STUDIES ON THE COMPONENTS OF RESISTANCE IN GROUNDNUT TO LATE LEAF SPOT DISEASE INCITED BY PHAEOISARIOPSIS PERSONATA (BERK AND CURT) V. ARX.

By

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Mr. T. Ratna Rajesh has satisfactorily procecuted the course of research and that the thesis entitled **STUDIES ON THE COMPONENTS OF RESISTANCE IN GROUNDNUT TO LATE LEAF SPOT DISEASE INCITED BY PHAEOISARIOPSIS PERSONATA (BERK AND CURT) V. ARX** submitted is the result of original research work and is of sufficiently high standard to warrant its presentation to the examination. I also certify that the thesis or part thereof has not been previously submitted by him for a degree of any University.

Date: 16th June, 1999

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CERTIFICATE

This is to certify that the thesis entitled **"STUDIES ON THE COMPONENTS OF RESISTANCE IN GROUNDNUT TO LATE LEAF SPOT DISEASE INCITED BY *Phaeoisariopsis personata* (Berk and Curt.) V. Arx"** submitted in partial fulfillment of the requirements for the degree of **MASTER OF SCIENCE IN AGRICULTURE** of the **Acharya N.G. Ranga Agricultural University, Hyderabad,** is a record of the bonafide research work carried out by **Mr. T. RATNA RAJESH** under our guidance and supervision. The subject of the thesis has been approved by the Student's Advisory Committee.

No part of the thesis has been submitted for any other degree or diploma. The published part has been fully acknowledged. All assistance and help received during the course of the investigations have been duly acknowledged by the author of the thesis.

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CONTENTS

LIST OF ILLUSTRATIONS

contd..

Figure	Title	Page No.

LIST OF PLATES

contd..

Plate No.	Title	Page No.

LIST OF TABLES

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DECLARATION

I, **T. RATNA RAJESH**, hereby declare that the thesis entitled **"STUDIES ON THE COMPONENTS OF RESISTANCE IN GROUNDNUT TO LATE LEAF SPOT DISEASE INCITED BY PHAEOISARIOPSIS PERSONATA (BERK AND CURT) V. ARX."** submitted to Acharya N G. Ranga Agricultural University for the degree of **Master of Science in Agriculture** is a bonafide record of work done by me during the period of research at International Crops Research Institute for Semi-Arid Tropics (ICRISAT), Patancheru, A.P., 502 324, India. This thesis has not formed in whole or in part, the basis for the award of any degree or diploma.

Date : 16th June, 1999

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ABSTRACT

Studies on the effect of temperature, humidity, inoculum concentration and age of the plant on the components of resistance *viz.*, incubation period, latent period for sporulation, number of leions per leaf , lesion diameter, per cent necrotic area, sporulation index, defoliation and disease score to late leaf spot were undertaken at ICRISAT, Patancheru. In all the studies, TMV2, ICGV 86590 and ICGV 86699 remained susceptible, moderately resistant and resistant respectively.

Over the range of temperatures tested (15, 20, 25, 30, 35^{O}C), the severity of disease was the highest at 25 ^{O}C in all the cvs with the shortest incubation period and latent peroid, the maximum number of lesions per leaf, per cent necrotic area, defoliation and disease score. At 30 and 35^{O}C, no significant amount of disease was observed. The severity of disease progression was the highest between 15 and 20^{O}C and the lowest between 20 and 25^{O}C.

Duration of wetness treatment to cause the maximum amount of disease was 16 h in all the cvs. The severity of the disease was the lowest in low humidity treatments (4,8 and 12 h) and the highest in high humidity treatments (16,20 and 24 h) with the

maximum at 16 h treatment with the shortest incubation period and latent period , the maximum number of lesions per leaf, per cent necrotic area, defoliation and disease score.

Inoculum concentration was found to have no effect on incubation period, latent period for sporulation and sporulation index. The optimum inoculum concentration to cause the highest amount of disease was 20,000 conidia ml^{-1} and there was no significant increase in the amount of disease with further higher concentration in all the three cvs with the maximum number of lesions per leaf, per cent necrotic area, defoliation and disease score. The amount of disease was very much restricted in lower concentration treatments (1000-7500 conidia ml^{-1}).

Twenty to fity day old plants were found to be most susceptible to late leaf spot infection and disease development in all the cvs. However in ICGV 86699, no significant interaction between plant age and late leaf spot infection was observed. Ten day old plants were found to be the most resistant followed by 60,70,80 and 90 day old palnts

Defective germination of conidia, delayed penetration and slow invasion of host tissue by the pathogen led to prolonged incubation period in the resistant cultivar, ICGV 86699. The differences between TMV2 and ICGV 86590 were not much for incubation period.

INTRODUCTION

CHAPTER I

INTRODUCTION

Groundnut (*Arachis hypogaea* L) is one of the world's major food legume crops It originated from South America, where the genus *Arachis* is widely distributed Groundnut is an important oil, food and forage crop generally distributed in tropical, subtropical and warm temperate zones World's groundnut production averages to 28 18 million t from harvest of approximately 21 15 million ha and in India it is 8 2 million t from 8 2 million ha (Production estimates and crop assessment division, USDA, Dec-1997) India, China and USA produce 70 per cent of the world's groundnut (Porter *et al* , 1982)

Late leaf spot (LLS) disease caused by *Phaeoisariopsis personata* (Berk and Curt) V Arx is globally wide spread, and is the most important foliar disease of groundnut LLS damages the plant by reducing the available photosynthetic area, by lesion formation, and by stimulating leaflet abscission Worldwide, yield losses range from 10 to 50 per cent or even more, but vary considerably from place to place and between the seasons Conservative estimates of yield losses caused by the disease are in the order of 0 5 tons per year for India alone, which is about seven per cent of the total production (T G Kelley, ICRISAT, Unpublished data, 1992)

