EFFECT OF TEMPERATURE AND HOST GENOTYPE ON COMPONENTS OF RESISTANCE TO GROUNDNUT RUST[1]

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ABSTRACT

The effects of temperature on incubation period, infection frequency, lesion diameter, leaf area damage, pustule rupture, and sporulation were quantified for six groundnut genotypes, representing rust-resistant and susceptible reactions using detached leaves. Rust developed on all groundnut genotypes at 10, 15, 20, 25 and 30°C but not at 35 and 40°C. Incubation period decreased with increase in temperature but in susceptible genotypes it again increased at 30°C. Infection frequencies were highest for susceptible genotypes at 20°C and for resistant genotypes at 30°C. Lesion diameters were smallest at 15°C but increased with the increase in temperature. Optimal temperature for lesion diameters was at 30°C for most genotypes. In susceptible genotypes, the optimal temperature for the leaf area damage occurred at 25°C. The optimal temperature for resistant genotypes was at 25 or 30°C. Nearly 100% pustules ruptured on susceptible genotypes at most temperatures. In resistant genotypes, the percentage of pustules ruptured was highest at 15°C but decreased at 20, 25, and 30°C with the exception of NC Ac 17090 at 30°C. Sporulation was highest on susceptible genotypes at 20 and 25°C. Resistant genotypes had high sporulation at 15 or 20°C but decreased at higher temperatures with the exception of NC Ac 17090. Resistant genotypes had longer incubation period, lower infection frequencies, smaller lesions, reduced number of ruptured pustules, lower sporulation, and leaf area damage than the susceptible genotypes. The differences between susceptible and resistant genotypes in some of the components were large only at certain temperatures.

Key Words: *Arachis hypogaea*, components of resistance, disease resistance, groundnut

RÉSUMÉ

Les effets de la température sur la période d'incubation, la fréquence d'infection, le diamètre des blessures, l'endommagement des feuilles, la déchirure des pustules, et la sporulation ont été évalués pour six génotypes d'arachide qui présentent sur les feuilles des réactions de résistance et susceptibilité à la rouille. La rouille se développe sur tous les génotypes à 10, 15, 20, 25 et 30°C, mas pas à 35 et 40°C. La période d'incubation diminue quand la température augmente, mais pour les génotypes susceptibles, elle augmente de nouveau à 30°C. Les fréquences d'infection sont les plus élevées à 20°C pour les génotypes susceptibles et à 30°C pour les génotypes résistants. Les diamètres des blessures sont les plus petits à 15°C, mais grandissent quand la température augmente. La température optimale pour le diamètre des blessures était

de 30°C pour la plupart des génotypes. La température optimale pour l'endommagement des feuilles était de 25°C pour les génotypes susceptibles et de 25-30°C pour les génotypes résistants. Prèsque 100% des pustules sont déchirées sur les génotypes susceptibles à toutes les températures. Pour les génotypes résistants, le pourcentage de ruptures est le plus haut à 15°C et diminue à 20, 25 et 30°C, sauf pour NC Ac 17090 où il est maximum à 30°C. La sporulation était la plus haute pour les génotypes susceptibles à 20 et 25°C. La sporulation des génotypes résistants est haute à 15 et 20°C et diminue quand la température monte, sauf pour Nc Ac 17090. Les génotypes résistants ont des périodes d'incubation plus longues, des fréquences d'infection plus basses, des blessures plus petites, moins de ruptures de pustules, des sporulations plus faibles, et moins de dommages aux feuilles que les génotypes susceptibles. Pour certaines composantes, les différences entre les génotypes résistants et susceptibles étaient élevées seulement à quelques températures.

Mots Clés: *Arachis hypogaea*, composants de résistance, résistance aux maladies, arachide

INTRODUCTION

Rust of groundnut (*Arachis hypogaea* L.), caused by *Puccinia arachidis* Speg., is an important disease in most major groundnut growing countries (Subrahmanyam *et al., 1985*). Several sources of resistance to rust have been identified (Subrahmanyam and McDonald, 1987). During our earlier investigations on components of resistance to groundnut rust, neither the size, nor the frequency of stomata were correlated with host resistance (Subrahmanyam and McDonald, 1987). Urediniospores germinated on leaf surfaces and the fungus entered through stomata irrespective of whether a genotype was immune, resistant or susceptible to rust. However, in immune genotypes the fungus died shortly after entering the substomatal cavity. Differences in resistance were associated with differences in extent of mycelial development within the cavity and within leaf tissues. Subrahmanyam *et al.* (1983a and 1983b) found that in rust-resistant genotypes the pathogen had increased incubation period, decreased infection frequency, and reduced pustule size, spore production, and spore germinability. However, little is known concerning the effects of temperature and its interaction with host genotypes on components of resistance to groundnut rust.

