Piece	Living Leaf Piece
Number of conidiophores with conidia/mm^2 3 days after first condiophores formed (a)	15	15	17	15	2	0.2	8	3
Calculated number of conidiophores with conidia on one leaf piece surface (600 mm^2) 3 days after first conidiophore formed. (b)	9000	4500	10000	1900	1200	60	5000	350
Number of conidiophores with conidia/mm^2 14 days after inoculation (a)	29	29	21	20	4	0.6	12	3
Calculated number of conidiophores with conidia on one leaf piece surface 600 sq.mm 14 days after inoculation (b)	17500	17500	12500	9000	2400	360	7500	1800

(a) For sporulating region on leaf surface only.
(b) Calculation on basis of area of sporulating region, and number of conidiophores/mm^2

TABLE 9

MEAN LATENT PERIODS (DAYS) FOR B.SQUAMOSA ON ONION LEAF PIECES INCUBATED UNDER VARIOUS CONDITIONS.

CONDITION	Period between inoculation and conidiophore formation		Period between inoculation and conidial formation	
	Necrotic Leaf Pieces (a)	Living Leaf Pieces (b)	Necrotic Leaf Pieces (a)	Living Leaf Pieces (b)
Incubator - continuous darkness 12°C.	5	5	5	5
Light 4 hrs., darkness 10hrs. 23-24°C.	3.5	4.5	3.6	4.5
Continuous darkness 25°C.	5	8.5	5	8.5
Continuous light 24-27°C.	3.4	5	5	5

(a) Average for 10 replicates.

(b) Average for 6 replicates.

lation started and 14 days after inoculation. Necrotic leaves generally gave rise to more conidiophores producing conidia than did healthy leaves. The most profuse sporulation was found in continuous darkness at 12°C. while continuous darkness at 25°C. gave rise to the least numbers of conidia. In continuous light numerous conidiophores were formed but few of these produced conidia especially on healthy leaves. The number of conidia produced per conidiophore was not accurately estimated as any attempt to move mature conidiophores dislodged all their conidia and it was virtually impossible to count the conidia on conidiophores in situ under the stereoscopic microscope. However, an average estimate of the number of conidia/spore cluster on a conidiophore is 100. These spore clusters were produced at the rate of one cluster per day for about 2 weeks after sporulation started, hence one conidiophore produces about 1500 conidia in a two-week period. The average upper surface area of a healthy leaf piece (600 sq.mm.) gave rise to about 12,000 conidiophores producing conidia after 14 days incubation in alternating light and dark conditions. About 10 million conidia would therefore be produced by such a leaf piece under similar conditions over a two-week period.

Conclusions

This experiment was essentially of a preliminary exploratory nature designed to indicate primarily the direction which subsequent experiments should take. A lack of plant material resulted in an unequal replication for the inoculated living leaf piece treatments and hence no statistical analysis of the results was attempted. No conclusions are therefore drawn on the effect of various conditions of temperature and light on latent periods of the fungus on necrotic and living leaf pieces.

In spite of the above mentioned limitations of this experiment it demonstrated adequately that B.squamosa does colonize and sporulate from necrotic onion leaf tissue. It also showed that B.squamosa's latent period is generally shorter on necrotic leaf pieces than it is on living leaf pieces for two of the conditions tested. Appendix Table 6 shows that for living leaf pieces the latent period was never less than four days in any of the conditions tested.

The experiment also demonstrated that B.squamosa has a high reproductive capacity. The number of spores produced on necrotic leaves were generally more than on living leaves suggesting that necrotic leaves may be of greater

importance in the epidemiology of this disease than has been formerly realized.

Indications from this experiment on which future experiments can be planned are that light and dark conditions seem to have a differential influence on conidiophore formation and conidial production as many conidiophores but few conidia were produced in continuous light. Few conidiophores, but all bearing conidia, were produced in continuous darkness and in alternating light and darkness large numbers of conidiophores all bearing conidia were produced. Temperature also appears to be important as the most conidia were formed at 12°C. and the least at 25°C. in continuous darkness.

4.3.3 The Effect of the Removal of Necrotic (Senescent) Leaf Tissue on the Infection of Healthy Onion Plants with Spores of B.squamosa and Subsequent Sporulation of the Fungus.

Introduction

Preceding experiments have shown that sporulation was abundant on necrotic tissue of both intact and excised onion leaves and that the latent period was longer on leaf tissue which was living prior to inoculation than on tissue which was necrotic prior to inoculation. Sporulation was found not to originate from necrotic tips resulting from the

disease but generally developed on chlorotic areas around lesions. These observations suggested that the chlorotic margin of lesions might offer an ideal site for the development and sporulation of B.squamosa originating from conidia formed on other necrotic tissues.

This present experiment was therefore performed to determine if sporulation was possible on leaves of healthy onion plants from which all necrotic tissue was removed, and which were isolated from extraneous sources of spores of B.squamosa, and also to determine the latent periods.

Materials and Methods

Three healthy plants (variety Spanish-G-Valencia) with their necrotic (senescent) leaves intact and 4 similar plants with their necrotic leaves removed were washed and then inoculated 4 hours before the start of the dark period with a spore suspension of Ste.Clothilde isolate 55 of B.squamosa. The isolate was grown on Bergquist-Lorbeer medium (Bergquist and Lorbeer 1970) at 12°C. The materials and methods used for inoculation was as in the preceding experiments.

Three plants with necrotic leaves intact were sprayed with sterile water and served as uninoculated controls.

Each plant was enclosed in a sterile test tube (35 mm x 250 mm) containing sterile sand and Hoagland's No. 1 nutrient solution. The tubes were capped with a 100 ml beaker to provide a humid environment. They were then placed in a growth chamber at 23-24°C, 14 hours light, 10 hours darkness. Test tubes were used as containers to allow easy observations on lesion formation and sporulation without removal of the plants from their containers and to reduce the chance of contamination by extraneous spores of B. squamosa.

Results

Appendix Table 7 summarizes the results of this experiment. All plants and most leaves developed lesions after a short incubation period of 20 hours and sporulation occurred from both the treatment with necrotic leaves removed and the treatment with necrotic leaves intact after periods varying from 3 to 10 days after inoculation.

Dieback occurred on only 6 leaves in the whole experiment. On 5 of these leaves, dieback developed from the coalescing of lesions which were concentrated near the tips of the leaves. In the other case there were only a few lesions near the leaf tip and dieback appeared to be similar

to the type described by other workers. No mycelium was observed on the lacunar surface of this leaf before sporulation occurred.

Older leaves became chlorotic and necrotic earlier than younger leaves even though the inoculum used was similar for all leaves. Table 10 gives the average latent periods for all leaves, leaf #1 being the oldest necrotic leaf and leaf #6 being the youngest leaf. There were generally more lesions on leaves of plants with all necrotic leaves removed than on those with necrotic leaves intact. Latent periods increased as leaves got younger.

Conclusions

This experiment showed that B.squamosa can infect healthy onion leaves on the plant and give rise to typical symptoms and sporulation after latent periods which were generally shorter on older leaves than on younger leaves.

4.4 The Identification of the Pathogen Following the Artifical Inoculation and the Sporulation of B.squamosa on onion leaves.

In the preceding experiments spores identified as conidia of B.squamosa (see Section 3.3.1) were artificially

TABLE 10

AVERAGE LATENT PERIODS OF B.SQUAMOSA ON LEAVES OF ONION PLANTS WITH NECROTIC (SENESCENT) LEAVES REMOVED AND INTACT

Leaf Number	Average Latent Periods on Plants with Necrotic Leaves Removed (a)	Average Latent Periods on Plants with Necrotic Leaves Intact (b)
1	0	3.33
2	4.5	4
3	5	5.33
4	6.25	8.33
5	9	-
6	-	-

(a) Mean of 4 plants

(b) Mean of 3 plants

Leaf No. 1 is the oldest necrotic leaf.