Crop protection from the diseases is one of the mandates given by humanity to plant pathologists The management of LLS disease is mainly based on the use of available host plant resistance and fungicidal spray Exploitation

of host plant resistance is the watchword of every agriculturist in developed and developing countries. Advances in screening (Subrahmanyam *et al.*, 1989) and breeding for resistance to LLS have resulted in release of a number of promising lines (Wynne *et al.*, 1991). However, the degree of resistance shown by a given genotype may vary between the locations, possibly owing to the differences in climate and / or pathogen race. So, it is essential to understand the components of resistance operating in a genotype and the way in which these are affected by various factors. This knowledge will help to develop cultivars with better resistance to LLS even when the environment favours rapid disease increase. Therefore, the present investigations were carried out to:

1. Study the effect of temperature and humidity on the components of resistance to late leaf spot on groundnut.

2. Study the effect of inoculum concentration on the components of resistance to late leaf spot on groundnut.

3. Study the effect of age of the groundnut plant on the components of resistance to late leaf spot on groundnut.

4. Surface studies on the inoculated leaves of different cultivars for spore germination and penetration of late leaf spot pathogen.

REVIEW OF LITERATURE

CHAPTER II

REVIEW OF LITERATURE

The literature relevant to the present investigations is reviewed in this chapter under the following sections:

1. General
2. Components of resistance
3. Histopathological and histochemical studies

2.1 GENERAL

2.1.1 Distribution

Late leaf spot (LLS) caused by *Phaeoisariopsis personata* (Berk and Curt) V.Arx is the most important disease of groundnut worldwide (Jackson and Bell, 1969; Feakin, 1973; Mc Donald *et al.*, 1985). This disease along with early leaf spot has also been referred to as *Mycosphaerella* leaf spots, *Cercospora* leaf spots, peanut cercosporiosis, tikka, viruela, brown spot and black spot (Jackson and Bell, 1969). LLS is commonly present wherever groundnut is grown (Feakin, 1973; Mc Donald *et al.*, 1985) and appears later in the season. The incidence and extent of damage caused by LLS can differ markedly between locations and seasons. In India, LLS is predominant than early leaf spot (Nath and Kulkarni, 1967; Subrahmanyam *et al.*, 1980).

2.1.2 Economic importance:

Late leaf spot together with early leaf spot and rust is the most serious disease of groundnut worldwide. Losses in yield vary from place to place and between seasons. Losses in yield of kernels around 10 per cent have been estimated form the Southern USA, where fungicide application is normally practiced, while over much of the semi-arid tropics where chemical control of leaf spots is very rarely practiced, losses in excess of 50 per cent are common (Jackson and Bell, 1969; Garren and Jackson, 1973). Sundaram (1965) observed a loss up to 22 per cent in yield compared to plots receiving fungicide sprays. Late leaf spot together with rust can cause >50 per cent yield losses (Subrahmanyam *et al.*, 1982) and pod yield losses due to early and late leaf spot pathogens may range from 10-60 per cent (Ghuges *et al.*, 1981). In India, Mehta and Mathur (1954) estimated a reduction in yield of groundnut from 20-50 per cent in severe cases, particularly in late maturing varieties. Leaf spots were alone responsible for more than a half of the total loss caused by diseases to this crop in India (Vasudeva, 1961). Sulaiman (1965) recorded a reduction in groundnut yield of 40 per cent due to leaf spot in Maharashtra and Siddaramaiah *et al.*, (1977a) found a loss of more than 50 per cent in Karnataka.

2.1.3 Symptomotology

Symptoms of the disease have been described by several workers (Woodrof, 1933; Porter *et al.*, 1982 and Mc Donald *et al.*, 1985). Host genotype and environmental conditions influence the symptoms of the disease. Late leaf spots are first recognizable as small chlorotic flecks that enlarge and become

light to dark brown lesions measuring from one to eight mm in diameter Lesions formed by *Phaeoisariopsis personata* tend to be smaller, more nearly circular and darker On the abaxial surface the lesions are black and slightly rough in appearance The fungus usually sporulates on the abaxial surface and the conidial tufts of the pathogen are macroscopically visible as raised circles The yellow halo which is present around early leaf spot is often less conspicuous or absent in LLS lesions The LLS pathogen also produces lesions on petioles, stems, stipules and pegs in the later stage of an epidemic These are oval to elongate and have more distinct margins than the leaflet lesions

2.1.3 The pathogen: *Phaeoisariopsis personata* (Berk and Curt) V Arx

Phaeoisariopsis personata (Berk and Curt)V Arx Proceedings of the Koninklijke Nederlandse Akademie 86(1), 15-24, 1983 (anamorph),

= *Cercosporidium personatum* (Berk & Curt) Deighton Mycological Papers 112, 71,1967

= *Cladosporium personata* Berk & Curt Grevillea 3, 106, 1875,

= *Cercospora personata* (Berk & Curt) Ellis & Everhart Journal of Mycology 1, 63, 1885,

= *Septogloeum arachidis* Racibolski, Zeitschrift fuer Pflanzenkrankheiten und Pflanzenschutz 8, 66, 1898,

= *Cercospora arachidis* P Hennings Hedwigia 41, 18, 1902,

= *Passalora personata* (Berk & Curt) Khan & Kamal Pakistan Journal of Science 13(4), 188, 1961

Mycosphaerella berkeleyi W A Jenkins Journal of Agricultural Research 56, 330, 1938 (teleomarph)

Description of the pathogen

The pertinent morphological characters of the anamorph are stroma dense, pseudo parenchymatous, upto 13 μ in diameter, conidiophores numerous, pale to olivaceous brown, smooth 1-3 geniculate, 10-100 × 3 0-6 5 μ in size, conidial scars conspicuous and prominently 2-3 μ wide The conidia are medium olivaceous cylindrical, obclavate usually straight or slightly curved, walls usually finely roughened, rounded at the apex, base shortly tapered with conspicuous hilum, number of septa vary from 1-9 and 3-4 septa are common

2.2 COMPONENTS OF RESISTANCE

2.2.1 Correlation among the components of resistance

Resistance to late leaf spot pathogen has been attributed to various morphological and anatomical characters of the host plant and to different chemical constituents of leaves and seeds It operates by prolonging incubation and latent periods, and by reducing number of lesions per unit area of leaf surface, defoliation, leaf area damage, size of the lesions and sporulation The components of resistance play an important role in imparting resistance to groundnut genotypes and thus are very useful criteria in selection of genotypes for breeding for LLS resistance In groundnut, however, the association among the components may not be always positive Nevill (1981) reported that on some genotypes that defoliate severely, lesions sporulate sparsely while on others they sporulate heavily Conversely, some genotypes defoliate heavily before 50 per cent of the lesions have began sporulating, while on others, lesions sporulate heavily before defoliation The importance of latent period for

sporulation has long been recognized (Zadokes,1972) and it is the most important component of resistance (Nevill,1980).