This paper describes investigations on the effects of temperature on incubation period, infection frequency, lesion diameter, leaf area damage, pustule rupture, and sporulation of six groundnut genotypes, representing rust-resistant and susceptible reactions, following monocyclic infection in the laboratory.

MATERIALS AND METHODS

Six groundnut genotypes were investigated. The genotypes, TMV 2 and J 11 were most susceptible to groundnut rust (scored 9 on a 9-point scale Subrahmanyam *et al.,* 1983a). The genotype, NC Ac 17090 was most resistant (score 2.2) followed by PI 405132 (score 2.4), EC 76446 (292) (score 2.8), and PI 259747 (score 3.0) (Subrahmanyam *et al.,* 1983a). Seeds were sown in 15 cm-diameter plastic pots in the greenhouse. Four seeds were sown in each pot and the seedlings were later thinned to two pot^{-1}. Five pots were used for each genotype. Air temperature in the greenhouse ranged from 25 to 30°C during the plant growth period. At 40 days after sowing, the middle leaf on the main stem of each plant was excised through the pulvinus and its area was measured by tracing its outline and measuring the area with a leaf area meter (Hayashi Denkoh Co. Ltd., Tokyo, Japan). The detached leaves from different genotypes were arranged with their petioles buried in sterilized river sand in plastic trays (56 x 25 x 5 cm) in randomized blocks with 10 replications of each genotype, one replicate of a treatment was one leaf. Sand in the trays was moistened with Hoagland's nutrient solution. Trays were covered with thin, plastic sheets and incubated before inoculation for 24 hr in a growth chamber (Percival Refrigeration and Mfg. Co., Boone, Iowa, USA) at 25°C with 12 hr photoperiod.

Urediniospores were produced on rooted, detached leaves of the rust susceptible genotype TMV 2 in a growth chamber. Urediniospores were harvested with a Cyclone spore collector (ERI Instrument Shop, Iowa State University,
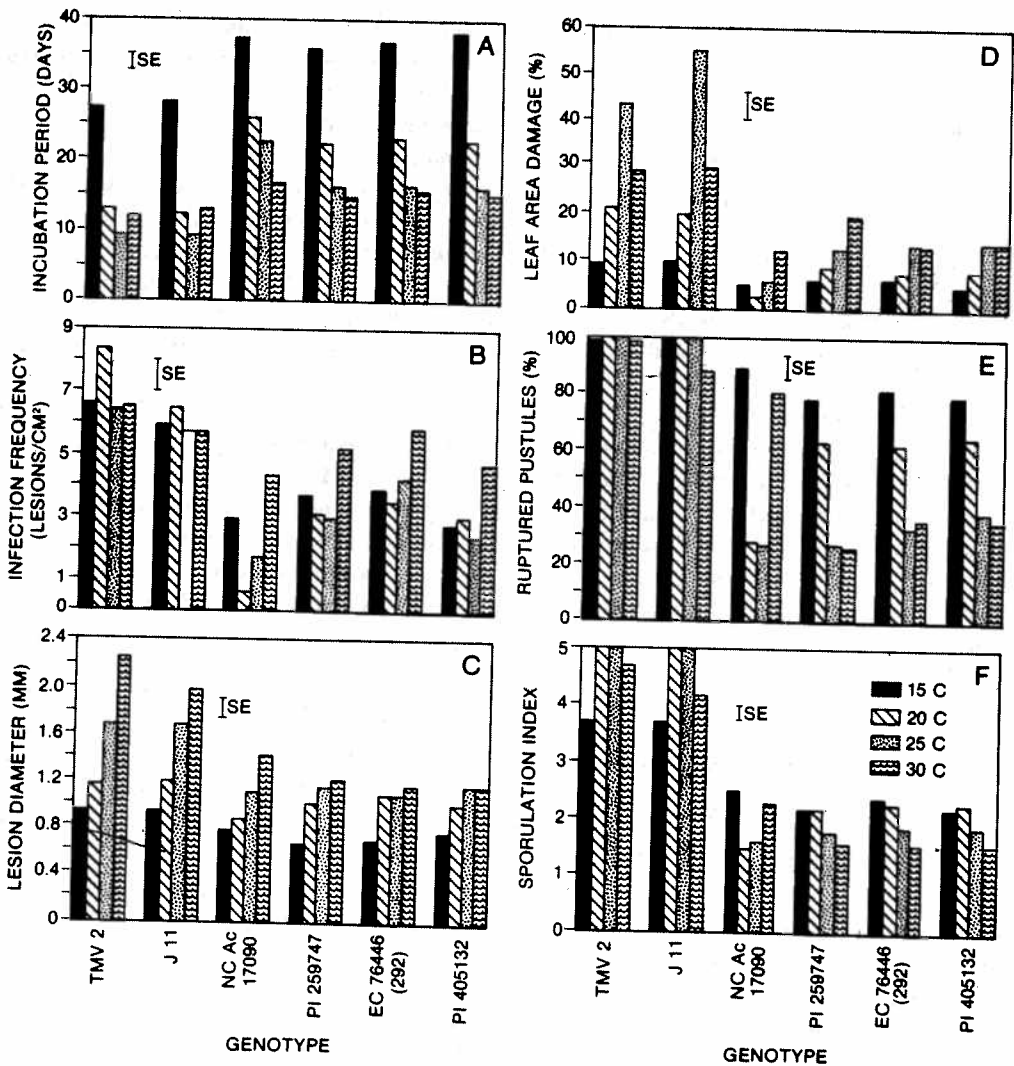
Figure 1. Effect of temperature on (A) incubation period, (B) infection frequency, (C) lesion diameter, (D) leaf area damage, (E) pustule rupture, and (F) sporulation of *Puccinia arachidis* on two susceptible (TMV 2 and J 11) and four resistant (NC Ac 17090, PI 259747, EC 76446 (292), and PI 405132) groundnut genotypes. Standard errors (SE) are shown in vertical bars for each disease parameter.

