

Effect of Temperature and Leaf Wetness Period on the Components of Resistance to Late Leaf Spot Disease in Groundnut

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A complete understanding of the epidemiological factors required for optimum for disease development facilitates the design of effective and reliable screening techniques and also disease prediction models. An attempt was made to study the effects of different temperatures (15-35°C) and leaf wetness periods (4-24 h) on the development of late leaf spot (LLS) in three groundnut genotypes differing in their susceptibility to LLS infection. Irrespective of the genotype, the disease progress evaluated based on different components of resistance was maximum between 15-20°C and minimum between 20-25°C. At temperatures $\geq 30^\circ\text{C}$, LLS development was insignificant. The overall severity of LLS increased with an increase in the leaf wetness period from 4 h to 12 h a day. Further increase of wetness period to 16 h resulted in a rapid increase in the severity. Thereafter, the disease severity gradually decreased with an increase in the wetness period. The effect of temperature and wetness periods on the individual component of disease quantification was not uniform compared between genotypes with different levels of susceptibility/resistance to LLS infection. The results of this study indicate that temperature and leaf wetness period are critical in late leaf spot screening programs since the expression of disease symptoms measured from disease initiation till defoliation, varied differently in the test genotypes with respect to change in these two parameters.

Keywords : *Arachis hypogaea*, *Cercosporidium personatum*, epidemiology, leaf defoliation, peanut

Late leaf spot (LLS) disease caused by *Phaeoisariopsis personata* (Berk and Curt) V. Arx is the most economically important disease of groundnut (*Arachis hypogaea* L.) in the semi-arid tropic regions (Subrahmanyam et al., 1984). Severity of LLS and resulting yield losses largely depends on the environmental conditions that prevail during the crop season and the yield losses may be up to 50% (McDonlad

et al., 1985). Disease management largely depend on the use of available host-plant resistance (HPR) and chemical fungicides. Exploitation of HPR is the watchword of every agriculturist in both developed and developing countries. Advances in screening and breeding for resistance against LLS have resulted in identification of a number of promising groundnut lines (Wynne et al., 1991; Subrahmanyam et al., 1995).

Temperature and leaf wetness are the two most important epidemiological factors required for development of several foliar fungal diseases in different crops and LLS of groundnut is one among them (Butler et al., 1994; Wadia and Butler, 1994). Quantification of LLS severity is based on multiple components, since it is difficult to quantify the disease based on a single component. Also, groundnut genotypes vary in their response to individual components of disease scoring, as the measurement of these components is at different stages starting from lesion appearance till defoliation of leaf lets (Pande et al., 2002). A complete understanding of these two key epidemiological factors on individual components of LLS scoring in different groundnut genotypes facilitates the refinement of the existing host-plant resistance screening techniques and also disease assessment methods.

In earlier studies, a temperature of 16-20°C favored the germination of *P. personata* conidia and there was a greater reduction in the germination at $>28^\circ\text{C}$ (Sommarlyta and Beute, 1986). The importance of extended periods of leaf wetness with intermittent dry periods for optimum disease development has been emphasized (Shew et al., 1988). Butler et al. (1994) observed that the number of lesions resulting from a fixed amount of inoculum was several times greater when the leaves were exposed to alternate wet and dry periods as compared with continuous wetness. However, in majority of these studies the effect of temperature and leaf wetness on expression of LLS was studied using a highly susceptible genotype.

Information on the combined effect of epidemiology and host resistance on different components of LLS quantification is essential for absolute identification of disease

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resistance or susceptibility, especially when the selection was based on a single component. The present study was undertaken to determine the effect of host resistance and temperature or wetness period on the individual components of resistance used for LLS quantification, using groundnut genotypes that are susceptible, moderately resistant and resistant to LLS infection.

