# SOME EFFECTS OF ENVIRONMENT ON SPORE PRODUCTION AND GERMINATION IN YELLOW RUST OF WHEAT

A Thesis submitted for the degree of

MASTER OF PHILOSOPHY

in the

University of Southampton

by

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1980

## ACKNOWLEDGEMENT

I wish to express my sincere gratitude to my supervisor Dr. J.G. Manners for his constant advice and encouragement during the course of my work.

I wish to thank Professor S.H. Crowdy for helping me during the preparation of my thesis and for allowing me to use the facilities in the Biology Department.

I would like to thank Dr. R.J. White for his help in the interpretation of statistical analysis.

I would like to convey my appreciation to my husband, Gopalan for his keen interest in my research and for typing my thesis.

I wish to thank The British Council for helping me towards my studies.

Finally, I wish to thank the technical staff of the Biology Department and my colleagues who made my stay at Southampton University, pleasant.

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## ABSTRACT

The effects of environment on the spread of yellow rust have long been studied but a predictive model for the disease has not been established.

Two years field experiments were carried out on the effects of environment on germination of yellow rust uredospores infecting wheat plants. Analysis of the field data indicated two important environmental factors viz. temperature and humidity affecting the viability of spores in the field.

In our preliminary experiments, spores collected from different leaves on the same day showed differences in germination indicating that apart from environmental influences there were other physiological factors that were affecting the germination of yellow rust uredospores. Experiments were conducted in the field to study the effect of age and position of leaf on spore germination. Significant differences were obtained from statistical analysis computed from the data obtained in the field. Spores collected from younger leaves with green healthy tissue always germinated far better than spores from old senescent leaves under similar environmental conditions. The effect of age of leaf on spore germination was demonstrated in experiments done in a controlled environment. The techniques adopted and the results obtained are discussed in detail.

Leaf position also had a significant effect on the germination of yellow rust spores. Our field results showed that spores collected from $L_3$, $L_4$ and $L_5$ germinate better than spores collected from the primary and second leaves. The study could not be extended to controlled conditions due to lack of time.

The importance of light in propagating yellow rust in winter months was confirmed. Better results were obtained under sodium light in glasshouse conditions than under high pressure mercury fluorescent lamps.

# CHAPTER ONE

## Literature Review

### A. Effect of Climatic Variation on Spore Production and Germination

The seasonal development and the geographic distribution of plant diseases is largely determined by the prevailing environment and soil conditions. These effective environmental factors have been extensively measured and analysed in the case of yellow rust of wheat caused by Puccinia striiformis (Hassebrauk,1970; Oakley,1978) and it has been established that the rate and extent of disease development are determined by pathogen-host-environment interaction.

a) Spore Production:

The relative success or failure of the pathogen is determined partly by the amount of inoculum produced, the liberation of the inoculum, the resistance of the inoculum to unfavourable conditions and factors affecting the germination and establishment of the pathogen in the host. After infection has occurred the pathogen has to sporulate to carry on its life-cycle. Environment plays a decisive role in the production of spores in a definite time. The time that elapses between inoculation and sporulation determines the number of spore germinations and naturally the number of generations produced by these spores decides the build up of the epidemics. Economy of space and time in spore production has an important significance in disease development.

Longevity of inoculum:

The longevity of inoculum affects the spread of disease. The duration of viability determines the potential effectiveness of the inoculum. A considerable time may elapse between spore

production and favourable conditions for germination and infection. The longevity of spores depends on the condition under which they are produced or stored. Becker(1928) showed that the longevity of spores of P.striiformis depended on temperature and relative humidity; spores lost their viability quickly at 25°c. Macer(1959) and Hughes and Macer(1964) found that P.striiformis spores dried and stored in vacuo at 1-3°c remained viable at least for 4 years. Kilpatrick(1971) stored uredospores of P.graminis in liquid nitrogen for ten years and found that some were still viable when removed from storage.

Temperature:

Temperature is a determining factor in the incidence of yellow rust. P.striiformis thrives mainly in cool regions. Temperature after infection directly affects the time required for the pathogen to sporulate. The rapidity of development is highly related with the prevailing temperature showing a linear relationship upto 18°c and then tends to fall off becoming non-linear. Above 24°c there is a rapid decline. The speed with which pustules are formed differs at different temperatures (Mehta (1923) & Melander, 1931). Tollenaar and Houston(1966) have shown that the temperature during uredospore production governs the maximum germination temperature of the spores. The temperature not only determines the length of time the pathogen takes to develop but also the kind of effect it produces on the host. Temperature is a controlling factor in plant resistance. Usually increasing temperature increases resistance but some races are different where resistance decreases with increase of temperature. Gassner and Straib (1940) have shown certain wheat varieties to be resistant to certain races of yellow rust as temp-

erature increased with the critical temperature for the change in each race-variety combination ranging between 15° and 24°c. Hence when the temperature deviates too for from the optimum, the occurrence of yellow rust may be prevented. The various temperature ranges reported for infection include 6 - 22°c (Shaner,1971), 9° - 15°C ( ZADOKS, 1965 ), 2° - 13°c (Sharp,1965). Infection in the field is said to occur from 4°- 17°c (Burleigh,1970).

Light:

In the field, light is rarely a limiting factor and probably not a determining factor in the development of yellow rust while in glasshouse condition light may be a limiting factor. After infection has taken place, light may affect growth and sporulation and the infection type produced by the yellow rust. Hwang(1942), Dillon-Weston(1932) and Stubbs(1967) reported both day - light and artificial light on the response of some wheat varieties to infection with P.striiformis. Bever(1934) demonstrated that light intensity affects the length of incubation period in Puccinia striiformis . Manners(1950) showed the infection type to decrease with a prolongation in photoperiod in a growth cabinet. Straib(1940) demonstrated that with in a certain range of light intensities, the reactions of race-variety combinations may or may not be affected. Those races which cause necrosis on certain varieties, may grow well under low light, produce less necrosis and ultimately sporulate ( Stubbs, 1967 ). The development of the host plant is dependent on light and therefore light may be an important factor in the prevalance and severity of infection.

Moisture:

Precipitation is one of the factors determining the occurrance of yellow rust. Availability of free water is necessary for the development of the pathogen and determines whether there can be a disease cycle at all. Burrage (1970) has reported infection of wheat leaves by black rust to occur only in the presence of free water on the leaf surface. Excess water in the form of rain may wash spores from the plant surface while dew deposits form gradually , creating favourable conditions for germ-tube growth and penetration of the stomata(Burrage,1969). The number of penetrations taking place per unit number of viable spores on the surface is determined by the duration of surface wetness; Yarwood(1961) showed that high humidity increased sporulation in Uromyces phaseoli and this is true of other rusts(Manners,1971). Below 50% relative humidity sporulation is absent, while there is an increase in sporulation with increasing humidity (Rapilly et.al,1970), Zadoks(1961) found an increasing reaction type with increasing humidity.

Other factors like nutrients and atmospheric ions are said to affect spore production and germination in yellow rust. Rusts develop better on thrifty plants and nitrogenous fertilizer is conducive to rust development (Gassner,1934 ). The effect of nutrients is reported to be quantitative rather than qualitative (Stakmann,1957 ).

b) Spore Germination:

Germination of the spore on the potential host is the first event in the formation of a new fungus colony and is a determining factor in the onset of colonization. The spores persisitence and the extent of infection and growth seem to depend largely on a suitable environment for yellow rust to flourish. The salient requirements of such an environment are briefly described below to provide an insight into the work dealt with in the latter part of the Thesis.

Temperature:

Extensive work has been carried out on the effect of temperature on the germination of yellow rust and extensive literature is readily available. It is established that germination only occurs at suitable temperatures and any deviation from that range preclude infection. Temperature thereby becomes a determining factor in abundance, speed and sometimes in the type of infection.

Erikson and Henning(1896) first recognized the effect of temperature during the production of uredospore on their germination potential. Straib(1940) observed higher germination temperature when spores were produced at a high temperature. Schroeder and Hassebrauk(1964) using only two fruiting temperatures confirmed Straib's observation.

The optimum temperature for the germination of yellow rust uredospores reported in the literature varies widely: 10°c (Straib, 1940), 7°c (Sharp,1965), 12°c (Manners,1950). The maximum temperature has been reported as 25°c by Straib(1940). In studies with yellow rust, the optimum and maximum temperatures of uredospore germination are frequently used among other factors.

Erikson and Henning(1896) noticed that the uredial stage of P.striiformis in wheat leaves survived sub-zero temperature. Hecke( 1911 ) noted a prolongation in latent period at low mean daily temperatures. Subsequent observations and extensive investigations confirmed the role of dormant uredomycelium in the over wintering of yellow rust. In the field yellow rust exposed to a fluctuating temperature is more likely to favour germination than a constant temperature is not established. A constant or mean temperature above 22-25°c inhibits germination and eliminates the yellow rust fungus.

Light:

Light has more effect on the longevity of spores than on germination( Hwang, 1942 ). Schroeder(1965) ascertained that the spores germinate much more rapidly and better in light than in the dark. Low light intensity during germination according to Hassebrauk and Schroeder(1964) lowers the germination percentage. Brown and Kochman(1964) observed an inverse relationship between intensity of light and germination in the case of P.graminis and P.coronata.

Light may also have a strong influence on the reaction to rusts. In the experiments of Gassner and Straib(1940) low light tended to increase the susceptibility of wheat varieties that were most susceptible at low temperatures. Hassebrauk and Schroeder(1964) reported germination to be faster under diffuse light at > 13°c than in the dark. McCracken and Burleigh( 1962 ) have reported higher germination under both unfiltered light and blue light than under red, green and yellow light. Changing light and or temperature levels have long been known to change the intermediate disease

reactions exhibited by some wheat cultivars to some yellow rust isolates. It may be argued that the effect of light or temperature is not directly on the host or the parasite but on the interaction between the two.

According to Wilhelm(1931) uredospores developing in plants exposed to low light intensities germinate neither in vitro not in vivo. Stroede(1933) indicated that both natural and artificial light retarded germination at temperatures between 11°& 17°c. Tollenaar and Houston(1968), however reported that exposure to light inhibitied germination at 6°c but promoted germination at 11°c.

Humidity:

Uredospores of yellow rust require liquid water or atleast very high relative humidities, for germination. In the field, dew and gentle rains are particularly conducive to abundant germination and consequent infection of leaves. Moisture provides a migratory pathway and access to substrates.

High air humidity during incubation is said to accelerate germination rate(Hassebrauk and Schroeder,1964). Straib(1940) reported the shortest incubation period in a saturated atmosphere especially in the case of yellow rust. Maddison and Manners(1972) found that low humidity during spore formation resulted in uredospores with poor and variable germination. If liquid water is absent very high humidites of the order of 99% are required for germination of uredospores and germ-tube growth. The necessity for the presence of water for infection to take place has been reported by Zadoks(1968) and Burrage(1969), but work by Burrage(1969) using very carefully controlled conditions has shown that P.graminis will germinate at high relative humidity and this is very probably

true of P.Striiformis also. Germinability has been shown to be inversely proportional to temperature within the range of 5°-18°c and directly proportional to humidity of the atmosphere (70-100%).

Chemical Nutrients:

Specific correlations of host nutrition with infection may be generalised as follows; abundant nitrogen favours infection while potash has an adverse effect and the influence of phosphorus is variable

Other Factors:

Erikson and Henning(1896) were of the opinion that the initiation of an epidemic was dependent on the hybernating mycelium rather than on surviving uredospores. Hassebrauk and Schroeder(1964) have reported that, though the barometric pressure had no influence on spore germination, anti-cyclonic weather enhanced sporulation. Sharp(1967) has shown a detrimental effect of atmospheric ions on the germination of yellow rust uredospores. Wilhelm(1931) assumed the capricious nature of yellow rust epidemics was due to sesitivity of yellow rust to various environmental factors and other workers like Straib( 1940 ) and Manners( 1950 ) have agreed with Wilhelm's assumption.

Yellow rust being an obligate parasite, disease is a consequence of interaction of a susceptible host and a favourable environment. The fluctuations in many environmental factors may affect the state of the host rather than the pathogen in the early stage (Manners,1971). It is becoming possible in a few situations at least to specify the relative importance of these factors. Knowledge of the relative role of the pathogen and environment needs a thorough consideration.

B. FIELD EPIDEMIOLOGY:

i) Introduction and Review of the Literature:

Yellow rust of wheat is one of the most important and wide spread rust disease of wheat in N.W.Europe and in other regions where wheat is grown under cool temperate conditions or wheat grown at high altitutdes in regions generally too hot,is often severely attacked.

Puccinia striiformis, a member of the uredinales is highly specialised with respect to host range. It commonly occurs on wheat, barley, rye and many grasses. A particular host cultivar is often susceptible only to a specified race or even isolate (Doling & Doodson,1970). The race is characterised by a particular combination of virulence genes. The basis of all physiologic race identification is their interaction to the host cultivar and they are known as "differentials". For many years the set of differentials derived by Gassner & Straib(1932) was used to identify race of P.striiformis but this set eventually inadequate has now been replaced by others(Johnson et.al.,1972).

Most cultivars are susceptible to all races under all conditions but some are resistant at particular stage of development and under certain conditions. Manners(1950) showed that winter wheat cultivars resistant to P.striiformis. at the seedling stage was also resistant at the adult stage whereas those cultivars susceptible at the seedling stage were either susceptible or resistant at adult stage. Batts(1957) and Doling(1957) evaluating the position agreed with Manners findings. Zadoks(1961) designated the type of resistance as 'overall' resistance when the plants were resistant

at both seedling and adult stage. It appears that adult plant resistance is often physiologically controlled. It is more frequently effective against all pathogenic races than overall resistance and more likely to provide durable resistance (Sharp, 1976) than overall resistance often controlled by a few major genes and highly race-specific. Priestly and Doling(1976) have identified specific virulences in P.striiformis using adult plants. These are similar to those identified by Zadoks(1961) and Stubbs(1972) with different cultivars.

The primary symptom of yellow rust is the appearance of yellow stripe like patches on the leaves. Therefore it is also known as stripe rust. These are orange-yellow uredosori, the elongate pustules or series of pustules though distributed at random on young plants are generally oriented parallel to the leaf vein in the adult plant. Heads and leaf sheaths and stems may also be attacked but the characteristic leaf stripe is typical of the disease. As yellow rust has no alternate host unlike other cereal rusts, it has to depend on air-borne uredospores as the only means of dispersal.

Development of an Epidemic:

Epidemics will occur when inoculum and a susceptible cultivar are brought together. The intensity of the epidemics will be determined by various factors such as presence of abundant inoculum near host plants, timely liberation, dissemination of inoculum and optimum temperature and moisture. Hot,dry weather checks epidemic development.

Development of a yellow-rust epidemic largely depends on initial source of inoculum,in the spring P.striiformis overwinters as mycelium, as already mentioned and fresh outbreaks will occur with the initial rise in temperature combined with wet weather. The resting uredospore has all the mechanisms in the proper biochemical state and is ready to germinate under favourable environmental and nutritive conditions. The severity of the disease in the previous season is an indication of an overwintering mycelium. Erikson and Henning(1894) considered that the severity and extent of an epidemic were dependent on hybernating mycelium rather than on surviving uredospores. Doling and Doodson(1970) in their work on incidence of cereal rust from 1958-67 observed that weather conditions during and immediately after harvest are of prime importance in influencing epidemics. Zadoks(1961) noted that yellow rust could survive satisfactorily as mycelium in infected leaves even withstanding frost. The attacks of yellow rust in 1931-32 have been attributed partly to conditions in the previous winter(King, 1952). Subsequent investigations of many more workers have shown overwintering to be the cause of an yellow rust epidemic in spring.

Dispersal of Spores:

The survey of inoculum at the beginning of a new season provides a rational point of departure for a disease forecast. In vast wheat growing areas where there is no local or regional source of inoculum the only source may be from a distant area. Wind is the only means of carrying spores hundreds of miles. The rapid dilution of a spore cloud with increasing distance from its source has been calculated and discussed by Gregory(1945). Distance, proportion of viable spores, trapping efficiencey, probability of finding a host and environment between them govern the infective potentiality of inoculum. Hirst(1953) has shown with his spore trap records abundant spores in the air during the afternoon. Low humidity, high wind speed and rain result in good spore dispersal in the afternoon (Hirst et.al.1955; Hirst,1959). Zadoks(1961) suggested the dispersal of spores in the case of an overwintering inoculum on leaves near the ground is due to rubbing of leaves together in the wind rather than through air. Hirst(1961) has found the spore of P.graminis to be dispersed by rain drops but on a dry day spore catches are supposed to be generally higher than on a humid day (Rapilly,1970).

Methods used in predicting epidemics:

Reviews on disease forcasting are given by Bourke (1970) Waggoner(1960) and James(1974). Wilhelm(1950) adopted the term "inoculum potential" with his work on tomato plants."Inoculum Potential" is defined as the number of independent infections that are likely to occur in a given situation in a population of susceptible healthy tissues.

Critical Stage Hypothesis:

Studies of loss in yield of small grain cereals due to disease suggest that loss may be either proportional to the area under the disease progress curve or if the shape of the curve is constant, loss will be proportional to disease at some specific stage of host development; this simplifies loss assessment. Chester(1946) has shown March to be a critical month for forecasting wheat leaf rust epidemics in the area in which he worked. He believes the weather to be rarely a limiting factor after this month. The critical stage system was found applicable to Rhynchosporium Scald of barley in England and Wales in 1967 where yield loss is equivalent to approximately 2/3$^{rd}$ of the percentage of flag leaf area visibly infected at the milky ripe stage of growth(James et.al.,1974). Similarly Large(1954), and Romig and Calpouzos(1970) have related certain stages in the growth of cereals to loss in yield without regarding the rate of infection. Doling and Doodson(1968) using a critical stage system equated loss empirically to the square root of percentage of yellow rust severity. King(1976) in his survey of the 1975 epidemics reported the major effect of yellow rust as a reduction in grain weight.

Progress of an Epidemic:

Van der Plank(1963) uses the term "compound interest disease" in the case of wheat rust because of the multiplication of the pathogen through successive generations in the course of an epidemic. It is therefore interesting to note the source of infection, points of outbreaks and the place where spores are deposited. Horsfall and Dimond (1960) are of the opinion that the

inoculum produced at the source is directly proportional to the amount of inoculum in the place of infection.

In the case of a source of inoculum at a considerable distances from the crop to be infected the pathogen must race against time for an epidemic to occur before the spores become non-viable while in the case of an overwintering mycelium, early multiplication is critical. One of the important characteristics of yellow rust is its date of appearance of the disease. The date of onset is determined by what happens before the onset. Rate of multiplication gives a clear picture at the development of the disease. It is independent of the epidemic upto onset since the proportion of the plant surface already infected is negligible. and becomes a direct reflection of the environmental conditions, the resistance or susceptibility of the host, the proportion of spores that germinate, efficiency of infection and spore production. Fast multiplication in moderately cool moist weather means fast spread of the disease. Van der Plank(1961) has shown the progress of infection with time to be sigmoid, if environmental conditions are constant. The early part of the curve is logarithmic. Later there is a falling off due to a large proportion of the foliage already being infected.

Similar methods are used to retard stem rust development(Stakman,1957;Harpenden,1956). Zadoks(1961) has suggested "cumulative spore counts as a measure of disease severity". Romig and Dirks(1966) in their work on P.graminis found the cumulative spore counts to be significant and suggest that they can be used to follow an epidemic. Van der Plank's equation $\log_e (x/1-x) = \mu$ has been widely used to characterise the progress

of rust epidemics as measured by number of spores(Romig&Dirks,1966).

Empirical Methods:

Translation of biological systems in mathematical terms is necessary for quantitative analysis of epidemics. Presently, multiple regression analysis is widely used as an useful tool in forecasting epidemics(Kranz,1974;Butt&Royle,1974) involving several disciplines.

Bourke(1970) has used statistical correlation in forecasting late blight of potato. A multiple regression equation between blight infection and weather has been developed by Schrodter and Ullrich(1965). Burleigh et.al.(1972) used regression analysis for estimating losses in yield due to wheat leaf rust. Romig and Dirk(1966) have effectively used regression analysis in predicting brown and black rust epidemic of wheat.

In recent years,a successful forecasting system developed for potato blight depends on selection of one or two climatic factors (Cox and Large,1960) usually a humidity factor affecting spore germination and sometimes sporulation and a temperature factor affecting whole life-cycle.

Zadoks and Risdjik(1974) have used simulation models in case of partial resistance. The whole technique of computer simulation is still at its preliminary stage.

Concluding Remarks:

The forecasting of disease is a contribution to the prediction of yields. Reliable forecasting can enable control measures to be taken at the optimum time with a reduction in the number of fungicidal sprays. Advantages of forecasting at an early stage gives opportunities for remedial cultivars or selecting an alternate crop or chemical control. In the case of yellow rust which is sensitive to environment, it has the disadvantage of the outcome being modified.

A uredospore of brown or black rust gives rise to an infection covering a definite leaf area which makes effective predictive models straightforward. In the case of yellow rust, because of its semi-systemic growth(Zadoks,1971) the construction of effective models is difficult. The influence of an expanding sporulating zone should be used in predicting models (Emge,1975).

The recognition of a critical stage in the growth of the plant or a critical period in the development of the epidemic has been successfully used in forecasting epidemic of yellow rust.

The prediction of epidemic depends on accuracy and range of forecast, availability of remedies and the ease with which they can be implemented.

# CHAPTER II

## EXPERIMENTAL METHODS

Experiment in the field indicated that infection was dependent on temperature, humidity, leaf age and position. These four variables were difficult to separate in the field and experiments were designed to evaluate the individual effects and their interaction on spore production, viability and capacity for infection using controlled environmental conditions where one variable could be altered at a time.

In order to obtain better spore production in winter, light experiments were carried out in the greenhouse. These experiments are also described in Chapter III..

a) Aim of the Experiments:

1. To observe the effect of both quality and quantity of light on spore production of yellow rust.
2. To ascertain the highest maximum temperature at which spores can survive.
3. To determine the effect of temperature on the viability of spores.
4. To study the effects of environment and the physiological condition of the plant on spore germination in the field.

b) Techniques and Procedures in Studying Spore Germination:

All experiments were carried out on the winter wheat cultivars Maris Beacon. Race 104E137 was the physiologic race of P.striiformis that was used in the study of environmental effects on yellow rust. This was the most prevalent race in Britain in

1972(Chamberlain et.al,1972). McGregor(1978) working on various wheat cultivars has shown Maris Beacon to be most susceptible to P.striiformis race 104E137. Maris Beacon is most susceptible to this race both at the seedling and at the mature plant stage.

Location:

Controlled Environment Experiments:

All controlled environment experiments were carried out either in the glasshouse or inside a Prestcold growth cabinet.

Light experiments were conducted in glasshouse no.5 at the Southampton University Biology Department. This glasshouse is ideal for the growth of yellow rust as it is maintained at 15°C. The glasshouse was kept cool by air being forced through ventilators. The experiments were carried out in the autumn and winter months. A layer of muslin was used to cover the 'perspex' box to reduce the total intensity of light given out by both the sunlight and artificial light. A sodium lamp and a mercury fluorescent lamp were the two artificial lights used in these experiments. A 16h day provided the length of photoperiod in all the experiments.

A Controlled environment cabinet was used for experiments on temperature and humidity. These cabinets can be set at the required temperature and photoperiod can be varied. In the studies on effect of temperature, spore germination both on leaf surface and on agar, the temperature was controlled to our requirements. The various day and night temperatures used in the experiments were 7-10°, 10-15°, 15-20° and 20-25°C respectively. The humidity was maintained at 70-80% during the day and 90% during the night. Fluorescent tubes were the artificial source of lighting. The lighting was adjusted to give a 16h photoperiod. The intensity measured with a

photometer was found to be an average 18018/$m^2$ lumens at plant level.

Numerous problems were encountered in experiments where the temperature was constant but the humidity had to be varied. In these growth cabinets certain humidities could not be obtained at certain temperatures. It was virtually impossible to attain 50% relative humidity at 15°C. This prevented us from demonstrating our field results completely under controlled conditions. The humidity could be raised to a higher percentage than was otherwise possible by continuously trickling water into the cabinet.

The air supply inside the cabinet is filtered thereby excluding spores and dirt from outside.

Growing Plant Materials:

Seed of Maris Beacon covered with a mercurial dressing was supplied by the National Institute of Agricultural Botany (NIAB) - Cambridge. Here the samples are ted for germination potential and purity by the 'trial branch' under field conditions.