Subrahmanyam *et al.*, (1982) screened a number of genotypes in glass house and found that the parameters *viz*., lesion diameter, per cent defoliation and sporulation gave highly significant correlation with field disease scale. Significant differences in lesion diameter on groundnut genotypes in field were reported (Subrahmanyam *et al.*,1982 and Walls *et al.*, 1985). Similarly significant differences in the amount of spore production of *Cercosporidium personatum* were reported among genotypes GPNC-343 and NC5 and susceptible check Nc3033 (Walls *et al.*, 1985). Differences in necrotic area produced on groundnut lines by LLS pathogen have also been observed (Iromue and Knauft ,1987).

Chiteka *et al.*, (1987) screened 116 genotypes of which the most resistant genotypes, UF 81206-1, UF 81206-2 and PI 203396 had both longer latent period and reduced sporulation. Similarly, Jogloy *et al.*, (1987) tested 20 breeding population for resistance to LLS and found that the resistant population had an increased latent period, decreased number of lesions, lesion size, defoliation and reduced spore production.

Greatest variability among genotypes for lesion diameter and latent period has been recorded by Chiteka *et al.*, (1988a). These workers (1988 b) also found that amount of sporulation, lesion size and latent period were highly correlated with each other and with per cent necrotic area. Based on this they concluded that these were the most important components of visual plant appearance score of which sporulation accounted for most of the variability in the score. Shokes and Gorbet (1991) reported that resistance was due to

decreased sporulation and lengthened latent period in resistant varieties (Southern runner and UF81206) as compared to susceptible variety (Florunner)

Recently Watson *et al.*, (1997) observed reduced lesion diameter and longer latent period for sporulation of 50 per cent lesions in partially resistant Southern Runner as compared to susceptible cultivar- Florunner. But there were no differences between spore germination, incubation period and number of lesions per leaf in the two varieties

2.2.2 Effect of temperature and humidity on the components of resistance

It is a well-known fact that temperature and humidity play an important role in the development of diseases. LLS development is highly influenced by these two weather parameters. In the earlier years, Wolf (1914) found no correlation between temperature and moisture and the prevalence of leaf spot. But in 1938, Jenkins reported in Georgia that cool, humid weather during the epiphytotic months favoured the spread and development of disease. Maublanc (1925) from Senegal and Kenknight (1941) from the USA, attributed the rapid spread and severity of leaf spots to heavy rainfall in August-September and in spring in their respective countries. LLS incidence was found relatively more in damp, warm weather and periods of heavy dew in North Carolina, USA (Research and Farming, 1943). Miller (1946) opined that the rapid spread of the leaf spot disease might be correlated with periods of heavy rainfall. Sulaiman and Agashe (1965) studied the influence of climate on the incidence of tikka disease of groundnut in Maharashtra and Andhra Pradesh and found that the minimum predisposing factors to disease development were, an average

maximum temperature of 29.3^{0}C, an average minimum temperature 23^{0}C, an average RH 81.8 per cent and an average rainfall of 240.8mm.

Relative humidity (RH) appears to be a better measure of all the moisture factors that affect leaf wetness and duration of leaf wetness in the crop canopy for the development of disease (Jenson and Boyle ,1965). It was also found that the period required for the maximum infection decreased with increasing temperature between 18 and 27 ^{0}C which implies that rate of infection increases with temperature but with adequate leaf wetness periods However, the final level of infection would not be affected by temperature between 20 and 27^{0}C.

Jenson and Boyle (1966) recognized the importance of leaf wetness to infection and made three important assumptions: (1) adequate inoculum is always present, (2) free water is necessary for spore germination and the speed of germination depends on temperature and (3) periods of RH greater than 95 per cent indicate periods of leaf wetness (this includes wetness from rains) This implies that under sufficient inoculum level, if favourable temperature persists along with long periods of leaf wetness (>95% RH) at least for 2-3 days, LLS development takes place. Ramakrishna and Appa Rao (1968) from Hyderabad , India, reported that a 72h period of high RH was ideal for infection and further development of leaf spot disease.

While evaluating the groundnut genotypes for resistance to leaf spot, Hassan and Beute (1977) found high environmental variations for lesion count per leaf. They found that plants grown continuously in greenhouses tended to develop more lesions than did plants grown outside for two weeks before inoculation. Shew and Beute (1984) reported the requirement of longer mist

periods up to eight days for increase in lesion numbers under greenhouse conditions

Germination of *Cercospora arachidicola* spores declined as RH was reduced from 100 through 98% and the germ tubes were longest at 22^{0}C (Alderman and Beute, 1986)

Temperature of 16-20^{0}C was found favourable for the germination of conidia of *Cercosporidium personatum* (Sommartya and Beute, 1986) They also studied the germination of different isolates of *Phaeoisariopsis personata in vitro* and found that the percentage germination of conidia decreased after 48h with increasing temperature between 16 and 32^{0}C The maximum reduction was at temperature greater then 28^{0}C Contrary to the reports of Jenson and Boyle (1966), Sommartya and Beute (1986) stated that the germination was the maximum at 20^{0}C and at this temperature more than 50% of the conidia germinated

Studies on the effect of duration of leaf wetness on infection at different temperatures revealed that in majority of genotypes the maximum number of lesions were obtained at 20^{0}C but differences between 20 and 24^{0}C were not large (Shew *et al.*, 1988) They pointed out the importance of long periods of leaf wetness with intermittent dry periods for the development of LLS lesions on inoculated leaves Resistance level of genotypes with high (PI 259747,Nc Ac17133), moderate (GPNc 343) and low (Nc 3033, Robut 33-1) resistance decreased with increasing temperature from 20 to 32^{0}C and also by decreasing high RH shorter than 12h/day.