Materials and Methods

Fungal culture. Single lesion isolate of *P. personata* available at legumes pathology laboratory, International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, India was used in the present study. The fungus was maintained on a susceptible host genotype TMV 2 following detached leaf technique (Subrahmanyam et al., 1983). Conidia were harvested from the sporulated lesions using a cyclone spore collector (Fisher Scientific Co., USA) in small glass vials (7.5 × 1.2 cm diameter) and stored at 4°C up to a period of 90 days.

Genotypes. Three groundnut genotypes, TMV 2 (susceptible to LLS), ICGV 86590 (moderately resistant to LLS) and ICGV 86699 (resistant to LLS) were used in studies conducted to determine the effects of temperature and leaf wetness period on the development of LLS.

Inoculum preparation. Fresh conidia of *P. personata* were evenly suspended in sterile distilled water (SDW) with 0.01% Tween 80 (Polyoxyethylene sorbiton monooleate) as a wetting agent. The concentration was adjusted to 2×10^4 conidia ml⁻¹ using a haemocytometer and observing under a light microscope. Freshly prepared conidial suspension was used as inoculum in all the experiments.

Artificial inoculation of groundnut plants for development of LLS. Thirty-day-old plants of the three test genotypes TMV 2, ICGV 86590 and ICGV 86699 were used for artificial inoculation. Five seeds were planted in 15 cm diameter pots filled with red alfisol, sand and farm yard manure (3:1:1), and trimmed to four after emergence. The pots were maintained in a greenhouse at $28 \pm 2^\circ\text{C}$. At the time of inoculation, the plants were uniformly sprayed with the inoculum using a hand-operated atomizer. Inoculated plants were allowed to air dry and transferred to dew chambers (Clifford, 1973) to maintain leaf wetness (RH 100%). After 16 h, plants were shifted to greenhouse/incubator for 8 h to provide dry period. The alternate wet (16 h) and dry (8 h) periods were maintained up to 8 days after inoculation (DAI) and then the dry period continued. Four plants in each pot were considered as one replication and the experiment was conducted with triplicates in each treatment and repeated once.

Effect of temperature on the development of LLS. The effect of different temperatures ranging from 15 to 35°C on the development of LLS was studied in the three test genotypes. Following pathogen inoculation, all the plants were transferred to dew chambers maintained at 15, 20, 25, 30 and 35°C, respectively. To provide the required dry period, plants in each dew chamber were transferred to incubator maintained at the same temperature and equipped with fluorescent lighting, for 8 h in a day. After 8

DAI, the plants were retained in the incubators at respective temperatures and adequately watered till end of the experiment. **Effect of leaf wetness period on the components of resistance.** The effect of leaf wetness period, from 4 h to 24 h a day, on the development of LLS in three different genotypes was studied under controlled environment conditions. The inoculated plants were air dried and exposed to different periods of leaf wetness i.e., 4, 8, 12, 20 and 24 h per day by transferring between dew chambers and greenhouse. Alternate wet and dry period treatments were continued up to 8 DAI, after which plants were left in the greenhouse. The temperature in both the dew chambers and greenhouse was maintained at 25°C throughout the experimentation period.

Disease scoring. In each inoculated plant, third and fourth fully expanded leaves from top were tagged prior to inoculation and used for disease scoring. The severity of LLS was measured based on the following components of resistance: a) incubation period (IP) - number of days from pathogen inoculation till appearance of the first lesion, b) latent period (LP) - number of days between pathogen inoculation and appearance of the first sporulating lesion (LS₁), or 50% of lesions sporulating (LS₅₀) (The lesions were considered to be sporulating when tufts of fascicles are visible on the lesions when observed under 20X magnification), c) lesion count (LC) - number of lesions developed on all the four leaflets of each tagged leaf (when two or more lesions coalesced during growth, a single count was recorded), d) lesion diameter (LD) - the diameter of five randomly selected lesions on each leaf was measured in mm, e) percentage necrotic area (PNA) - measured by comparing with standardized pictorial charts depicting leaves with known percentages of their areas affected (Hassan and Beute, 1977), f) percentage defoliation (DEF) - calculated based on the number of leaflets defoliated in each tagged leaf, and g) disease score (DS) - measured on a 1-9 rating scale (Subrahmanyam et al., 1982) by considering the four plants in each replication.