Seeds of Maris Beacon were either sown in trays and then transferred to little pots for experimental use or were planted directly in 6cm pots in John Innes No.2. compost. All the controlled environment experiment plants were raised in the glass house and transferred to growth cabinets only after inoculation. As the primary leaf greatly varied in their size from plant to plant, the second leaf was used for all experimental work. The plants were sprayed with 'Milstem' two days prior to inoculation to prevent infection by powdery mildew.

Multiplication of the Inoculum:

The vaccum-dried spores used in the production of yellow rust inoculum was obtained from NIAB-Cambridge. The isolate was No.69/163 originally isolated from Maris Beacon(Priestly,Pers.Comm.) in Cambridgeshire and the race was confirmed as 104E137.

These spores and fresh samples used in subsequent experiments were multiplied on Maris Beacon seedlings grown in the glasshouse. Spores were harvested by suction. Spores were also produced inside the growth cabinet for experiments on viability and to examine the germination of spores produced by different ages of leaves spores were stored in ampoules(ref.P.21). Spores from a single ampoule were normally used to inoculate a set of experimental plants but whenever there was a shortage of spores from a single ampoule, the spores from two different ampoules were mixed together before inoculation.

Isolation of Yellow Rust:

Experiments carried out on the isolation bench(Jenkyn et.al.,1973) at times got contaminated with brown rust from adjacent experiments in nearby pots. On such occassions to isolate the yellow rust inoculum from brown rust, a chinese barley cultivar Fong Tien was used. Manners(1950) working on the physiologic race survey of yellow rust found this cultivar to be resistant to brown rust but susceptible to most of the races of yellow rust. The barley variety was once again obtained from NIAB. Pure cultures of yellow rust were obtained by inoculating the plants with contaminated yellow rust spores. The pure yellow rust inoculum obtained from the barley plants was re-inoculated on to wheat seedlings before being used for experimental work. The isolation bench was used in the production of the inoculum.

Water condensed on the plastic domes used to cover the pots on the isolation bench and prevented the leaves from being dry. When dry leaves were required, special isolation chambers were used. The isolation chambers are made up of cylindrical metal frames. The frame work was covered with cellophane bound with strips of sellotape. The pots were placed over sand benches and covered with the isolation chamber.

Inoculation of Seedlings:

The plants were inoculated by three different methods.

Heavy Inoculation with a Paint-brush:

This method of inoculation was largely used throughout the course of this work. This is very useful specially for the production of inoculum. Yellow rust inoculum was diluted with talc roughly in a ratio of 1:20 and the diluted mixture was gently applied to the complete adaxial surface of the leaf with a sterile paint-brush. Russell(1975) has shown germination to be better on the adaxial surface than on the abaxial surface of the leaf. Though there cannot be an even deposition of spores, this result in a uniform infection of the whole leaf. The deposition of the spore may be dense even compared to other types of inoculation described below.

Point Inoculation using Scalpel:

Scalpel inoculation permits a heavy inoculum to be used and makes it possible to obtain a heavier and more even infection than that obtained by other methods(Manners,1971). The scalpel is cleaned with alcohol, heated over a flame and allowed to cool before use. The leaf to be inoculated is wetted prior to inoculation. A small amount of inoculum is picked on the metal edge of the scalpel and deposited gently on the adaxial surface of the leaf. A dense inoc-

ulum is thereby left on the leaf surface. Allen(1955) found that percentage germination was inversely related to the quantity of spores present (mg.spores/ml.water). There was a significant decrease in rate of germination at 1500 spores/$cm^2$.
Doling and Priestley(1974) have shown that infection type is constant once a certain density of inoculum is reached. Inoculation by scalpel always results in this density being exceeded. The method is useful where a uniform infection is required.

A lighter infection following inoculation by this technique can be obtained by turning the inoculated leaf upside down and gently tapping on the top of the leaf surface. This enables any loose spores not adhering to the leaf surface to fall off.

Inoculation with a Settling Tower:

A settling-tower was used for the quantitative inoculation of leaf blades. In temperature experiments where the germination of spores was studied on the leaf surface, quantitative spore deposition was attained on the second leaf with the help of a settling tower.

The pots are placed in a circle beside the inoculation disc. A cylindrical tower of about 55cm in height and 22cm in diameter with a small hole at the side rests on the top of the disc. The top of the cylinder is closed with a disc covered in aluminium foil. A glass tube bent upwards at right angles (13mm) from the distal end is attached to the nozzle of a pipette filter. A weighed quantity of spores is placed in the right angle and the tube is inserted through the hole in the cylinder so that its nozzle reaches the centre of the tower. The spores are forcibly dispersed upwards by blowing the pipette-filter. Opening and

closing of the shutter in the middle of the tower regulates spore deposition and avoids spore clumps being deposited on the leaf surface.

Spraying Technique:

This method was used for inoculation in the field where a large number of plants had to be inoculated. The plants were sprayed with a diluted spore/talc mixture in a 'squeezy' bottle. Prior to inoculation, the plants were sprinkled with water. Then the plants were covered with a polythene sheet. The spores were sprayed evenly across the plot by retaining the polythene sheet at the top during spraying. This prevents the spores from being blown away by wind or washed off by rain.

Incubation:

After inoculation, potted plants were sprinkled with water and covered with plastic domes. The domes were sealed air-tight with clipper's tape. The pots were left in a cold room for 48h to ensure 100% relative humidity and a deposition of liquid water which is essential for yellow rust infection. Whenever polythene boxes were used, they were sealed with plastic sheets. Plants were mostly inoculated in the mornings. After the prescribed time in the cold room, the plants were brought back and incubated in either the glass house or growth cabinet.

Germination of Spores on Agar:

Manners(1950) and Hassebrauk and Schroeder(1964) working on yellow rust germination on agar have observed a relationship between the quality of agar and rate of spore germination. Preliminary germination tests were carried out on 2% Difco-Bacto agar and 1.2% Oxoid no.3 agar made up with

distilled water. After several observations, it was decided to use 2% Difco-Bacto agar in studies on spore germination because germination percentage was more consistent.

For each batch of medium, 20g Difco-Bacto agar were suspended in a litre of distilled water placed in a media bottle and autoclaved. Small amount of molten-agar were poured in to sterile glass Petri-dishes. Once the agar had set, the Petri-dishes were stored in refrigerator at 5°C to prevent in dessication. Fresh agar plates stored in the refrigerator could be used for fifteen days without any desiccation. Used Petri-dishes were soaked over-night in 'extran' solution and washed with water. They were again cleaned in alcohol and dried before use. Petri-dishes from the same batch were used in all the experiments on spore germination.

Spores were seeded on to agar plates with very fine paint-brushes. On certain occassions the spores from the leaves were dusted on to agar-plates. There was dense deposition of spores on such occasions. Schroeder(1965) has shown self-inhibition of very dense spore suspensions. Manners(1950) has shown better germination with an increase in density of yellow rust spores within certain limits. Tollenaar and Houston(1966) reported good germination at densities of up to 5,400 spores per $cm^2$ with spores collected from the field. Allen(1955) found that percentage germination was inversely related to the quantity of spores present (mg spores/ml water). Significant decrease in rate of germination was observed at 1,500 spores/$cm^2$.

The agar-plates were incubated in the dark at 5°, 10° or 15°C. The spore counts were made under the low power of the

microscope after 24h. About fifty spores per field and six such field were counted. In all three hundred spores per Petri-dish were counted for germination. Spores clustered together were not scored for germination.

Germination of Spores on Leaf Blades:

To determine the presence of spores and infection after incubation, an imprint of the inoculated leaves was taken. Necloidine solution was applied with a glass rod on the adaxial surface and when the solution dried, the necolidine was pealed off from the leaf. The peel was immediately immersed in lactophenol-cotton blue to stain the mycelium and prevent the epidemal strips from curling. The peel was mounted in glycerol and examined under low power of microscope. Full epidermal strips of the leaf imprint were never obtained as they used to break in between and it might results in false results in case of studies of germination and penetration of the fungus infecting the leaf.

As it was difficult to get good peels without curling, the peel method was used only as a guide to determine the presence of spores and infection. In experiments to assess the percentage germination and infection on leaf surface at different temperatures, a leaf clearing and staining technique was used.

Leaf Clearing and Staining Technique:

The inoculated leaves after incubation for 24h at the experimental temperature was cut into small pieces and immersed in chloral hydrate solution (5g chloral hydrate in 2ml water) (Shipton & Brown,1962). Small quantities of the solution were placed in Petri-dishes. The leaves were cut into small pieces and immersed in the chloral hydrate solution.

The leaves were allowed to remain for a week in the solution. The cleared leaf sections were dried on a filter paper to remove chloral hydrate and stained with lactophenol-acid fuchsin.

Preparation of the Stain:

Brown and Shipton(1962) have used alcoholic lactophenol - cotton blue to stain their pieces. In this case both the spore and the mycelium are stained dark blue. But by using aniline blue (solution A) with acid fuchsin (solution B), the mycelium takes a light purplish stain while the spores are dark purplish in colour(El Azzebbi & Manners, personal communication).

Solution 'A' was prepared by mixing 0.2g of aniline blue and 100 ml of lactophenol with 100 ml of distilled water. In solution 'B' 0.15g of acid fuchsin is dissolved in 100 ml distilled water. The final stain is prepared by taking 3 ml of solution A in a conical flask and adding 10 ml of solution 'B' and 18 ml of distilled water to it. Solution 'A' and solution 'B' can be prepared and kept for a long time but the final mixing should be done immediately before staining.

A small amount of the stain is placed in a Petri-dish. The dried leaf-pieces are immersed in the stain for 30 minutes. The pieces are then mounted in glycerol. The mounted leaf sections can be maintained for a period of two months in a dark place without losing the stain. Then the stain begins to fade.

The readings that were taken under the microscope were number of spores germinated(germ), number of spores causing infection(penetration) and number of ungerminated spores(ungerm) There is no true appresorium formation in yellow rust(Allen,1935) Therefore, whenever a germ tube rested on a stoma, an infection

was recorded.

Dividing the total number of spores causing infection by the total number of germinating spores (Infection/Germination) enables the proportion of germinating spores causing infection to be obtained.

Latent period was defined as the time taken from the time of inoculation to the eruption of pustules and were recorded in days.

No pustule counts were made in any experiment but the rapidity with which the pustules were produced under different experimental conditions was observed.

Spore Collections:

Spore collections were made by dusting infected leaves on to aluminium foils or by a suction device.

Suction Method:

Priestly and Doling(1972) first developed this method. Suction is created inside a millipore filter holder (1.2 m) by sending an air-flow of 10 $l.min^{-1}$ from a small air-compressor. The holder when placed on an infected leaf surface sucks the spores through the pore. Some spores stick to the sides of the holder but a higher air-flow of 50 $l.min^{-1}$ minimises the losses.

For the collection of spores for freeze-drying(P.20) the pots were carefully brought inside an air-chamber previously cleaned with alcohol. A sheet of aluminium foil was spread under the plants and the plants were gently shaken so that the spores fell directly on to the aluminium foil. Aliquots amounts of these spores were placed in ampoules and freeze-dried in an Edward's centrifugal freeze-drier. A small quantity of spores is placed

in an ampoule constricted in the centre. The ampoule is connected to the secondary header of the freeze-drier. Vacuum is created by drawing the pressure out of the pump and water-vapour is absorbed by $P_2O_5$. After 12h. the ampoule bearing the spores in vacuum is sealed. The ampoules are labelled and stored in a refrigerator at 5°C.

Spore Counts:

Spore counts were usually made in the Coulter counter (Priestly & Doling,1972) but sometimes spores were weighed in a microbalance(Johnson and Bowyer,1974).

Coulter Counter Method:

A 2% sodium chloride electrolyte solution was prepared and passed through a glass-fibre filter. Particles greater than 0.5μm could be filtered off leaving a clear solution of sodium-chloride; 50 ml of the solution which were measured in a beaker and the spores from a millipore filter were suspended in to the beaker. The beaker was then placed in an ultrasonic bath for 15 seconds to break up clumps of spores. The suspension was then passed through a sintered glass filter to prevent large particles of debris from blocking the hole of the Coulter tube. The beaker and sintered glass filtered were always rinsed with the electrolyte. In this work, a Coulter tube with an internal diameter of 250μm was employed. 1μl of the electrolyte was counted at a time and ten such counts were made for every sample

# CHAPTER III

## Influence of Light in Spore Production of Yellow Rust

### Introduction:

Propagation of yellow rust in the glasshouse in the winter months has always been a problem. The response of wheat cultivars to infection with P.striiformis under different light treatments has been studied by McGregor and Manners (1978). They showed a decrease in the production of yellow rust spores with decreasing light intensity. Bever (1934) demonstrated that decreasing light intensity increased incubation period. Manners (1950) showed a decrease in infection type with a prolongation in photoperiod in a growth cabinet. Straib (1940) obtained a decrease in infection type with increasing light intensity. Stubbs (1967) combining the above two observations suggests the effect of the total quality of light to be the product of duration and intensity of light. Not much has been reported about the influence of the quality of light. An investigation was undertaken to ascertain the effect of supplementary sources of light on the quantity of spore production in the greenhouse.

### Preliminary Experiments with Sodium Light:

1. Experiments with wheat:

The experiment originally started in the month of December with the object of producing large amounts of yellow rust inoculum to be vacuum-dried for future work.

Seeds of Maris Beacon were sown in 12 cm diameter pots in the glasshouse. About seven plants per pot were grown at 15°C.

When the second leaves had completely unfolded, they were inoculated with P.striiformis race 104E137 diluted with talc, using a paint-brush. Of the sixteen pots that were inoculated and incubated in the cold room for 48h, eight pots were placed under sodium light in glasshouse no.3 which is maintained at a higher temperature of 20°C (± 2°C) and the remaining pots were placed under mercury vapour lamps in glasshouse no.5 maintained at 15°C (±2°C). This is the coolest greenhouse suitable at Southampton for the growth of yellow rust. It was observed that yellow rust grew lavishly under sodium light and 18°C in a relatively short time while it took longer for the spore to be produced under the mercury vapour lamp. Whether the temperature alone hastened the spore production or the type of light or the difference in intensities had a share in the rate of spore production remained to be seen. Therefore it was decided to design an experiment to study exclusively the effect of different types of lights in spore production under similar conditions.

2. Experiments with barley:

Bever (1934) has suggested that rust development on barley changed inversely with the total quantity of light, as the leaf tissue of barley seedlings is very thin permitting considerable light to pass through. The effect of excessive light was found to be lethal only to the extent of inhibiting sporulation.

The chinese variety Fong Tien (P.20) which was used at times to obtain pure cultures of yellow rust from material that were infected by brown rust showed similar reactions to Bever's observation. When inoculated barley seedlings were put under sodium light, no spores were produced while the plants germinated at a lower intensity with only a 6h photoperiod supplied by

a mercury vapour lamp. The plants under sodium were infected as the pustules could be seen but beyond that no sporulation was observed. The reasons for poor germination under sodium light in contrast to the results with wheat are not clear.

Germination on Agar:

The spores produced under the two lights in the preliminary experiments were seeded on to previously prepared 2% plain Difco-Bacto agar plates and incubated at 5°,10° and 15°C respectively. Spores from different leaves were collected. Spore counts were made after 24h. In all cases, if the length of the germ-tube exceeded the smallest diameter of the spores, the spores were scored as germinated. The results are tabulated as follows:

The mean of three replicates taken after 24h:

| Temperature(°C) | % Germination | |
|---|---|---|
| | Sodium | Mercury |
| 5 | 43 | 34 |
| 10 | 62 | 45 |
| 15 | 36 | 29 |

Table I

Once again there was a difference in germination of the spores produced under the two lights but it remained to be seen whether it was the effect of light or temperature. Comparison of the spores produced at the same temperature were not possible since in the second experiment no spores were produced under sodium light.

Study of Light/Temperature Interaction:

Experimental Design:

All experiments were carried out on a sand-bench along the wall facing the south in glasshouse no.5. The sand was always kept moist thereby maintaining a high humidity inside the 'perspex' box used in the experiment.

The experimental pots were placed inside the plastic boxes and the whole box was covered with a single layer of muslin to minimise the total effect of light on spore production. The reflection of light by the aluminium foil on the leaf surface proved detrimental in supporting the inoculated leaves,so boat-shaped corrugated plastic sheets were carefully held below the inoculated leaves with the help of sellotape. This permitted the exposure of leaves at the required light intensity to receive the same dose and provided an easy means of collecting the spores off the leaves without much loss. The spores were collected by gently tapping the inoculated leaves and allowing them to fall on to the plastic sheets.

Sodium and mercury vapour lamp were the two different types of lights used in the experiments. Both lights were fitted on the same side of the glasshouse, a bench in a cool position being chosen. Interference between the two lights was reduced by fixing them at opposite ends of the glasshouse bench. A hardboard partition was fixed at the side of each set of plants to prevent adjacent lights on neighbouring pots of the benches from illuminating the experimental plants and spores.

About six experimental pots with three or four plants in each pot were placed in a plastic box. Ten replicates per

experiment were taken and collections from extra leaves were used in case of emergency.

Selection and Inoculation:

In both the experiments, seeds of Maris Beacon were grown in 6 cm. pots under ordinary conditions in glasshouse no.5. When the second leaf had completely unfolded, the length and maximum width of the leaves were measured. A jeweller's tag with the replicate number and the date was tied around each leaf. The leaves were inoculated with P.striiformis with the help of a sterile paint-brush. The freeze-dried spores were diluted with talc and the diluted mixture was gently applied on the adaxial surface of the leaves. The inoculated plants were sprinkled with water and placed inside the'perspex' boxes which were covered with plastic sheet and sealed air-tight with a clipper's tape. The boxes were kept in the cold room for 48h as this ensures 100% relative humidity. After 48h the plants were brought back to the greenhouse and were put on the sand-bench under the two different types of lights.

Types of Light Treatments:

Two types of experiments were conducted with the plants under the control of two different light conditions. In the first set of experiments (A), both the sodium and mercury vapour lamp were adjusted at the same height; 1.2m from the plant level. The intensities produced by the two lights at the same height were measured with an Eel's photometer. There was a great difference in the two intensities. Sodium light gave an intensity of 10550 lumens/$m^2$ at 1.2m from plant level while that of mercury vapour lamp was only 6474 lumens/$m^2$.

In the following experiment (B) the lights were adjusted such that they produced the same intensity at plant level. This was done by placing the sodium light at twice the distance of the mercury vapour lamp. The vapour lamp was about 46cms above the plants while the sodium was nearly 90cms above the plant level. The vapour lamp was too near the plants. The intensities were once again determined with an Ell's photometer to ensure the same intensity in both cases (10185 lumens/$m^2$). The wavelengths of the two-lights are as shown in the graph(P.38 ).

A thermo-hygrrograph was positioned adjacent to the plastic boxes which recorded the temperature and humidity prevailing inside the glasshouse. Average temperature and humidity could be obtained from weekly charts which recorded every two hours. A thermometer placed inside the plastic box read at random time intervals always showed a difference of +1° or 2°C from the temperature outside the box.

Spore collections were made on the $12^{th}$ day but sporulation continued till the $16^{th}$ day. Even on the $12^{th}$ day, the whole area of leaf was usually colonized especially in experiment 'A' under sodium light. In experiment 'B', the whole leaf area was colonised under both light treatments but there was a difference in spore production. Spore counts were made in a coulter counter (P.28). The spores were suspended in an electrolyte and 1ml of the electrolyte was allowed to pass through the capillary at a time. Number of spores present in that 1ml could be directly read on the counter. An average of eight readings was taken for each replicate. Some of the spores were seeded on to Difco-Bacto agar plates and incubated at 10°C to determine viability as indicated by percentage germination

after 24h.

Results:

No eleborate statistical analysis was necessary for calculations as there were few variables and the results were clear-cut. The data from the light experiments were even then analysed using the t-test to compare the means of spores produced from the leaves under two different lights. To test the significant difference, 't' was calculated as follows:

$$t = \frac{\bar{x}_1 - \bar{x}_2}{sed}$$

where $\bar{x}_1$ and $\bar{x}_2$ are the mean of the spores collected from individual leaves under the two lights and 'sed' is the standard error of difference between the two means.

The 5% critical value for t with 10 d.f. is 3.7 and our observed t value in experiment 'A' 14.46 and 4.3 in experiment 'B' shows the means to be significantly different.

The results of spores produced are as shown in Fig.4.

The relationship between both the intensity and quality of light to spore production can be clearly seen from the graph(P.38).

There was a substantial difference in latent period between the two treatments. In experiment 'A' where both the lights were at the same height from the plant, the latent period was atleast 2-3 days shorter under sodium light than under mercury vapour lamp.

In experiment 'B' the difference in latent period was restricted to one day. A greater proportion of the leaves were infected

under sodium light than under mercury. The greater difference in latent period in experiment 'A' was probably due to the high intensity of the sodium light, in experiment 'B' the intensity of both the lights were the same.

Pustules began to appear on the 7th day in plants under sodium light. Though no pustule counts were made, visual observation showed the rate of pustule formation and the ultimate pustule density to be greater under sodium than under mercury.

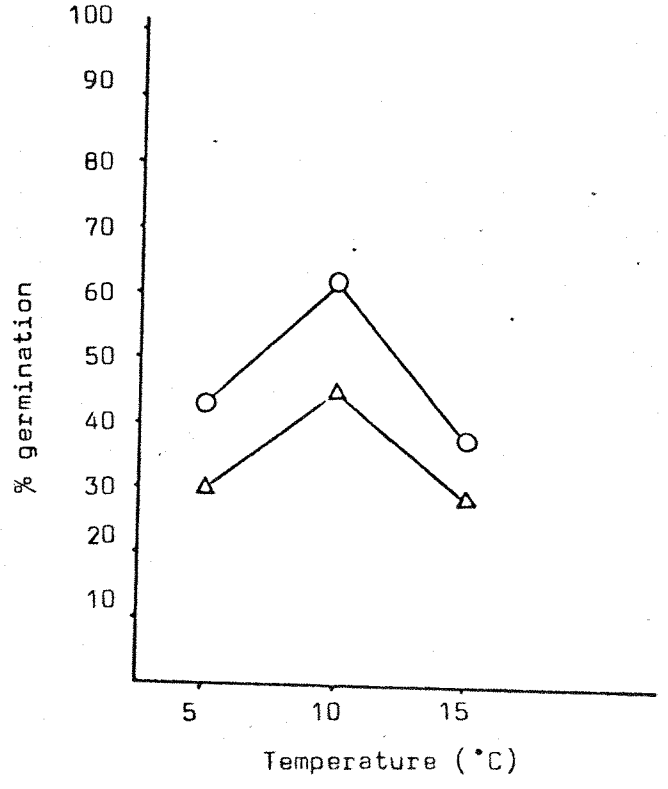
Spore Production per Leaf:

Leaf area was obtained by multiplying length x breadth and production of spores per leaf was calculated by multiplying the readings on the Coulter counter.

Spore production per unit area was obtained by dividing the total number of spores produced by the leaf by area of the leaf. ( no. of spores/area of the leaf).

The disease spreads on individual leaves faster under sodium light. The rate of spread was linear up to the 16th day of incubation and then there was a steady decline until it ceased completely. This is very true in the case of experiment 'A'. Spore collections were made on 14th day after inoculation. Though no spore collections were made specifically to determine the period of linear relationship, it was observed from the quantity of spores produced that there was a steady decline in the number of spore produced after 18th day after inoculation. McGregor and Manners (1978) reported largest spore production 12 days after inoculation.

The rate at which the pustules were produced differed greatly with time and light treatment.