Leaf No. 6 is the youngest leaf.

inoculated onto onion leaves and gave rise to symptoms essentially like the disease in the field. Sporulation also occurred after various latent periods. The conidiophores and conidia produced were examined microscopically and were found to have all the characteristics of B.squamosa, (see Section 3.3.1). All the steps implicit in Koch's postulates were therefore carried out and it can be concluded that B.squamosa was the pathogen responsible for part or all of the disease syndrome observed at Ste. Clothilde.

4.5 Soil Inoculation Experiments

The problem of the origin of the initial inoculum for the Ste.Clothilde epiphytotic was a relatively important one for this project and hence two experiments were set up with the hope that their results would provide a solution to this problem.

In the first experiment sclerotia of Ste.Clothilde isolate #125 of B.squamosa were placed in 5 pots each of undetermined varieties of onion plants grown from bulbs in the greenhouse and in the growth chamber (under the same conditions as described in Section 5.3.2). The plants were about one month old and 50 sclerotia were placed in and on the surface of the soil in each pot near to the plant. The

plants were observed regularly for symptoms of B.squamosa leaf disease but none developed and the treated plants appeared as healthy as nearby untreated plants. This result is in agreement with results of similar experiments reported by Page (1957).

It was thought that perhaps the initial inoculum at Ste.Clothilde might have arisen from conidia of B.squamosa produced on sclerotia in the soil or on soil debris after a high temperature stimulus following a period of freezing temperatures and so, in the second experiment, 30 sclerotia of the same Ste.Clothilde isolate used above were placed in approximately 20 ml. samples of soil in small petri dishes. There were three types of soil: sand, muck-soil, and a greenhouse sand-clay-loam mixture. The soil samples in the petri dishes were autoclaved for about 14 hours at 15 p.s.i. pressure. After seeding with inoculum, the petri dishes were placed in a deep freeze (-16°C.) in sets of 9 comprising 3 replicates of each soil type. One set was left in the dark at 25°C to act as a control and it was proposed to remove one similar set from the deep freeze at two weekly intervals, place them in the incubator at 27°C. and observe for sporulation of B.squamosa. However, there was profuse sporulation of contaminant fungi two days after the first two sets were removed from the deep freeze and the experiment

was abandoned. This experiment could not be repeated due to the limited time available.

No conclusions can be drawn on the origin of the initial inoculum at Ste.Clothilde from the results of these soil inoculation experiments.

4.6 Histological Studies on Infected Leaves

Background

Hancock and Lorbeer 1964 and Hancock et al 1964a have indicated that Botrytis squamosa mycelium spreads rapidly on the "favourable" lacunar surface of the infected onion leaf after penetrating the deep seated lesions incited by its enzymes. They also imply that when the mycelium reaches the leaf tip it causes dieback symptoms five to twelve days after the initial leaf spotting. Some histological observations were therefore made to determine whether or not appreciable lacunar growth of Ste.Clothilde isolates of Botrytis squamosa preceded tip dieback symptoms on the leaves of artificially inoculated plants.

Materials and Methods

These investigations may be divided into three phases:

1) The examination of the unstained lacunar surfaces of leaves of artifically infected 3-month old onion plants at various times after lesion development for the presence of mycelium, using a steroscopic microscope.

2) The examination of cylindrical leaf pieces which were artificially inoculated on their external surfaces with conidia of B.squamosa. These leaf pieces were placed in petri dishes in which high humidities were maintained by moist filter paper. The petri dishes were placed under the same four environmental conditions as described in section 4.3.2 and the development of the mycelium on the lacunar surfaces of the excised leaves was noted for each of these conditions.

3) The examination of stained leaf tissue at various stages of infection for the presence of mycelium in and on lesions, in healthy leaf tissue, on the external surface of the leaf, and on the leaf lacunar surface. Leaves used were from inoculated plants kept in the greenhouse and in the growth chamber and also from inoculated leaf pieces. The leaf tissues selected for examination were generally lesioned tissue of which either freehand transverse sections, or five cm. square sections, incorporating healthy tissue as well, were made using new razor blades. The leaf sections were cleared for twenty-four hours at about 75°C. in a 95:5 (v/v) Dioxan: proprionic acid mixture (Tichelaar 1967). They

were then washed in distilled water, stained in Cotton blue in lactophenol and examined under the microscope.

Results

1. Examination of inoculated plants

The following observations were made:

a) In older leaves which were chlorotic or necrotic before inoculation, B.squamosa mycelium was seen growing on the leaf lacunar surfaces three days after inoculation.

b) No mycelium was observed to grow on the lacunar surfaces of leaves which were healthy prior to inoculation until necrosis occurred at which stage mycelium in the lacunae was restricted to the necrotic areas and generally appeared in the leaf lacunae simultaneously with the occurrence of sporulation on the outer leaf surfaces.

c) No mycelium grew in the leaf lacunar surfaces of predominantly green tissue.

d) Leaves which had developed a few lesions only and were still predominantly green but whose tips became necrotic 6-9 days after lesion development also had no mycelial growth on the lacunar surfaces.

e) Most lesions did not show a strict dieback but the same effect was achieved by the relatively rapid coalescence of chlorotic areas around lesions, especially near the tip.

Sporulation was generally from inter-lesion areas and not from the lesions themselves and there tended to be a concentration of conidiophores and conidia near the lesion margins.

2. Examination of inoculated leaf pieces

The following observations were made:

a) Continuous Light 24-27°C and Alternating Light and Darkness 23-24°C.

The growth of B.squamosa mycelium on the lacunar surfaces of the leaf pieces was restricted to the necrotic portion of the lacunar surface. The mycelial front moved at a rate of approximately 4 mm/day from the cut ends of uninoculated control leaf pieces.

b) 12°C. Incubator - Continuous Darkness

B.squamosa mycelium rapidly spread over the whole lacunar surface in two days, i.e. well before the mycelium on the outer leaf surface could do so. The mycelium on the outer surface moved at an average rate of approximately 10 mm/day. The uninoculated controls remained perfectly green for over three weeks.

c) Continuous Darkness 24°C.

Two days after inoculation mycelium grew over the whole lacunar leaf surface. The rate of mycelial growth over the external leaf surface was similar to that in the low temper-

ature incubator. The uninoculated control treatments became completely chlorotic after one week.

3. Examination of stained leaf tissue

The following observations were made:

a) No mycelium was seen on either external surfaces, lacunar surfaces or on the lesions themselves of living leaves of three day infected plants. The morphology of the lesions were in agreement with former published results and were consistent with an enzyme degradation hypothesis.

b) Mycelium was observed in most lesions which were over two days old, the mycelium was inter and intra-cellular and seemed to be localized internally in the lesion. No mycelium was seen in apparently healthy tissues surrounding the lesions.

c) One week after inoculation when there was dieback symptoms on some leaves, the lesions on these leaves had mycelium internally in the lesions proper and externally on the epidermis. The mycelium did not extend past the margins of the lesions, there being a concentration of mycelium both internally and on the epidermis at the junction of the lesions with apparently healthy tissue. There was no mycelium on the lacunar surfaces of the numerous stained lesions and adjacent healthy tissues examined. Figures 44 and 45 illustrate these results.

FIGURE 44 and FIGURE 45

FIGURE 44

Transverse section of onion leaf in lesioned area showing disorganized tissue with mycelium in necrotic area.

FIGURE 45

Surface view of lesioned area of onion leaf showing mycelium concentrated in margin of lesion. No mycelium is in healthy tissue on left, lesioned tissue is on right.