Results

Effect of temperature on the components of resistance to LLS. Among the different temperatures tested for their effect on development of LLS in three genotypes ICGV 86699, ICGV 86590 and TMV 2, no disease development was observed in any of the genotype at 35°C. At 30°C, only a few lesions developed, but the disease severity was insignificant. At the three other temperatures i.e., 15°C, 20°C and 25°C the measured components of resistance in the three genotypes were as follows (Fig. 1):

a) Incubation period (IP): In the three test genotypes, IP was shortest at 25°C, longest at 15°C and was almost similar at 20°C and 25°C. At any given temperature, there were significant differences in IP when compared among the three genotypes.

b) Latent period (LS₁ and LS₅₀): No sporulation was observed on LLS lesions developed in ICGV 86699. In the other two genotypes TMV 2 and ICGV 86590, both LS₁

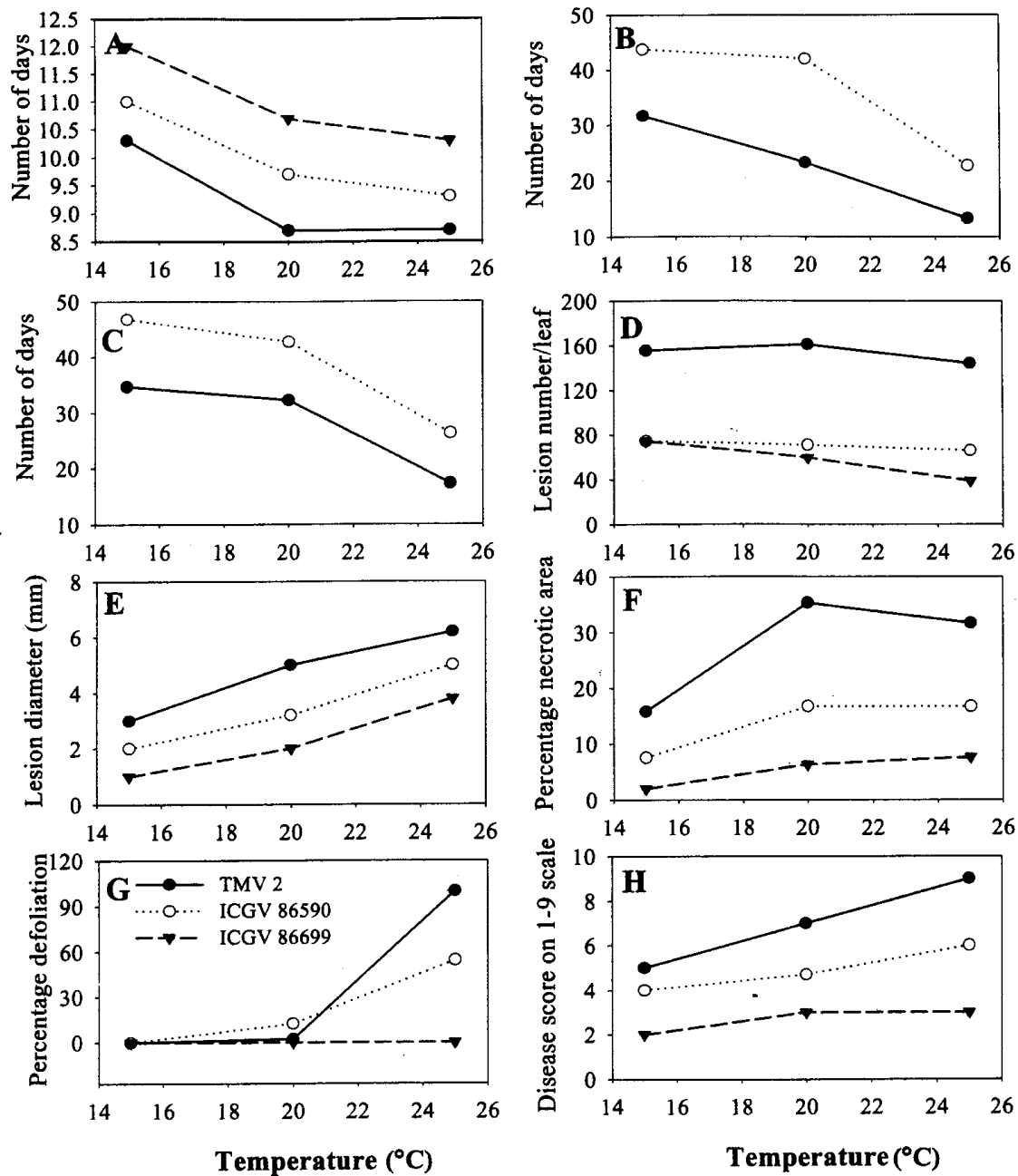


Fig. 1. Effect of temperature on the development of late leaf spot in groundnut, measured based on the different components of resistance. (A) Incubation period, (B) Latent period (LS_1), (C) Latent period (LS_{50}), (D) Lesion count, (E) Lesion diameter, (F) Percentage necrotic area, (G) Percentage defoliation, and (H) Disease score on 1-9 rating scale. Sporulation was not observed in ICGV 86699 at all the temperatures. Lesion count presented was measured at 3 weeks after inoculation (WAI), when there was no increase in lesion number in TMV 2. Lesion diameter, percentage necrotic area and percentage defoliation were at 4 WAI and disease score at was at 5 WAI, when the recorded value of these components was maximum in TMV 2.

and LS_{50} significantly decreased with increase of temperature from 15°C to 25°C. Of all the different treatments, TMV 2 incubated at 25°C showed the minimum latent period.

