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Requirements of leaf wetness and temperature for infection of groundnut by rust

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Experiments are described to quantify the effects of temperature and duration of leaf wetness on infection of groundnut by *Puccinia arachidis*. After inoculation, a minimum period of leaf wetness, m, was necessary for infection. When leaf wetness duration was greater than m, lesion density increased with increasing wetness duration to an asymptote, D_{max}. The principal effects of temperature were on m and D_{max}. The value of m decreased linearly from 6 h, as temperature increased from 15 to 25 C and increased slightly at temperatures greater than 25 C. D_{max} increased with temperature from zero at 8 C to a maximum at 22 C, and decreased to zero again at about 30 C. The experimental results were used to produce a set of curves relating an infection index to leaf wetness duration at different temperatures. The implications for infection of groundnut crops are discussed in relation to the climate at Patancheru in southern India.

INTRODUCTION

Groundnut (*Arachis hypogaea*) is a major oil-seed crop of the semi-arid tropics, where the main constraints to production include unreliable rainfall, diseases and pests. Rust, caused by *Puccinia arachidis*, occurs globally and has been estimated to cause yield losses in excess of 50% in certain locations (Subrahmanyam *et al.*, 1980). Chemical control of rust and other foliar diseases is common in the USA, but widespread use of fungicides is not possible for resource-poor farmers in many tropical regions. In recent years much effort has been put into breeding for resistance to rust (e.g. Subrahmanyam *et al.*, 1983; Reddy *et al.*, 1987), and a number of promising resistant lines have been released already (Subrahmanyam *et al.*, 1985). However, it has been observed that resistance identified in one location may break down elsewhere; this may be due to differences in climate and/or pathogen race.

A thorough understanding of the way in which weather affects disease should lead to improved screening methods for resistance breeding programmes, as well as reliable systems for forecasting disease epidemics. Such systems would enable timely and efficient use of fungicides. This is of particular importance where the possible number of applications is severely restricted by cost, pesticide availability or limited water supply in remote areas. Reduced pesticide use will also lessen environmental damage and enhance the stability of farming systems.

The effect of temperature on percentage germination of urediniospores has been examined by Malliah & Rao (1979) and Subrahmanyam *et al.* (1988). They found similar relationships, with the optimum temperature for germination between 20 and 25 C; however in West Africa the optimum temperature was found to be 27 C (Savary, 1985). Malliah & Rao (1979) also observed the speed of spore germination at constant temperature. At 20 and 25 C they found that germination started within 2 h of preparing a spore suspension and was close to the maximum after 6 h.

The importance of water to infection by rust pathogens has long been recognized and the requirement of liquid water for germination of *P. arachidis* urediniospores has been demonstrated *in vitro* (Malliah & Rao, 1979) and on groundnut leaves (Cook, 1980). After spore germination on leaves, germ-tube growth terminates over stomata where appressoria form. Not all germ tubes form appressoria, and formation is less likely on leaf surfaces which are water repellent (Cook, 1980).

Although it is well established that leaf wetness is necessary for infection by *P. arachidis*, the

periods of leaf wetness necessary for different degrees of infection have not been quantified. This paper describes an investigation in controlled environments of how temperature and leaf wetness affect rust infection in groundnut. The results are discussed in relation to weather patterns at Patancheru in the south of India.

MATERIALS AND METHODS

Groundnut plants (cv. TMV 2) were grown in 13-cm diameter pots in a glasshouse. The sterilized potting medium was 50% loam, 25% sand and 25% compost, and Broughton's nutrient solution (Broughton & Dilworth, 1971) was applied every 2 weeks. Four-week-old plants (two per pot) were used for inoculation experiments.

Rust inoculum was obtained by multiplying locally collected urediniospores on groundnut leaves (cv. TMV 2) and harvesting with a cyclone spore collector. Spores were stored at 4 C.