Lanmou and Blizoua (1989) reported that on detached leaves at least six days were required for the establishment of lesions at 27^{0}C and 100 per cent RH

and on potted plants the infection efficiency was the highest when a daily rythemicity in RH (70-100%) was simulated. They concluded that, long period under humidity saturated conditions, preferably spread out over time was required for the development of *cercospora* leaf spot epidemic.

Infection was very rapid at 23^{0}C when the leaf wetness was provided continuously for five nights (Butler, 1990). These results confirmed earlier findings of Shew *et al.*, (1988) that infection occurred with intermittent periods of surface moisture. He therefore concluded that dominant variable affecting infection at a particular temperature was the total number of hours of leaf wetness.

In controlled environment experiments, Alderman and Nutter (1994) found that the minimum of four hours of RH >95 per cent per day was required for conidial production by *C. personatum* and the highest number of conidia was produced when lesions were subjected to a daily period of 16h or more at >95 per cent RH. The optimum temperature for spore production was around 20^{0}C.

Butler *et al.*, (1994) found that the temperature response curves for conidial germination and infection were similar with the optimum close to 20^{0}C and the minimum and the maximum temperature of about 8 and 34^{0}C respectively. They found that the number of lesions resulting from a fixed amount of inoculum was several times greater if the leaves were exposed to alternate wet and dry periods as compared with continuous wetness.

Wadia and Butler (1994) reported that latent period for *C. personatum* ranged from 13-38 days in susceptible variety TMV2, between temperatures 12 and 33^{0}C confirming the findings of Nevill (1981) that latent period for

P. personata was 14.6 days at 25^{0}C in TMV2 They established cardinal temperature as T min -10^{0}C, T opt- close to 25^{0}C and T max-35^{0}C by relating the rate of pathogen development (1/LP) to temperature. These workers (1995) further observed an increase in mean conidial length and number of septa when the humidity increased from 96 through 100 per cent.

2.2.3 Effect of inoculum concentration on the components of resistance

Hassan and Beute (1977) screened about 16 cultivars for resistance to *C. arachidicola* at three inoculum levels (15,000, 10,000 and 5000 conidia ml^{-1}) and found that there were consistent cultivar differences in number of lesions

Components of resistance such as number of lesions, time to leaflet loss by *C. arachidicola* and *C. personatum* were influenced by concentration of inoculum applied to the leaves (Nevill, 1981). Similar results were obtained for other host-pathogen systems - *Solanum spp* to *Phytophthora infestans* (Guzman, 1964) and winter barley cultivar -Vulcan to *Rhynosporium secalis* (Habgood, 1972).

Shew and Beute(1984) found increase in lesion number with increasing spore concentration from 12,500 to 1,00,000 condia ml^{-1} in the cultivar Nc3033.

2.2.4 Effect of plant age on the components of resistance

The amount and extent of infection depends on age of plants, rate of plant growth, method of cultivation and the length of the peanut rotation cycle (Miller, 1946). Subrahmanyam *et al.*, (1982) observed significant interaction between plant age and genotype for all the parameters except sporulation. Fifty day old plants showed higher per cent defoliation, per cent necrotic area,

infection frequency, and lesion diameter than 30 days old plants of different genotypes.

Savary and Van Santen (1992) while working on primary gradients of LLS in field reported heavy defoliation in case of younger plants whereas in older crops accessibility was reduced by high foliage density and high leaf area index. Contrary to this, Comacho de Torres and Suberco (1993) reported that younger plants were the most resistant to *C. arachidicola* independent of the cultivar (Red star, Bolivia pintado and Tarapoto)

2.3 HISTOPATHOLOGICAL AND HISTOCHEMICAL STUDIES

2.3.1 Histopathological studies

Little has been carried out on the mechanisms of resistance. The host response to LLS pathogen widely varies from highly resistant to highly susceptible reaction. The resistance is influenced by the morphological and physiological characters of the host. It is also noted that the number of lesions, necrotic area and extent of sporulation vary greatly from susceptible to resistant cultivars. This may be due to reduced number of penetrations, reduced stomatal index, increased thickness of the cuticle/epidermis and/or histochemical changes within the host in response to infection

Jenkins (1938) reported that infection by LLS pathogen was accomplished through either leaf surfaces. The resistance in wild *Arachis* sp. appeared to be associated with small stomatal apertures (Decurz and Upadhyaya, 1961 and Gibbons and Bailey, 1967) Hemingsway (1957) suggested that the more susceptibility of sequentially branched early maturing variety was due to higher proportion of stomata of "penetrable size" on the

dorsal leaf surface. He also proposed that greater amount of palisade tissue might account for slower rate of pathogen growth on alternatively branched culitivars. Similarly Mazzani *et al.*, (1972) found resistance to LLS in cultivars with small stomatal apertures and also observed that leaf spot counts were higher on cultivars with large light green leaves. Contrary to these reports, Cook (1981) observed no difference in conidial germination on 12 peanut cultivars and also observed that variation in stomatal density and stomatal length were not related to resistance to infection.

Abdou *et al.*, (1974) compared post germination behaviour of *C. personatum* on the leaf surface of wild species and cultivated genotypes and found that germ tubes apparently were not attracted towards the stomata in resistant genotypes but were attracted towards stomata in susceptible genotypes. They reported that conidia and germ tubes lost their stainability and became transparent on resistant genotypes. They also noted that resistance after penetration was associated with the formation of barrier in advance around the infection site in the form of cell wall swelling and thickening and the deposition of pectic substances on the cell wall and in the intercellular spaces.