c) Lesion count: The average number of lesions developed on each tagged leaf was significantly ($P=0.05$) higher in TMV 2 than in ICGV 86590 and ICGV 86699 at the three temperatures 15°C, 20°C and 25°C. In contrast to the higher incubation period at 15°C and 20°C compared to 25°C, the lesion number was observed to be higher at 15°C and 20°C than at 25°C in the three test genotypes. In TMV 2 and

ICGV 86590 the difference in the number of lesions developed at different temperatures was <15%, whereas the LC was >90% higher at 15°C compared to 25°C.

d) Lesion diameter: Significant ($P=0.05$) differences in LD were recorded between the temperatures, irrespective of the genotype. LD was maximum at 25°C, followed by 20°C and 15°C.

e) Percentage necrotic area (PNA): In all the genotypes PNA increased according to temperature. However, the increase in PNA from 15°C to 20°C was high compared to

Table 1. ANOVA interactions for the effect of temperature on the development of late leaf spot in groundnut

Component	Interaction	SEM (±)	LSD ($P=0.05$)
Incubation period	genotype	0.102	0.294
	temperature	0.132	0.380
	genotype × temperature	0.228	0.658
Latent period (LS ₁)	genotype	0.196	0.567
	temperature	0.253	0.732
	genotype × temperature	0.439	1.268
Latent period (LS ₅₀)	genotype	0.094	0.272
	temperature	0.122	0.352
	genotype × temperature	0.211	0.609
Lesion count	genotype	2.790	8.050
	temperature	3.600	10.390
	genotype × temperature	6.230	17.990
Lesion diameter	genotype	0.037	0.017
	temperature	0.048	0.138
	genotype × temperature	0.083	0.238
Percentage necrotic area	genotype	0.656	1.895
	temperature	0.847	2.446
	genotype × temperature	1.467	4.237
Percentage defoliation	genotype	1.361	3.930
	temperature	1.757	5.074
	genotype × temperature	3.043	8.790
Disease score	genotype	0.039	0.111
	temperature	0.050	0.144
	genotype × temperature	0.086	0.249

that from 20°C and 25°C except for ICGV 86590, where PNA remained the same both at 20°C and 25°C.