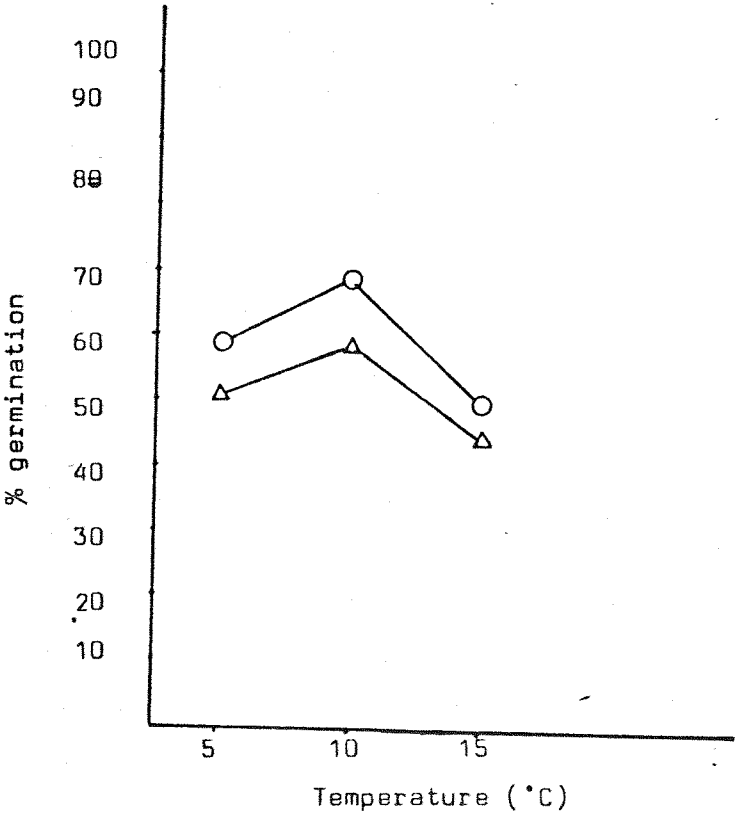



germination of spores from two different intensities of light

Sodium O-O

Mercury △-△

Fig.1




Germination of spores from same intensity of light

Sodium O—O

Mercury. △—△

Fig. 2

# PLANT IRRADIATION EQUIPMENT

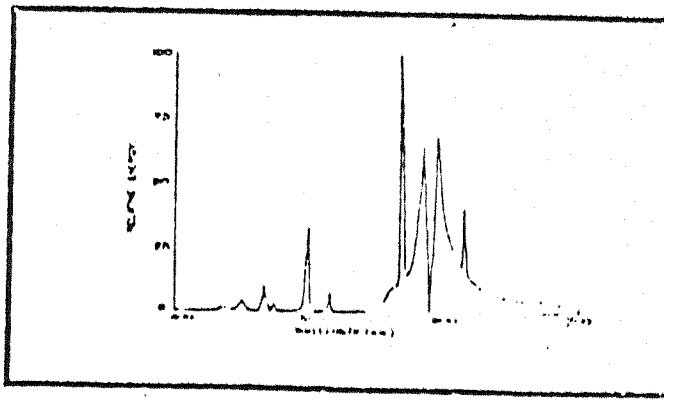
## LAMP DIMENSIONS

| Cat. No. | Diameter | Overall Length | Light Centre Length Objective | Arc Length Objective |
|---|---|---|---|---|
| HD26435 | mm 50 ± 2 | mm 292 | mm 175 | mm 90 |

The OSRAM SOLARCOLOUR high pressure sodium lamp is guaranteed to give a life of 4000 hours provided it is correctly operated.

## HIGH PRESSURE SODIUM LAMP DATA

| Type | Cap | Watts | Objective Life | Lumens Output Initial | Lighting Design Lumens |
|---|---|---|---|---|---|
| SON/T | G.E.S. | 400 | 4000 hours | 47,000 | 45,000 |

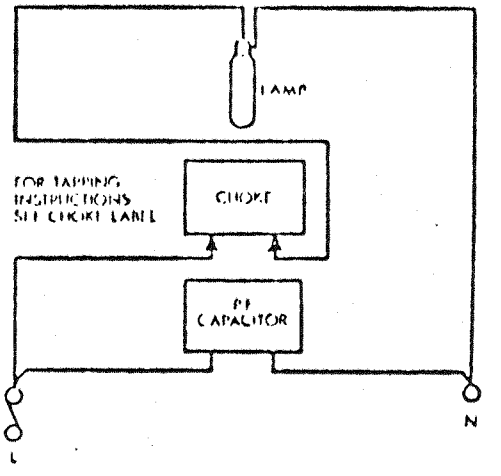


## SUGGESTED MOUNTING HEIGHTS AND SPACING FACTORS FOR HD71022S FITTING

| Illuminance | Mounting Height m (ft) | "LARGE BLOCK" SCHEME Area/Lamp m² (ft²) | Dimensions of Area m (ft) × m (ft) | Mounting Height m (ft) | Single or Double Rows of Lamps Area/Lamp m (ft) | Dimensions of Area m (ft)* × m (ft) |
|---|---|---|---|---|---|---|
| 6 500 | 2·4 (7·9) | 5·5 (59) | 2·6 (8·5) × 2·1 (6·9) | 2·0 (6·6) | 3·0 (41) / 3·3 (35) | 2·0 (6·5) × 1·9 (6·2) / 1·4 (4·6) × 2·5 (8·2)** |
| 10 000 | 2·0 (6·5) | 3·6 (39) | 2·4 (7·9) × 1·5 (4·9) | 1·8 (5·1) | 2·1 (23) / 1·8 (19) | 1·75 (5·7) × 1·2 (3·9) / 1·2 (3·9) × 1·5 (4·9)** |
| 13 000 | 1·8 (5·9) | 2·8 (30) | 2·3 (7·5) × 1·2 (3·9) | 1·8 (5·1) | 1·7 (18) / 1·4 (15) | 1·4 (4·6) × 1·2 (3·9) / 0·9 (3·0) × 1·6 (5·2)** |

BY PERMISSION OF THE ELECTRICITY COUNCIL

## A TYPICAL CIRCUIT FOR MBF/U AND MBFR/U LAMP




## PLANT IRRADIATOR DIMENSIONS

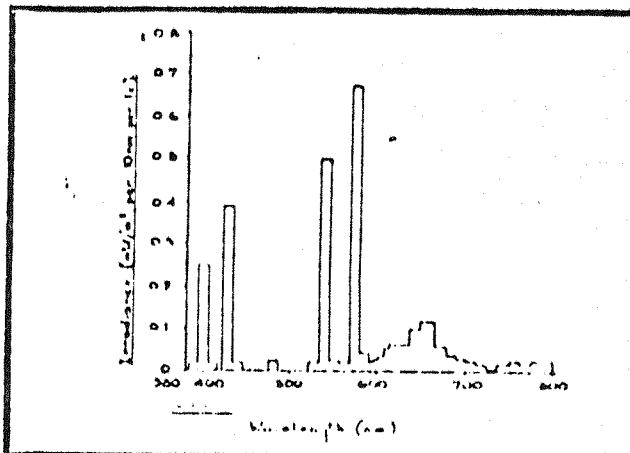
| Cat. No. | Description | Height in. | Width in. | Length in. | Weight lb. |
|---|---|---|---|---|---|
| HD71022M | Moulded plant irradiator with G.E.S. lampholder | 6¼ | 15 | 16 | 4 |
| Z1681 | 400W choke | 6 | 4¼ | 4¼ | 14 |
| Z1775X | 20 mfd capacitor | 2 | 3 | 6 | 2¼ |

## LAMP DIMENSIONS

| Cat. No. | Diameter mm | Overall Length mm | Light Centre Length Objective mm |
|---|---|---|---|
| HD26435 | 120 ± 1·5 | 210 ± 5 | 177 |

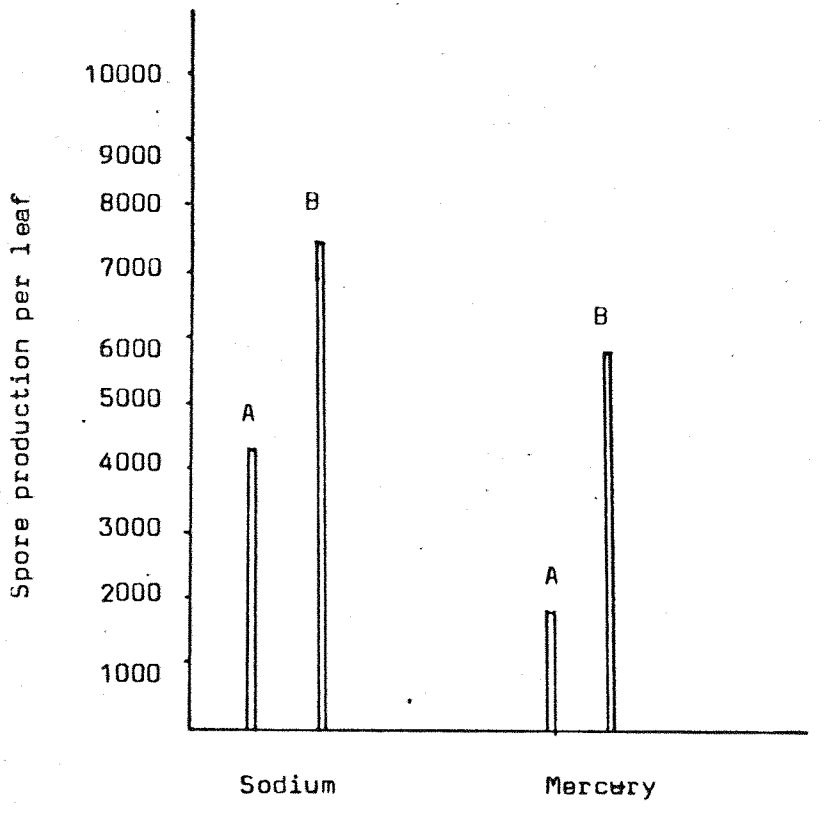
## HIGH PRESSURE MERCURY FLUORESCENT LAMP DATA

| Type | Watts | Objective Life | Lumen Output Initial | Light Design Lumens |
|---|---|---|---|---|
| MBF/U | 400 | 7500 hours | 21,000 | 20,000 |




SPECTRAL ENERGY DISTRIBUTION CURVE FOR 400W MBF/U LAMP

Fig. 3




Mean spore production under two different lights (10 replicates)

A = Different intensity

B = Same intensity

Fig. 4

Discussion:

Spore production results are a mean of ten leaves per light treatment. Spore production gave very large differences between the two lights, sodium light giving better results in both the experiments. The difference once again lessened considerably when the plants were subjected to the same intensity of light. Moreover, the plants appeared to age more rapidly when the mercury vapour lamp was placed close to the box in experiment 'B'.

A difference in germination was also observed when the spores were collected from the leaves under two different lights and seeded on to Difco-Bacto agar plates and incubated at 5°,10° and 15°C respectively. The spore counts were made after 24 hours. Spores collected from plants under sodium light germinated better than the ones collected from plants under mercury in both experiment A and B. [Fig. 1 & 2.]

Influence of quality of light on the development of yellow rust on barley has been studied by Stubbs(1967). He found "Topper" a variety though susceptible to P.striiformis to be completely resistant under mercury vapour lamp at 15°C. In experiment 'B' where the plants were subjected to the same intensity of light from the two lamps, the plants under mercury vapour lamp still produced fewer spores than plants under sodium light. Perhaps the wheat plants in the present experiments tended to show partial resistance to P.striiformis under mercury.

The effect of wavelength of light on photoresponses has been studied by various workers. Maddison and Manners(1970) showed a reduction in uredospores germination of yellow rust spores exposed to u.v. irradiation. Bush(1967) observed a reduction in

Uredospore germination of P.graminis when exposed to near u.v. radiation (300-400nm) while Lucas et.al.(1975) with their work on phyto-control of P.graminis spore germination have shown the responses to various wavelengths of light. They associated inhibition by the uredospores to high intensities from blue and for red light to similarities in the phytochrome system of higher plants.

There is a difference in the wavelengths of the two lamps used in the experiments (Fig. 3 ) Light from the sodium lamps is predominantly made up of a single wavelength in the orange-yellow part of the visible spectrum (600nm) while the mercury vapour lamp shows some production in the long u.v. region ( 400nm; .1nm=$10^{-9}$m) as well as a band in the blue part of the visible spectrum. In experiment 'A', the difference in intensity between the two lights resulted in a particularly good spore production under sodium lamp. In experiment 'B',although the lights were adjusted to give the same intensity, spore production under mercury vapour lamp was still less than that under identical conditions using sodium lamp. This latter result could be explained by the following observation. In order to obtain the same intensity, the mercury vapour lamp had to be adjusted at half the distance of sodium lamp. At this distance plants were exposed to a high level of radiations from the high energy u.v. region in the mercury vapour lamp. The mercury fluorescent lamp has hard glass bulb but this may not absorb completely high energy u.v. irradiations. Specially at a shorter distance from plant level. The approximate energy emitted by the two different lamps may be obtained by the equation

$$E = Nh\nu$$

Where E is the energy of light, h is Planck's constant ($6.625 \times 10^{24}$), $\nu$ is the frequency ($\nu = c/\lambda$ = speed of light/wavlength of light) and N = number of molecules ($6.024 \times 10^{23}$).
Using the above equations, approximate levels of yellow and violet regions of the visible spectrum has been calculated as 47.67 k.cal/mole and 71.5 K.cal/mole ( K.cal/mole is the unit of energy).
Since mercury vapour lamp shows some absorption in the longer u.v. region, the energy emitted by it is higher than that obtained from sodium lamp. The plant may have differential absorption because of photoresponses of the pigment present on the leaf surface. The increase in energy given by mercury vapour lamp may in part be responsible for inhibiting the germination(Dillon-Weston,1932,b) or may retard photosynthesis in plants. The net loss in photosynthesis may result from heat given out by exposure to longer u.v. absorption in the mercury fluorescent lamp.

From the above results it can be suggested that a relatively high intensity of light is essential in production of yellow rust uredospores. In the winter months in Britain supplementation of daylight by artificial light is necessary. Besides intensity of light it is also essential to note that the quality of light in Yellow rust spore cultures. Light may not be a limiting factor but is certainly an essential factor in multiplication of the inoculum.

# CHAPTER IV

## Influence of Temperature on Pathogen and Host-pathogen Interaction

### Introduction:

Temperature has long been shown to be a limiting factor for growth and survival of the pathogen. The limits within which the spores can germinate are wide. Mean value affects the growth rate and extreme influences the survival of hyphae and propagules. In the field, spread of the disease to epidemic proportion has been shown to be a direct consequence of ideal temperature variations in leaf temperature and ambient temperature have been reported in the field by workers on microclimate on leaf surface(Burrage, 1971 a; Raschke,1958; Linacre,1972). The effect of temperature on the host resulting in net loss in photosynthesis and carbondioxide assimilation have been shown by Drake et al.(1970).

In the field the plants are exposed to a fluctuating temprature and thereby allows the survival of host and the pathogen but prolonged period of extreme temperature kills the pathogen completely.

Our analysis of field results carried out in August-September 1978, showed temperature to be the most important factor affecting germination of yellow rust uredospores. Therefore it was decided to study the effect of various temperatures both on survival and viability of spores.

The effect of temperature can be on the host or the pathogen or both. In principle it would have been ideal to treat the plants under the same conditions both before and after inoculation. Due to shortage of space in growth cabinets, it was decided to grow the plants in the greenhouse and transfer them into growth

cabinets after inoculation. Experiments at different temperatures could not be performed simultaneously as there was only one cabinet available. Therefore the experiments were carried out one after another. A high level of humidity was maintained by placing a large amount of plant material inside the cabinet and trickling water into the cabinet continuously.

Germination and penetration on the leaf surface were ascertained at different temperatures.

Materials and Methods:

Seeds of Maris Beacon were sown in 6cm pots. About seven seeds were sown in John Innes no.2. compost in glasshouse no.5.. All the experimental plants were grown under similar conditions and when the second leaf had completely unfolded itself, each plant were transferred in to 4cm pots. About ten plants per experiment was used.

The plants were inoculated with the help of a settling tower (P.22). This gave an even deposition of the spores. About 3mg. of freeze-dried spores were allowed to fall on to experimental leaves, through a tower, arranged in a circle over the metal disc. Prior to inoculation the leaves were dampened and the inoculated pots were placed in perspex box, sealed air-tight with a plastic sheet and incubated for 24h. at the controlled temperatures.

In an experiment to determine the viability of spores at different temperatures, the plants were inoculated with a paint-brush and left in the cold room for 24h. They were then transferred to the controlled environment cabinet for spore production at a particular temperature. The spores produced at the experimental temperature were used in incubating plants for

ascertaining the viability of spores at different temperatures. Ten plants were inoculated with a settling tower and incubated at the experimental temperature for 24h.

Ten other plants inoculated with spores produced at a particular temperature and inoculated through a settling-tower were exposed to a higher temperature of 26°C for 2h. and were transferred back to the experimental temperature.

Leaf Clearing and Staining:

After 24h. the leaves from individual pots were cut into small pieces and immersed in chloralhydrate solution contained in Petri dishes. The Petri dishes were labelled with relevant details to avoid any confusion. After a week the cleared leaf sections were stained in acid fuchsin/aniline blue for 15-30 min. After staining, the sections were mounted in glycerol. The details of leaf clearing and staining technique has been described in P.

The stained section could be conveniently left for a week or two in a dark place before spore counts were made without the stain fading. The different observations that were made were %germination, penetration and ungerminated spores. The propartion of germinating spores causing infection could be obtained by dividing the number of spores out of spores penetrating by the number of spores germinating.

About 800-900 spores were counted in all under each conditions. A thermohygrograph placed inside the growth cabinet gave recordings of the prevailing temperatures and humidity. Humidity was always above 80%. The photoperiod by fluorescent tubes was 16h a day.

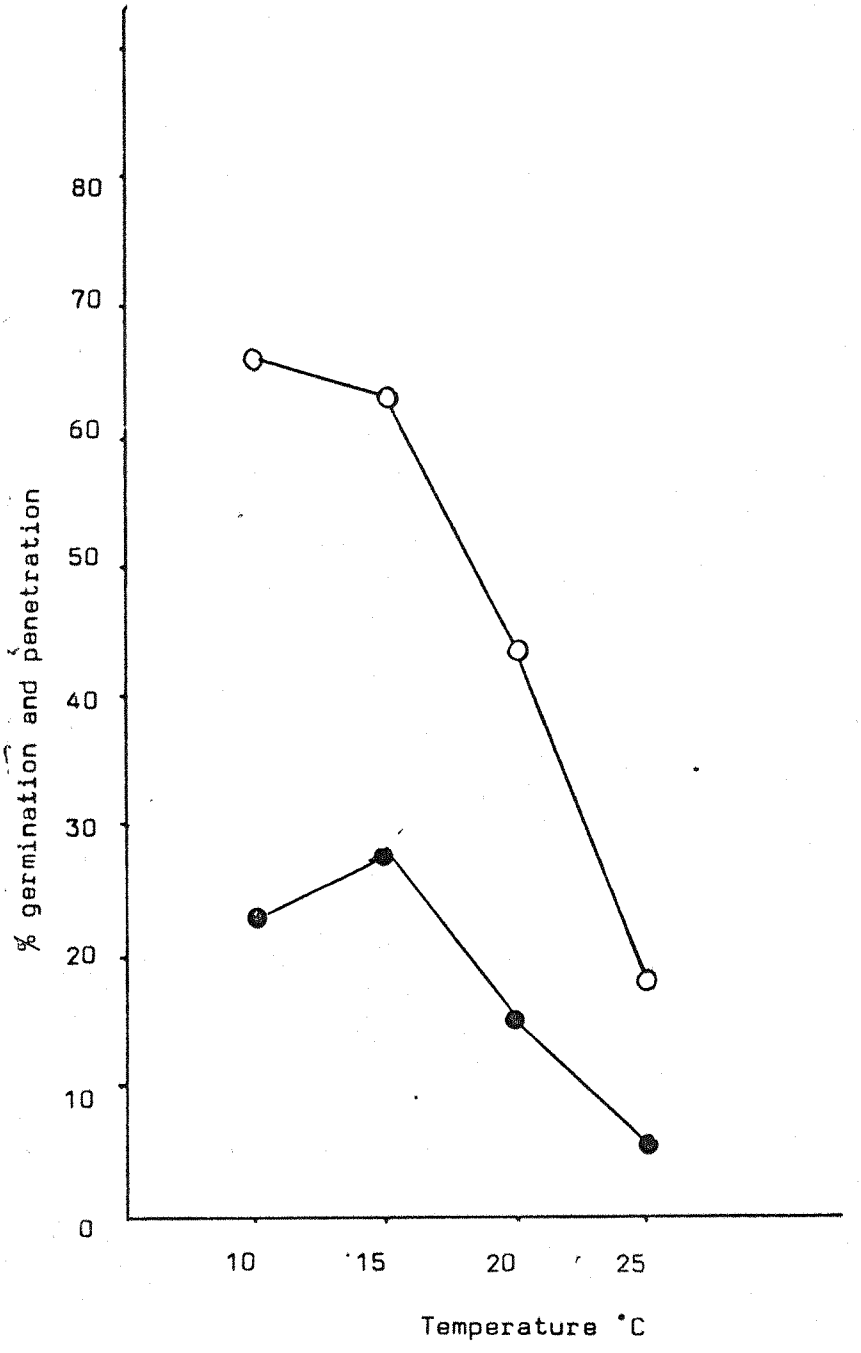
Results and Conclusion:

Survival of Spores:

The leaves inoculated with freeze-dried spores collected from the glasshouse showed a definite reaction to temperature. No statistical analysis were made as ten replicates were mixed together during the course of leaf cleaning and staining technique The results as shown in figure 5. are a mean of total number of spores counted from sections of ten leaves. Germination was best at 10° ± 1°C followed by 15° ± 1°C. No substantial difference was noticed in rate of germination at the two temperature but the number of spores causing infection was more at 15°C. Spores germinated quite well at 20°C but very poor germination were recorded at 25°C. This trend of linear relationship upto a certain temperture (18°C) and then a non-linear relationship has been observed throughout the course of this work. The number of spores causing infection after a given time interval was greater at 15° and 20°C than at 10°C but even that was low in case of 25°C. This clearly indicates that temperature affects penetration and the rate of spore production

Viability of spores produced at different temperatures:

Many workers have reported temperature during ureoospore production to influence the effect of temperature at the time of germination on germination behaviour(Straib,1940). Contrary to the results observed by many workers, no substantial difference was observed in the rate of germination between leaves inoculated with spores produced at the same temperature and leaves inoculated with spores produced at a standard temperatures (Fig.6). A slight improvement in percentage germination and penetration was observed both in case of 15° and 20°C .




Germination and penetration expressed as % of germinated spores

O = Germination of spores at different temperature

● = Penetration of spores at different temperatures

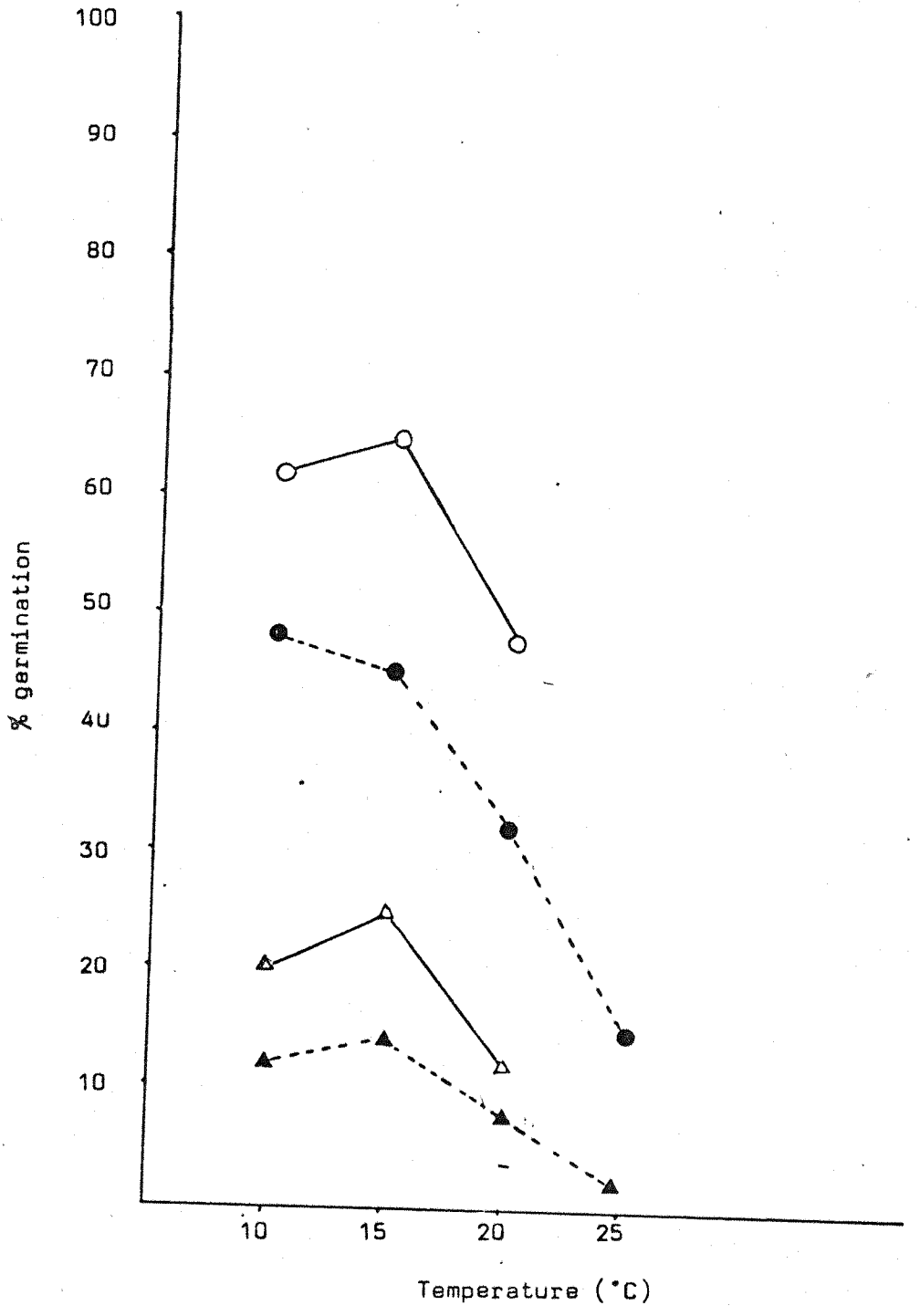
Fig. 5

As no spores could be produced for collection at 25°C, the viability test could not be performed at 25°C. The results are tabulated as shown in Fig. 6.