FIGURE 44

FIGURE 45

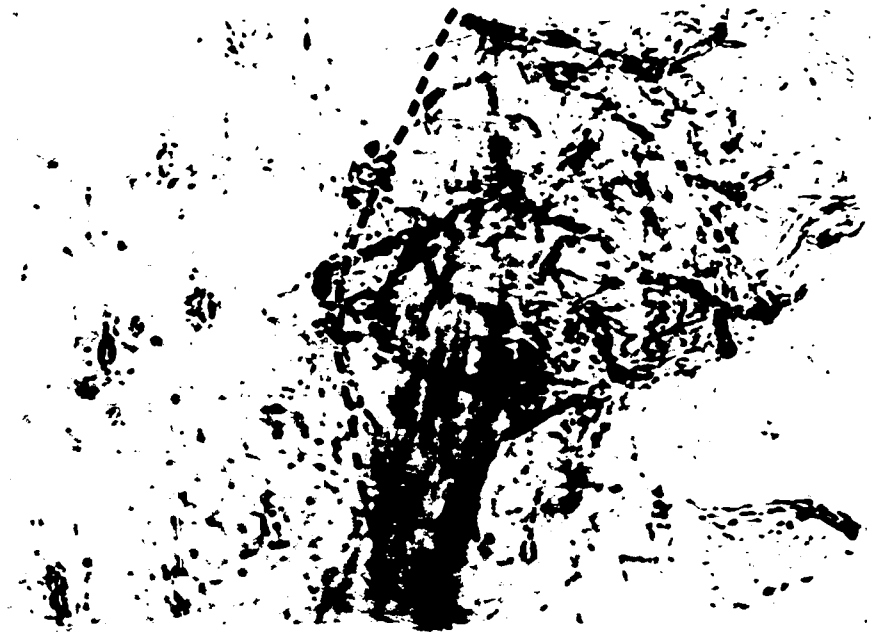
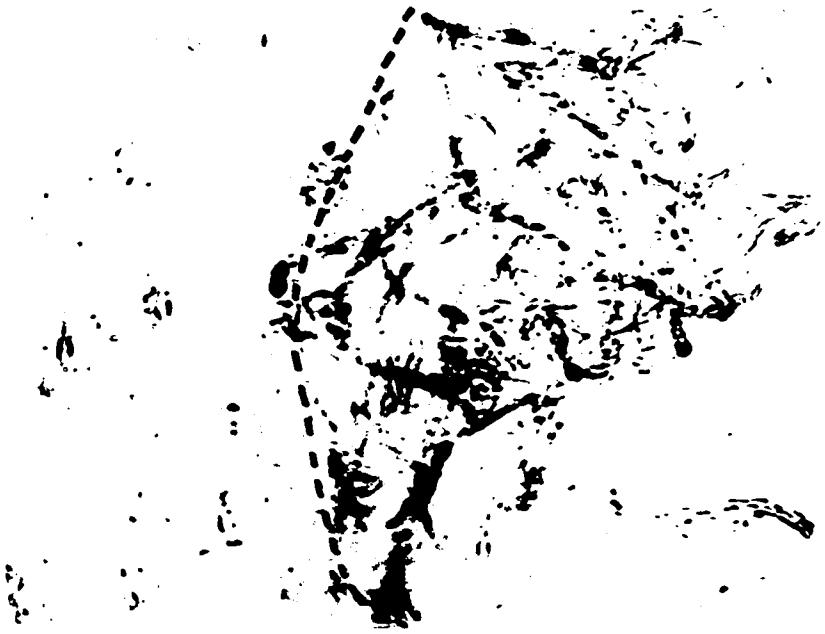


FIGURE 44

FIGURE 45



d) Older necrotic leaf tissue from infected leaf pieces and from whole onion plants showed B.squamosa mycelium ramifying both inter and intra-cellularly throughout the necrotic tissue. The mycelium grew on both the external leaf surfaces and on the leaf lacunar surfaces. Conidiophores with bulbous bases seemed to originate from stomatal apertures as stated by Page (1957). Figure 46 illustrates these results.

Conclusions

The observations made here do not support Hancock et al's hypothesis of dieback being caused by the rapid spread of mycelium to the leaf tip through the leaf lacunae. Dieback, caused by a coalescing of chlorotic halos around closely spaced lesions, was the predominant type seen in the inoculation experiments.

4.7 The Effect of Puncturing Leaves on the Development of Physiogenic Dieback

Engle et al (1965), reported that field grown onions of the F1 hybrid Epoch developed tipburn symptoms after 24 hours when their leaves were randomly punctured from 1-20 times with a sterile pin 1 mm. in diameter. They found that 10 or more of these punctures were sufficient to cause dieback symptoms. It was decided to carry out a

FIGURE 46

FIGURE 46

Conidiophores on epidermis of two adjacent leaf pieces.

FIGURE 46

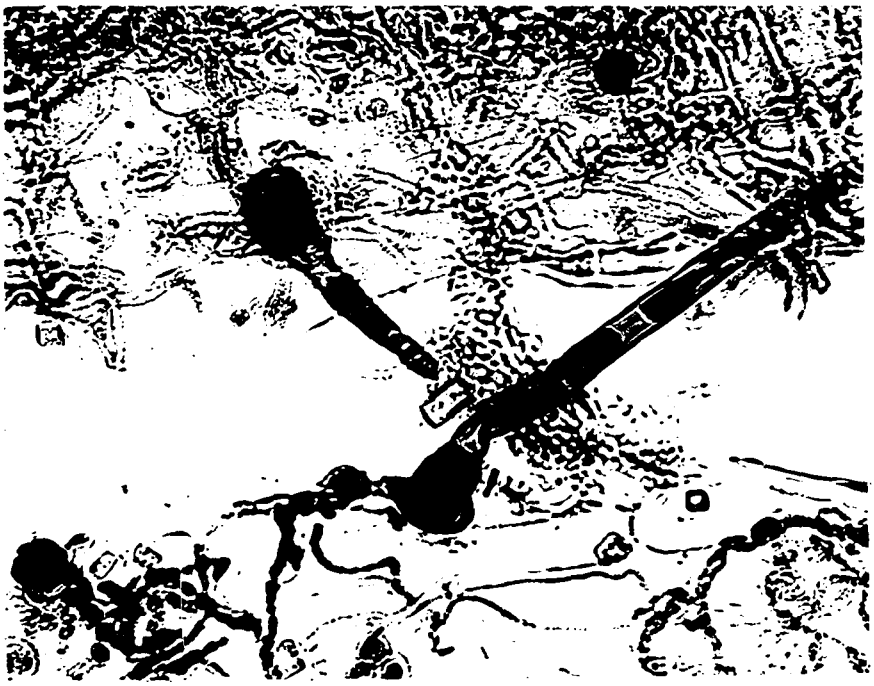
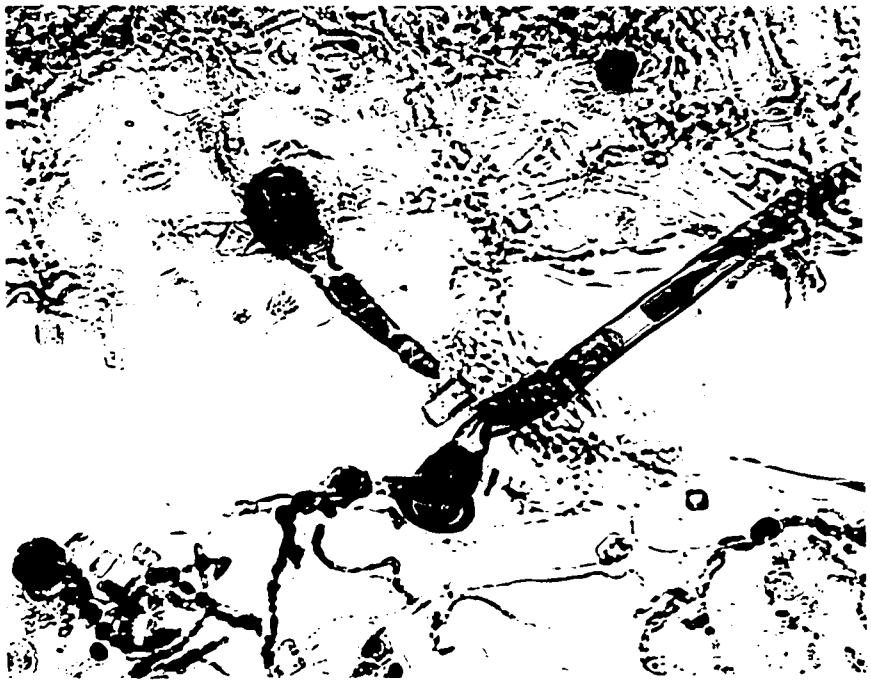


FIGURE 46



modification of this experiment as if mechanically induced dieback could be confirmed for the onion varieties used in the inoculation studies of this project. It could offer an explanation of some aspects of the epidemiology of the B.squamosa induced leaf blight disease of onions.