Longer incubation period, reduced sporulation, stomatal exclusion and absence of directed growth of germ tubes towards the stomata are some of the components of host resistance (Nevill, 1981). Kaur and Dhillon(1988) reported that the epidermal and mesophyll cells were shrunken or collapsed in LLS pathogen infection. The damage to protoplast was more obvious than to the cell walls.

Thicker epidermis, smaller stomata and compact palisade in resistant leaves were accounted for fewer penetration sites of the pathogen in resistant

cultivars (Basra *et al.*, 1985) Mayee and Suryawanshi (1995) reported that late appearance of symptoms in resistant cultivars (NC Ac 17133, PI-405132, 259747,381622 and 390595) to LLS was due to prolonged incubation period because of delayed appearance of mesophyll mass below epidermis

Recently Bera *et al.*, (1997) reported that susceptible genotype possessed wider stomata than resistant / tolerant genotype in lower surface of the normal leaf. They also reported more distortion of mesophyll tissue in susceptible genotype than in resistant / tolerant genotype and opined that thick palisade and spongy tissue in resistant / tolerant genotype might have allowed limited and slow rate of pathogen growth

2.3.2 Histochemical studies

A low level of magnesium was either directly or indirectly responsible for increased susceptibility (Bledsoe *et al.*, 1946). Yenni (1970) found that healthy tissues of all cultivars tested had higher magnesium content than in diseased leaves.

The histochemical localizations revealed a gradual depletion of polysaccharides, proteins, ascorbic acid and nucleic acids from the diseased host tissue at the site of contact with the pathogen and their subsequent accumulation in the pathogen in the later stages of disease developement (Kaur and Dhillon, 1988).

The level of nitrogen was low and phosphorus and that of potassium was high in the resistant cultivars than in the susceptible cultivars at all the growth stages and the elements decreased with the age of the plant in all the cultivars. Similarly the level of zinc was high and level of iron was low in resistant cultivars (Jagalan and Sindhan, 1988). High levels of zinc may be responsible

for resistance because deficiency is thought to restrict RNA synthesis which in turn inhibit protein synthesis and lead to poor growth and increased susceptibility to various pathogens (Mogle and Mayee, 1981)

Sindhan and Parashar (1996) reported that resistant cultivars had higher phenolic contents and lower reduced and non-reducing sugars, and higher P, K, Zn & Cu compared to N, Mn, and Fe. After infection the total phenols increased in all the cultivars and the carbohydrates decreased

MATERIALS &METHODS

CHAPTER III

MATERIALS AND METHODS

The investigations on the quantification of components and mechanisms of resistance in groundnut to LLS were carried out at International Crops Reaserch Institute For Semiarid Tropics (ICRISAT), Patancheru, Andhra Pradesh, 502 324, India. The materials and methods used in the present investigation are broadly described under the following heads:

1. General
2. Experimental design
3. Green house experiments
4. Observations
5. Histopathological investigations

3.1 GENERAL

Three groundnut cultivars (cvs.), TMV 2, ICGV 86590, ICGV 86699 representing susceptible, moderately resistant and resistant respectively were selected to study the effect of temperature, humidity, spore concentration and the age of the plant on the components of resistance. Further, histopathological investigations were conducted to further quantify the components of resistance in these cultivars.

3.1.1 Plant material

Groundnut plants of all the three cvs. were grown in 15-cm diameter plastic pots in a greenhouse. The potting medium consisted of 60 per cent, Alfisol, 20 per cent sand and 20 per cent compost. Healthy plants were maintained in the greenhouse. Four weeks old plants (two/ pot) were used for inoculation.

3.1.2 Collection and multiplication of inoculum

3.1.2.1 The pathogen:

Single lesion isolate of LLS pathogen of groundnut available in groundnut pathology laboratory at ICRISAT-Patancheru was used in these investigations. Inoculum of this pathogen was maintained on the detached leaves of a susceptible groundnut genotype, TMV2. The inoculum was harvested with a cyclone spore collector and stored at 4^0C.

3.1.2.2 Detached leaf techniques for inoculum production

3.1.2.3 Inoculation

Mature, undamaged, apparently healthy leaves of green house-grown groundnut plants were excised through the pulvinus base from the middle portion of the main stem . The leaves were thoroughly washed and arranged in plastic trays (55cm long x 27.5 cm wide and 5 cm deep) with their petioles buried in steam sterilized (15 lbs for 30 min) sand. There were four leaves per row and six rows per tray. Trays were covered with clear polyethylene bags (62 x 38 cm) with the open ends partially sealed with cellophane tape to maintain high relative humidity and the trays were kept in percival incubators at 25^0C and 12h photoperid (4000 lux). After 24 h, the trays were removed from the incubator chambers and the leaves were sprayed on both surfaces with spore suspension (20,000 conidia ml^{-1}) using a plastic atomizer. The trays were then returned to the incubator (Foster *et al.*, 1980).

3.1.2.4 Spore collection

LLS lesions developed and sporulation was observed in two weeks after inoculation in all the leaves. Then the spores were collected from the lesions using a

cyclone spore collector (Fisher Scientific Co., USA) in small glass vials (7.5 cm x 2.0 cm diameter).

3.1.2.5 Inoculum preparation

For all the experiments (except for the study of effect of spore concentration) the inoculum was prepared in the following way

The spores were suspended in distilled water to which a few drops (10 drops 1000 ml^{-1}) of Tween-80, (Polyoxyethylene sorbiton mono-oleate) wetting agent were added (Melouk and Banks, 1978). The spore suspension was stirred well using a magnetic stirrer (model 213, Fisher Scientific Co., USA) to make inoculum uniform. The spore concentration was adjusted to 20,000 conidia ml^{-1} using a haemocytometer

3.2 EXPERIMENTAL DESIGN

All the experiments were carried out under greenhouse conditions. Experiments were conducted in a completely randomized block design with three replications. Each replication contained two healthy plants. On each plant the third or fourth fully expanded leaf from top was tagged prior to inoculation to study the components of resistance.