During the course of spore production, the influence of temperature was observed to have a remarkable effect on latent period and rate of spore production. The latent period was shortest at 20°C with only 10 days while it took about 14 days for the eruption of pustules at 10°C. The rate of spread of the disease was faster at 20°C than at the lower temperature but the total production of spores was longest at 10°C. At 25°C the incubation period was prolonged to 15 days and few pustules that eventually appeared on the leaf surface failed to sporulate well. As a result enough spores could not be collected to inoculate through a settling tower. The leaves began to age faster at higher temperatures while at 10°C although the entire area of the leaves were colonised, they could produce spores for a longer period.

Not much difference in germination was observed between leaves inoculated with spores collected at the various experimental temperatures. There was only a slight shift in temperature. Whether this has anything to do with the pre-inoculation treatment as observed by Sharp(1965) is not known. As stated before all plants prior to inoculation were grown in a glasshouse maintained at 15°C (± 2°C).

In studies on exposure of spores to higher temperature, though only one temperature was used for a fixed interval of 2h. the results showed a negative production trend under all temperatures.




germ. / germ. = viability of spores produced at the same temperature

germ. / germ. = Exposure of spores to a higher temperature (26°C) for 2 hours.

Fig. 6

There was a substantial decrease in percentage germination and penetration even by exposing the plants for only 2h to a higher temperature. The plants were exposed immediately after inoculation to a higher temperature and then transferred back to the standard experimental temperature. Whether this has anything to do for a considerable difference in germination could not be proved as experiments were'nt carried out by exposing the inoculated leaves at a later period instead of immediately after inoculation. Germination percentages were the same as observed in the case of survival of spores at different temperatures. The spores germinated the best at 10° & 15°C followed by 20°C. Leaves inoculated with spores collected in the glasshouse were exposed for 2h at 26°C and transferred back to 25°C as no spores could be produced at 25°C. The germination was very low as observed before (Fig. 6 ).

Discussion:

Clear-cut differences in their response to the relative effects of temperature and incubation period in a saturated atmosphere on uredospore production and on the initiation of infection by these spores P.striiformis were observed by Straib (1940). His results showed an optimum of 10°C and a maximum germination temperature of 25°C. Our results are in agreement with this observation as best germination was obtained at 10°C while some germination did occur at 25°C accounting for the maximum germination at which the spores can survive for a certain length of time. Manners(1950) and Schroeder and Hassebrauk(1964) showed the spores to be viable even after being held at 25°C in the dark

for several hours and returned to 10°C. This clearly indicates that a hot, dry, sunny weather and a heat wave can check an epidemic.

Eriksson and Henning(1891) first recognised the effect of temperature during the production of uredospores on their germination potential. Straib(1940) noted that the temperature during uredospore production had a marked influence on the rate of germination. For instance he observed a maximum temperature higher by 2-3°C with a fructification temperature of 20-25°C when compared with a fructification temperature of 8-12°C. Tollenaar and Houston(1966) have shown maximum germination temperature to be governed by the temperature during uredospore production. His highest maximum temperature at which spores germinated was 24.5°C. Sharp(1965) showed a shift to greater susceptibility in two differentcultivars pre-conditioned at 24°C in comparison with plants maintained at 15°C. We failed to notice any such difference: this may be due to the difference in pre-conditioning phase as observed by Sharp, Gsssner & Straib(1940) observed a minimum latent period at 20°C. Our results are in agreement with his observation. Exposure of yellow rust toahigher temperature indicates that the spores of yellow rust are tolerant to fairly high temperatures and are viable when brought back to lower temperatures. This result is in agreement with many observations made in the field epidemiology by many workers(Tollenaar & Houston 1967, Ellingboe 1968).

Temperature therefore is a controlling factor and the total duration of a given temperature to which the spores are exposed is as important as the maximum and minimum temperature

for both survival and viability of yellow rust uredospores. This is very true under field conditions where the changes in temperature may encourage or delay the occurrance of an epidemic as in the summer of 1947 in which, following prolonged high temperatures, the fungus was absent from a large area so that the epidemic was slow and late in developing the following year though the weather was favourable(Manners, 1971).

# CHAPTER V

## Effect of Age of Leaf on Germination of Yellow Rust

### Introduction:

Yellow rust of wheat caused by P.striiformis can occur on plants of all stages and on different ages of leaves both under glasshouse and field conditions. The rust is especially important as it is known that the disease spreads rapidly at certain growth stages with favourable environmental factors(James et.al.,1974). Straib(1939) working on the reaction of wheat to yellow rust found that new cultures acquired resistance with advancing age and temperature; others were susceptible at any age and temperature or uniformly resistant at all temperatures.

In young leaves there is a random distribution of the uredosori while serial lesions develops appearing like strips on the green leaf tissue of older leaves. Guyot(1948) has reported a change in colour, dimension and arrangement of the uredosori with increasing age of the host tissue. Sporulation is known to be better at the distal end than at the base of the leaf (Russell,1976).

The results of our work carried out in the summer of 1979 showed a significant effect on both host age and position of individual leaf on spore germination. As there was insufficient time to demonstrate both these results under laboratory conditions, it was decided to study the effect of age of leaf on spore germination under a controlled environment.

The field experiment results were used as a basis for the selection of the condition employed in the controlled

environment cabinets. Our computed analysis of field data showed temperature and humidity to be the two important factors affecting germination. Differences in mean temperature or average temperature over 5 days brought about differences in germination with a decrease in germination at a higher mean temperature. Relative humidity below 75% resulted in decreased germination. It was always the combined effects of temperature and humidity that seemed to affect germination. Therefore the experiment was designed to work under a combination of temperature and humidity factors in a controlled environment growth cabinet.

Materials and Methods:

Seeds of Maris Beacon were sown in 6cm pots and grown in glasshouse no.5. Three plants per pot were used during the course of the experiments. Twenty five pots were sown at a time. The seeds supplied by the Plant Breeding Institute germinated well. The second leaf was used for experimental purpose. The plants were sprayed with milstem prior to inoculation.

Inoculation:

The plants were inoculated with freeze-dried uredospores of P.striiformis with a sterile paint brush on the adaxial surface of the leaf. The plants after inoculation were sprinkled with water and placed in 'perspex' boxes. The plastic boxes were left in the cold room for 24h and were brought back and left in the glasshouse maintained at 15°C under artificial light for 6 days. Then they were transferred back to the controlled environment cabinet. This was mainly done to ensure sporulation at higher temperatures.

Experimental Design:

The plants were divided into two groups. The first set of experiments was inoculated when the second leaf was of the same length as the primary leaf (S=P). In the second set of experiments, the plants were inoculated after allowing a gap of 7 days after the second leaf had reached the length of the primary leaf (S=P+7). This enabled us to have a clear differentiation in the age of the leaf as the leaves in the second set of experiment were closer to the senescence stage.

A set of pots was sown with Maris Beacon seeds in order to measure the length of different leaves of both when S=P and S=P+7 and finally when the second leaf eventually ceased to increase in length. There was a great variation in the length of the primary leaves leading to differences in the length of the second leaves. Of the ten leaves that were measured, the second leaf equalled the primary approximately on the $12^{th}$ day when they were on an average 6.5cm in length. On the $19^{th}$ day, after an interval of seven days the leaf had grown to a length of 16.5cm and the average of the final length of the leaves was 22cm. The results of these test plants were very similar to those for the experimental plants. About ten replicates per experiment were used. A jeweller's tag was fixed around each experimental leaf stating the age of the leaf and the replicate number. The spores were collected on the $12^{th}$ day of incubation and seeded on to previously prepared labelled Difco-Bacto agar plates with a sterile paint-brush. Care was taken to use a specific labelled paint-brush for each replicate to avoid contamination of spores from different leaves. The agar plates were

incubated at 10°C and the spore counts were made after 24h. About fifty spore per field and three hundred spores in all were counted per Petri dish. If the length of the germ-tube exceeded the smallest diameter of the spores, the spores were scored as germinated.

A diurnally varying temperature was used in the growth cabinet. The various temperatures used were 5/10°, 10/15°, 15/20°, 20/25°C respectively. The temperature could not be maintained reliably at 5°C and there was a fluctuation of ± 2/3°C at this temperature. At other temperature levels, the temperature remained consistent as read from the thermohygrograph stationed inside the growth cabinet. The wet and dry bulb thermometer was used to check the thermohygrograph always showed the expected temperature levels. The humidity though adjusted to be maintained at 70-90% was always in the range of 80-90%

The various humidities originally designed for the experiment were 90-95%, 70-90%, 50-80% at a constant temperature 15/20°C and humidity levels of 70-90% with extreme temperatures (25-20°C). None of these experiments could be carried out but for the first one as the controlled cabinet could not be adjusted to the required level of humidity. The humidity could not be varied especially in these growth cabinets and at the most could be set only in higher ranges of humidity level. This prevented us from demonstrating our results from field experiments under laboratory conditions.

The fluorescent lights inside the growth cabinet provided a 16h photoperiod.

Results:

The student t-test was performed to compare the significance of age of leaf and effect of temperature on the age of leaf and spore germination.

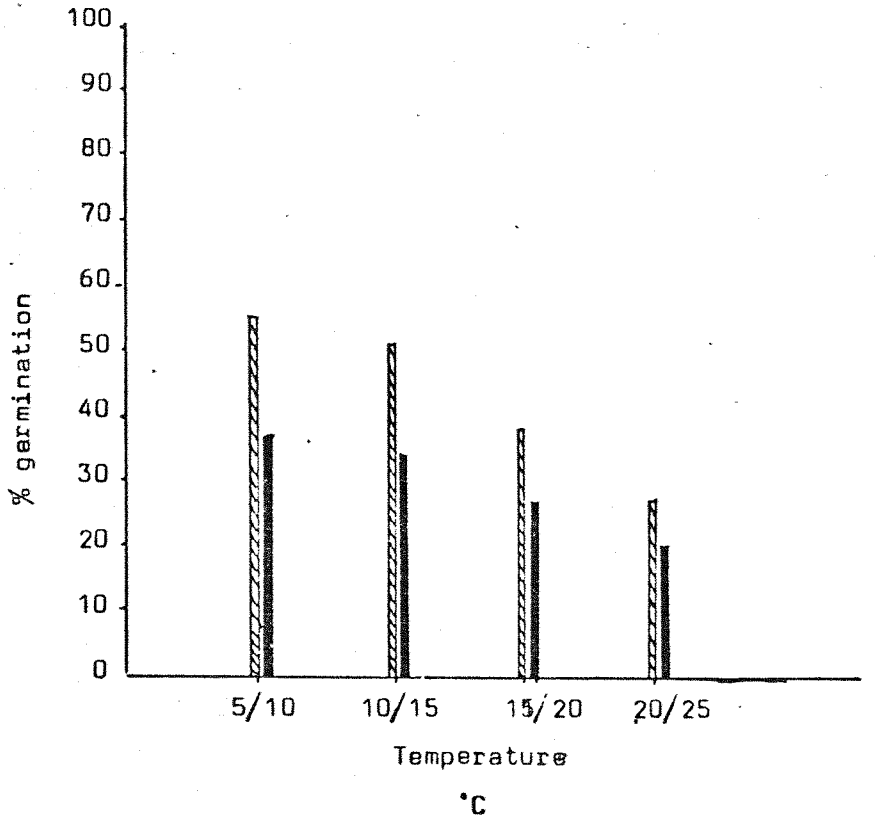
The 5% critical value for t with 10 d.f. is 3.17. When the means of germination of spores from S=P and S=P+7 were compared individually for value of t at four different temperatures, the results turned out to be very significant under all temperatures. But the t value decreased in comparison as the temperature increased or in other words the difference in germination of spores collected from S=P and S=P+7 narrowed gradually. The t values that were obtained in the analysis were: [Fig.7.]

| Temp.°C. | t-value |
|---|---|
| 5/10°C | 18.20 |
| 10/15°C | 13.30 |
| 15/20°C | 7.78 |
| 20/25°C | 6.10 |

The diurnally varying temperature used during the course of the experiment perhaps helped in increasing the t value at higher temperature as it is the mean temperature that is known to affect germination of spores. The analysis showed the age of the leaf to be significant both in the field and laboratory conditions. The effect of temperature also turned out to be significant on the same age group of plants, the significant difference being clearly shown in comparison of the data from a low temperature and the one from a higher temperature.

Discussion:

Leaves of different ages were all grown under similar conditions. The various ages of the leaves were made available




Mean percentage germination of spores collected from two different ages of leaves

S = P + 7.

S = P.

Fig. 7

at the same time by sowing some pots earlier. Inoculation for both S=P and S=P+7 were carried out on the same day from the same ampoule of freeze-dried spores.

The effect of age of leaf on spore production and germination was very marked at all temperatures. Though the plants were inoculated on the same day S=P always produced pustules earlier than S=P+7. There was at least a difference of two days in each case. In one of the experiments (10/15°C) where actual pustule counts were made on the 9$^{th}$ day a wide difference was revealed. Pustules on the leaf surface of all the ten replicates in both the cases counted with the naked eye gave a mean of 31 pustules in S=P and 22 pustules in S=P+7. The rates at which they were formed were so different that even visual observations showed a difference in density and consequently production of spores. The percentage area of the leaf being colonised remained approximately the same both in S=P and S=P+7 at all temperatures but a definate difference in the quantity of spores being produced was observed. No spore counts were made but visual observation during collection indicated a difference in quantity. The plants remained healthy and continued sporulating for 30/35 days at lower temperatures of 5/10° & 10/15°C. In the case of 15/20°C the rate of production of spores on the leaf surface itself showed a clear difference even before collection. The latent period was shorter in this case with the pustules appearing on the leaf as early as the 7$^{th}$ day and by the 10$^{th}$ day the plants began to sporulate. S=P+7 failed to show the luxurious growth of yellow rust as seen on the leaves of S=P. The plants continued producing the spores up to 18/20 days until they died completely. In

20/25°, inspite of the plants being left in the greenhouse for six days after inoculation, S=P+7 produced enough spores only to seed on agar-plates but no collection could be made while S=P produced spores which when tapped on to aluminium foil could be collected. This lasted only for three days. The latent period was prolonged to 15 days after inoculation. The extent of infection was consistently less as indicated by the systemic index which declined with increasing development of the leaf. The difference in number of pustules in both S=P and S=P+7 indicates that the old tissue is unable to support large amount of the fungus as the young green tissue.

The rate of spread of the disease on to new leaves was greater in case of S=P at all temperature while at lower temperature a larger proportion of the seedlings picked up infection in case of both S=P and S=P+7.

Statistical analysis reveals a substantial difference when the germination of spores collected from S=P and those from S=P+7 on agar plates under all temperatures. In addition to the effect of temperature, age of leaf also had a significant effect on germination. This clearly indicates the importance of physiological status of the host at the time of spore production and there by its effect on spore germination.

Reaction of temperature on age of leaf, spore production and germination:

Comparison of seedlings of similar age incubated under different conditions showed that sporulation and spread of the pathogen were limited at higher temperatures. The effect of

of temperature on the host is shown by the fact that the plants remained healthy and could promote sporulation at lower temperatures for longer lengths of time than at higher temperature. The effect of temperature was very clearly seen in initiating the disease quickly and in a faster rate of spread of the disease.

In case of 5/10° and 10/15°C, the latent period was 12-14 days after inoculation while at 15/20°C it lasted for only 10 days. The latent period at 20/25°C was prolonged to 15 days showing a non-linear relationship between temperature and yellow rust infection.

The rate of pustule formation was again related to temperature showing a steady increase up to 15/20°C but with a steep reduction at 20/25°C . Area of the leaf being colonised and the spread of the disease with in a given time was greatest and best at 15/20°C but the total proportion of plants being infected was greater at 10/15°C. The plants aged quickly at higher temperatures as shown by the difference in number of days sporulation continued at different temperatures. This effect of temperature on ageing of plants was demonstrated very clearly at 20/25°C at which temperature the leaves senesced even before the spread of the disease to the entire cross-section of the leaves.

Germination of spores produced at different temperatures from S=P & S=P+7 also showed a substantial difference in germination on agar plates. Spores produced at 10/15 and 5/10°C germinated well (60%). Spores produced at 15/20°C gave 45% germination while the spores produced at 20/25°C germinated only up to 25%. This

result confirms with our field analysis which indicate that temperature affects the germination of yellow rust uredospores.

One experiment carried out with humidity set at 95% (±2%) gave a germination of 60%. It did not provide a good comparison for the effect of humidity on germination as other experiments on humidity had to be abandoned. But the difference in germination of spores from S=P and S=P+7 was marked.

The results of the field experiment conducted under laboratory conditions clarified the importance of the age of the host and its effect on germination. Analysis of both the field data and the results obtained from controlled environment gave encouraging results and is in agreement with some of the work of other workers(Dickinson & Wallace,1976). The vigour of the host plant and the amount of leaf tissue available for colonisation is more in the case of young leaves. The nutrition produced by these leaves in time and the type of exudates that accumulate may affect the germination of spores. Components of the exudates may be antagonistic to the survival of the pathogen. Preece(1976) reports a change in the relationship between host plants and their parasites with advancing age. The presence of micro-organisms colonising the older leaf tissue may also be one of the reasons for the difference in germination.

Literature is available in plenty regarding the environmental factors influencing germination and temperature and humidity have been shown to be the two important factors. Though inability to vary humidity in a controlled environment meant that conclusive evidence was not forthcoming, the analysis of the field data shows that temperature and humidity have a combined effect on germination.

For instance best germination results were not obtained on days when both temperature and humidity were low or when humidity was near saturation with a higher temperature. On the contrary good germination percentages resulted from spores collected on low temperatures (mean 12°C) with high percentage of humidity (mean 80%). Differences in spore production indicates the relation of incidence on young leaves at favourable temperature to severity of the disease.

It is clear that the physiological age of the leaf on which uredospores are being produced as well as the temperature and humidity at which spore production recurs, are factors of epidemiological importance and should be taken into account when predicting models of disease development are being constructed.

# CHAPTER VI

# FIELD WORK

## a.) Weather Measurements:

### Introduction:

In forecasting epidemics of yellow rust of wheat, it is essential to know the environmental conditions prevailing both before and at the time of the appearance of spores. Broadly speaking it is generally well-known that a low optimum and maximum temperature with bright days encourage infection while very high temperatures jeapordise the survival of the spores. The occurrance and development of yellow rust being intimately related to the prevailing climate greatly suggest the need for metereological data for the forecasting of the disease.

### Description of the Instruments:

The various instruments that were used in the field were as follows:

### Thermohygrograph:

A thermohygrograph in a Stevenson screen was caliberated to give the recordings within $\pm$ 2°C and $\pm$5% relative humidity. Simultaneous records of temperature and relative humidity are obtained on a dual channel chart paper changed weekly.

Temperature is measured by means of a curved bimetallic helix, coiling and uncoiling with changes in temperature. One end of the bimetallic strip is attached to the instrument case through a pivot mechanism and the other end to a pen arm by means of a special linkage.

Relative humidity is measured by means of a bundle of

human hair looped through the pen linkage and firmly attached to the instrument case. Changes in relative humiditiy cause the hair to expand or contract, thereby moving the pen arm linkage.

Rain gauge:

The function used was average daily rainfall in millimetres siphoning automatically after each 0.2 in. or 5mm of rain. Rainfall is collected and led into a funnel and then to a chamber containing a hollow metal float, to the upper axis of which is attached the pen-arm.

As the chamber fills, the float rises until it reaches a sufficient height to release the tip, when the weight of water contained causes the chamber to tilt on the knife-edge sending a surge of water through the siphon tube. When the chamber is empty it is restored to its original position by its counter-weight. The siphoning period is about 15 seconds. (Fig. 9.)

Surface wetness recorder:

Hirst(1957) was the first to relate the duration of surface wetness to the germination of plant pathogens. The test surface is made of a block of expanded polystyrene cut into a tetrahedral shape at the end of 1/4" ( 6 mm)diameter rod. An arm carrying a paddle immersed in a small oil filled tank in front of the beam reduces the oscillation of the beam. A dial records changes in the equilibrium of the beam on daily chart placed in a rotating drum at the left of the instrument. (Fig. 8.)

Anemometer:

Wind speed measurements are made with in a 3 cup anemo-

meter. The cups are attached to the shaft which has a ball bearing as its main support. The speed of rotation of the cups is directly proportional to the speed of the wind. Rate of rotation of the three cup assembly of the anemometer depends on the intensity of the wind speed which is recorded directly onachart that can be changed every month.

The direction of the wind is determined by using the air-foil.

Solar Radiation Integrator:

A Lucas solar radiation integrator was used to measure the total radiation per day. A silicon light-sensitive semi--conductor with in a diffuser provide necessary angular response. Variations in ambient temperature is compensated by a thermal senser. A potentiometer is fitted for temperature compensation adjustment. The total amount of radiation was directly recorded after 24h.

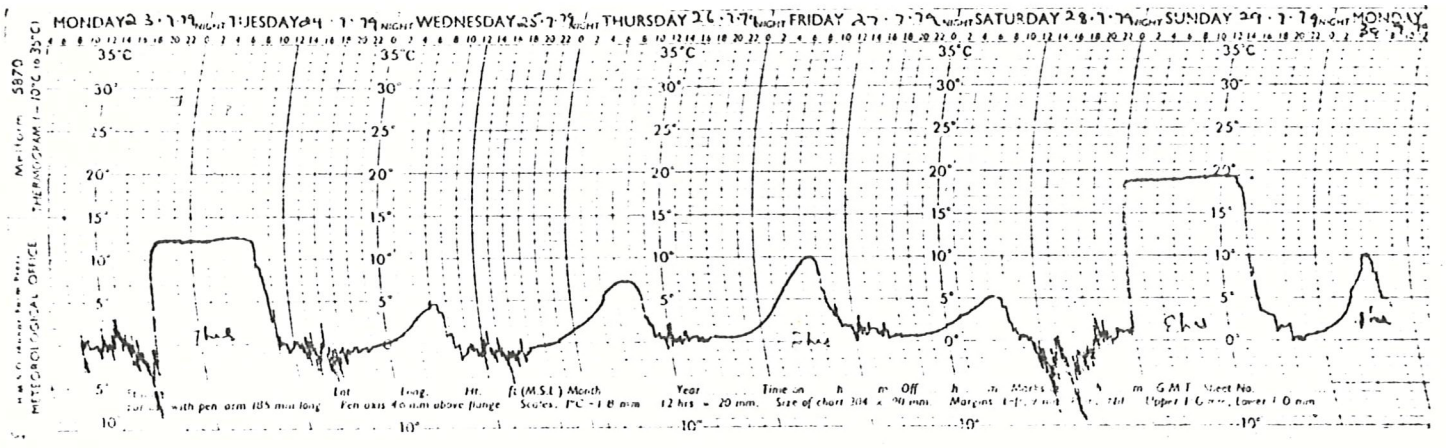
General comments on the use of the instruments:

The first set of field experiments lacked the necessary metereological instruments. The thermohygrograph that was insta,-lled failed to function properly, leading to a number of missing readings. So all the different weather data were obtained from Metereological office in Southampton. The following year the various instruments were adjusted properly to give reliable recordings and they were placed in suitable positions in the plot before the experiments began.