Ames, Iowa, USA) and suspended in sterile distilled water containing Tween-80 (polyoxyethylene sorbitan monooleate)(10 drops 1,000 ml^{-1}). The suspension was adjusted to 50,000 spores ml^{-1}. Leaves were inoculated with the urediniospore suspension sprayed on with a plastic atomizer. Leaves were then incubated initially in the dark for a 24 hr period at 25°C and were incubated subsequently at 10, 15, 20, 25, 30, 35 or 40°C with a 12 hr photoperiod until the end of the experiment.

From 8 days after inoculation, leaves were examined daily and numbers of visible uredinia, irrespective of whether ruptured or unruptured, were recorded. Ten days after the cessation of daily increase in number of uredinia, leaves were scanned through a stereomicroscope (at a magnification of 70 X), and numbers of ruptured and non-ruptured uredinia were recorded. The degree of sporulation was measured on a 5-point scale (1 = no sporulation and 5 = extensive sporulation). The diameters of five randomly

selected uredinia on each leaflet of the test leaf (i.e., 20 pustules leaf^{-1}) were measured using an ocular micrometer. The percentage of the area of the test leaves damaged by rust, which included yellowing and necrosis, was estimated by comparison with diagrams depicting leaves with known percentages (0.5, 1, 2, 5, 10, 20, 35, 50, 75, and 100%) of area affected.

From these data the following disease characters were determined: 1) *Incubation period:* number of days between inoculation and appearance of 50% of the pustules, 2) *Infection frequency:* final number of pustules per cm^2 of leaf area, 3) *Lesion diameter:* mean diameter (in mm) of a random sample of uredinia, 4) *Percentage leaf area damaged:* area of inoculated leaf damaged by rust as a percentage of total leaf area, 5) *Ruptured pustules:* percentages of ruptured pustules for each genotype at all temperatures, 6) *Sporulation scores:* for each genotype at all temperatures.

Three successive experiments were performed. The data from all experiments were combined and an analysis of variance was carried out for each disease character.

RESULTS

Rust developed on all groundnut genotypes at all test temperatures except 35 and 40°C. Leaves incubated at these temperatures were examined until 60 days after inoculation but no visible rust development was observed. At 10°C, symptoms appeared very late on all genotypes (incubation period over 50 days), and meaningful data could not be obtained because of the development of water-soaked necrotic lesions on the leaflets and subsequent colonization of leaflets by saprophytic fungi (predominantly *Alternaria* spp.) in the dead tissues. Hence, only data from 15, 20, 25 and 30°C are presented in this report (Fig. 1 A to F).