Materials and Methods

Three-month old plants of the onion varieties Nugget and Spanish G.Valencia which were grown in the growth chamber were used. Five plants of each variety were randomly punctured with a sterile needle 1/2 mm in diameter 25 times around the circumference of each healthy leaf to give holes of about the same size as many B.squamosa induced lesions. Five control plants of each variety were left unpunctured. There were five pots per variety each containing a treated and an untreated control plant. After puncturing, the plants were placed in a greenhouse (see section 4.2) and watered at 2 days intervals. Daily observations for the onset of tip dieback symptoms were carried out and the experiment was terminated after 14 days.

Results

Table 11 summarizes the results of this experiment.

No dieback developed on any punctured leaf after 24 hours. The oldest punctured leaves of all plants developed

TABLE 11

PERIODS BETWEEN PUNCTURING AND DIEBACK FOR OLDEST AND 2ND OLDEST LEAVES OF FIVE PLANTS EACH OF NUGGET AND SPANISH G.VALENCIA VARIETIES OF ONION.

		PERIOD BETWEEN INJURY AND DIEBACK (days)			
		OLDEST LEAF (a)		2nd OLDEST LEAF (b)	
Onion Variety	Replicate	Treated	Control	Treated	Control
Nugget	1	2	7	2	-
	2	4	7	-	-
	3	7	7	-	-
	4	7	7	9	-
	5	9	7	-	-
Spanish G.Valencia	6	7	10	-	-
	7	9	10	-	-
	8	9	10	4	-
	9	4	8	8	-
	10	4	8	6	-
	MEAN	6	8	6	-

A dash indicates that there was no dieback symptoms.

(a) and (b) living.

dieback symptoms but not significantly earlier than the dieback symptoms observed on unpunctured control leaves.

Tip dieback symptoms were observed on the second oldest punctured leaves of some plants of both varieties tested after periods ranging from 2 to 9 days. There was no tip dieback on the second oldest leaves of the control plants.

It was observed that all the punctures developed chlorotic halos after an average period of about 4 days. These halos were reminiscent of the halos around B.squamosa lesions, however the punctures were not sufficiently close to one another for the chlorotic areas to coalesce. Stemphylium sp. was observed sporulating on most necrotic tips after one week and a few punctures supported the sporulation of Penicillium sp. after two days.

Conclusions

This experiment did not confirm the results of Engle et al's experiments as no dieback symptoms developed one day after puncturing of the leaves. The experiment does suggest however that mechanical injury of the second oldest healthy leaf of the varieties tested can give rise to dieback symptoms 2 to 9 days after that injury.

5. INTEGRATION OF OBSERVATIONS AT STE.CLOTHILDE WITH RESULTS OF LABORATORY OBSERVATIONS

The epidemiology of parasitic disease may be defined as the science of increase in populations of parasitic pathogens on the population of the host, it is concerned with all host, pathogen and environmental factors and conditions which play significant roles in the development and spread of epidemics with the aim of elucidating the manner in which these operate in the overall causative complex. In this study, an epiphytotic of leaf blight disease of onions was followed at Ste.Clothilde and observations were made on the relative importance of various environmental, pathogen, and host factors on the onset and development of this epiphytotic; laboratory studies which were designed to provide answers to some of the problems posed by the field observations were also carried out and the data presented separately. This section combines the observations of Ste.Clothilde with the results of the laboratory experiments to elucidate the pathogen, host and environmental factors which influenced the onion leaf blight epiphytotic at Ste.Clothilde.

Pathogen Factors

The epiphytotic was of the explosive annual type, r, the relative infection rate for the period between the first and last survey being 0.31 per unit per day. The

relative infection rate for the period between the first and second survey was 0.1379 per unit per day while that for the period between the second and third survey was 0.4186 per unit per day. These relative infection rates were all calculated using Van der Plank's equation:

$$r = \frac{2.30}{t_2-t_1}\left(\log_{10}\frac{X_2}{1-X_2} - \log_{10}\frac{X_1}{1-X_1}\right)$$

as the epiphytotic had passed the logarithmic stage of increase at the time the surveys were carried out. R, the basic infection rate corrected for latent periods, was 0.492 for the second survey date. The latent period at that time was 3 days as deduced from the graphs of spores trappped at Ste.Clothilde and from laboratory experiments. The threshold value, as deduced from the dosage response curves of the Ste.Clothilde experiments, was approximately 1700 cumulative spores. This threshold value is very similar to the one obtained by Segall and Newhall (1960). Their threshold value for an unspecified Botrytis species was 1,094. In the laboratory inoculation experiments approximately 700 spores of B.squamosa were required to produce one lesion on older leaves. On younger leaves the number of spores required to produce one lesion was much higher varying from 80,000 to 1,500.

The reproductive capacity of B.squamosa was not determined in terms of the number of conidia produced per individual infection, and the number of conidiophores producing conidia on intact necrotic onion leaf surfaces was not estimated. However, that this fungus has an immense reproductive capacity is indicated by the fact that approximately 10,000 conidiophores were produced on 600 sq.mms. of detached formerly necrotic leaf pieces 6 days after inoculation. The number of conidia produced by these conidiophores at that time would be approximately 2 million. On one infected necrotic leaf in the field at least as many conidia as these are produced daily for infectious periods of over three weeks duration when environmental conditions are favourable.

The latent period for B.squamosa, when there were favourable environmental conditions in the field, was estimated to be 3 days. Inoculation experiments in the laboratory showed that latent periods varied from 3 days on senescent necrotic leaves to 9 days on healthy leaves of infected plants. The latent period for formerly healthy leaves was never less than 4 days even under the apparently optimal environmental conditions for infection, incubation and sporulation under which these experiments were carried out. Latent periods also apparently increased as leaves got younger.

No conclusion could be drawn on the ability of B.squamosa to survive in the soil. However, sclerotia were observed on naturally infected leaves taken from Ste.Clothilde and also on artifically infected leaves and leaf pieces and the possibility exists that the fungus does overwinter in the soil in the form of sclerotia.

This fungus is apparently capable of both saprophytic and parasitic survival on onion leaves as is shown by the results of the inoculation experiments and the very explosive nature of the Ste.Clothilde epiphytotic can be explained in terms of the saprophytic build up of vast quantities of B.squamosa inoculum on necrotic older leaves of onion plants which inoculum then parasitized healthy onion leaves.

Host Factors

The most important host factor in the epidemiology of B.squamosa leaf blight disease of onions is probably host resistance and susceptibility. Other factors such as growth type of host, density and purity of host stand and alternate hosts are relatively unimportant.