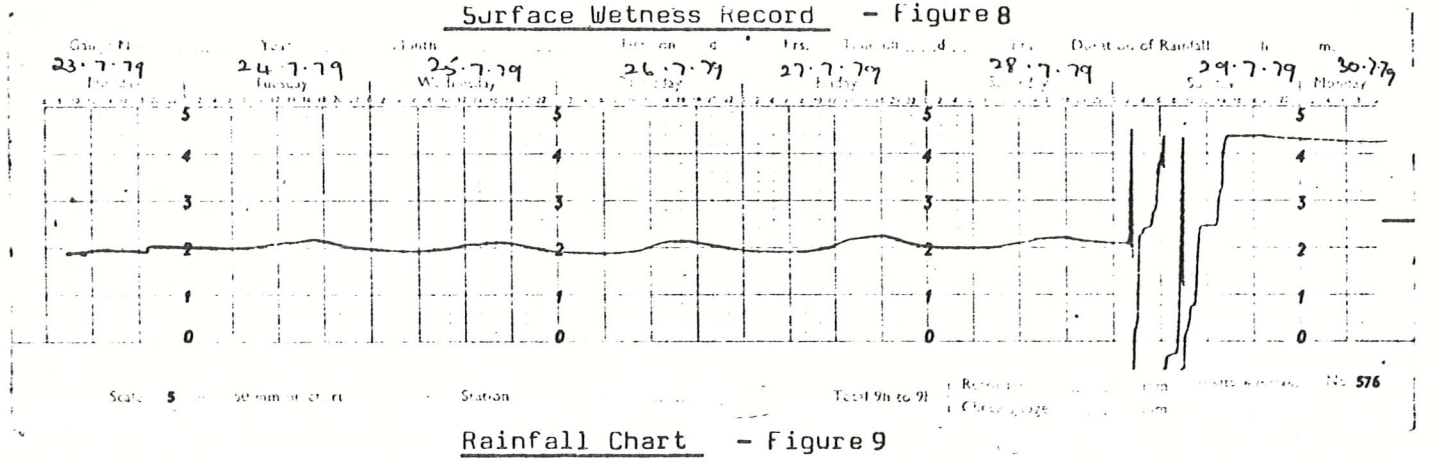
A Thermohygrograph was placed inside the cage near the plot but the exact temperature and humidity within the canopy was not measured. There is always a difference of 2-3°C between

the temperature in the canopy and the ambient temperature(Burrage, 1971). The thermohygrograph functioned reliably and the readings from the chart were taken every 2h.

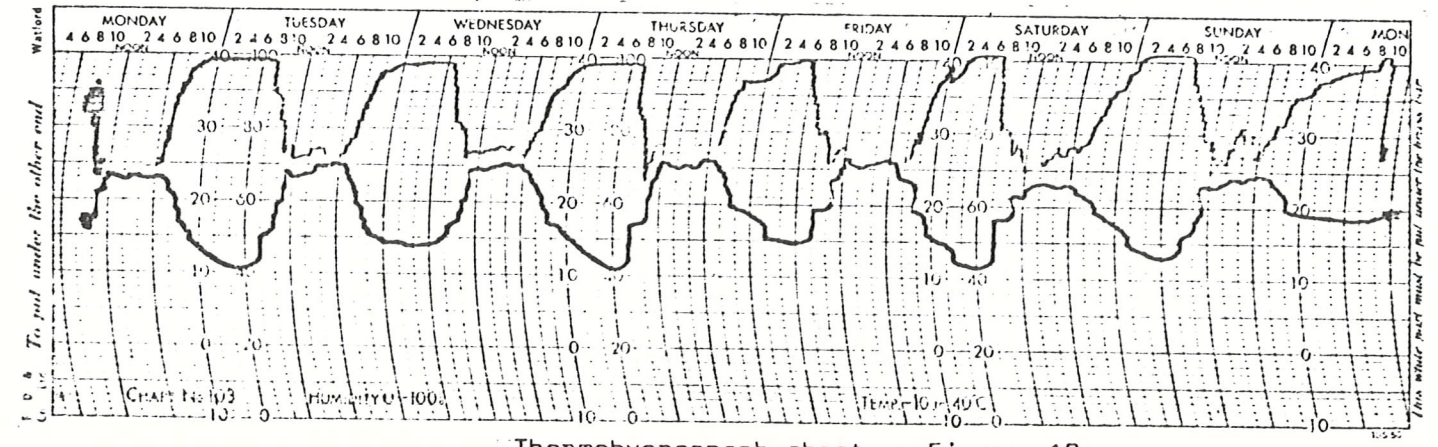
The rain gauge worked very well and recorded properly all the readings. Average rainfall was calculated from the chart and recorded every 2h. The surface wetness recorded was used only to record a day as a wet day or a dry day as there was a difference between evaporation from the leaf surface and that from the polystyrene used in the instrument. The polystyrene is said to evaporate faster than the leaves. The average number of hours recorded above 10°C on the chart was used to indicate surface wetness as the temperature chart was used, since charts developed for this instruments were not available. Depending on the number of hours it was decided to be a wet or a dry day. The print out of the solar radiation integrator didn't function. Therefore the readings could be obtained for a period of 24h only. In the case of the Anemometer there were missing readings due to some unknown reason. Therefore the readings were taken from meterological office.




Surface Wetness Record - Figure 8




Rainfall Chart - Figure 9




Thermohygrograph chart - Figure 10

## B. General Materials and Methods:

### 1) Introduction:

Yellow rust of wheat being confined predominantly to cool temperate regions. The possibility of a fluctuation in climatic conditions could explain in part the variable pattern of epidemic development. Yellow rust is most sensitive to environmental parameters. The growth of the host cultivars and their susceptibility to yellow rust infection is determined by combinations of temperature, moisture, light, air-movement, pressure, genetic constituents, nutrition and the age of the plant.

Field experiments were conducted in the first phase to determine the relationship between environment and the incidence of the disease. Epidemic development is a result of an increase in proportion of the leaves infected and the percentage area of the leaf affected. The general effects of weather on the prevalance and severity of yellow-rust are fairly well-known but the evaluation of the relative importance of the individual factors on the exact way in which these factors affect the final results seems to be a problem.

Germination behaviour is of primary importance for studies of pathogenicity. In the present investigation, the influence of climatic factors was mainly evaluated by determining the percentage germination on agar plates of spores collected from the field. In the preliminary experiments undertaken in May 1978, there was a great variation in the percentage germination of spores collected in the field. It was clear that factors other than climate

were involved, so the 1979 experiment was designed so as to make it possible to disentangle the various types of factor. Non-environmental factors which were considered included age and position of leaf, since the work in the previous year suggested that they played an important role in determining germination percentage.

2. Location and Design of the Plot:

A square plot 3m x 3m in the Botanic garden of Southampton University was chosen to be the site of the experiment. The square plot was situated with in a wire mesh cage designed to prevent disturbance from birds. Seeds of Maris Beacon were sown in the plot and an artificial epidemic of yellow rust was created by spraying the plants with Puccinia striiformis race 104E137.

3. Efficiency of the Plot:

Though the experiment was designed to be carried out on a winter wheat, the plot was rotavated and rolled in April 1978 for the first year's field work since the research programme did not commence until spring 1978. Wheat seeds dressed with an organomercury seed dressing and B.H.C. were obtained from the Plant Breeding Institute-Cambridge and were sown with the help of a Stanhay single row seed drill on 30$^{th}$ April 1978. The seeds were re-sown whenever necessary.

I.C.I. No.3 fertilizer (N:P:K = 9:24:24) was used to enrich the soil and a top dressing of Nitram at the rate of 250 kg. ha$^{-1}$. was applied before sowing the seeds.

The primary leaf began appearing on 14th May. When the wheat plants were in 12th stage of growth (Feeke's Scale-2nd leaf unfolded), the plot was sprayed with 2% 'Milstem' to prevent the plants from being attacked by powdery mildew. Two days after the spray of 'Milstem', the plot was inoculated with experimental disease.

4. Inoculation:

Vacuum-dried spores of yellow rust, race 104 E 137 was supplied by the National Institute of Agricultural Botany-Cambridge These spores were diluted with talc in the ratio of 1:20 and was placed in a 'squeeze' bottle. The inoculation was carried out in the latter part of the evening when it had become relatively cool on 15th May. Before inoculation, the wheat plants were sprayed with water to dampen the leaves. A polythene sheet was spread over the plants. The spore-talc mixture was gently spread across the field to get an even distribution of the inoculum. The polythene sheet was anchored over the plants to avoid the inoculum being blown away by wind. Infection of wheat leaves by uredospores of yellow rust can occur only in the presence of free water on the leaf surface as this is required by the pre-penetration phases of infection. The polythene sheet was left over the plants overnight to create a saturated atmosphere for the plants to get free water and thereby for the inoculum to pick up infection. As the following day happened to be cool, the polythene sheet was left covering the plot for a period of 48h.

After 15 days as there was no trace of rust development the plants were re-inoculated in a similar manner. No reason could be identified for the failure of infection.

5. Experimental Design:

a) The Germination of Spores Collected from Various Leaves:

Although the failure of rust infection in the first instance cannot be accounted for, the re-inoculation did initiate the primary infection. The type of weather that followed the release of spores may have influenced the germination potential. This observation shows the adaptation of the pathogen to a fairly precise set of environmental conditions for optimum growth is apparant. The outbreak of this primary infection later developed into epidemic proportions.

In the first set of preliminary experiments, thirteen observations were made from 13$^{th}$ August 1978. Spores from the field were daily collected in the evening from leaves of five different plants at random which were seeded on to three previously prepared Oxoid No.3 agar plates with a sterile paint-brush. These agar plates were then incubated in the dark at 5°, 10° & 15°C respectively. Spore counts were made after a period of 24h. About fifty spores per field were assessed and six such fields were counted in each Petri-dish. Care was taken to avoid clumping of spores and clumped spores when present were not considered for spore counts. If the length of the germ-tube exceeded the smallest diameter of the spore the spores were scored as germinated.

b) The Germination of Spores Collected from Different Ages of Leaves and Spores:

Burrage(1970) working on P.graminis reported a relationship between germination rate and total germination, and pustle age and duration between spore collections. Cochrane(1945) using uredospores of Phragmidium mucronatum of different ages attributed differences in germination rate and capacity to the degree of maturity of spores. Manners(1978) believes the rate of ageing of pustules to be the result of age of leaf. The above workers have shown matured spores to germinate better than the freshly formed spores.

The physiological condition of the leaf is another factor which has now been reported to affect the germination of yellow rust uredospores. This might account for the difference in the percentage germination between spores collected on the same day from two different leaves and incubated under similar conditions. Therefore a standard technique was adopted to study the effect of germination in relation to the age of the spores and age of the leaves.

The second experiment began on 14$^{th}$ October 1978 and seventeen observations were made in all. The site of the experiment was once again the same plot as described in Materials and Methods section (Refer P. 71 ) with change in mode of our technique. All the metereological instruments were adjusted and set near the plot. The charts were changed weekly and the readings were calculated for each period of 24h.

Ten experimental plants were selected at random from different corners of the plot. These ten plants were divided

into two groups, 'A' and 'B'. 'A' denoted shaken leaves where after the spores had been collected daily and seeded on to agar plates, the leaves were shaken vigorously to remove any loose spores adhering to the leaves. On plants marked B, the excess spores were allowed to remain on the leaves after collection so that the freshly formed spores were always mingled with the old and matured ones. This arrangement differentiated between young spores forming a fresh on the surface of shaken leaves and a mixture of young and matured spores obtained from unshaken ones.

To differentiate the age of leaf, second, third and fourth leaves were selected at random. The selection was quite limited as the infection had picked up considerably and the leaves selected had quiet a deal of infection on them. The leaves were labelled as young, matured and senescent according to the physiological condition of the leaves. A jeweller's tag with the details about the nature of the leaf and spores was tied around the experimental leaves. The spores were seeded on to previously prepared agar-plates with a paint-brush. Spore collections once again were made in the evenings. These agar plates were incubated at 5°, 10° & 15° C respectively. Spore counts were made after 12h and 24h. Spores were evenly spread on agar surface preventing spore clusters as for as possible. Fifty spores per field and three hundred spores in all were counted per Petri-dish. The length of the germ-tube determined the germination of the spores.

c) The Germination of Spores from Leaves of Different Age and Position:

James(1969) suggested that the assessment of a disease on individual leaves is required to give a clear picture of the spread of an epidemic as the severity of infection is supposed to differ considerably from leaf to leaf on the same plant. The method is less prone to error than others as leaves are assessed individually for disease incidence. Last(1953) found that upper leaves of wheat were less prone to mildew than were the lower leaves. Ohm and Shaner(1976) has shown pustule size in P.striiformis to be larger on the flag leaf than on first and second leaves below the flag leaf. Russell(1976) has shown higher percentage germination on the adaxial surface of the flag than on that of the second leaf. There have been reports that position of leaf may induce or retard the germination of spores. Khairi and Preece(1979) with their work on powdery mildew of wheat have shown the effect of leaf position on germination percentage to be highly significant and conversely the differences within groups of leaves of the same numbers were not significant. Our objective was also to examine the potential interaction between position of leaf, age of leaf, environment and germination.

The investigations began on 8$^{th}$ June 1979 on spores grown in the field. The plot was again in the University Botanic garden inside the cage but an adjacent plot to the one used in 1978 was chosen to be the site of experiment. The size of the plot remained the same i.e. 3mx3m. The seeds of Maris Beacon were sown this time in winter . Development of the crop was favoured by the frost and snow during winter and the mild wet spring. The plants were healthier than the one grown in summer and this considerably helped the spread of the disease.

The plot was rotavated and rolled in November'78. Seeds of Maris Beacon dressed with organomercury seed dressing were sown in the first week of December'78 with stanhay single row seed drill and re-sown by hand whenever necessary. The soil was enriched by the addition of I.C.I. No.3. fertilizer and a top dressing of Nitram.

All the metereological instruments were calibrated and arranged near the plot. The charts were changed on every Monday morning. Care was taken to record the weather data regularly. Only the Solar Radiation Integrator had to be placed inside the glasshouse in the Botanic garden with the recording head clipped outside the glasshouse at the top of the roof as there was no provision inside the cage. The total amount of radiation was directly read from integrator every 24h.

A very cold winter and limitation of sunlight delayed the germination of the seeds. Only in April'79 did primary leaves begin to appear. Another dressing of I.C.I. fertilizer was added to the soil. When the second leaf had completely unfolded, the plants in the plot were inoculated. Just 2 days prior to inoculation, the plot was sprayed with 'Milstem' as a routine measure to prevent the natural infection of powdery mildew on wheat plants.

The plants were inoculated on 14$^{th}$ April 1979 in the latter part of the evening when it was cool. The inoculation was carried out in the same manner as described before (P. 71 ). Once again the cold weather and the shorter photoperiods prolonged the incubation period. In April-May, mean temperatures remained very low (5-7°C) restricting the growth of the pathogen while a warmer period towards the end of May and the beginning of June provided more

suitable conditions for fructification and the general development of the disease. Within a few weeks this led to the propagation of the disease throughout the plot.

By the time the epidemic had fully developed not many primary leaves were available for the examination of effect of leaf position as such leaves were drying.

The experiment lasted for nearly fifty days. Ten different shoots that were attacked by yellow rust were selected at first. Five of these were original experimental plants and the other five were used as duplicate ones in case of need. These five plants were called A, B, C, D and E respectively. Contrary to usual occurrance of 7 leaves in each shoot, only five leaves were observed throughout the plot. The leaves of individual plants were numbered in an ascending order from the primary leaf($L_1$) to the flag leaf($L_5$). This not only gives an idea of positional effect but also accounts for the effect of age in relation to environment and its response on spore germination. At the start of the experiment it was mainly $L_2$ and $L_3$ which had a generous growth of the fungus, $L_1$ occassionally adding to the boom.

As our previous results indicate no significant difference in germination of spores of different ages, it was decided to shake off all experimental leaves after daily collection of spores from the field. As a result, studies were made on freshly formed spores. The spores were collected daily in the morning and seeded on to previously prepared 2% Difco-Bacto agar plates with a sterile paint-brush. Utmost care was taken not to contaminate the Petri-dishes with spores from different leaves. About ten different paint-brushes were used which were all clearly labelled with the plant

and leaf numbers. The Petri-dishes were also labelled to avoid any confusion. The paint-brushes were cleaned with alcohol and dried in the oven at 20°C after use. Jewellers tags bearing the plant and leaf numbers were tied around each leaf of the experimental plant. The Petri-dishes were incubated at 5°, 10° and 15° C respectively. Spore counts were made after 24h. The assessment of germination was as described in the General Material and Methods section(P.72). The experiment lasted for nearly two months at the end of which time, the plants in the plot were completely infected with yellow rust. A few hot days in the last week of July made way for the appearance of aphids and prevented us from making any more observations.

## C. Multiple Regression Analysis:

### 1.Introduction:

The preliminary information from a series of plotted graphs in which the dependent variable was plotted against independent variables showed a sort of linear relationship. But the multiplicity of weather factors (independent variables) showing gross relationship with germination had to be separated to draw a correlation between the most important weather factor and germination of yellow rust. A multiplicity of environmental factors affect the progress of a disease with time and within certain area which with mathematical equations can help in predicting the outbreaks of epidemics.

Multiple Regression analysis has been widely used in the prediction of epidemics(Butt & Royle 1974; Burleigh et.al. 1972). Cox and Large(1960) working on potato blight epidemics has shown the importance of selecting just one or two climatic factors for predicting epidemics. The mathematical modelling helps to interpret biological features in mathematical terms. A mathematical model of the multiple regression type was used in expressing the effect of environment on the pathogen and host cultivar and other physiological factors relevant in the development of the disease.

### 2. Evaluation of the Data:

The results recorded in the field experiments were not suitable for fitting directly into the equation. Moreover, there was a vast amount of data and an initial analysis was achieved by various statistical techniques. The multiple regression analysis was initially used for screening some of the weather factors and

selecting the most important ones and assigning values to them for future analysis for better explanation. Regression equation for biological values were also computed immaterial of the climatological variables.

The experiments were carried out under field conditions where both host and the pathogen were exposed to natural weather prevailing at that time of the year. Two years of field experiments were carried out in summer. Meterological parameters were measured by placing equipments at the site of the experiment. No devices for measuring the micro-climate was used. The experiment was also designed to give certain information about the physiological status of the host. The events measured in the field were evaluated under controlled conditions but certain draw backs in the controlled cabinets prevented us from obtaining a complete correlation.

3. Preliminary Data Treatment:

A linear correlation (r) was established between two variables $x_1$ and $x_2$ for $n$ pairs of observations. 'r' is computed as,

$$r = \frac{x_1 x_2 - \frac{(x_1)(x_2)}{n}}{\left(x_1^2 - \frac{(x_1)^2}{n}\right)\left(x_2^2 - (x_2)^2\right)}$$

where $x_1$ and $x_2$ are two independent variables.

In the 1979 field data healthy and senescent leaves were assigned the numbers 1 and 0. The preliminary analysis brought no significant difference in germination at 5° and 10°C. So further analysis were all carried out for 10°C. As the effect of weather on individual replicate leaves gave approximately the

same results, the percentage germination from the same leaf number of different groups were combined for each leaf position. There was a wide difference in the total number of replicates, $L_1$ having the least. Therefore a correction was made for leaf position.

4. Correction Factor for Leaf Position:

The mean percentage germination was calculated individually for all the individual leaves of different groups by dividing total % germination by number of cases. From this the overall mean was calculated and by subtracting the individual mean for each leaf from over all mean for all the five leaves, a correction factor could be introduced in the analysis (e.g. mean of $L_1+L_2+L_3+L_4+L_5$ - mean of $L_1$). Table II shows the correction factor for each leaf.

Analysis of variance : Healthy leaves.
Effect of leaf number on germination at 10° giving correction for leaf position:

| Leaf no. | Total no. of leaves | Mean value | Correction for leaf no. |
|---|---|---|---|
| Group 1 | 5 | 23.4 | +28.8 |
| Group 2 | 12 | 35.8 | +16.3 |
| Group 3 | 68 | 55.5 | - 3.3 |
| Group 4 | 92 | 56.3 | - 4.1 |
| Group 5 | 141 | 50.3 | + 1.8 |
| Total | 318 | 52.2 | |

Table II

5. Analysis of Weather Data:

The weather factors used in correlation were temperature, humidity, rainfall, surface wetness, sunlight and speed of the wind. These six weather factors were further subdivided to give twelve independent variables interacting with the dependent variable. An interrelationship between these various weather factors was also established and their significant values are tabulated as shown in Table III . The factors considered were:

Temperature - maxima, minima, daily mean, average over 5 days.

Relative humidity- daily mean, hours of humidity below 75% and average humidity over 5 days.

Rainfall - amount of rainfall and duration.

Surface wetness - duration.

Sunlight - daily recorded units of energy

Wind - speed in Km

Temperature:

The temperature data obtained from a thermohygrograph at standard Stevenson's screen height were analysed as follows: the trace of the thermohygrograph read every two hours was used in calculating the average daily temperature. The daily minimum and maximum temperature were also extracted from the thermohygrograph charts and the computer was programmed to calculate the average temperature over a period of 5 days. Straib(1940), Tollenaar and Houston(1966) have reported the importance of temperature at the time of spore production and they have shown a higher optimum germination temperature for spores produced at a higher temperature. Burrage(1972) has shown a difference of +2°-3° C

between temperature at plant level and ambient temperature.

Humidity:

Similarly to temperature the humidity data was extracted from the thermohygrograph charts. Average humidity was calculated from 2h. traces of the thermohygrograph charts. But instead of minimum and maximum being calculated humidity was computed as number of hours below 75%. Burrage(1978) has shown a diurnal relative humidity, humidity cycle being highest at night and lowest during day time.

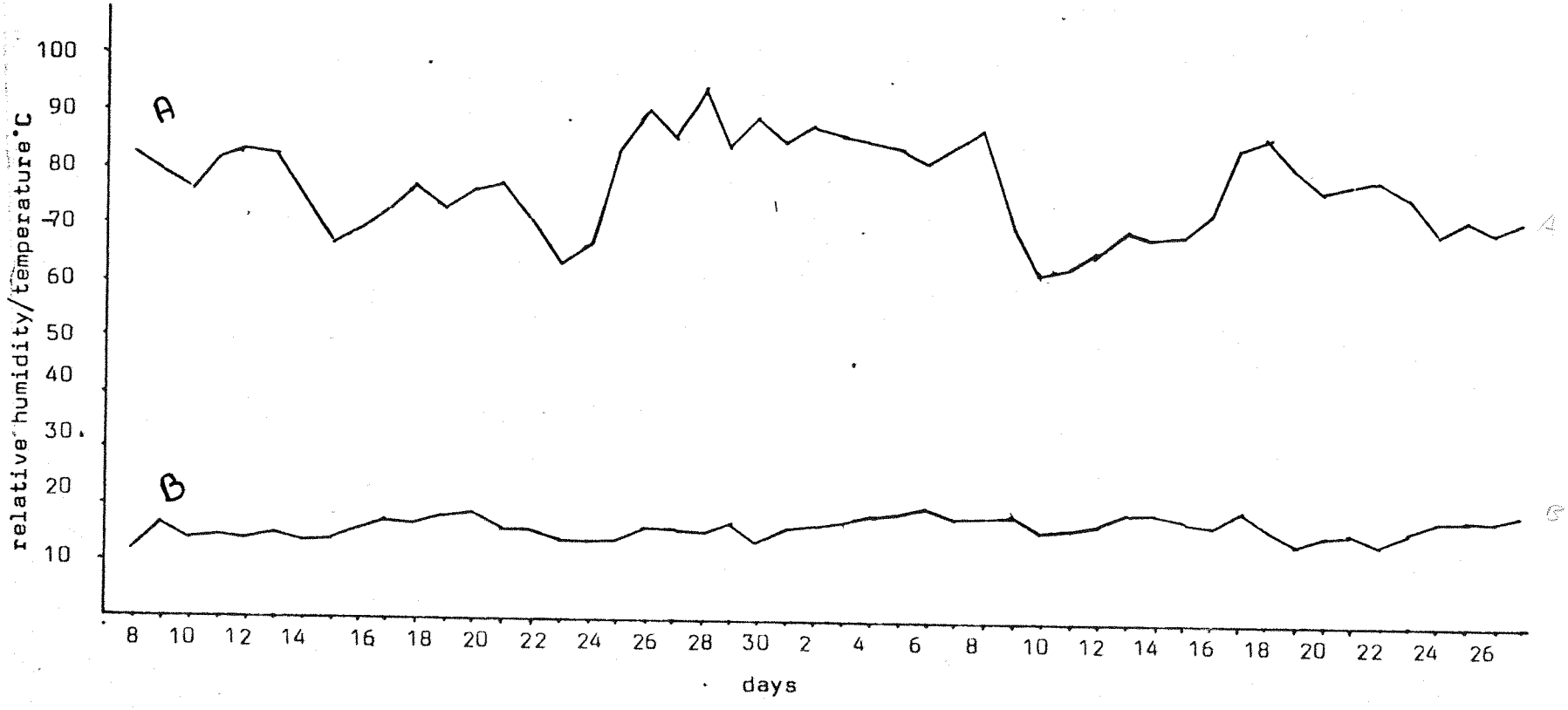
Speed of the Wind:

The meqn speed of the wind per day expressed in Km was directly obtained from the Meterological office.