3.3 GLASS HOUSE EXPERIMENTS

3.3.1 Inoculation

A spore concentration of 20,000 conidia ml^{-1} was used for all the experiments except for the study of effect of spore concentration in which different spore concentrations were used. Conidial suspension was prepared as already described. Immediately after preparation of conidial suspension, the plants were uniformly inoculated with atomizer. Inoculated plants were kept in dew-chambers to ensure complete wetting of leaves. Next day morning the plants were shifted to the greenhouse. Thus, the plants were kept in dew-chambers in the night (16h for wet

period) and in greenhouse during day (8h for dry period) for six days and then the plants were permanently shifted to glasshouse for the rest of the experiment

3.3.2 Studies on the effect of temperature on the components of resistance

The components of resistance to LLS were studied at 15, 20, 25, 30 and 35°C Six dew chambers and six incubators were adjusted to maintain 15,20,25,30 and 35°C for this study. The plants were kept in the dew chambers at night for wet period and moved to incubator during the day for dry period. Care was taken to keep the plants in the same temperature while shifting from and to dew chambers for six days Then the plants were permanently shifted to incubators at the respective temperature till the end of the experiment.

3.3.3 Studies on the effect of humidity on the components of resistance

Immediately after inoculation the plants were kept in dew chamber for leaf wetness for different durations of 4, 8, 12, 16, 20 and 24 h per day. The plants were taken out of the dew chambers as per the treatment and shifted to greenhouse everyday The wet and dry treatments were given for six days and after that, the plants were permanently shifted to greenhouse till the end of the experiment

3.3.4 Studies on the effect of inoculum concentration on the components of resistance

To study the effect of inoculum concentration, the plants were inoculated with different concentrations of conidial suspension (1,000, 2,500, 5,000, 7,500, 10,000, 15,000, 20,000 and 25,000 condia ml^{-1}) Immediately after inoculation, the plants were kept in dew chamber for wet period during nights and in green house for dry period during day. The dew chamber treatment was continued for six days and after that the plants were permanently shifted to greenhouse till the end of the experiment.

3.3.5 Studies on the effect of age of the plant on the components of resistance:

To study the effect of the age of the groundnut plant on the components of LLS resistance, different age groups of plants ranging from 10 to 90DAS were maintained by staggered sowing at 10 days interval. The plants of all ages were inoculated at a time with a conidial suspension containing 20,000 conidia ml^{-1}. Then, dew chamber treatment was given as in the case of other experiments for six days and after that the plants were permanently shifted to green house for the rest of the experiment

3.4 OBSERVATIONS:

The components of resistance studied were incubation period (IP), latent period (LS), lesion count (LC), lesion diameter (LD), necrotic area (NA), defoliation (DEF) and disease score (DS). Scoring was done at weekly intervals soon after IP was observed in all the experiments. The scoring was continued up to 100 days after sowing.

3.4.1 Incubation period (IP): IP was recorded by counting the number of days from inoculation to the appearance of first symptoms. All the leaves of the plants were observed daily to record IP.

3.4.2 Latent period (LS): Latent period was recorded by counting number of days from inoculation to appearance of first sporulating lesion (LS1), and 50 per cent of lesions sporulating (LS50) everyday with the help of a 20X magnifying lens. The lesions were considered to be sporulating tufts of fascicles visible on the lesions

3.5 Surface studies on the inoculated leaves

3.5.1 Plant material

Histopathological studies were conducted by adopting detached leaf technique The leaves were collected and maintained as described in section 3 1 2 2

3.5.2 Inoculation

The detached leaves were inoculated with a conidial suspension containing 50,000 conidia per ml with the help of an atomizer Immediately after inoculation, the trays containing detached leaves were covered with polythene bags and incubated at 25^{0}C in an incubator (Percival Co , Boone, Iowa, USA) Sufficient leaf wetness (RH> 95 %)was maintained at least for 16 h in the trays throughout the experiment

3.5.3 Collection of leaf samples

Samples were collected everyday at 0800h and 1600h, starting from four h after inoculation, till the appearance of first lesion The leaflets were cut into small bits (1× 1 cm) after the midrib was incised

3.5.4 Processing of leaf samples

3.5.4.1 Fixation and clearing

The leaf samples were fixed and cleared in a solution containing glacial acetic acid and absolute ethanol in 2 1 ratio (Johanson, 1940) The samples were left in the solution for 36h After that, the samples were decanted and stained

3.5.4.2 Staining

After clearing, the leaf samples were stained in lactophenol coton blue (0 1%) for about five min and then the stained samples were observed under the light microscope

Table 1: Sporulation index (after incubating the leaves for 48-72h at 25°C in moist chamber) (Pande *et al.*, 1995-ICRISAT- unpublished)

Rating	Reaction	Percentage of lesion area covered with fascicles with conidia
1	No sporulation	0
2	Sparse	Upto 10
3	Slight	11- 20
4	Moderate	21-30
5	Fair	31-40
6	Moderately high	41-50
7	High	51-60
8	Very high	61-80
9	Dense	81- 100

Table 2: A 9- point scale for screening of ground genotypes for resistance to the late leaf spot disease(Subrahmanyam *et al.*, 1982)

Score	Description
1	No disease
2	Lesions present largely on lower leaves, no defoliation
3	Lesions present largely on lower leaves, very few on middle leaves, defoliation of some leaflets evident on lower leaves
4	Lesions on lower and middle leaves but severe on lower leaves defoliation of some leaflets evident on lower leaves
5	Lesions present on all lower and middle leaves; over 50% defoliation of lower leaves
6	Severe lesions on lower and middle leaves; lesions present but less severe on top leaves; extensive defoliation on lower leaves; defoliation of some leaflet evident on some middle leaves
7	Lesions on all leaves but less severe on top leaves; defoliation of all lower and some middle leaves
8	Defoliation of all lower and middle leaves; severe lesions on top leaves; some defoliation of top leaves evident
9	All most all leaves defoliated, leaving bare stems; some leaflets may remain, but show severe leafspots