A series of experiments was carried out to examine how different periods of leaf wetness affect infection. There were six treatments in each experiment, and two pots were allocated to each treatment. On each plant the third and fourth leaves from the top were tagged for inoculation, giving a total of eight inoculated leaves per treatment. Immediately before inoculating, a suspension of urediniospores was prepared in distilled water with a few drops of Tween 80 wetting agent. The spore concentration was determined with a haemocytometer and adjusted to approximately 50 000 spores/ml. The suspension was sprayed with an atomizer, ensuring that both sides of the tagged leaves were completely wetted. Immediately after inoculation, each pair of plants was covered with a polyethylene bag and placed in a controlled temperature cabinet (Percival) in the dark. The same cabinet was used successively, and was set to one of six temperatures between 15 and 30 C for each experiment. At the end of predetermined periods (ranging from 3 to 48 h), two pots were removed and the leaves dried in front of a fan before being transferred to a glasshouse. Coolers were operated in the glasshouse to prevent the air temperature exceeding 35 C, and the minimum temperature was usually between 15 and 20 C. The humidity in the glasshouse depended largely on the external ambient conditions, but minimum daytime values were commonly between 30 and 50%. Plants in the glasshouse were observed and the number of rust lesions on inoculated leaves was counted daily. When the number of lesions stopped increasing, the inoculated leaves were removed and their areas determined with a leaf area meter (Delta-T Devices Ltd, Cambridge, UK). Experiments at each temperature were repeated at least three times.

The effect of temperature on the germination of urediniospores was assessed in a separate experiment. A suspension of spores was prepared (50 000/ml) and one drop placed on each of 12 glass slides. The slides were placed in small humid chambers to prevent the drop drying, and these were placed on a thermogradient plate (the plate was designed to examine the effect of temperature on seed germination and is described by Garcia-Huidobro *et al.*, 1982). There were two slides at each of six temperatures between 8 and 34 C, and their temperatures were monitored with thermocouples connected to a data logger (Model CX21, Campbell Scientific Inc., Logan, Utah, USA). After 24 h, when maximum germination would have occurred, the slides were removed and examined microscopically. About 300 spores were counted to obtain numbers for germinated and non-germinated spores.

Further experiments were carried out to examine the effect of temperature on infection of groundnut with a constant wetness period. The procedure for leaf inoculation was as described above, and twelve pots were placed in each of six controlled temperature cabinets in the dark. The treatment temperatures (between 10 and 30 C) were checked hourly with thermocouples attached to a digital thermometer. All plants were removed after 30 h and transferred to the glasshouse, and symptom development was assessed as before. The procedure was repeated three times.

RESULTS

The number of lesions per inoculated leaf usually increased each day for about 1 week after symptoms first appeared, but then commonly declined as adjacent lesions grew and coalesced. The maximum lesion number on each leaf was used to calculate the mean lesion density for each treatment.

Wetness period

When the same experiment was repeated, absolute lesion densities often differed, so results were normalized to allow comparisons of relative differences between treatments in all experiments. An asymptotic relationship was found (Fig. 1) between lesion density, D, and periods (in h) of leaf wetness, W, with a minimum period of

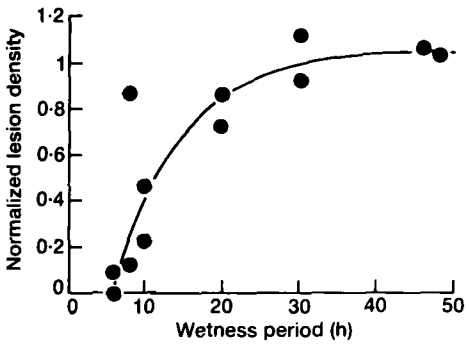

Fig. 1. The relationship between groundnut rust lesion density and period of leaf wetness at a constant temperature (20 C) for inoculated leaves. The fitted line is given by Equation 1 (see text for details).