f) Percentage defoliation (DEF): Temperature had a profound effect on DEF in ICGV 86590 and TMV 2, and no defoliation occurred at 15°C. At 20°C, DEF was significantly ($P=0.05$) higher in ICGV 86590 than TMV 2 and at 25°C the defoliation was 100% in TMV 2 compared to 54.2% in ICGV 86590. No defoliation was observed in ICGV 86699 at all the tested temperatures.

g) Disease score (DS): Based on the DS at 6 WAI, the effect of temperature on development of LLS was observed to be more prominent in TMV 2 than in ICGV 86590 and ICGV 86699. In TMV 2 and ICGV 86590 the disease score was maximum at 25°C and was significantly ($P=0.05$) high compared to 20°C, and at both these temperatures the disease score in ICGV 86699 remained the same.

The mean values of a particular component measured were compared within the genotype, temperature or genotype × temperature treatments using LSD at a significance level of 5% ($P=0.05$) (Table 1). The standard error of mean values (SEM) for individual or combined effect of the two treatments, genotype and temperature, were also mentioned.

Effect of leaf wetness period on the components of resistance to LLS. The effect of leaf wetness period on the development of LLS in the three test genotypes was as follows (Fig. 2):

a) Incubation period: In the three test genotypes, TMV 2, ICGV 86590 and ICGV 86699 there was a gradual decrease in the IP, with an increase of leaf wetness period from 4 h to 16 h per day. Further increase in the leaf wetness period resulted in an increase of IP and is more prominent in ICGV 86699.

b) Latent period (LS₁ and LS₅₀): There was no sporulation in ICGV 86699 in all the treatments, and in ICGV 86590 and TMV 2 at 4 h leaf wetness period. In TMV 2 both LS₁ and LS₅₀ were similar at 16 h and 20 h of leaf wetness periods, and were significantly less compared to 12 h and 24 h wetness periods. In ICGV 86590, LS₁ and LS₅₀ were observed to be significantly ($P=0.05$) less at 16 h wetness period compared to any other wetness treatment. Sporulation was not observed in ICGV 86699 at any of the wetness period tested.

c) Lesion count: The number of lesions per leaf was observed to be greatly influenced by the wetness period. In the three test genotypes, lesion count was significantly ($P=0.05$) higher when plants were exposed to 16 h leaf wetness period per day following pathogen inoculation, compared to all other wetness periods tested.

d) Lesion diameter: There was gradual increase in LD with increase in leaf wetness periods up to 16 h, in all the three genotypes. Further increase in wetness period reduced LD to a greater extent in ICGV 86699, and doesn't had any effect in TMV 2 and ICGV 86590.

e) Percentage necrotic area: In the three test genotypes, PNA increased when the leaf wetness period was increased from 4 h to 16 h. In TMV 2 and ICGV 86590 further increase in leaf wetness period to 24 h significantly increased the PNA. In ICGV 86699 a sudden decrease was observed in PNA, when the wetness period was increased from 16 h to 20 h.

f) Percentage defoliation: In TMV 2 and ICGV 86590, upto 12 h of leaf wetness period no defoliation was observed and at 16 h wetness period 100% and 56.3% leaflets defoliated. In these two genotypes, both at 20 h and 24 h of wetness period treatments, DEF was significantly ($P=0.05$) less compared to 16 h leaf wetness period. In ICGV 86699, no defoliation occurred in all the wetness treatments.

g) Disease score: In the three test genotypes, an increase in DS was recorded with increase in leaf wetness period from 4 h to 16 h, followed by a decrease in DS with further increase in wetness period.