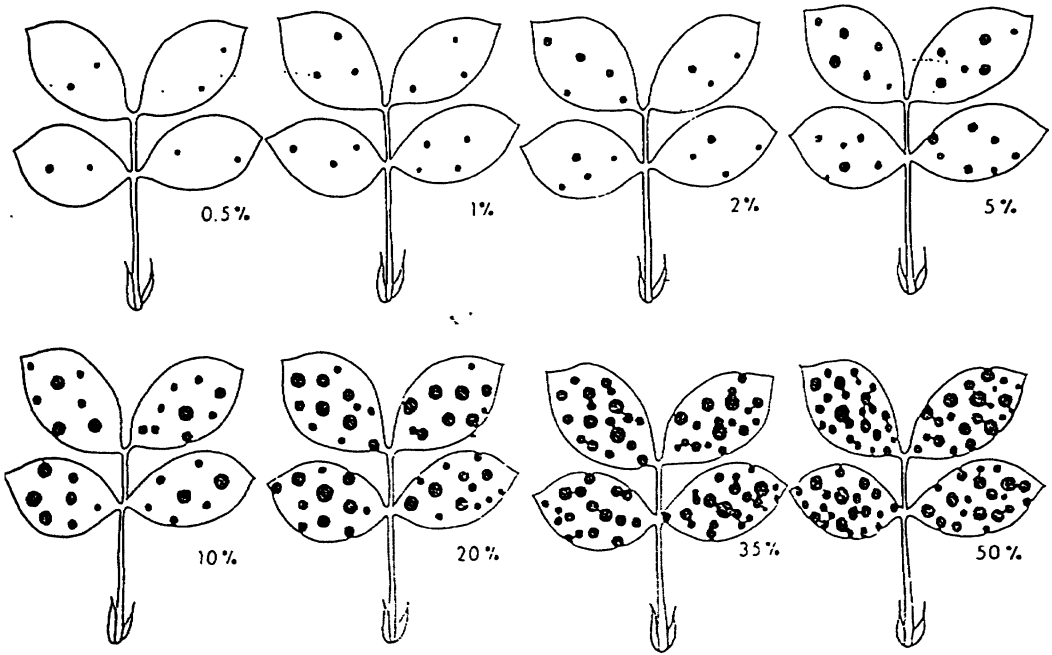
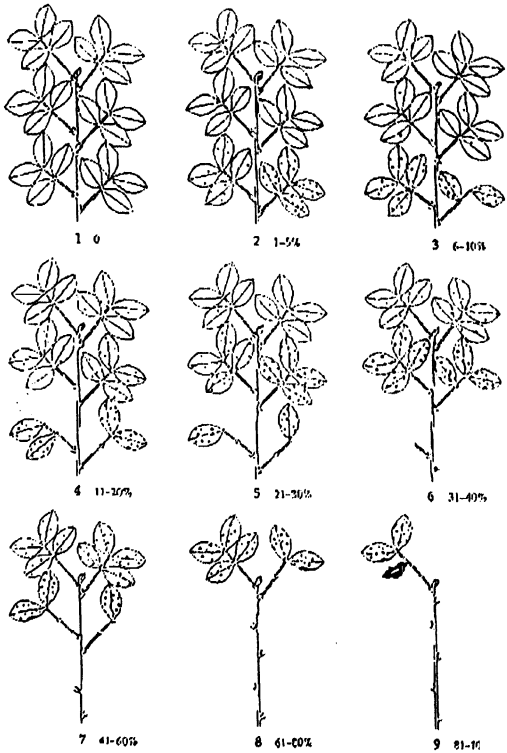


Fig.1: Standardized pictorial chart showing per cent necrotic area caused by late leaf spot disease

Fig.2 : Modified 9-point scale for evaluation of late leaf spot



Source: Subrahmanyam et al., 1995

RESULTS

CHAPTER IV

RESULTS

Investigations were carried out to study the effect of temperature, relative humidity, inoculum concentration and age of the plant on the components of late leaf spot. Histopathological investigations were also carried out to further quantify the components of resistance to LLS. The results obtained from the above investigations are presented in this chapter.

4.1 Effect of temperature on the components of resistance to LLS

Different temperatures (15,20,25,30 and 35°C) were selected to study their effect on the components of resistance. At 35°C there was no disease and plants eventually died. At 30°C, even though lesions were observed the amount of disease was insignificant.

4.1.1 Incubation period

The incubation period (IP) was shortest at 25°C and longest at 15°C in all the cvs (Table 3). Among the cvs, TMV2 showed shortest IP (8.67 days) at 25°C and ICGV 86590 showed longest IP (10.67 days) at 20°C. Significant differences were found between cultivars at all the temperatures. However there was no significant difference between the IP's at 20 and 25°C in all the cultivars. No significant difference was observed between TMV2 and ICGV 86590 at 25°C.

4.1.2 Latent period (LS 1 and LS 50)

No sporulation was observed at all the temperatures in the cv ICGV 86699. Significant differences were seen between the temperatures for both LS 1 (days from inoculation to the appearance of first sporulating lesion) and LS 50 (50% lesions sporulating) in TMV2 and ICGV 86590 and also between these two cvs

Table 3 : Effect of temperature on incubation period, latent period and sporulation index.