Multiple Linear Regression Analysis:

A correlation was obtained between all the independent variables by using regression analysis as the independent variables should be independent of one another(Butt & Royle, 1974). A high correlation was obtained between mean temperature and humidity(0.99). Correlation coefficients between the weather factors are tabulated in Table III .

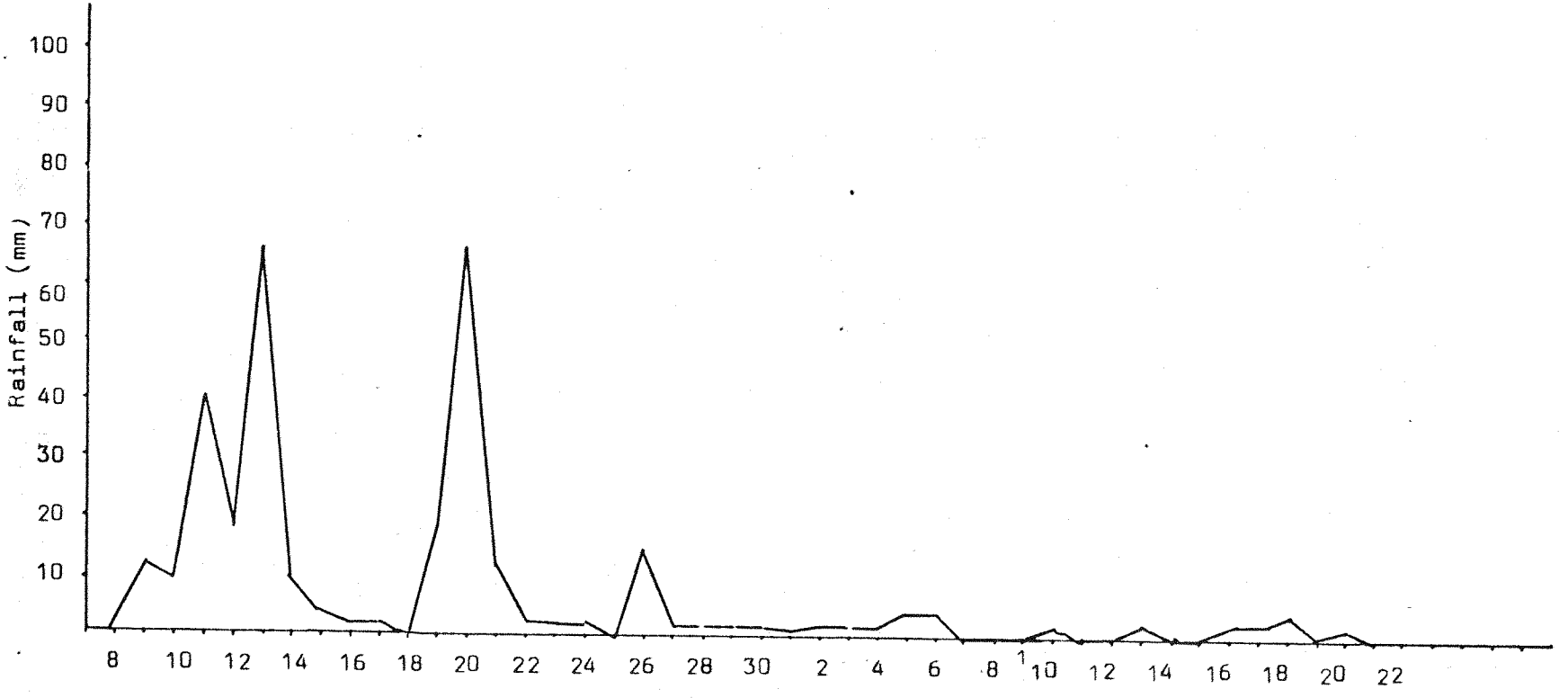
The evaluation of the 12 independent variables in relation to a dependent variable was achieved by using the stepwise selection programme (SPSS). To begin with a single linear regression is established between all the different variables. The association of a dependent variable with that of an independent variable is expressed as $y=a+b_1x_1$, where 'y' is a predicted value of the dependent variable (%germination),'a'is a constant, 'b' is the slope for the variable x and $x_1$ is one of the independent




Weather data extracted for June/July 1979

A- Relative humidity- mean relative humidity (%)  B- Temperature- mean daily temperature

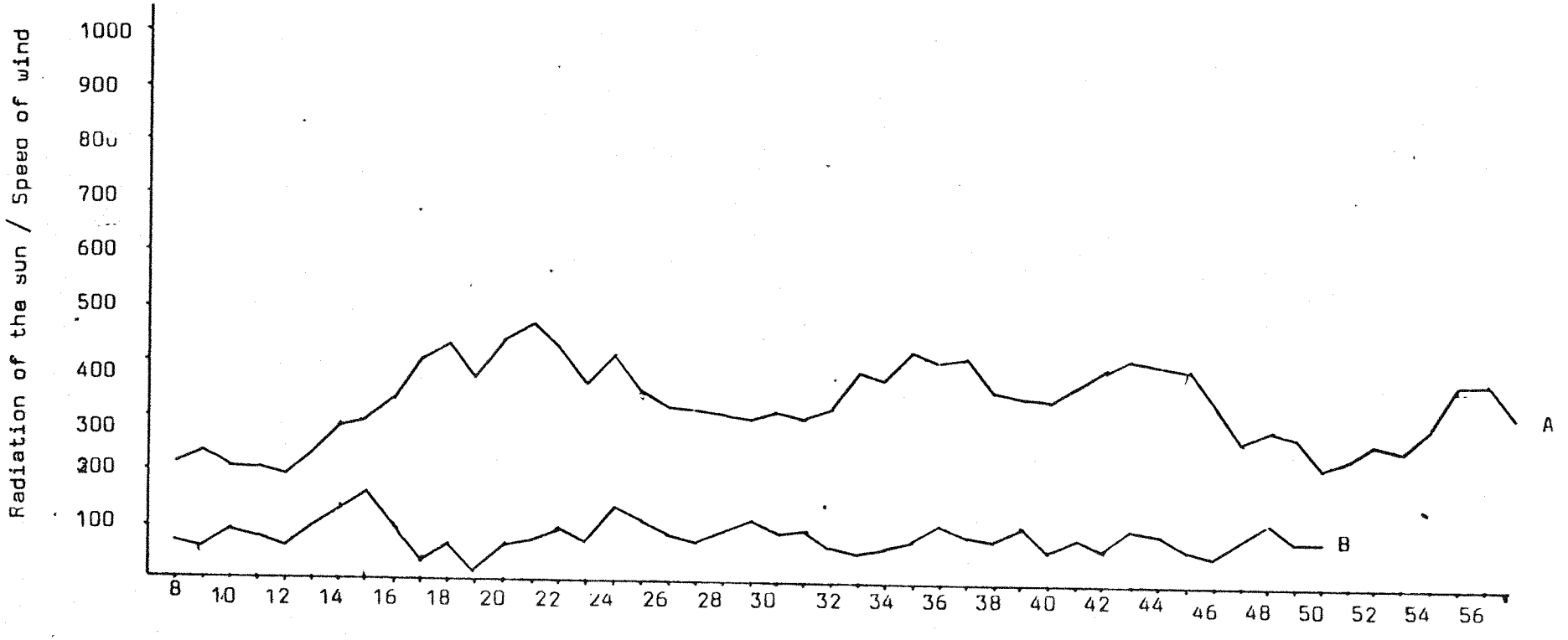
Fig. 11




Weather data extracted for June/July 1979

Rainfall (mm)

Fig. 12




Extracted Weather Data - June and July, 1979

A = Radiation of the sun ($mwh.cm^{-2}$)

B = Speed of wind ( $Km.sec^{-1}$)

Fig. 13

variables included in the regression. Similarly the results were analysed for 'n' pairs of independent variables.

It is well-known that a high percentage of humidity is essential for germination of spores and for infection in particular. But in the field, the plants are exposed to a fluctuation in climatic condition and therefore a variation in humidity. Hart(1926) and Rosen et.al.(1940) have shown greatest longevity of spores at 50% relative humidity. Humidity influences survival and growth and affects spore liberation. Mean humidity over a period of five days was also calculated for the weather data.

Rainfall:

The average daily rainfall in millimetreswas obtained from rain-gauge charts. The total number of hours it rained per day was also noted and included in the weather data.

Surface Wetness:

The surface wetness recorder pen was adjusted to be at 0° (base line) and the number of hours the trace on the chart was above 10°C was used asawetness function.

| | GERM10 | MINTEMP | MAXTEMP | MEANTEMP | RAINHRS | RAINMM | MEANHUM | LOWHUM | RADIATN | WIND | WETNESS | TEMPM5 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| GERM10 | 1.00000 | -0.90066 | -0.90066 | -0.88232 | -0.48516 | -0.39883 | 0.46021 | -0.48511 | -0.04916 | 0.20406 | -0.11493 | -0.95455 |
| MINTEMP | -0.90066 | 1.00000 | 1.00000 | 0.87790 | 0.22832 | 0.33580 | -0.75112 | 0.72099 | 0.22034 | 0.10307 | -0.21922 | 0.88795 |
| MAXTEMP | -0.90066 | 1.00000 | 1.00000 | 0.87790 | 0.22832 | 0.33580 | -0.75112 | 0.72099 | 0.22034 | 0.10307 | -0.21822 | 0.88795 |
| MEANTEMP | -0.88232 | 0.87790 | 0.87790 | 1.00000 | 0.15816 | 0.25640 | -0.54772 | 0.52730 | 0.46968 | -0.35830 | -0.31022 | 0.74502 |
| RAINHRS | -0.48516 | 0.22832 | 0.22832 | 0.15816 | 1.00000 | 0.83966 | 0.40325 | -0.40148 | -0.75874 | -0.03722 | 0.54365 | 0.62174 |
| RAINMM | -0.39883 | 0.33580 | 0.33580 | 0.25640 | 0.83966 | 1.00000 | 0.24237 | -0.34602 | -0.53737 | 0.15027 | 0.02105 | 0.55278 |
| MEANHUM | 0.46021 | -0.75112 | -0.75112 | -0.54772 | 0.40325 | 0.24237 | 1.00000 | -0.98096 | -0.50539 | -0.36717 | 0.41928 | -0.43297 |
| LOWHUM | -0.48511 | 0.72099 | 0.72099 | 0.52730 | -0.40148 | -0.34602 | -0.98096 | 1.00000 | 0.48178 | 0.29344 | -0.26827 | 0.44010 |
| RADIATN | -0.04916 | 0.22034 | 0.22034 | 0.46968 | -0.75874 | -0.53737 | -0.50539 | 0.48178 | 1.00000 | -0.37686 | -0.71477 | -0.19223 |
| WIND | 0.20406 | 0.10307 | 0.10307 | -0.35830 | -0.03722 | 0.15027 | -0.36717 | 0.29344 | -0.37686 | 1.00000 | -0.09746 | 0.04871 |
| WETNESS | -0.11493 | -0.21922 | -0.21822 | -0.31022 | 0.54365 | 0.02105 | 0.41928 | -0.26827 | -0.71477 | -0.09746 | 1.00000 | 0.19207 |
| TEMPM5 | -0.95455 | 0.88795 | 0.88795 | 0.74502 | 0.62174 | 0.55278 | -0.43297 | 0.44010 | -0.19223 | 0.04871 | 0.19207 | 1.00000 |
| HUMM5 | 0.76469 | -0.84341 | -0.84841 | -0.52349 | -0.48041 | -0.49496 | 0.59249 | -0.57274 | 0.28206 | -0.46900 | -0.11380 | -0.90212 |

Correlation Coefficient between various weather factors

Table : III

The single factor having the highest correlation with the dependent variable is selected in the single, linear regression equation calculating the significance of the F value. The subsequent steps follow a similar pattern until the number of degrees of freedom is reduced to one. Every independent variable was accounted for in the regression equation with the residual variable not being accounted. The variable which gave the most significant correlation was tested in the multiple regression equation to determine the significance of the variable.

The independent variables measured the effects of mete logical factors on spore germination. By using multiple regression analysis, the highly correlated independent variables were estimated independent of correlation between themselves. The model selected the independent variables according to their merit of estimating the correlation with out duplicating the factors.

The Correlation of Weather with Germination of Spores:

The data collected from the field in 1978 with weather data obtained from Metereological office were analysed using stepwise multiple regression analysis. Effect of weather on spores collected from different ages of leaves were individually analysed. Fairly good correlations were obtained in case of weather and germination of spores. Spore counts made after 12h and 24h were analysed individually in the case of all ages of leaves. No difference in correlation of weather was obtained between germination after 12h and 24h. Daily temperature in most of the cases had the highest F value showing it to be the most significant factor affecting germination. A further analysis was made by omitting the results

towards the end of the season when the leaves had naturally senesced and the results had nothing much to do with weather. Once again temperature turned out to be the major significant factor affecting germination. The effect of temperature remained nearly the same on spores collected and germinated from young, mature or senescent leaves. The data subjected to a stepdown selection method of multiple regression analysis eliminated all but three independent variables with significant partial regression coefficients accounting for differences in germination of spores. High correlations were obtained between germination and spores collected from young leaves with a low correlation coefficients between germination and spores collected from senescent leaves.

The analysis of the 1979 data gave very useful information on the influence of weather on spore germination. Germination was greatly influenced by the prevailing weather with a higher percentage on a cool, moist, day. A correlation coefficient between the different climatological paramaters were established. A high correlation was noted between mean temperature and humidity (0.99). As the multiple regression does not evaluate the merit of these parameters according to their correlation, the significance of the individual parameter is assessed independently. Interactions between environment and leaf position were significant. The analysis of germination of spores from individual leaves showed daily mean temperature or average temperature over a period of five days to be highly significant, germination decreasing with higher mean temperature (20-22°C). Longer hours of low humidity below 75% had a negative effect on spore germination. Total amount of

rainfall per day had a positive effect while surface wetness was found important in the case of $L_4$. The necessity of free water for germination is indicated by the significance of mean humidity and rainfall though they were less significant than temperature The significant interaction between temperature and humidity shows that germination results from their combined effects. When the data from individual leaves were corrected for leaf position, it was again the daily mean temperature that was most significant followed by a negative effect of low humidity (hours of humidity below 75%). There was a slight alteration in the order of significance Just in one case radiation of the sun had an effect on leaf position but wind, minimum or maximum temperature for a few hours during the day were unimportant in the germination of yellow rust uredospores.

In the analysis of effect of average temperature and average humidity over a period of five days, average temperature turned out to be the most significant factor. No significant differences were obtained between germination at 5° and 10°C. The higher the temperature of spore production the more the difference in germination at 5° & 10°C narrowed down. The effect of weather explained 56% of the variation in the data.

Analysis of Leaf Position and Age on Germination:

As the climatological variables explained only a proportion of the significant correlation, the effects of position and age of leaf were analysed separately. As all the leaves in the field developed infection at different time because of the different position on the plant a correction factor was introduced by subtracting the overall mean percentage for all the leaves ($L_1 \ldots L_5$)

from mean % germination for individual leaves ($L_1$)(ref.P.93) and incorporating the values in the analysis to correct for the effect of leaf on germination. The position of leaves were also analysed individually without introducing any correction factor. Though the position of leaf itself in a way is related to age of leaf on a shoot with the upper leaves being younger than the lower leaves, the data was divided into healthy and senescent leaves and analysed separately. Healthy leaves were defined as leaves that were green regardless of their position and senescent denoted leaves turning yellow or brown.

Factorial analysis of variance showed the effect of position of leaf on spore germination to be highly significant both before and after correction for leaf position. Germination of spores collected from $L_4$ was significantly better than that from other leaves (56.34) with germination of spores from $L_3$ and $L_5$ being 55.5 and 50.3.. Germination of spores collected from $L_2$ and $L_1$ had a low correlation coefficient ($L_2$ 20.4, $L_1$ 35.8). Spores collected from different groups of leaves of the same position did not vary significantly indicating clearly the importance of position of leaf despite the place or area where they are grown. The effect of weather on particular days did not change the effect of position of leaf on germination. The position of leaf in part explained 50% of correlation coefficient with germination.

Our analysis of germination of spores collected from healthy and senescent leaves showed the effect of age of leaf on germination. A significant difference in germination was obtained as in the analysis of the previous year results, spores collected from healthy leaves germinating better than senescent leaves.

But a slight alteration was indicated in the order of position of leaf in relation to its effect on germination. In case of senescent leaves, it was $L_5$ which germinated the best followed by $L_4$ and $L_3$, $L_2$ and $L_1$ giving poor germination. This may be due to variations in the age of leaf as only the colour of the leaf was used as an indication.

Effect of leaf no., date on germination at 10°C for healthy leaves:

| | Sum of squares | DF | Mean square | F |
|---|---|---|---|---|
| Leaf no. | 15344.977 | 22 | 7672.398 | 157.665 |
| Date | 33556.414 | 41 | 798962 | 16.418 |
| Leaf no, date | 586.554 | 9 | 65.173 | 1.339 |
| Explained | 41910.715 | 53 | 790.768 | 16.250 |
| Residual | 12846.941 | 264 | 48.663 | |
| Total | 54757.656 | 317 | 172.737 | |

Table IV

From the above table it is clear that the leaf no. and weather factors are significant individually but the low 'F' value (1.339) shows that there is no interaction between the two. Similar results were obtained for senescent leaves.

## 6. Results:

### Effect of Environment on Spore Germination:

In the preliminary experiments, no metereolgical instruments were used but generally the type of day was noted. Obviously a great variation in the germination of spores collected on different dates resulted normally with the spores collected on a cool, bright day giving a high percentage germination and falling drastically on a warm,sunny day. Occassional showers did enhance the rate of germination. Besides the direct effect of climate on spore germination, it was noted that there was a difference in percentage germination of spores collected on the same day and exposed to similar conditions but from two different leaves.

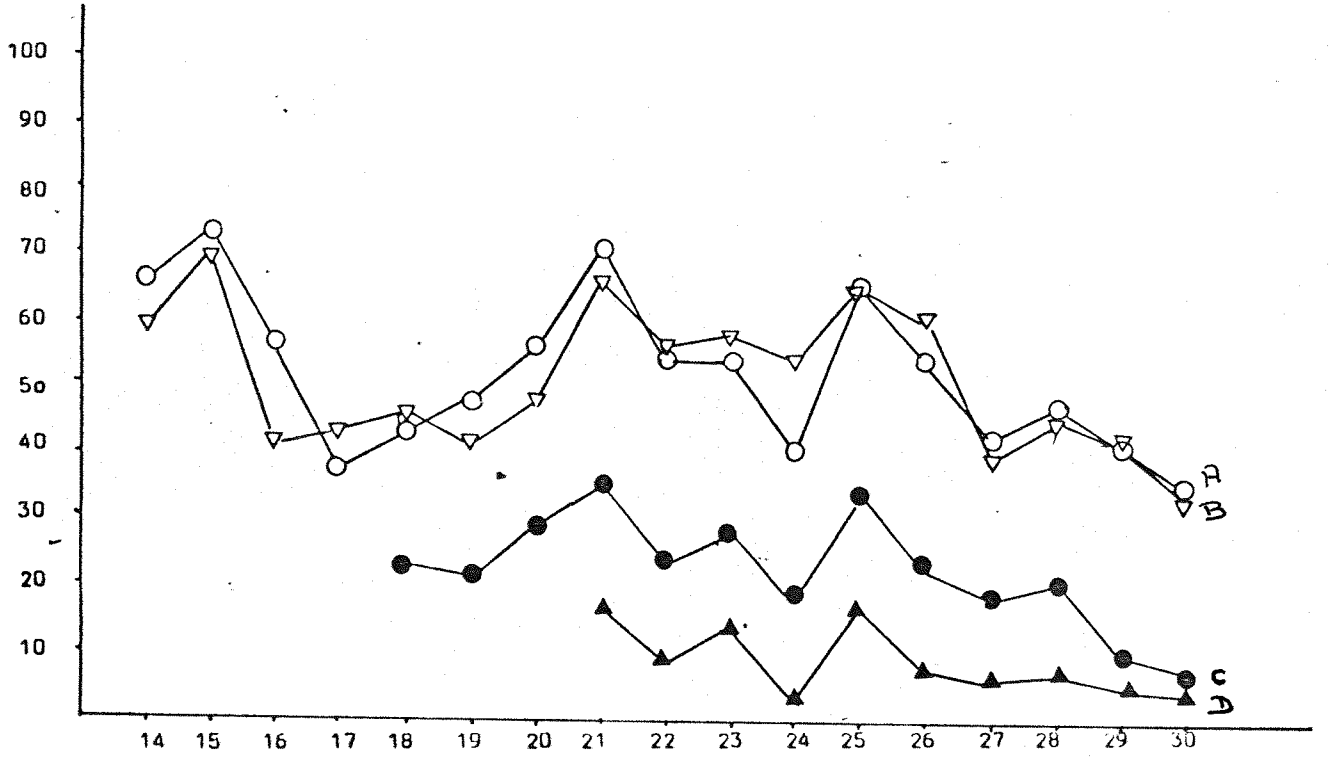
In the experiments carried out in 1979, the plot became completely infected by 10$^{th}$ of July and even the upper leaves began to senesce; it became clear that it would not be possible to continue the experiment for as long as it had been hoped, and it had to be discontinued. In all 17 observations were made. There were some missing data in the metereological readings recorded in the field. A complete set of weather data was therefore obtained from Southampton Metereological Observatory. In addition to the metereological data obtained from the observatory, notes were made on the general weather type each day. The readings were recorded hourly and the daily mean was calculated. Both the weather data and the germination percentage were fed onto the computer to determine the correlations. The computer analysis is discussed under multiple regression analysis.

After the second inoculation, the latent period was 10-12 days, after which interval the pustules began to appear on the second and third leaves in the field. As the leaf aged, the long broad yellow stripes were seen on the leaf surfaces. Numerous plants in the field became affected simultaneously. The leaves bearing yellow stripes began to sporulate 14 days after inoculation. The rate of progress of the disease was very rapid and the entire length and breadth of the leaves were colonised.

The weather data obtained from the Metereological office clearly shows the mean daily temperature of 8-10°C to have favoured the growth of yellow rust on Maris Beacon.

Environment had a role to play in the germination of spores as well. Germination varied from 10 to 90% on young leaves. Spores collected on a cool bright day germinated the best. Hot, sunny days decreased the germination percentage as noticed in the preliminary observations. Both occassional rain and dew enhanced the percentage germination of yellow rust uredospores. Spores incubated at 10°C (±1°C) germinated the best in all ages of leaves. Germination percentage of spores incubated at 5 and 15°C were nearly the same. (Fig. 14, 15).

The 1979 field work gave positive results for future work. But for the missing readings in the anemometer charts, all other metereological data were analysed for every two hours and the daily mean calculated from the chart. Both the field and the weather data were computed and establishment of their correlations are discussed under multiple regression analysis.