The survey data from Ste.Clothilde showed that older leaves had more lesions than younger leaves and this indicated either that older leaves were more susceptible to

the disease than younger leaves or that the older larger leaves were more exposed to inoculum than the younger ones. However, a correlation analysis of the data showed that only in the early stages of the disease was there a significant correlation between lesions and leaf number. This suggested that the older leaves were more susceptible than the younger ones in the early stages of the epidemic but as inoculum increased and environmental conditions became more favourable for the pathogen there was no differential susceptibility and the pathogen was able to infect all types of leaves equally well. The laboratory inoculation experiments gave a similar result to the Ste.Clothilde surveys in that older leaves generally had more lesions than younger leaves; however, the difference was not significant.

The most significant factor with respect to the relative susceptibility of leaves, however, was that the fungus was capable of infecting and sporulating on leaves which were necrotic prior to inoculation. In inoculation experiments involving the inoculation of healthy and necrotic leaves it was consistently observed that necrotic leaves generally supported sporulation of B.squamosa after a shorter latent period than leaves which were apparently healthy prior to inoculation. Also, the reproductive capacity of

the fungus was generally higher on leaves which were necrotic prior to inoculation than on leaves which were healthy prior to inoculation.

When healthy leaves were artificially inoculated, dieback generally resulted from the coalescing of closely spaced lesions rather than from the growth of the fungus through the lacunar space of the leaves to the leaf tips as postulated by Hancock et al 1965. Dieback, also, did not seem to generally result from mechanical injury as shown by the results of the leaf injury experiment.

The incubation period for lesion formation was found to be about one day in most cases.

Environmental Factors

The environmental factors which characterized the onset of the disease were vastly different from those which obtained during its latter development. The available facts suggest that the first symptoms observed were probably physiological in origin and subsequent symptoms were incited by B.squamosa. A summary of the meteorological conditions which characterized the onset and the development of the disease at Ste. Clothilde is given in another section of this report (3.3.6.10). The results of the laboratory inoculation experiments generally indicated that high temperatures were

important in the development of this disease as plants kept in the greenhouse at high temperatures developed symptoms and gave rise to sporulation before plants kept at the relatively low growth cabinet temperatures. Plants kept at 12°C. in the dark in the low temperature incubator did not sporulate or develop typical symptoms until they were removed to the greenhouse. These results contrast strongly with the results of the leaf piece inoculation experiments which showed that leaf pieces kept at 12°C generally gave rise to more sporulation than those kept under other temperature and light conditions.

The influence of light on this disease is not clear. One experiment indicated that light was not necessary for sporulation especially for necrotic leaf pieces. Indeed, this experiment indicated that a dark period is necessary for the copious formation of conidia while light is necessary for the abundant formation of conidiophores. More work on this aspect of the epidemiology of B.squamosa leaf blight is necessary as the results of these exploratory experiments all indicate that light might play a significant role in the reproductive capacity of this fungus and this strenghthens the observation made on the Ste.Clothilde phase of this investigation that more spores were produced in short and intermittent sunlight periods than in long periods of sunlight.

Relative humidity is the only other environmental factor on which some limited conclusions can be drawn from the laboratory experiments. It was observed that on the mornings on which sporulation was noted in the plant inoculation experiment there was no apparent moisture droplets on sporulating necrotic leaves although drops of moisture were seen on the inner plastic bag surface and guttation droplets were seen on healthy leaf tips. This seems to indicate that perhaps high humidity is as effective as dew in stimulating sporulation and free moisture may not be required.

6. DISCUSSION AND CONCLUSIONS

A great deal of confusion still exists in the literature as to the causal agent of the disease symptons on onion leaves variously described as leaf blight, leaf fleck, blast, and tip dieback of onions. This project was therefore carried out mainly to determine the causal agent of the disease under Ste.Clothilde conditions in the summer of 1969 and also to determine some of the epidemiologically important factors of this disease.

The majority of the results, both of the field observations and the laboratory experiments strongly suggests that the epiphytotic at Ste.Clothilde was the result, first, of adverse environment conditions which caused the physiological onion blast disease to develop, and then B.squamosa leaf blight disease followed the initial blast. The evidence for this conclusion is derived from the following facts:

The weather conditions at Ste.Clothilde at the onset of the disease were not generally favourable for a pathogenic disease and in most respects were consistent with the conditions which Jones postulated to be favourable for the physiogenic onion leaf blast disease.

The dosage response curves of the field data, the inoculation experiment results, and Hancock and Lorbeer's results all indicate that the threshold value of spores required to cause appreciable disease is quite high, thus it is reasonable to expect that if the early stages were caused by a pathogen there would be quite a large number of spores trapped. This was not found to be so as spore trap data indicated that the concentration of B.squamosa spores necessary to cause appreciable infection were only trapped about one week after the first disease symptoms were observed.

No primary infection foci were observed at the onset of the disease. All plants were uniformly affected at that stage.

The disease survey data at Ste.Clothilde indicated that older leaves were more susceptible to lesion development than younger leaves in the early stages of the epidemic, but that there was no significant difference in susceptibility between leaves of different ages in the latter stages. Laboratory experiments, however, indicated that leaves of all ages are susceptible to B.squamosa as was also reported by Hancock and Lorbeer 1965. The occurrence of lesions on the older leaves only during the early stages of the disease

when all leaves were susceptible to B.squamosa indicates that the disease was mainly ofa physiological nature at that stage.

Isolations from infected plants at Ste.Clothilde on the 29th of July confirmed that B.squamosa was the pathogen at that time as 1) many lesions gave rise to B.squamosa when they were cultured on PDA, 2) all of the Ste. Clothilde isolates which were tested caused typical leaf blight symptoms in subsequent inoculation experiments and 3) the fungus which was reisolated had all the diagnostic characteristics of B.squamosa.

Meteorological data from Ste.Clothilde indicated that the latent period for spore production by B.squamosa during periods of favourable environmental conditions was only 3 days. In laboratory experiments where environmental conditions should have favoured the fungus, the latent periods recorded were never less than 4 days for leaves which were healthy prior to inoculation. However, the latent period for conidial production on leaves which were necrotic prior to inoculation was 3 days. These facts suggested the following hypothesis for the onset and development of the onion leaf disease observed at Ste.Clothilde.

The onset of the Ste.Clothilde epidemic was most likely due to unfavourable weather conditions which resulted in onion blast disease. Environmental conditions became more favourable for the pathogen after the onset of the blast symptoms and B.squamosa spores of undetermined origin, but most probably derived from overwintering sclerotia of the previous season, infected older necrotic onion leaves. The inoculum of B.squamosa conidia then increased mainly on necrotic onion leaves, being released by hygroscopic movements of conidiophores and were disseminated by wind to other necrotic leaves where new infections were started.

On the first survey date the inoculum was not high enough for the fungus to infect all leaves of the onion plant and so the oldest, most exposed leaves developed more lesions than younger leaves. However, necrotic leaves were still preferentially infected and provided the majority of inoculum. This stage of the epidemic was characterized by regular peaks and troughs of daily spore concentrations, the peaks followed each other in regular sequence corresponding to the latent period of the fungus on necrotic tissues and were seemingly dependent on favourable weather conditions. Around the time of the second survey date the high inoculum developed was able to infect all leaves irrespective of age and one week after this the regular pattern of peaks and troughs in daily spore concentrations was obscured. This is

thought to be due to inconstant latent periods and environmental factor fluctuations.

Ozone has been found by Engle et al (1965) to be incitants of a tipburn disease of onions with similar symptoms to those caused by B.squamosa. Rich, et al (1969) found that crop damaging periods of ambient ozone occurred one day in four during the growing period in Connecticut. Manning, et al (1969) in Massachusetts found that ozone caused damage on potato leaves which resembled the flecks caused by B.cinerea and that this ozone injury appeared to increase the susceptibility of potato leaves to infection by B.cinerea.