All groundnut genotypes showed longest incubation periods at 15°C (Fig. 1 A). In rust-resistant genotypes, the incubation period decreased with increase in temperature. While in susceptible genotypes incubation period decreased from about 28 days at 15°C to 9.3 days at 25°C, there was an increase in incubation period at 30°C. Rust-susceptible genotypes had significantly (P ≤ 0.01) shorter incubation periods than the rust-resistant genotypes at all

temperatures. Among the resistant genotypes, NC Ac 17090, the most resistant to groundnut rust had longer incubation periods at 20, 25, and 30°C than other genotypes (Fig. 1 A).

Infection frequencies (Fig. 1 B) on susceptible genotypes were high at 20°C; however, there were no differences at other temperatures. Infection frequencies on resistant genotypes were highest at 30°C. Infection frequencies were significantly (P ≤ 0.01) higher on susceptible genotypes than on resistant genotypes, especially at 15, 20, and 25°C. Optimal temperature for infection frequency on TMV 2 (susceptible genotypes) was at 20°C and for resistant genotypes at 30°C (Fig. 1 B).

On both the susceptible and resistant genotypes, lesions were smallest at 15°C (Fig. 1 C) and gradually increased in diameter with increase in temperature. The lesions were largest on both the susceptible and resistant genotypes at 30°C. Resistant genotypes had significantly ((P ≤ 0.01) smaller lesions than the susceptible genotypes, especially at 25 and 30°C (Fig. 1 C).

Leaf area damaged (Fig. 1 D) on susceptible genotypes was lowest at 15°C, but significantly ((P ≤ 0.01) increased at 20 and 25°C. However, the percentage leaf area affected was lower at 30°C. The most optimal temperature for leaf area damage on susceptible genotypes was at 25°C. On resistant genotypes, the percent leaf area damaged was lower at low temperatures but increased at higher temperatures. The resistant genotypes had significantly (P ≤ 0.01) lower percentage of leaf area damaged than the susceptible genotypes at all temperatures (Fig. 1 D).

The percentages of ruptured pustules (Fig. 1 E) were close to 100% for susceptible genotypes at all temperatures with an exception of J 11 at 30°C. There were marked effects of temperature on percentages of pustules ruptured on resistant genotypes. The resistant genotypes showed highest percentages of pustules ruptured at 15°C but decreased at other temperatures with the exception of NC Ac 17090 which showed a significant (P ≤ 0.01) increase at 30°C. The susceptible genotypes had highest percentages of ruptured pustules than the resistant genotypes and the differences were most evident at 20, 25 and 30°C (Fig. 1 E).

For the susceptible genotypes, the highest sporulation (score 5) occurred at 20 and 25°C

(Fig. 1 F) but significantly decreased at 15 and 30°C. Sporulation on resistant genotypes was high at 15 and 20°C but significantly decreased at 25 and 30°C with the exception of NC Ac 17090. This genotype had high sporulation at 15 and 30°C and low at 20 and 25°C. The resistant genotypes had significantly (P ≤ 0.01) lower sporulation than the susceptible genotypes at all temperatures (Fig. 1 F).

DISCUSSION

Disease development in plants involves various inter-related processes each of which may be influenced by environmental factors as well as host and pathogen genotypes. Temperature in the range of 20–25°C was reported to be optimum for urediniospore germination of groundnut rust (Subrahmanyam and McDonald, 1987). Light (5000 lux and above) was found to inhibit urediniospore germination (Subrahmanyam and McDonald, 1987). Availability of water on the leaf surfaces for over 6 hr is necessary for urediniospore germination and infection (Liang-gao, 1987). In the present investigation, leaves were inoculated and incubated at high relative humidity in the dark for 24 hr at 25°C thus providing optimum conditions for infection. The leaves were subsequently incubated at different temperatures to investigate the effects of temperature on rust development in the post-penetration phase of host-pathogen interaction. The components of resistance analyzed in this investigation are considered to be of great value in a genetical as well as epidemiological perspective (Parlevlier, 1979; Zadoks and Schein, 1979).