Least significance difference and SEM values for genotype and wetness treatments and genotype × wetness interaction

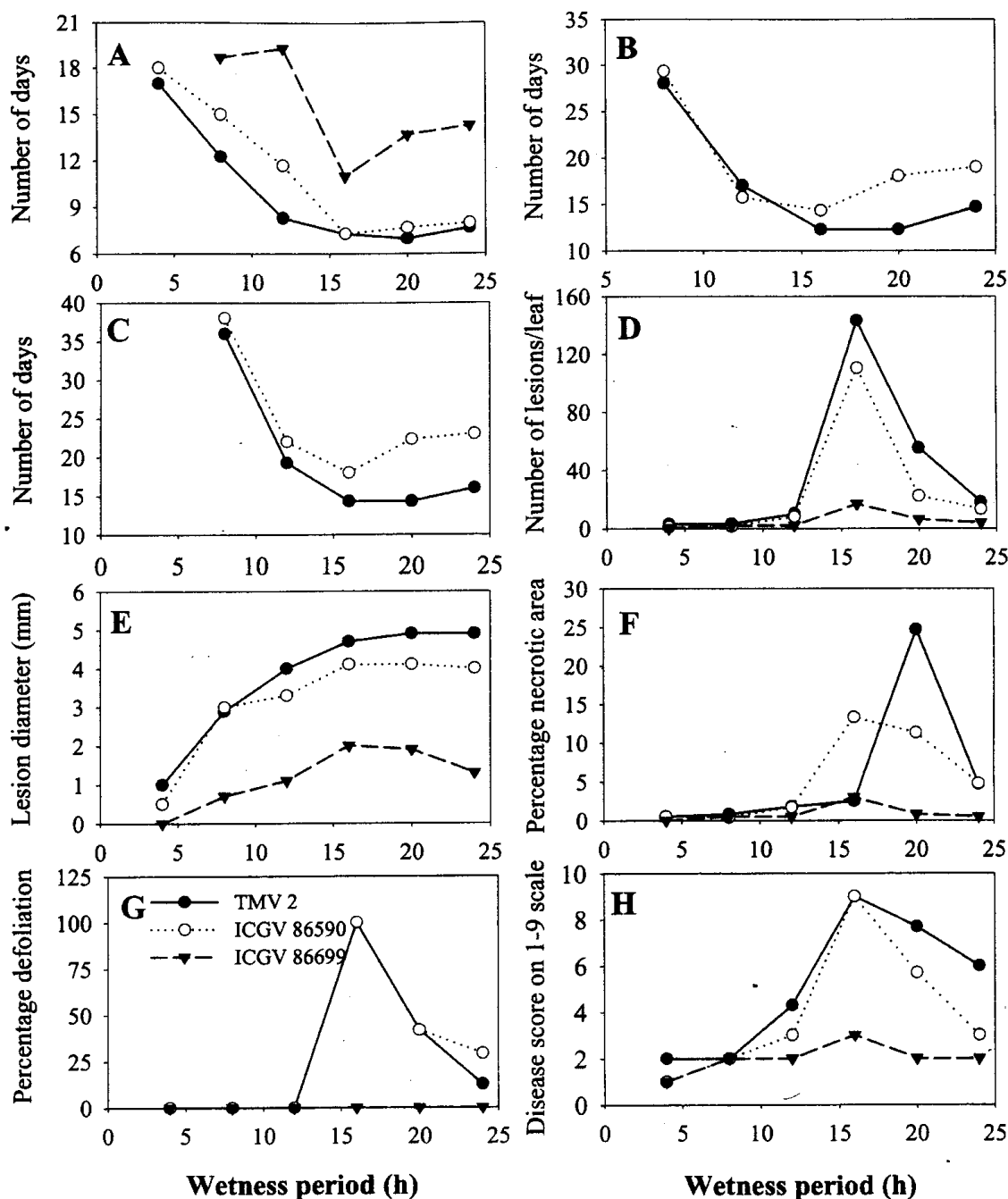


Fig. 2. Effect of leaf wetness period on the development of late leaf spot (LLS) in groundnut. The following components of resistance were measured for evaluation of development of LLS. (A) Incubation period, (B) Latent period (LS₁), (C) Latent period (LS₅₀), (D) Lesion count, (E) Lesion diameter, (F) Percentage necrotic area, (G) Percentage defoliation, and (H) Disease score on 1-9 rating scale. Lesion count was at 3 weeks after inoculation (WAI), when the increase in lesion number was first stopped in TMV 2. Lesion diameter, percentage necrotic area and percentage defoliation were at 4 WAI, just before 100% defoliation was observed in TMV 2. Disease score was at 5 WAI, when maximum disease score was recorded in TMV 2.

were provided in Table 2 for comparison of these different treatments.

Correlations among components of resistance. IP, LS₁ and LS₅₀ had a significant ($P=0.01$) negative correlation to different treatments of both temperature and humidity. Though insignificant, LC was negatively correlated to temperature and positively correlated with wetness periods. LD had a significant ($P=0.01$) positive correlation to both temperature and humidity. Other components PNA, DEF

and DS were more positively correlated to temperature rather than leaf wetness (data on correlation coefficients not shown).

Discussion

The present study aimed to determine the effects of different temperatures and leaf wetness periods on the components of resistance to LLS in three different genotypes. It was

Table 2. ANOVA interactions for the effect of leaf wetness period (LWP) on the development of late leaf spot in groundnut

Component	Interaction	SEM (\pm)	LSD ($P=0.05$)
Incubation period	genotype	0.124	0.356
	leaf wetness period	0.176	0.504