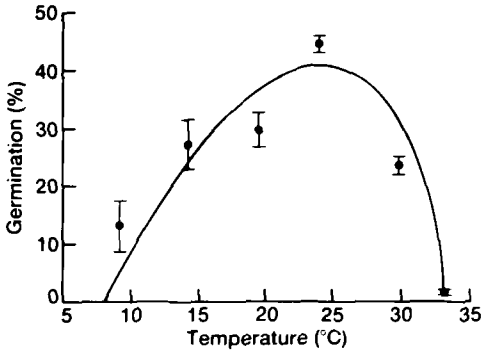

Fig. 3. The effect of temperature on percentage germination of *Puccinia arachadis* urediniospores on glass slides.

Table 1. Parameter[a] values with standard errors (S.E.), obtained by fitting Equation 1 to data relating groundnut rust lesion density, D, to periods of leaf wetness at different temperatures

Temperature (C)	D_{max}	S.E.	b	S.E.	m	S.E.
15	1·05	0·07	0·114	0·029	6·44	0·45
20	1·05	0·06	0·115	0·026	5·75	0·46
22	1·11	0·11	0·181	0·047	4·18	0·38
25	1·08	0·12	0·188	0·062	3·39	0·57
27	1·03	0·21	0·159	0·078	4·34	0·72
29	1·09	0·14	0·148	0·049	4·47	0·36

[a] D_{max} = asymptote; b = rate of change in lesion density with increasing duration of leaf wetness; m = minimum duration of leaf wetness required for infection.

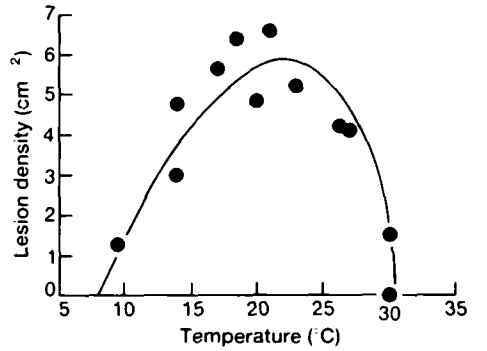

Fig. 4. The relationship between groundnut rust lesion density (number of lesions cm^{-2}) and temperature at the time of infection. After inoculation, groundnut leaves were maintained wet for 30 h in a controlled-temperature cabinet in the dark. The fitted line is given by Equation 2 (see text for details).

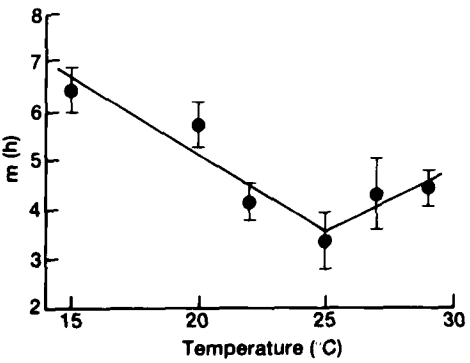

Fig. 2. The effect of temperature on the minimum time of leaf wetness required for rust infection in groundnut (parameter m, Equation 1). Standard errors obtained from the fitting procedure are indicated by bars.

wetness, m, within which no infection occurred. This is described by the equation

$$D = D_{max}\{1 - \exp[-b(W - m)]\}, \qquad (1)$$

where D_{max} is the asymptote and b is a measure of the rate of change of D with W. Some points in Fig. 1 have values greater than 1 because of the method used to normalize the data. Wetness periods in each experiment were selected to exceed that at which the asymptote is reached. The asymptote was taken as the average value of points obtained at wetness periods beyond which there was no consistent increase in lesion density. Other points were normalized with respect to this value.