No visible rust pustules or lesions were observed at 35 or 40°C even at 60 days after inoculation indicating that temperatures of 35°C and above inhibited groundnut rust development. However, in this study we did not determine whether the pathogen was killed or remained quiescent in the host tissues at these high temperatures. Rust developed at 10°C on all genotypes but the incubation period was in excess of 50 days. In susceptible genotypes the incubation period was highest at 15°C and decreased with the increase in temperature but again increased at 30°C as observed by other workers (Liang-Gao, 1987; Mayee, 1987; Savary, 1987). However, in resistant genotypes no such increase in incubation period was observed at 30°C. The rust-susceptible genotypes had significantly shorter incubation periods than the rust-resistant genotypes. These results clearly indicate that temperature and host genotype play an important role in determining the length of incubation period. Optimal temperature for infection frequencies on susceptible genotypes was 20°C and on resistant genotypes at 30°C. A coefficient of variation (36.8%) observed for infection frequency suggesting that this variable may be less accurate in measuring the genotypic (Parlevlier and Kuiper, 1977), and temperature (Savary, 1987) effects on rust development. In this investigation, the inoculated leaves were initially incubated at optimal temperature (25°C) to facilitate urediniospore germination and subsequently transferred to other temperatures. This might have contributed to less accuracy in assessing the effects of temperature on infection frequency. There was an increase in lesion diameter with increase in temperature on both the resistant and susceptible genotypes indicating that higher temperatures are favourable for the growth of the pathogen in host tissues. It is interesting to note that although the resistant genotypes had smaller lesions at 15°C, a high percentage of them were ruptured with high sporulation. The resistant genotypes had lower sporulation than the susceptible genotypes at all temperatures. The most optimal temperature for leaf area damage on susceptible genotypes was at 25°C. On resistant genotypes, leaf area damage was high at higher temperatures. The resistant genotypes had significantly lower percentage of leaf area damaged than the susceptible genotypes at all temperatures. Among the rust-resistant genotypes, NC Ac 17090 behaved distinctly different from other genotypes in infection frequency, leaf area damage, pustule rupture and sporulation, and may have mechanisms different from other resistant genotypes, in host-pathogen-environment interaction.

Temperature and other environmental factors may have direct bearing on epidemic development. Studies conducted at the International Crops Research Institute for the Semi-Arid Tropics, Patancheru, Andhra Pradesh (Subrahmanyan and McDonald, 1987) and other locations in India (Mayee, 1987) have shown that rust development is very rapid and the disease is severe on groundnut

crops grown during the rainy season (June-October) when temperatures are not extreme. However, rust development is slow and less severe during the postrainy season (December-May). During the early part of the postrainy season (December-February), day temperatures are optimum (25-30°C) for rust development while night temperatures are low (<15°C). In the later part of the season (April-May) day temperatures normally exceed 40°C and the night temperatures are around 30°C thus slowing rust development and severity. In the present investigation, groundnut leaves incubated at 35 and 40°C did not show any rust development even after 60 days of incubation. Although rust appeared at 10 and 15°C, the development was very slow and lesions were very small. These results clearly indicate that temperatures at 35°C and above or at 15°C and below are not conducive to rust development. In the rainy season crops it was estimated that the pathogen completes 6–9 reproductive cycles, while on postrainy season crops it has only 1–4 cycles (Mayee, 1987). In addition to temperature, other environmental factors such as rainfall and relative humidity also influence groundnut rust development (Mallaiah, 1976; Mayee and Kokate, 1987; Siddaramaiah *et al.*, 1980).

The present investigation shows that on resistant genotypes the pathogen fails to successfully invade the host tissues at all infection sites resulting in low infection frequency. Even if the pathogen invades, the host reaction slows down the disease development resulting in longer incubation period, smaller lesions with sparse sporulation as were found in previous reports (Lin, 1982; Sokhi and Jhooty, 1982; Subrahmanyan *et al.*, 1983a and 1983b; Mayee, 1987). The differences between susceptible and resistant genotypes in some of these components were large only at certain temperatures. For example, the susceptible and resistant genotypes could be best differentiated for incubation period and infection frequency at 15, 20, and 25°C, for lesion diameter at 25 and 30°C, and for leaf area damage at 20, 25 and 30°C. The effects of temperature and host genotype on rust development is especially important in disease resistance breeding programmes. Temperatures beyond optimal range may be less accurate for differentiating the genotypic effects on rust development.

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