The possibility therefore exists that ozone could have been one of the incitants of the disease symptoms observed at Ste.Clothilde and might have been one of the factors responsible for the rapid rate of increase of the disease in early August when rainstorm conditions could have given rise to appreciable ambient ozone concentrations. However, no monitoring of ozone concentrations was carried out and no valid conclusions can be drawn on the effect of ozone on the Ste.Clothilde epidemic especially as the other

studies quoted above were all carried out near industrial centres where pollutant levels are expected to be high. One factor, however, which indicates that there might have been appreciable concentrations of ozone at Ste.Clothilde during the first two weeks of August was that the rubber holders of the dew meter pencils were severely cracked around this period indicating the presence of oxidants in the atmosphere (see Bradley and Haagen-Smit 1950).

The very significant correlations between number of lesions on the leaf with maximum number of lesions and total number of lesions per plant indicates that, if this relationship is confirmed in future surveys, disease intensities could be estimated from lesion counts on one leaf only of a plant thereby increasing the accuracy of estimates of disease intensity by increasing the number of samples which could be made at any one time.

The exploratory series of laboratory experiments have uncovered some aspects of the biology of the Ste.Clothilde isolates of B.squamosa which have not been seen in the literature. One of these is the ability of these isolates to exist both saprophytically and parasitically on onion leaves. The saprophytic existence of a Botrytis species is a common occurrence but parasitic existence of

this genus is somewhat rare. B.squamosa is an exception to this generality as it has long been recognised as a specific parasite of onion leaves and this is probably the first report of its saprophytic existence on necrotic onion leaves.

The sporulation of B.squamosa is an aspect of the biology of B.squamosa which has been only superficially investigated here. The most interesting aspect of light stimulation of sporulation was that light and dark periods appear to act differentially in the stimulation of the formation of conidia and conidiophores respectively on leaf pieces. This aspect of the biology of B.squamosa should be more fully investigated especially along the lines of elucidating the biochemical pathways involved.

These experiments have failed to find evidence to support Hancock et al's hypothesis that onion leaf dieback, as incited by B.squamosa, is caused by the rapid growth of the fungal mycelium in the lacunar area of the onion leaves, for in these experiments tip dieback has been found to be caused by the coalescing of lesions in most cases. Of relevance here is the fact that Engle et al's findings that ten or more needle pricks on onion leaves cause tip dieback has not been substantiated by the results of the leaf

injury experiment.

The disease survey data showed that there was no correlation between disease intensity and yield and another section of this thesis (3.3.7) discusses possible reasons for this. However, in spite of the limitations imposed by the method used in measuring yields, it seems likely that chemical control of this disease is not necessary if the disease occurs late in the growing season as yields are not likely to be adversely affected by high incidence of the disease in the late stages of growth of the plant. A chemical spray program could be very effective, however, when there are high disease intensities around the 6 leaf stage of growth of the onions at which stage, as stated by Baker and Wilcox, defoliation causes most losses. The spore trap data indicates that spores are formed during night periods and are liberated in large quantities in the early hours of the day. This suggests that the application of protective chemical sprays could be more effectively timed either in the evenings or early in the mornings. Also the fact that fallen necrotic leaves support sporulation of B.squamosa on the ground suggests that eradicant spraying could also be directed at these necrotic leaves as well as at healthy leaves. Simard et al (1968) developed an empirical method for forecasting the date of onset of the B.squamosa leaf

blight disease and to determine the optimum dates for spray applications. The analysis of the Ste.Clothilde data generally supported their hypothesis.

In conclusion, this study has uncovered some new aspects of the biology of B.squamosa which apparently have great influence on the epidemiology on the leaf blight disease of onions incited by that fungus. Time has been a limiting factor in these studies but, notwithstanding this, indications are that more extensive research into the physiology of B.squamosa host:parasite relations could make some important contributions to the literature of host:parasite relations in plant pathology.

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8. SUMMARY

An explosive epidemic of leaf blight disease of onions, ($r = 0.3107$) occurred at Ste.Clothilde during the summer of 1969. An epidemiological study of this disease was carried out in two phases.

In the first phase various meteorological data were taken and a modification of the Theis and Calpouzos seven-day dew meter was used to record dew duration. This modification was designed to allow the testing of 5 pencils at a time and to give more accurate readings.

From disease survey data significant regression factors were obtained between number of spots on the leaf with the largest number of spots and the number of spots per plant.

There was no correlation between disease intensity and yield.

The absence of infection foci and the fact that meteorological conditions were generally unfavourable at the time when the first disease symptoms were noticed implied that the initial symptoms were physiological. B.squamosa was later isolated from diseased leaves and was also identified on spore trap slides. These spores showed a diurnal perio-

dicity and the maximum number of spores trapped per day was 30 x $10^2/14.4$ m^3.

The plotting of graphs with various meteorological data, spores trapped/day and disease progress curves for the whole period under study indicated that:

1) Relatively long periods of temperature over 27°C. was an important factor in this disease.
2) The disease was affected unfavourably by minimum temperatures of 16°C. or less.
3) Fluctuations in relative humidities lead to spore release by causing hygroscopic movements of the conidiophores of B.squamosa.
4) There was a latent period of 3 days during part of the epidemic.

In the second phase, it was found that:

1) The Ste. Clothilde B.squamosa isolates could infect necrotic as well as healthy leaves.
2) The latent period on leaves which were necrotic at inoculation was about 3 days while the latent period on leaves that were healthy at inoculation was never less than 4 days.
3) The infectious period was over 3 weeks.

4) The incubation period was one day.

5) Light and dark periods apparently affect conidiophore and conidial formation differently.

6) Dieback in many cases developed from the coalescing of chlorotic areas around lesions and not from the rapid spread of mycelium through the lacunar spaces of the leaves or from mechanical injury.

9. APPENDIX

HIRST AUTOMATIC VOLUMETRIC SPORE TRAP: BURKARD RECORDING. VOLUMETRIC SPORE TRAP. METHOD OF COUNTING AND CALCULATING CONCENTRATIONS.

A microscope with a mechanical stage having vernier scales calibrated in millimetres is essential. A squared graticule is placed in one eyepiece and calibrated with a stage micrometer. For pollens and spores $> 20\mu$ a magnification of approximately 250 is recommended, for spores $< 20\mu$, many of which are hyaline, a magnification of 500 (long working distance oil immersion objective) is necessary. The width of traverse in which spores are to be counted is determined by size of spore and the number present.

In both traps the adhesive surface is moved at 2 mm per hour past the orifice which is 2 mm wide. Immediately the trap is started part of the adhesive beneath the orifice is moved away and only after one hour will the deposit behind the orifice represent the true concentration, since all points are behind the orifice for one hour. The converse is true at the other end of the adhesive (daily in Hirst trap; weekly in Burkard trap). There is thus a 2 mm band of decreasing concentration (wedge) at either end of the spore trace. Average concentrations over 24 hour periods are most easily estimated by long traverses parallel to the

direction of movement. If hourly concentrations are wanted, counts are made on traverses at right angles to the direction of movement at positions representing the required times. First it is necessary to find the middle of wedge of spore deposit, a narrow traverse here represents half the concentration 30 minutes after the trap was started. If this is added to a corresponding estimate at the end of the previous days deposit, the total represents the mean hourly concentration at the time of change. Thereafter counts at 2, 4, or 8 mm intervals represent times 1, 2, or 4 hours apart.

The volume drawn through both traps is 10 litres per minute or 0.6 cubic metres per hour. The dimensions of the trap orifice are 14 x 2 mms but as slight scattering of the deposit occurs, the actual length of traverses at right angles to movement should be greater than 14 mms. The adhesive film is moved at 2 mms per hour and the orifice is 2 mms wide, hence spores caught in any hour can be spread over a 4 mm wide band. However, spores along any straight line across the surface (14 mm axis of orifice) will all have been deposited within one hour. It is therefore reasonable to regard counts on narrow traverses as the mean for

an hour but as the width of the traverse is increased so is the time at which the spores could have been trapped.