Fitted values of D_{max}, b and m are given in Table 1 for each temperature. Values of D_{max} are all

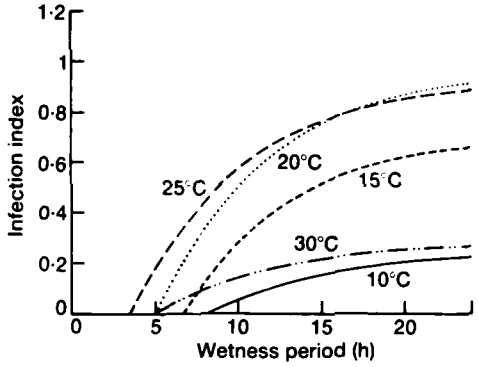

Fig. 5. Curves showing relative amounts of groundnut rust infection (the infection index) calculated for inoculated leaves at various temperatures with different periods of leaf wetness. The curves were generated using Equations 1 and 2. In Equation 1 the rate parameter, b, was assumed to remain constant with temperature and m, the minimum wetness period for infection, was obtained from linear relationships with temperature (Fig. 2) (see text for details).

slightly greater than 1, but none of the differences is statistically significant. There is some indication that values of b increase with temperature between 15 and 25 C, but differences are not significant. For values of m (Fig. 2), there is a significant decrease between 15 and 25 C ($P < 0{\cdot}01$) and an indication (although not statistically significant) of an increase between 25 and 29 C. In carrying out the experiments, a consistent increase was noticed in the period of wetness required to obtain lesions when comparing 25 C with greater temperatures. We believe that the fitted lines in Fig. 2 are realistic, because they reflect this increase, although changes in m between 22 and 29 C are slight.

Temperature

Temperature has a marked effect on the percentage germination of urediniospores on glass slides (Fig. 3). There was no germination below 8 C or above 34 C, and maximum germination (about 45%) occurred at 24 C.

When inoculated plants were provided with 30 h of leaf wetness at different temperatures, maximum lesion densities were obtained between 17 and 25 C, and very few lesions developed after incubation at around 10 or 30 C. The data (Fig. 4) display a classical response curve for biological systems with three cardinal temperatures: the optimum (T_{opt}), maximum (T_{max}) and minimum (T_{min}). The equation for the curve (Reed *et al.*, 1976) is:

$$Y = a(T - T_{min})(T_{max} - T)^{\beta} \quad (2)$$

where $a = y_{max}/[(T_{opt} - T_{min})(T_{max} - T_{opt})^{\beta}]$

and $\beta = (T_{max} - T_{opt})/(T_{opt} - T_{min})$.

Parameter values are $T_{max} = 30{\cdot}5$°C, $T_{min} = 8$°C and $T_{opt} = 22$°C.

Equations 1 and 2 were used to produce a series of response curves which account for the effects of both temperature and wetness periods on infection (Fig. 5). The result gives an infection index which varies between 0 (no infection) and 1 (maximum infection). The mean value of b from all determinations in Table 2 (0·15) was used in Equation 1, based on the assumption that b is independent of temperature. The value of m was assumed to decrease linearly with increasing temperature below 25 C, and to increase linearly above 25 C according to the fitted regression lines in Fig. 2. The following procedure was used to calculate the infection index. At each temperature, a normalized value of y was obtained from Equation 2, with $y_{max} = 1$. This provided an estimate, on a scale between 0 and 1, of the relative amount of infection at different temperatures with non-limiting wetness (Equation 2 describes the results of experiments with 30 h of wetness). This value of y ($0 < y < 1$) was assigned to the asymptote (D_{max}) in Equation 1, and represents the maximum possible infection with non-limiting wetness at the chosen temperature. Equation 1 was then used to obtain values of D (equivalent to the infection index) for different wetness periods up to 24 h. There would be little change in the index if the wetness periods were extended beyond 24 h, as may happen with continuously wet weather for more than 1 day. The result (Fig. 5) provides the means of assessing the effect of weather on groundnut rust infection. Daily values of the infection index may be obtained from the period of leaf wetness and the average temperature during that period.