	genotype \times leaf wetness period	0.304	0.873
Latent period (LS_1)	genotype	0.154	0.441
	leaf wetness period	0.218	0.624
	genotype \times leaf wetness period	0.377	1.081
Latent period (LS_{50})	genotype	0.064	0.184
	leaf wetness period	0.091	0.260
	genotype \times leaf wetness period	0.157	0.451
Lesion count	genotype	3.820	10.970
	leaf wetness period	5.410	15.510
	genotype \times leaf wetness period	9.370	26.870
Lesion diameter	genotype	0.022	0.622
	leaf wetness period	0.031	0.088
	genotype \times leaf wetness period	0.053	0.152
Percentage necrotic area	genotype	0.692	1.985
	leaf wetness period	0.979	2.807
	genotype \times leaf wetness period	1.695	4.362
Percentage defoliation	genotype	3.50	10.030
	leaf wetness period	4.940	14.800
	genotype \times leaf wetness period	8.560	24.560
Disease score	genotype	0.147	0.421
	leaf wetness period	0.208	0.596
	genotype \times leaf wetness period	0.360	1.033

observed that the response of different components of resistance to these two epidemiological factors varied according to the genotype. The overall disease severity was maximum at 25°C followed by at 20°C and 15°C in all the three genotypes. Insignificant disease development at 30°C was in agreement with earlier reports. Sommartya and Beute (1986) observed a greater reduction in conidial germination of *P. personata* at temperatures >28°C. The minimum period of exposure to high temperatures that irreversibly inhibits the post-infection development of LLS is not known, but lesion on leaves that were exposed to 32°C for 28 days and then transferred to lower temperatures did not resume development within two weeks (Shew et al., 1988). Under controlled environment conditions, a temperature of >30°C coupled with high RH effectively reduced the spore production on lesions than the recommended dosage of chlorothalonil applied at 7-10 day intervals (Labrinos and Nutter Jr., 1993).