If for example the spores on a 100μ wide traverse are counted they represent the mean concentration over an hour (actually 63 minutes) and since the orifice is 2 mm wide, need multiplying by 20 to represent the total catch during one hour, and by $\frac{1.6}{0.6}$ to correct for the estimated number per cubic metre. For traverses 100μ wide parallel to slide movement, the fraction of the trace scanned is $\frac{100}{14000} = 1/140$. The volume of air samples in 24 hours is 24 x 0.6 - 14.4 cu.metres. Thus the final correction factor is to multiply by $\frac{140}{14.4} = 9.72$. These correction factors assume that the trap is 100% efficient, but efficiency varies with wind and spore size and although corrections can be made on the basis of wind tunnel tests (see references) these are seldom done. The efficiency of the trap is $70 \pm 20\%$, thus the correction given above will give concentrations below the true value.

APPENDIX TABLE 1

SIMPLE REGRESSION ANALYSES OF SURVEY DATA USED TO DETERMINE FACTORS FOR COVERTING NUMBER OF SPOTS ON A PARTICULAR LEAF TO NUMBER OF SPOTS/PLANT

Variables		Survey 1				Survey 2				Survey 3				Significance of Regression Coefficient R		
		Test of Significance for regression analysis		Regression Coefficient		Test of Significance for regression analysis		Regression Coefficient		Test of Significance for regression analysis		Regression Coefficient				
y	X	F	Sig.	β	Sig.	F	Sig.	β	Sig.	F	Sig.	β	Sig.	P	F	R
1. Max. spots per leaf	spots per plant	37.06	.005	.9070	.01	22.64	.005	.9777	.01	3.03	.005	.9891	.01	.05 .025	5.32 7.57	.666 -
2. Total spots on 4 youngest leaves	"	-	NS	-	NS	15.72	.005	.8137	.01	15.83	.005	.8146	.01	.01 .005	11.26 14.69	.798 -
3. Spots on 3rd youngest leaf	"	-	NS	-	NS	12.81	.01	.7669	.05	7.42	.05	.6934	.05			
4. Spots on 4th youngest leaf	"	4.464	NS	.5998	NS	26.54	.005	.8775	.01	30.96	.005	.8911	.01			

APPENDIX TABLE 2

MULTIPLE CORRELATION ANALYSIS USED TO DERIVE CORRECTION FACTORS TO CONVERT NUMBER OF SPOTS ON 4TH LEAF AND NUMBER OF SPOTS ON LEAF WITH MOST SPOTS TO NUMBER OF SPOTS PER PLANT

	Test of Significance for Multiple Correlation Analysis		Significant values of F (8 df)			Partial Correlation Coefficients				Significant values of π (7 df)	
			Probabilities			X_2 fixed Spots on 4th leaf vs total spots per plant		X_1 fixed Most spots per leaf vs total spots per plant		Probabilities	
	F	Significance	.05	.01	.005	y1-2	Significance	y2-1	Significance	.05	.01
Survey #1	82.17	.005	4.74	9.55	12.40	0.7747	.05	0.8955	0.1	.758	.855
Survey #2	51.0	.005	4.74	9.55	12.40	0.8424	.05	0.9883	.01	.758	.855
Survey #3	38.67	.005	4.74	9.55	12.40	0.189	NS	0 .9139	.01	.758	.855

y = total spots per plant

X_1 = spots on 4th leaf

X_2 = maximum spots per plant

APPENDIX TABLE 3a

Time	Spore Trap Data for Longitudinal Traverses Spores counted between hourly grids x 4.8 JULY 1969												
	19	20	21	22	23	24	25	26	27	28	29	30	31
8 - 9	0	0	0	0	0	0	5	0	0	0	29	0	0
9 - 10	0	0	0	5	5	0	10	0	0	34	29	0	19
10 - 11	0	0	0	0	5	0	19	10	0	53	24	0	5
11 - 12	5	0	0	0	5	19	19	0	0	29	43	5	10
12 - 1 PM	0	0	0	0	10	82	34	0	5	29	53	0	14
1 - 2	5	0	0	20	10	14	34	14	0	53	34	0	5
2 - 3	0	0	0	0	0	5	10	34	0	24	58	0	0
3 - 4	0	0	0	5	14	10	29	14	0	24	38	0	0
4 - 5	0	0	0	0	5	19	19	5	0	10	10	0	0
5 - 6	0	0	0	0	10	10	14	19	0	5	0	0	10
6 - 7	0	0	0	5	14	14	19	0	0	0	0	0	0
7 - 8	0	0	0	5	0	0	10	0	0	0	0	0	0
8 - 9	0	0	0	0	5	0	5	5	0	5	0	5	5
9 - 10	0	0	0	0	14	0	0	5	0	5	0	0	0
10 - 11	0	0	0	0	5	0	0	0	0	0	0	0	0
11 - 12	0	0	0	0	0	0	0	0	0	0	0	0	0
12 - 1 AM	0	0	0	0	0	0	0	0	0	0	0	0	0
1 - 2	0	0	0	0	10	5	0	0	0	14	0	0	0
2 - 3	0	0	0	0	0	5	0	0	0	10	0	0	5
3 - 4	0	0	0	0	0	0	0	0	0	0	0	5	0
4 - 5	0	0	0	0	0	5	0	0	0	0	5	0	0
5 - 6	0	0	0	0	0	0	0	0	0	0	0	0	0
6 - 7	0	0	0	0	0	0	0	0	0	10	0	0	5
7 - 8	0	0	0	0	0	0	0	0	0	0	0	0	0
TOTAL	10	0	0	40	112	188	227	106	5	305	323	15	78

APPENDIX TABLE 3b

TIME	Spore Trap Data for Longitudinal Traverses - Spores Trapped between Hourly Grids x 4.8 A U G U S T 1969									
	1	2	3	4	5	6	7	8	9	10
8 - 9	0	0	0	0	0	14	14	106	5	182
9 - 10	34	0	0	43	5	77	38	197	10	115
10 - 11	518	0	19	283	24	144	58	86	38	24
11 - 12	408	24	394	336	19	62	130	43	19	206
12 - 1 PM	197	139	101	245	0	82	408	24	29	182
1 - 2	82	34	149	72	62	77	197	14	19	245
2 - 3	72	48	57	130	10	101	110	19	53	134
3 - 4	72	29	67	58	29	14	144	5	29	106
4 - 5	139	5	43	163	14	24	221	5	14	144
5 - 6	91	14	34	178	0	0	58	0	5	106
6 - 7	5	5	19	254	5	5	86	10	0	43
7 - 8	62	19	10	82	0	48	34	5	0	5
8 - 9	58	38	29	14	5	24	0	5	5	5
9 - 10	5	0	14	53	5	0	48	14	0	0
10 - 11	5	0	0	19	0	0	130	5	0	19
11 - 12	0	0	0	0	0	0	312	5	0	14
12 - 1 AM	0	0	0	0	0	0	206	14	5	24
1 - 2	0	0	0	0	0	5	120	5	5	0
2 - 3	0	0	5	0	0	0	154	10	0	14
3 - 4	0	0	0	0	0	0	336	5	0	0
4 - 5	0	0	0	5	0	0	115	0	5	0
5 - 6	0	0	10	0	0	0	5	5	34	5
6 - 7	0	0	0	0	0	0	19	5	67	10
7 - 8	14	0	0	10	0	0	58	10	125	10
TOTAL	1930	355	951	1955	178	677	3001	597	467	1593

APPENDIX TABLE 3c

Spore Trap Data for Longitudinal Traverses
- Spores Trapped between Hourly Grids x 4.8