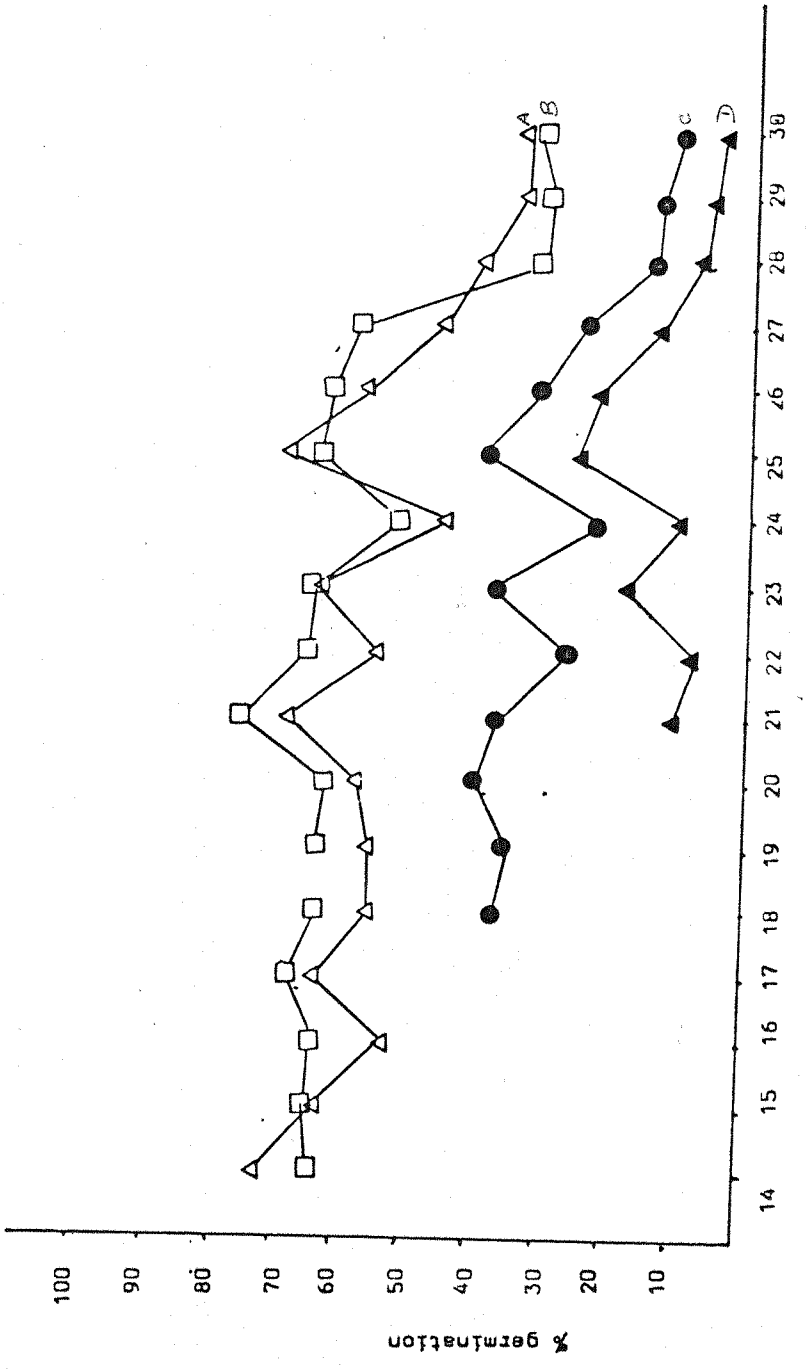



24 hours

%germination of spores collected from different leaves (shaken)

Fig. 14

A - 5°C
B - 15°C
C - 20°C
D - 25°C




A - 5°C
B - 15°C
C - 20°C
D - 25°C

% germination on different days (unshaken)

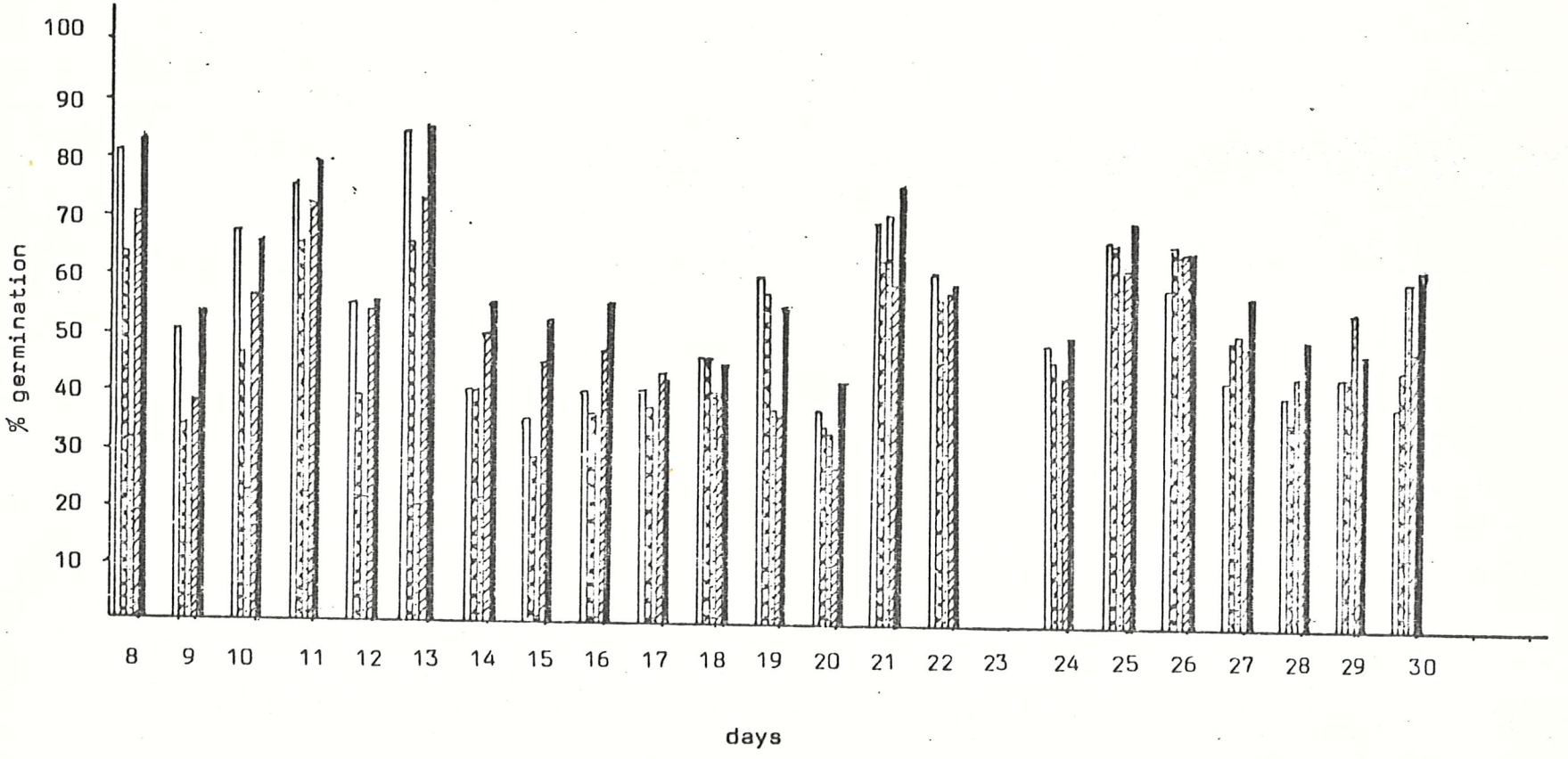
Fig. 15

This year there was a prolongation of latent period with the pustules appearing nearly 35 days after inoculation. Once again it was mainly due to a daily low mean temperature and a limited amount of sunlight. The rate at which the pustules were produced were slow and their distribution less dense for the first week but with the onset of a warmer weather towards the first week of June, prominent stripes were seen on leaf surfaces and there was a rapid progress of the disease thereafter.

The entire leaf surface was colonised in most instances and more often than not it was the tip of the leaves that were infected, an observation in agreement with Russell's report of better germination at the distal than at the basal end of the leaf. Occassionally the spores produced at the distal end failed to infect the 3$^{rd}$, 4$^{th}$ and 5$^{th}$ leaves.

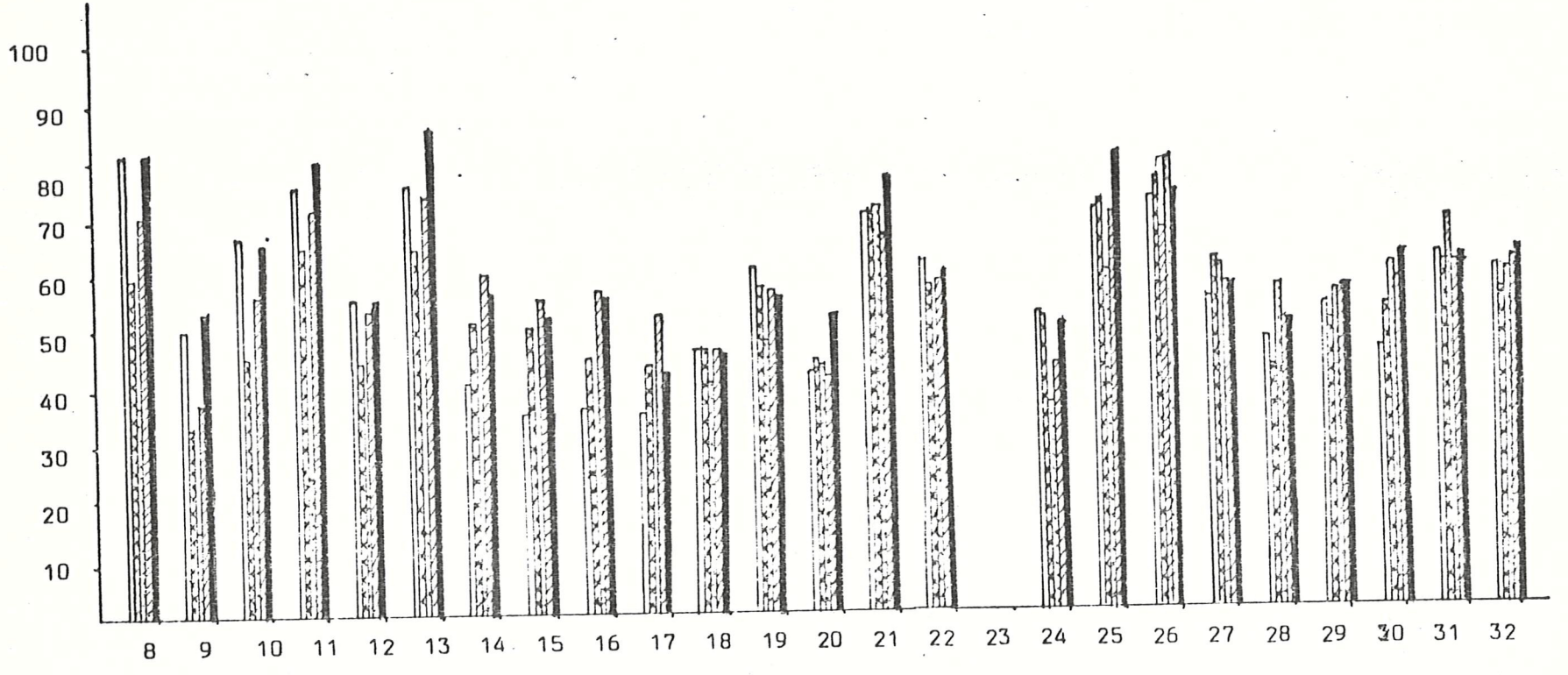
There was a good dissemination of the spores as a great amount of infection was built up on the flag leaves. Warmer weather promoted the rapid spread of the disease.

Certain unavoidable discrepancies were encountered during the course of the experiment. Not all leaves produced spores at the same time. As a result there was a difference in the prevailing weather during the course of spore production on individual leaves. When a marked plant leaf senesced and failed to produce spores, a duplicate leaf was used which sometimes caused a difference in age of the leaf. In analysis, germination results were separated according to whether the spores had been collecded from healthy or senescent leaves. The physiological factors like age of leaf and the age of spores that could affect the rate of germination in



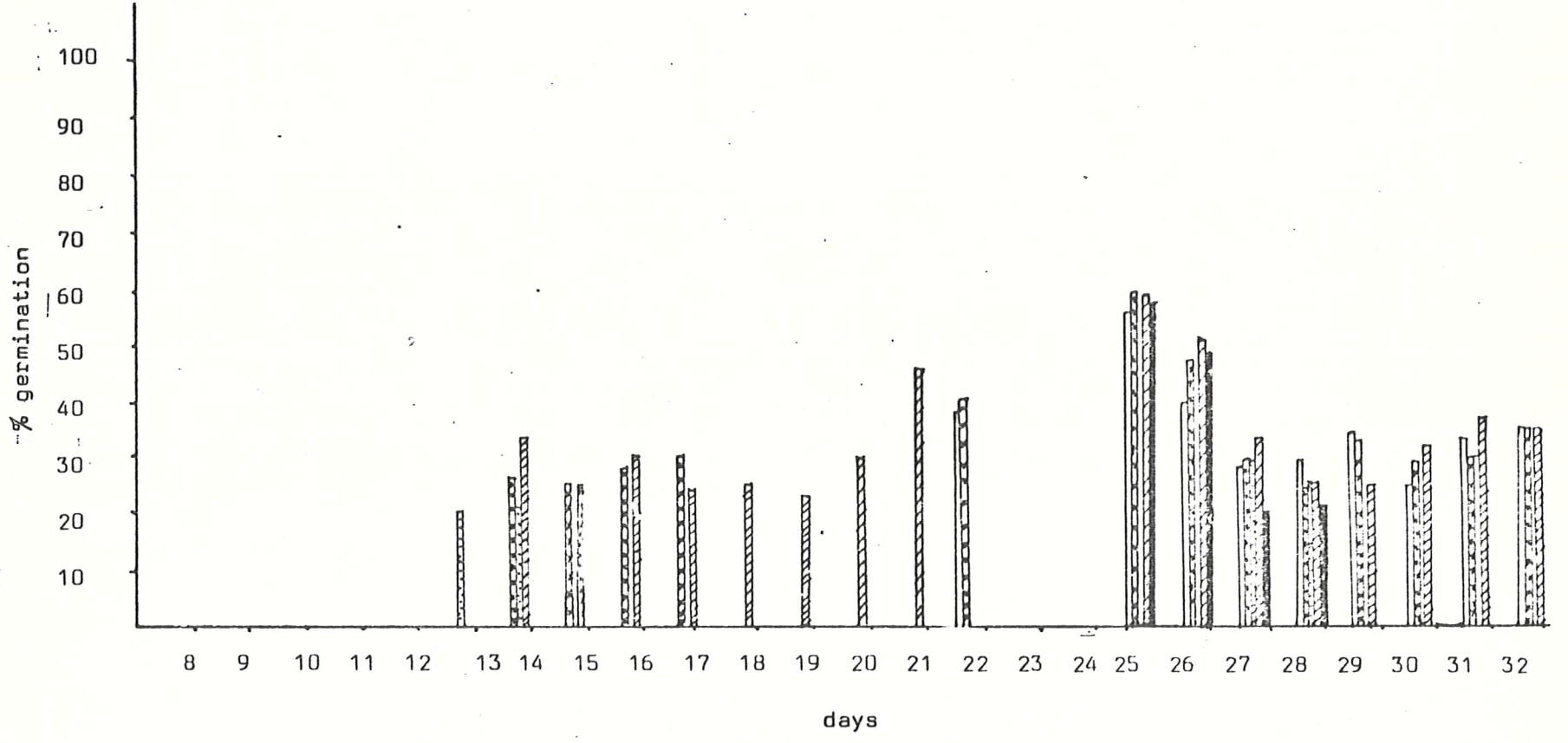

Total percentage germination of spores from five different shoots

Fig. 16




Percentage of germination spores collected from healthy leaves of five different shoots.

Fig. 17




Percentage germination of spores collected from senescent leaves of five different shoots.

Fig. 18

relation to environment are discussed below.

Effect of Age of Leaf and Spores on Germination:

Regarding the spores collected from group A (young spores) (Ref. Materials and Methods) and B (matured spores), the variation was within the 5% range. No significant difference was obtained on any day as contrary to the situation observed by Burrage in case of P.graminis. On the contrary, newly formed spores collected from group A plants germinated better, though not significantly better than those from group B. The results are tabulated in Table V & VI

Age of leaf, on the contrary, had a definite effect on the germination of spores. Spores collected from young leaves always germinated the best under all weather conditions and in all the three different temperatures. Spores collected from matured leaves did germinate well but the percentage germination of the spores collected from the senescent leaves was much lower than the spores from healthy leaves. This was true in case of both A and B (Fig. 19 & 20) clearly indicating the significance of physiological conditions of the leaf for the satisfactory germination of yellow rust uredospores.

Mean % germination of spores, seventeen days observation of shaken leaves:

| Age of leaf | 5° 12hrs | 5° 24hrs | 15° 12hrs | 15° 24hrs | 20° 12hrs | 20° 24hrs | 25° 12hrs | 25° 24hrs | 30° 12hrs | 30° 24hrs |
|---|---|---|---|---|---|---|---|---|---|---|
| Young | 64.5 | 78.6 | 69.3 | 86.8 | 29.6 | 42.9 | 13.5 | 20.0 | 1.40 | 5.00 |
| Mature | 56.7 | 70.2 | 60.2 | 74.5 | 29.6 | 38.3 | 11.2 | 18.8 | 2.60 | 3.00 |
| Senescent. | 15.0 | 19.0 | 17.5 | 21.1 | 5.8 | 8.0 | 2.3 | 3.0 | 0.2 | 0.1 |

Table V

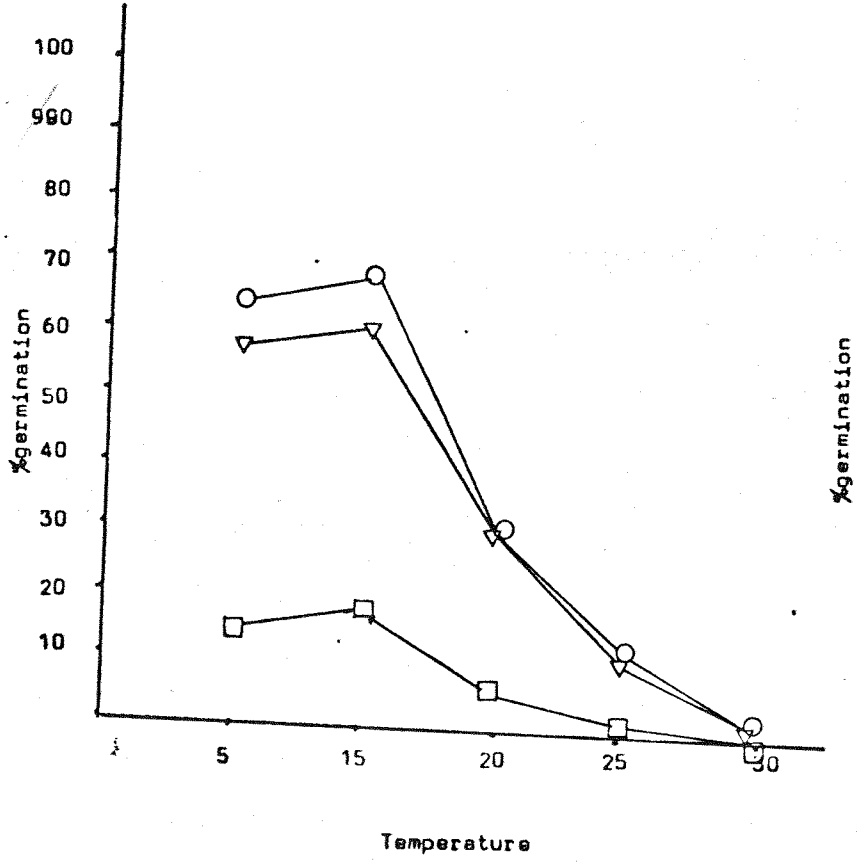
Readings taken after intervals of 12 and 24 hrs that was incubated at various temperatures.

Mean % germination of spores: seventeen days observations of unshaken leaves :

| Age of leaves | 5° 12hrs | 5° 24hrs. | 15° 12hrs | 15° 24hrs | 20° 12hrs | 20° 24hrs | 25° 12hrs | 25° 24hrs | 30° 12hrs | 30° 24hrs |
|---|---|---|---|---|---|---|---|---|---|---|
| Young | 61.3 | 71.8 | 70.0 | 82.8 | 28.3 | 35.7 | 8.4 | 14.2 | 0.7 | 2.4 |
| Mature | 54.0 | 65.3 | 60.5 | 70.0 | 25.3 | 33.6 | 10.4 | 15.0 | 1.4 | 4.0 |
| Senescent | 12.7 | 16.7 | 14.3 | 20.0 | 4.7 | 7.5 | 0.5 | 2.3 | - | - |

Table VI

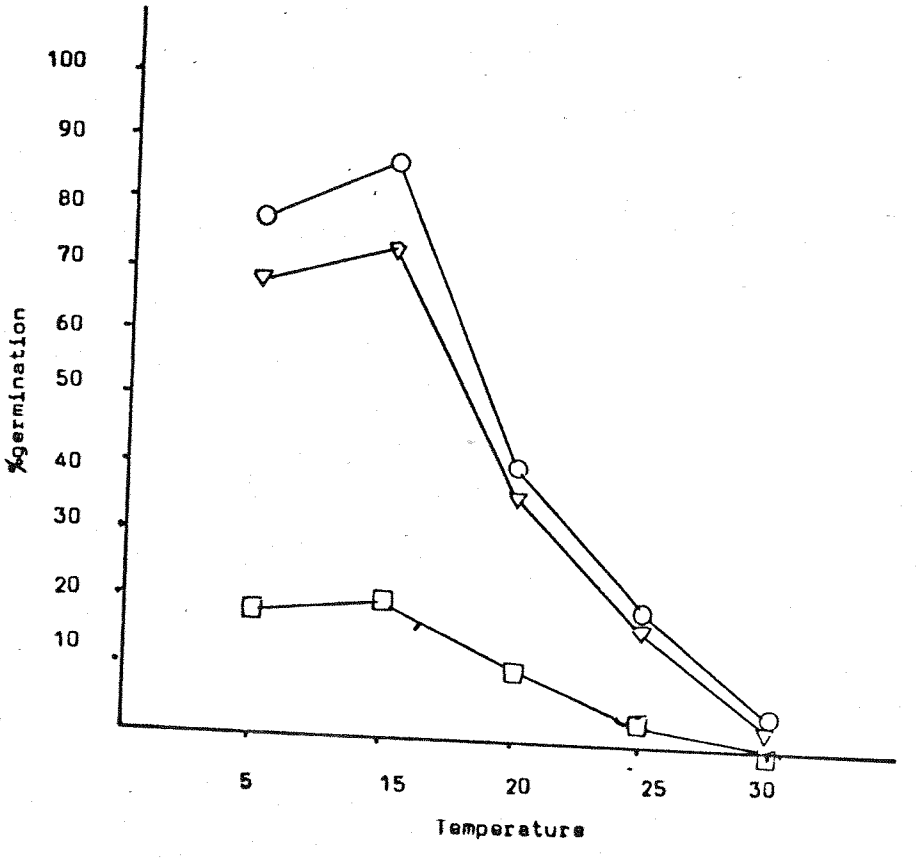
% germination of spores collected from unshaken leaves




after 12 hours

○ young △ mature □ senescent

Fig. 19




after 24 hours

● young ▲ mature ■ senescent

Fig. 20

% germination of spores collected from shaken leaves

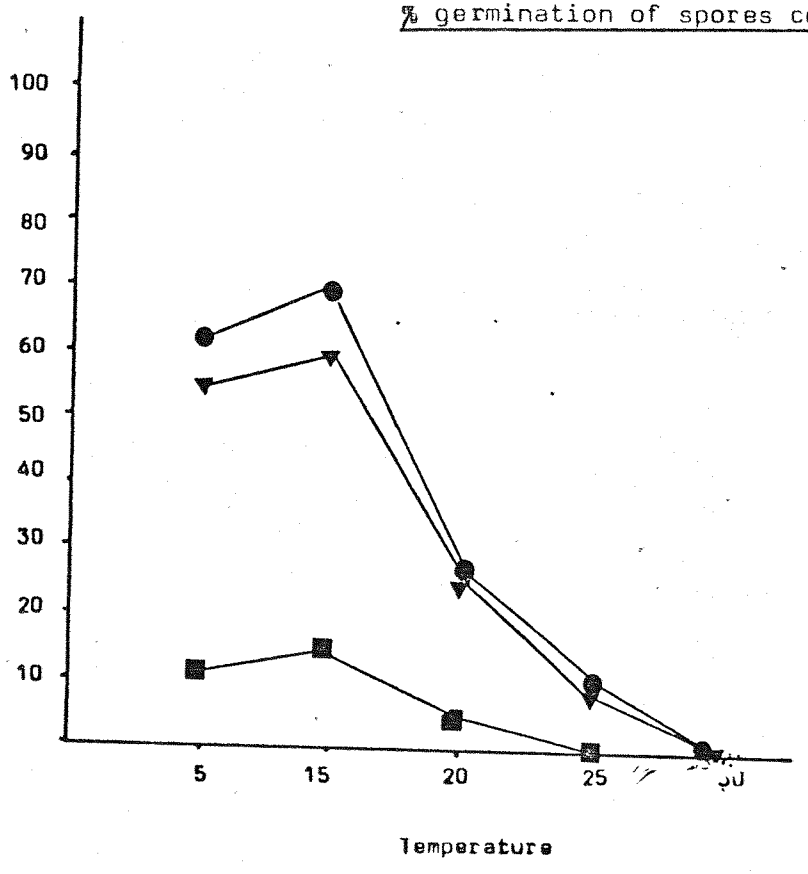



Fig. 21

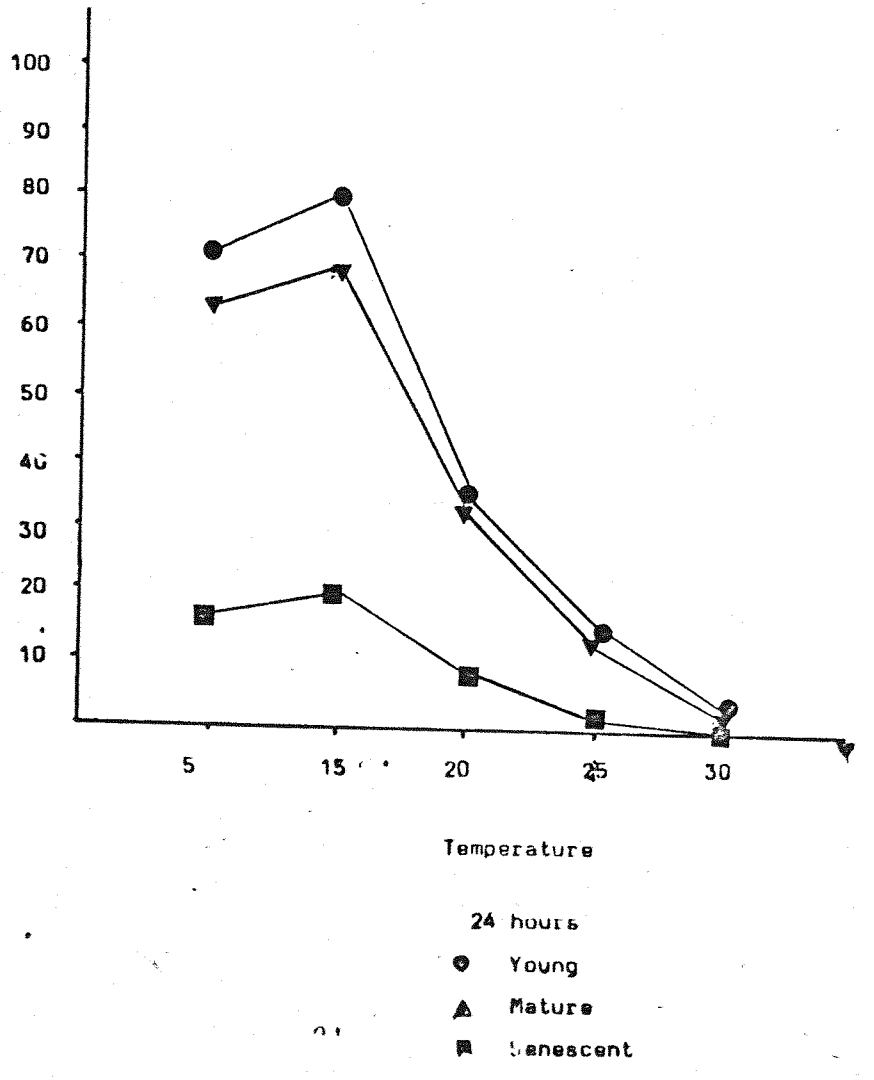



Fig. 22

Effect of Age and Position of Leaves on Germination:

Considering the germination of spores collected from individual leaves according to their position, spores collected from primary leaves ($L_1$) always resulted in poor germination with only about 20% even on a favpurable day. Spores collected from second leaf ($L_2$) germinated anywhere between 36-40% immaterial of the climatic conditions. $L_3$ and $L_5$ germinated well resulting in 65-80% on ideal weather conditions while $L_4$ showed 90% germination on such occassion. Compensation for positional effects also resulted in similar results. The same leaf numbers from different groups of plants always gave approximately the same percentage germination. Germination of spores collected from healthy leaves was always higher than the ones from senescent leaves. Germination at 10°C though not significantly different was better than 5°C. These results clearly shows that besides environment, the individual leaves of a shoot play an important role in the germination of yellow rust. This may be due to a multiplicity of non-environmental factors like presence of micro-organisms on leaf surface, leaf exudates or the difference in micro-climate prevailing on the surface of individual leaves because of the position.

Statistical Analysis:

Multiple Regression is an invaluable analytical tool in a study of natural field events. Significant correlations may have immediate practical value. The effects pf environmental factors are often of decisive importance in disease development and they have been taken into account in disease forecasting (Krause et.al.,1975) and they are factors over which no control

can be exercised. Epidemics of yellow rust in winter wheat have also been recorded only one in six years but they are associated with the breakdown of resistant cultivars to new virulent races of the pathogen.

Our analysis of the data gave significant correlations. Most of our correlations were in agreement with Preece's(1976) work on conidial germination of Erysiphae graminis. The weather during the field work was fluctuating and very suitable for comparing the relationship between dependent and independent variables. The statistical analysis provided a reliable correlation between germination, germination and humidity.

Yellow rust of wheat is very sensitive to external factors and changes in the atmosphere and direct statistical analysis may not supply the complete evidence of the relationship with weather but the statistical analysis helps in screening rationally the chosen variables measured during disease developed and that are assumed to influence the germination; the final analysis being limited by a single weather factor, temperature in combination with humidity shows a linear relationship with germination of yellow rust uredospores.

Analysis of position of leaf and age of leaf suggest a significant effect on germination. Spores from younger leaves germinate better than from older leaves. Spores from $L_4$ germinated better than spores from any of the other four leaves. These results are in agreement with those of previous workers on different crops (James et.al.,1969). The results of germination of spores from $L_4$ indicates the stage of growth where the yellow rust germinate

| Leaf position | Temp $M_5$ | Wetness | Wind | Radiation | Mean Hum. | Min. Temp. | Max. Temp. | Mean Tem. | Rain hrs. | Rain min. | Low Hum. $M_5$ |
|---|---|---|---|---|---|---|---|---|---|---|---|
| $L_1$ | (-) 1 | | | | | | | | | | |
| $L_2$ | | | | (+) 3 | (-) 2 | | | (+) 4 | | | (-) 1 |
| $L_3$ | (+) 6 | (-) 4 | 7 | | 8 | | | 2 | 5 | (+) 3 | (-) 1 |
| $L_4$ | (-) 2 | (+) 1 | (+) 4 | | | | | | | (+) 3 | |
| $L_5$ | (-) 3 | | | | | | | | (+) 1 | (-) 3 | (-) 2 |
| Leaf corr. for leaf position. Healthy | | | | | | | | (-) 1 | (+) 3 | | (-) 2 |
| Leaf corr. for leaf position. Senescent | | | | 4 | | | | (-) 1 | (+) 2 | | (-) 3 |

Table VII

Correlation of Weather with Germination

(significant factors selected by computer analysis)

| | Temp. $M_5$ & Mean Temp. | Mean Hum. & Low Humid. | Rain in hrs & mm.. | Wetness | Radi-ation |
|---|---|---|---|---|---|
| $L_1$ | (-) 1 | | | | |
| $L_2$ | (+) 4 | (-) 1 | | | (+) 3 |
| $L_3$ | (-) 2 | (-) 1 | (+) 3 | (-) 4 | |
| $L_4$ | (-) 2 | | (+) 3 | (+) 1 | (+) 4 |
| $L_5$ | (-) 3 | (-) 4 | (+) 1 | | |
| Leaves corr. for leaf position | (-) 1 | (-) 2 | (+) 3 | | |
| Healthy Leaves corr. for leaf postion | (-) 1 | (-) 3 | (+) 2 | | (+) 4 |
| Senescent Diff. in germ. | (-) 1 | | | | |
| Min.temp/Max.temp./ wind/Hum.$M_5$ | not significant. | | | | |

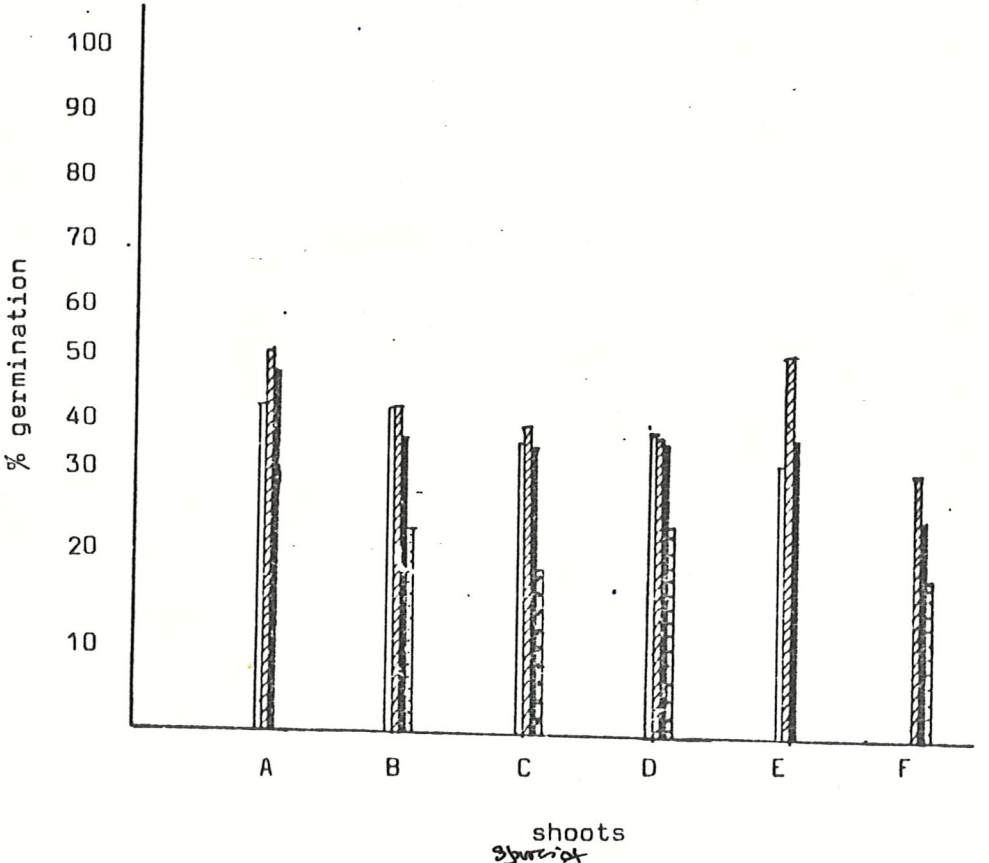
Table VIII

Analysis showing the highly significant correlation factors

the best.

Such results can help in assessing the loss in yield or help in forecasting systems.

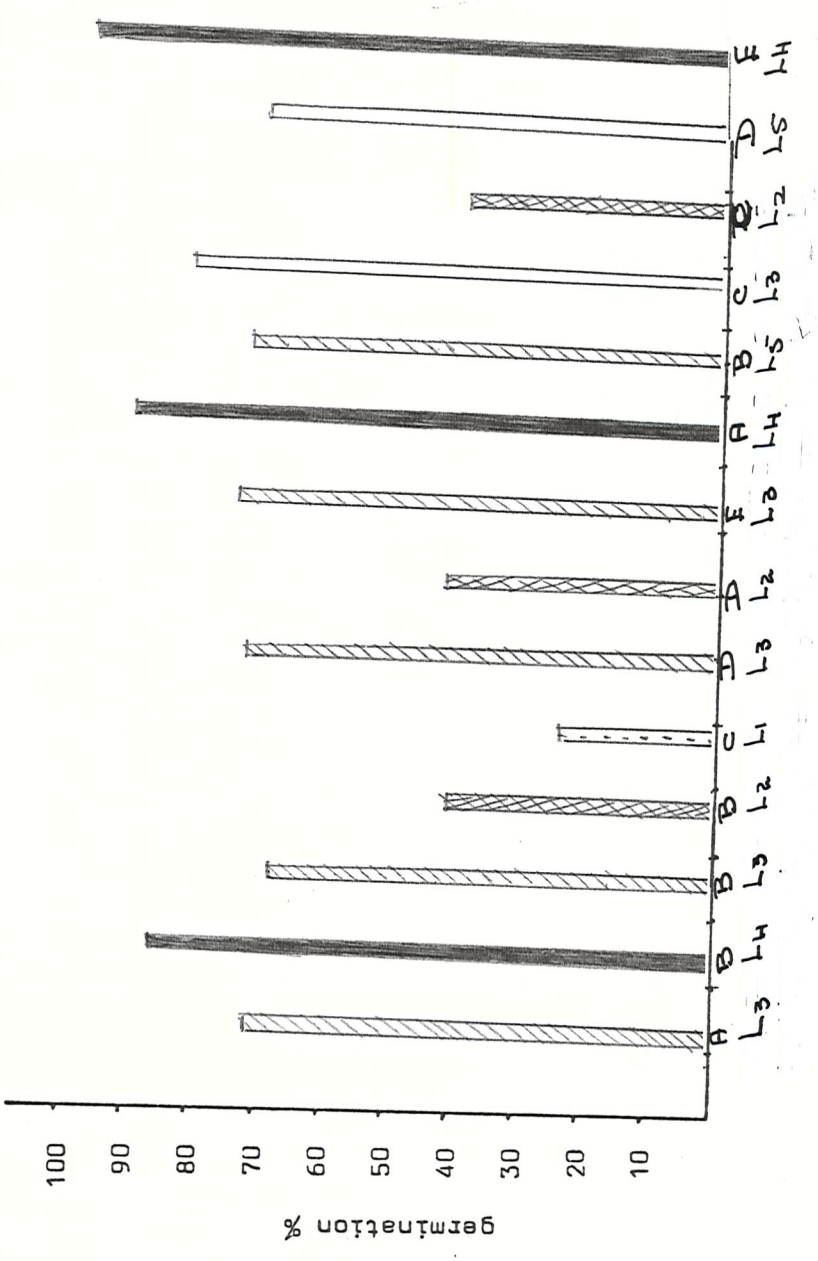
The results of the field experiments were confirmed under controlled environmental conditions. The experiment designed to give conclusive results could not be completed at the controlled growth cabinets did not have the facility of adjusting changes in humidity. Therefore the effects of temperature and age of leaf alone could be performed and the results are discussed in the chapter on controlled environment experiments.




Total % germination of five different leaves of the experimental shoots for a period of 50 days. (A--F; 5 different shoots)

$L_1$ $L_2$ $L_3$ $L_4$ $L_5$

Fig. 23




Spore bearing leaves of different shoots

% germination of spores collected from different ages of leaves and incubated at 10°C.

Fig. 24

D. Discussion of Field Results:

1. Effect of Environment in Relation to Spore Germination:

Environment has a significant influence on uredospore germination. Environment influences the photosynthesis, transpiration and metabolism of the host plants. These in turn determine the availability of nutrient material both for the host tissue and the pathogen. The fluctuation in environmental factors results in change of state of the host and consequently affect the germination of spores.

Rapilly(1979) has reviewed the quantitative epidemiology of yellow rust paying particular attention to the effects of climate, host, host populations and the parasite itself. Gassner and Straib(1944) and Sharp(1965) have shown the effect of climatic conditions during spore formation to play a role in the potential spore germination. Response of yellow rust germination to environment has been widely reported. Short term storage conditions also appear to influence germinability. This may be of significance in the field where the type of weather that follows the release of spores may influence the germination potential. Sharp (1965) and Hassebrauk & Schroeder(1964) have shown low mean temperature (7°-12°C) to favour germination. Burrage(1970) has demonstrated the importance of 100% relative humidity in germination studies. Our analysis revealed temperature to be most significant with humidity and rain being important as well.

2. Effect of Age of Leaf in Relation to Spore Germination:

Burrage(1970) with his work on P.graminis has shown an increase in rate of germination with age of the pustule, germination percentage after 1h increasing throughout. Burrage made spore collections at 2 day intervals and sprayed water to remove the remaining spores after collection. In our case, the collections were made daily and the leaves were tapped to remove the spores. The spores were collected until the leaf senesced and could produce no more spores. The difference may be due to the way in which pustules are produced in case of yellow rust and black rust. In P.striiformis a number of pustules are produced simultaneously from a single infecting spore while in P.graminis they are produced sequentially. Manners(1976) believes considerable variation for the rate of ageing of pustules to be most certainly due to ageing of leaf.

Severity of disease development in yellow rust has been studied at various growth stages of the host(Manners,1950). Not much work has been done on the effect of age of leaf on spore germination in yellow rust, though comparable work has been done on fungi attacking other plants. The effect of age of seedling on conidial production has been shown in Pine by Rowan(1972). The susceptibility of Pinus teada seedlings to infection by Coronartium fusiformae increased with increasing age. Dickinson and Crute(1974) have shown a reduction in the incidence of Bremia lactucae affecting lettuce with increasing age and development of the seedlings. They have shown differences between the physiological states of cotyledons of different ages, mycelium

being sparser in older cotyledons and sporulation less dense. Cohen(1952) suggested that leaf age has an effect on penetration by Bremia and that on older leaves there are proportionally more stomatal penetrations which would offset the spore mycellium growth and sporulation. Mence & Pegg(1971) found that resistance of pea leaves to Perenospora viciae increased with age but declined at senescence. Leech(1931) showed that young cotyledons and newly formed leaves of beet were most susceptible to Perenospora schatii, the susceptibility declining with increasing maturity. Lowing and Acha(1959) reported a substantial difference in the growth rates of hyphae of Phytophthora infestans in plants of different ages to be due to changes in the total carbohydrate in whole plant or is associated with fluctuations in environment.

Guyot(1948) reports the colour of uredosori like their dimensions and arrangement to depend on the age of the host tissue. Dickinson and Wallace(1976) with their study on rusted wheat flag leaves suggest that spores are able to germinate and penetrate very early in the life of the green leaf. The amount of newly formed tissue available for colonisation varies with season and according to nutrients available.

Thorne(1960), Hughes and Evans(1962) indicate a decline in 'Net Assimilation Rate' (NAR) with increasing age even when external conditions are unchanged. The rate of respiration shows a tendency to decrease gradually with increasing age of plants. These physiological changes occuring with increasing age of the leaves seems to have brought about a wide difference in the percentage germination of spores collected from different ages of leaves.

Results obtained from the field experiments were further clarified by studying them under controlled conditions in a growth cabinet. The analysis of the controlled cabinet results showed a significant difference in germination of spores collected from different ages of leaves (S=P and S=P+7) at all temperatures (P. 5%) The effect of temperature on the same age of leaf (S=P or S=P+7) was also significant with germination being good at 10°C. Temperature therefore affects the physiological condition of the host and inturn the germination potential of the pathogen.

3. Position of Leaf in Relation to Spore Germination:

Hecke(1928) observed the daily low mean temperature to prolong the latent period up to five months. Gassner and Steaib (1944) have reported shortest latent period at 20°C. The difference in latent period in 1978 and 1979 was mainly due to the occurrance of warmer days in 1978 after inoculation and very cool days of April/ May in '79 prolonged the latent period.

Effect of position of leaf on germination in various instances have been associated with surface micro-organisms existing on different leaves(Dickinson,1976), variation in micro-climate prevailing on the leaf surface(Burrage,1971) and the physiological status of different leaves. James(1960,62) reports levels of infection on $3^{rd}$ and $4^{th}$ leaves to be the same but significant differences in $1^{st}$ and $2^{nd}$ leaves with his flag leaf being numbered as $L_1$. This result he justifies by the physiological evidence that most of the carbohydrate for the grain was produced by $L_1$ (flag) and $L_2$. Thorne and French (1958) showed the flag leaf and $2^{nd}$ leaf to contribute 59 and 16% respectively of the grain dry matter.

Thorne and French(1958) have shown yellow rust to infect larger % area of flag leaf than of the second leaf ($L_4$) but the latter to have a greater total area infected because it is larger leaf suggesting epidemiology of yellow rust to differ significantly from that of other diseases. This suggestion is in agreement with our observation that spores collected from $L_4$ germinated best and varied significantly from $L_3$ and $L_5$. Russell(1974) suggest the complex interaction of different leaves to P.striiformis to be due to more than one mechanism of inhibition or stimulation of spore germination.

Leaf surface temperature has attracted the interest of a numbers of workers(Wallace & Clum,1938; Raschke,1958; Burrage, 1971-a). Raschke(1958) reported variations of leaf temperature showing a leaf to be cooler at the periphery than in the central area. Burrage(1969) demonstrated a relationship between the requirements of P.graminis and the nature of dew which occurred. He has reported surface humidity to be higher than the air surrounding the leaf. Royle and Burrage(1971) found a relationship with surface wetness recordings, exchanges of energy being influenced by crop structure which results in a differece in air temperature and humidity and at plant level. In the course of this work no measurements were made at plant level and the results therefore cannot be discussed on the basis of micro-climate prevailing on the surface of the leaves. It can be mentioned in a broad sense that the upper leaves are in an advantageous position in relation to weather and perhaps tends to supply nutrition to the pathogen resulting in good germination.

The importance of surface microorganisms in relation to the germination and early growth of leaf infection by fungi have been studied by Brown(1922-a), Kovacs & Sizeoke(1956) etc.. Dickinson(1976) isolated many fungi from washed wheat flag leaves planted on tap water agar, less on green leaves than on older senescing samples. The distribution of fungi on the leaf surface have shown some patterns of colonisation as well. These surface organisms may enhance germination or colonise substantial volume of the tissue. Purnell(1976) has shown in swede mildew two interacting substances affecting infection.

Estimation of crop loss due to disease have been equated to severity on individual leaves at different growth stages. James et.al.(1968) assessing individually the top leaves of plants attacked by Rhynchosporium secalis in the milky ripe stage provided a reliable estimate of loss because these two leaves produce most of the dry matter in the grain. Thorne(1960) has associated flag leaf with high photosynthesis production and larger leaf area after the emergence of the ear.

Due to various physiological changes occurring with in the host plant because of micro-climate at plant level or the exudations of various micro-organisms on the leaf surface, significant differences in percentage germination of spores from five different leaves of the same plant gives scope for the future examination of the position of leaf in relation to yellow rust epidemic. The results could not be confirmed under laboratory conditions mainly due to lack of time.

# CHAPTER VI

## General Discussion

Discussions have been included at the end of each chapter covering the results and analysis of the work and these results have been compared with observations made by other workers. Significant results from computer analysis were obtained from field work carried out in 1978 and 1979. The results could not be conclusively confirmed under laboratory condition both due to lack of availability of proper facilities and shortage of time. The results from the field work has given a clear lead for future work. The favourable weather prevailing during the course of the field work in 1978 and 1979 permitted the growth of yellow rust and enabled us to compile the necessary data for the analysis. The results can be broadly divided as (1) environmental effects and (2) physiological effects of the host on the growth of the pathogen.

Environmental Effects:

Environment plays an important role in the relationship of host and pathogen and in fact in influences a balance between host and pathogen. The course of an epidemic in turn responds sensitivity to external factors and the rates of spread of the pathogen with time can be expressed best by equating it to environmental parameters. Mathematical models expressing the effect of environment along with other relevant factors on the development of the disease can help in forecasting an epidemic. The combination of all the results generally reveals that temperature and humidity together in combination affects the growth of the pathogen and their correlation coefficient shows a good relationship between the two.

With the variables being limited to two parameters good prediction can be achieved(Kranz & Royle,1976). The environmental effect, being a consequence of natural causes, cannot be altered experimentally. Therefore analysis of the weather conditions and a knowledge of the ideal conditions for the growth of yellow rust make the prediction of an epidemic possible and this can certainly help in spray programmes and economic savings.

Physiological Effects:

Physiological effects are to some extent a consequence of environmental effects on the host as the pathogen depends on the host for its nutrition. Effect of age of leaf on the growth of yellow rust was very significant both in the field and under laboratory conditions. In the controlled environment experiments, the effect of temperature on the host and pathogen turned out to be significant immaterial of the age of the host. Therefore temperature affects the physiological status of the host for example by causing loss in photosynthesis and carbohydrate manufacture. According to availability of nutrient substances there is a loss in formation of new tissues and the general vigour of the host plants(Dickinson,1978). The age of the host affects the quality and quantity of the exuades accumulated and yellow rust which is an obligate parasite is directly affected by these physiological changes in the host. Therefore it multiplies faster on young leaves than on older and matured ones.

The positional effect of individual leaves of a shoot also showed significant results and this can help in forecasting an epidemic. The occurrance of the epidemic has been shown to be critical at certain stages of growth by various workers(James et.al,1974; Last,1951). Rate of germination being significantly

different of spores collected from 4$^{th}$ leaf indicates yellow rust to survive and spread faster at this stage of growth. But this could not be studied under laboratory conditions and it cannot be concluded that the presence of surface organisms may also be one of the reasons as shown by many workers(Ruscoe,1971). The positive results paves the way for future work that can help in forecasting the disease before a severe damage is caused.

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