DISCUSSION

Given adequate inoculum, the number of lesions which develop with a fixed period of leaf wetness is temperature dependent. The principal effects of temperature are on the asymptote (D_{max}) and the delay (m) in Equation 1. The value of m defines the minimum period of leaf wetness required for lesions to develop, and is important in interpreting the effect of patterns of leaf wetness which occur in the field. Low minimum temperatures, which are often associated with dew, will require relatively long periods of leaf wetness for infec-

Table 2. Long-term averages (1974–89) of monthly mean values of minimum air temperature (T_a), relative humidity at 0715 h (RH) and rain days (days with more than 1 mm rain) at ICRISAT Center, Patancheru, South India

	Jan.	Feb.	Mar.	Apr.	May	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.	Dec.
T_a (°C)	14·2	16·8	19·6	23·1	25·3	23·9	22·6	22·1	21·9	19·6	16·2	13·6
RH (%)	87	76	65	58	54	77	87	89	90	86	87	86
Rain days	1	1	1	2	3	10	15	14	12	6	2	1

tion. In addition the maximum possible number of lesions (given adequate leaf wetness) is reduced as the temperature decreases from the optimum.

The relationships of response to wetness and temperature for infection by *P. arachidis* are similar in form to those for *P. hordei* which causes barley brown rust (Beresford, 1986). In the case of *P. hordei*, the main effect of temperature was also on the delay and asymptote of curves relating lesion number to leaf wetness periods. Beresford (1986) found no consistent effect of temperature on the slope of these curves, so the response of the two fungi is similar in this respect also.

It appears that a continuous period of leaf wetness is necessary from the time of inoculation. If the inoculated groundnut leaves are dried for just 1 h and then rewetted and kept wet for 30 h at the optimum temperature, very few lesions develop. The results presented here on the effect of temperature on uredinospore germination are consistent with the findings of others in India (Malliah & Rao, 1979; Subrahmanyam *et al.*, 1988). The germination response to temperature (Fig. 3) is similar in form to the infection response (Fig. 4) but the cardinal temperatures are slightly different. The most marked difference is in T_{max} which is about 3°C lower for infection than for germination. It is possible that processes such as germ-tube growth, appressorium formation or host penetration respond to temperature in a slightly different way from germination. However, the effect of temperature on germination will largely determine the limits within which the response of infection can occur because spores which do not germinate cannot infect the host.

When considering how weather affects rust infection, patterns of temperature and leaf wetness in nature must be examined. Also, since light inhibits germination of urediniospores (Subrahmanyam *et al.*, 1988), only night-time conditions of temperature and leaf wetness will affect rust infection. For example, at Patancheru in southern India the occurrence of substantial dew is normally confined to a period of several months which begins towards the end of the monsoon season, when clear skies are accompanied by high relative humidity at night. At the beginning of the monsoon season the atmosphere is usually too dry for dew, and as the season progresses complete cloud cover is common. During this time most surface water on leaves is from rain, and the timing of showers is important in determining if the vegetation remains wet during the night.

During the rainy season (June to September), night-time temperatures are always close to the optimum for rust infection (Table 2). The frequency of rain (and the time of showers during the day) will determine whether there are adequate periods of wetness for rust infection. In years when rainfall distribution is very non-uniform, long dry periods will occur and wetness will probably limit rust development.

During the dry season (October–March), when groundnut is grown on stored soil moisture or with irrigation, night-time temperatures are commonly suboptimal and will limit rust development, particularly from November to January (Table 2). The amount of dew usually diminishes after October and there is normally little after January when the relative humidity remains low during the night (indicated by RH at 0715 h in Table 2). After October, therefore, rust infection will be limited partly by low temperature and partly by short wetness periods. The relative importance of wetness in limiting infection will increase between January and March.

The relationships presented here between rust infection, leaf wetness and temperature are of direct use in simulation modelling. Significant progress has already been made in modelling the development of rust in groundnut (Savary *et al.*, 1990) and the results from this study could be incorporated in such a model to strengthen that part concerned with the infection process.

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