	A U G U S T 1969										
TIME	11	12	13	14	15	16	17	18	19	20	21
8 - 9	72	5	0	14	0	5	0	14	0	0	0
9 - 10	91	58	10	19	48	82	0	19	0	0	5
10 - 11	77	34	29	129	283	24	10	14	0	0	14
11 - 12	24	44	53	220	192	19	10	0	48	14	10
12 - 1 PM	43	10	10	125	62	0	19	0	470	14	24
1 - 2	10	19	10	144	72	0	14	0	101	10	24
2 - 3	10	19	48	?	29	5	5	19	82	10	14
3 - 4	19	5	48	?	5	14	10	19	48	19	5
4 - 5	10	5	19	?	96	62	14	0	29	10	10
5 - 6	19	34	10	67	19	96	0	0	33	10	5
6 - 7	5	0	0	43	34	62	0	0	5	5	5
7 - 8	5	0	0	24	82	34	5	0	24	0	10
8 - 9	5	5	10	0	53	5	5	5	19	0	0
9 - 10	0	10	0	0	0	10	0	0	0	5	0
10 - 11	5	0	14	0	0	19	0	5	0	0	0
11 - 12	5	0	5	0	0	144	0	0	10	0	0
12 - 1 AM	0	0	10	0	0	221	0	0	0	0	0
1 - 2	0	0	10	0	0	24	0	0	0	0	0
2 - 3	0	0	5	0	0	10	0	10	0	0	0
3 - 4	0	0	0	0	0	58	5	0	0	0	0
4 - 5	0	0	5	0	0	53	5	0	0	10	0
5 - 6	0	0	0	5	0	19	19	0	0	0	0
6 - 7	0	0	5	5	0	14	0	5	5	0	0
7 - 8	0	0	0	0	5	0	5	0	0	0	0
TOTAL	400	248	301	805	980	980	126	110	874	97	126

? = spores obscured by debris

APPENDIX TABLE 4

CORRELATION ANALYSIS OF DISEASE SURVEY DATA

	t Test for Multiple Correlation Analysis		Simple Correlation Coefficients				Partial Correlation Coefficients			
			Spots vs leaf no. 1-2		Spots vs dieback y-1		X_2 fixed Spots vs dieback		X_1 fixed Leaf No. vs dieback	
	t	Signi-ficance	1-2	Signi-ficance	y-1	Signi-ficance	y1-2	Signi-ficance	y2-1	Signi-ficance
30th July	11.846	.001	.3891	.05	.4086	.05	.1436	N-S	.8896	.01
4th August	6.170	.001	.3633	N-S	.5072	.01	.3817	N-S	.7170	.01
12th August	1.709	N-S	.1395	N-S	.2182	N-S	.1703	N-S	.6642	.01

	Significant values	
Probabilities	t	
.05	2.021	0.385
.01	2.704	0.467
.001	3.551	

y = dieback + 1/2

X_1 = spots per leaf

X_2 = leaf no.

= 40

APPENDIX TABLE 5 a

LATENT PERIODS (DAYS) FOR B.SQUAMOSA ON ONION LEAF PIECES INCUBATED UNDER VARIOUS CONDITIONS

Condition	Period between inoculation and conidiophore formation				Period between inoculation and conidial formation			
	Necrotic Pieces Replicates		Living Pieces Replicates		Necrotic Pieces Replicates		Living Pieces Replicates	
	(a)	(b)	(a)	(b)	(a)	(b)	(a)	(b)
Incubator, continuous darkness 12°C.	5	5	5	5	5	5	5	5
	5	4	5	5	5	4	5	5
	5	7	5		5	7	5	
	5	4	5		5	4	5	
	5	4			5	4		
Mean	4.9		5		4.9		5	
light 14 hrs. darkness 10 hrs. 23-24°C.	3	4	5	4	3	4	5	4
	4	5	5	4	4	5	5	4
	4	3	5		4	4	5	
	3	3	4		3	3	4	
	3	3			3	3		
Mean	3.5		4.5		3.6		4.5	

(a) = original experiment
(b) = repetition of experiment

APPENDIX TABLE 5 b

LATENT PERIODS (DAYS) FOR B.SQUAMOSA ON ONION LEAF PIECES INCUBATED UNDER VARIOUS CONDITIONS

Condition	Period between inoculation and conidiophore formation				Period between inoculation and conidial formation			
	Necrotic Pieces Replicates		Living Pieces Replicates		Necrotic Pieces Replicates		Living Pieces Replicates	
	(a)	(b)	(a)	(b)	(a)	(b)	(a)	(b)
Continuous darkness 25°C.	5	6	8	9	5	6	8	9
	7	6	8	9	7	6	8	9
	3	3	8		3	3	8	
	5	6	9		5	6	9	
	3	6			3	6		
Mean	5		8.5		5		8.5	
Continuous light 24-27°C.	3	4	5	5	5	5	5	5
	4	3	5	5	5	5	5	5
	4	3	5		5	5	5	
	3	3	5		5	5	5	
	4	3			5	5		
Mean	3.4		5		5		5	

(a) = original experiment
(b) = repetition of experiment

APPENDIX TABLE 6 a

LATENT AND INCUBATION PERIODS AND NUMBER OF LESIONS ON INDIVIDUAL LEAVES OF PLANTS WITH NECROTIC LEAVES REMOVED AND PLANTS WITH NECROTIC LEAVES INTACT.

Replicate	Leaf No.	PLANTS WITH NECROTIC LEAVES REMOVED			
		Number of Lesions	Incubation Period (Hours)	Latent Period (Days)	Time between lesion formation and tip dieback (Days)
1	1	-	-	-	-
	2	81	20	6	-
	3	85	20	4	-
	4	74	20	4	-
	5	26	20	-	-
	6	0	0	-	-
Mean per leaf		53	20	4.7	-
2	1	-	-	-	-
	2	120	20	4	-
	3	106	20	6	-
	4	62	20	8	-
	5	7	20	-	-
	6	0	-	-	-
Mean per leaf		59	20	6	-
3	1	-	-	-	-
	2	101	20	4	-
	3	96	20	6	5
	4	79	20	9	6
	5	22	20	10	5
	6	0	-	-	-
Mean per leaf		60	20	7.1	5.3
4	1	-	-	-	-
	2	75	20	4	-
	3	84	20	4	-
	4	69	20	4	-
	5	54	20	8	5
	6	1	20	-	-
Mean per leaf		57	20	5	5

Leaf No. 1 is the oldest (necrotic) leaf.
A dash indicates that there was either no symptoms or no sporulation.

APPENDIX TABLE 6 b

LATENT AND INCUBATION PERIODS AND NUMBER OF LESIONS ON INDIVIDUAL LEAVES OF PLANTS WITH NECROTIC LEAVES REMOVED AND PLANTS WITH NECROTIC LEAVES INTACT.

Replicate	Leaf No.	PLANTS WITH NECROTIC LEAVES INTACT			
		Number of Lesions	Incubation Period (Hours)	Latent Period (Days)	Time between lesion formation and tip dieback (Days)
1	1	?	?	4	-
	2	33	20	4	-
	3	16	20	6	-
	4	24	20	8	-
	5	0	-	-	-
	6	0	-	-	-
Mean per leaf		15	20	5.5	-
2	1	?	?	3	-
	2	87	20	4	-
	3	54	20	4	-
	4	60	20	4	-
	5	1	20	8	5
	6	0	-	-	-
Mean per leaf		40	20	4.7	5
3	1	?	?	3	-
	2	30	20	4	-
	3	53	20	6	-
	4	28	20	9	6
	5	3	20	-	-
	6	0	-	-	-
Mean per leaf		23	20	5.5	6

Leaf No. 1 is the oldest (necrotic) leaf.
A dash indicates that there was either no symptoms or no sporulation.