Among the different components of resistance, latent period was considered as the important component for assuming the resistant reactions of different genotypes to LLS infection (Nevill, 1980 and Chiteka et al., 1998). In the present study, temperature was observed to highly influence the latent periods. Both LS_1 and LS_{50} were shortest at 25°C

and longest at 15°C. These results are in agreement with the earlier observations, that latent periods are longer at high temperatures and minimum between 20°C and 30°C (Wadia and Butler, 1994).

In all the genotypes and treatments, increase in LC continued up to ≥ 20 DAI. In the three genotypes, increase in LC was inhibited early at 25°C compared to 20°C and 15°C. This is because of the rapid disease progress at 25°C. As the disease progresses, the lesion size increase and often two or more lesions coalesce to give an appearance of a single lesion. Hence, increase in LC apparently stopped in TMV 2 and ICGV 86590 from 3 WAI. In ICGV 86699, resistance to LLS is associated with the development of small non-sporulating lesions, hence increase in the number of lesions was noticed even after 6 WAI.

Based on the different components of resistance the effect of temperature on *P. personata* infection and disease progress was high between 15 to 20°C than between 20 to 25°C. In similar studies under controlled environment conditions, infection by *P. personata* was optimum when the plants were exposed to 20°C coupled with >93% RH for 12 h a day up to 6 DAI (Shew et al., 1988). Butler et al. (1994) reported that temperatures close to 20°C were optimum for LLS development with 8°C and 34°C, being

the minimum and maximum. In the three test genotypes, there were no significant differences between 20°C and 25°C for IP, LC and PNA, but the disease severity at 20°C was less than at 25°C in terms of LS_{10} , LS_{50} , LD, DEF and DS. Even though the PNA was almost similar at 20 and 25°C measured at 4 WAI, DEF was more in 25°C at that point of time.

The reaction of different genotypes to duration of leaf wetness followed similar trends and maximum disease development was at 16 h in all the genotypes. Leaf wetness/high RH favors groundnut infection by *P. personata* (Rapilly, 1983). The need for alternate wet and dry periods for optimum disease development was emphasized by several studies (Butler et al., 1994; Lannou and Blizoua, 1989). The enhanced penetration under interruption wetting was due to hydrotropism, which directs the germ tubes towards stomata and leaf wetness leads to non-directional growth of germ tubes (Hemingway, 1954; Cook, 1981). Similar results were reported with *Cercospora beticola* (Rathaiiah, 1977), *C. musae* (Goos and Tschirch, 1963) and *C. medicaginis* (Baxter, 1956). The growth of germ tube of *C. arachidicola*, stopped when RH was of <65% during the dry period and resumed in subsequent wet periods (Alderman and Beute, 1986). When the RH was 30-40%, during the dry period, the germ tube failed to grow during further wetness periods. In the present study, the RH varied between 70-80% during the dry periods. Shew et al. (1988) observed maximum lesion number when the leaves were exposed to continuous wetness period. These observations were contradicted by further researchers since in the high humidity chambers used by Shew et al., RH varied between 93 and 99% and there is a possibility of subsequent drying of the leaves after wetting on each evening and the artificial illumination provided might have enhanced the evaporation from the leaves (Butler et al., 1994). Even though no significant differences observed in IP and LD and between 16 and 20 h wetness periods in TMV 2 and ICGV 86590, the severity disease was more at 16 h treatment with higher LN, PNA and DEF. These results support the need for intermittent wet (16 h) and dry (8 h) periods for optimum development of LLS. However, the response of individual components to alterations in wetness periods, for example PNA was not uniform between the three genotypes.

The results obtained in the present study indicate that following inoculation, 25°C temperature coupled with 16 h of leaf wetness period per day favors the maximum disease development in genotypes with varying disease levels of disease resistance. However, the individual components of resistance in the three genotypes didn't respond uniformly to changes in temperature and wetness period. It was concluded that temperature and leaf wetness periods have a crucial role in determining the resistance/susceptible

reactions of the test genotypes, particularly when evaluated based on individual components of resistance.

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