	Incubation period (IP)[a]				Latent peroid - (LS1)[b]				Latent period - (LS50)[c]				Sporulation index (SI)[d]			
Temperature[b]C*	15	20	25	Cv. mean**	15	20	25	Cv. mean	15	20	25	Cv. mean	15	20	25	Cv. mean
Cultivar																
TMV2(S)	10.33	8.67	8.67	5.63	31.67	23.33	13.33	13.67	34.67	32.33	17.33	16.87	4.93	9	9	4.59
ICGV 86590(MR)	11	9.67	9.33	6	43.67	42	22.67	21.67	49.67	46.67	26.33	24.53	2.93	8	9	3.99
ICGV86699(R)	12	10.67	10.33	6.6	–	–	–	–	–	–	–	–	1	1	1	0.6
Treat.mean	10.78	9.56	9.22		25.11	21.77	12		28.11	26.66	14.55		2.95	6	6.33	
	SEM	CD(0.05)			SEM	CD(0.05)			SEM	CD(0.05)			SEM	CD(0.05)		
Cultivar	0.102	0.294			0.196	0.567			0.094	0.272			0.037	0.017		
Temperature	0.132	0.38			0.253	0.732			0.122	0.352			0.048	0.138		
Interaction	0.228	0.658			0.439	1.268			0.211	0.609			0.083	0.238		
CV%	4.8				6.5				2.6				4.7			

All the figures are average of three replications

* = No significant amount of disease was observed at 30 and 35°C

** = Mean of five temperatures (15, 20, 25, 30, 35)

a = Incubation period as number of days from inoculation to the appearence of first lesion

b = Latent period as number of days inoculation to the appearence of first sporulating lesion

c = Latent period as number of days inoculation to the appearence of 50% lesions sporulating

d = Sporulation index on 1-9 scale 2 WAI, when maximum amount of sporulation was observed in susceptible cultivar- TMV2

S = Susceptible cultivar MR = Moderately resistant cultivar R = Resistant cultivar

(Table 3). LS 1 and LS 50 were shortest at 25°C and longest at 15°C in all the cultivars. TMV2 showed shortest LS1 (13.33 days) and LS50 (17.33 days) at 25°C.

4.1.3 Sporulation Index

Sporulation index (SI) was almost nil in ICGV 86699 at all the temperatures studied (Table 3). Highest sporulation (9) was recorded 2 weeks after inoculation (WAI) at 20 and 25°C in TMV2 and at 25°C in ICGV 86590. Significant differences were observed between 20 and 25°C in ICGV 86590. Significant differences were found among cultivars at 15°C. At 15°C, SI reached maximum at 5 WAI in TMV2 and 8 WAI in ICGV 86590. At 20°C, ICGV 86590 showed maximum SI (9) at 3 WAI.

4.1.4 Lesion number

The number of lesions per leaf was significantly higher in TMV2 (155.2, 160.8 and 144.2) than in ICGV 86590 (74.3, 70.7 and 66) and ICGV 86699 (74.2, 59.3 and 38.7) at 15,20 and 25°C respectively (Table 4). In susceptible TMV2, the number of lesions per leaf increased upto 3 WAI at all the temperatures and no significant differences were found among the temperatures. In ICGV 86590 also, no significant differences were found among the temperatures. However lesion number was increased upto 6 WAI at 15°C.

4.1.5 Lesion diameter

Maximum lesion diameter (6.23 mm) was observed in TMV2 5 WAI at 25°C. At 5 WAI significant differences were observed between the temperatures and also in between the cultivars (Table 4). TMV2 and ICGV 86699 showed highest (3, 5 and 6.23 mm) and lowest (1, 2 and 3.8 mm) lesion diameters respectively at 15, 20 and 25°C. Highest lesion diameter was noticed at 25°C and lowest at 15°C in all the cultivars.

Table 4 : Effect of temperature on lesion number, lesion diameter, per cent necrotic area and defoliation

	Lesion number per leaf [a]				Lesion diameter [b]				Per cent necrotic area [c]				Defoliation [d]			
Temperature°C*	15	20	25	Cv.mean**	15	20	25	Cv.mean	15	20	25	Cv.mean	15	20	25	Cv.mean
Cultivar																
TMV2(S)	155.2	160.8	144.2	92.1	3	5	6.23	3.25	15.83	35.33	31.67	16.67	0	2.5	100	37.5
ICGV86590(MR)	74.3	70.7	66	42.4	2	3.2	5	2.04	7.5	16.67	16.67	8.17	0	12.5	70.83	16.66
ICGV86699(R)	74.2	59.3	38.7	34.44	1	2	3.8	1.39	2	6.33	7.58	3.18	0	0	25	5
Treat.mean	101.2	96.93	82.96		2.06	3.4	5.01		8.44	19.44	18.64		0	8.33	65.27	
	SEM	CD(0.05)			SEM	CD(0.05)			SEM	CD(0.05)			SEM	CD(0.05)		
Cultivar	2.79	8.05			0.037	0.107			0.656	1.895			1.361	3.93		
Temperature	3.6	10.39			0.048	0.138			0.847	2.446			1.757	5.074		
Interaction	6.23	17.99			0.083	0.238			1.467	4.237			3.043	8.79		
CV%	19.9				4.7				28.1				36.5			

All the figures are average of three replications

* = No significant amount of disease was observed at 30 and 35°C

** = Mean of five temperatures (15, 20, 25, 30, 35)

a = Number of lesions per leaf 3WAI, when the increase in the lesion number was first stopped in susceptible cultivar-TMV2

b = Lesion diameter 5WAI, just before 100% defoliation was observed in the susceptible cultivar

c = Percentage necrotic area 4WAI, just before 100% defoliation was observed in the susceptible cultivar

d = Defoliation 6WAI, when 100% defoliation was first observed in the susceptible cultivar.

S = Susceptible cultivar MR = Moderately resistant cultivar

4.1.6 Per cent necrotic area

The per cent necrotic area (PNA) per leaf was significantly higher in TMV2 than all the cultivars at all temperatures (Table 4). The PNA was lowest at 15°C. Eventhough the PNA was maximum in all the cultivars at 20°C, it was on par with 25°C. ICGV 86699 did not show any significant difference between the temperatures. Cultivar differences were significant at 15, 20 and 25°C with highest PNA (15.83, 35.33 and 31.67) in TMV2 and lowest PNA (2, 6.33 and 7.58) in ICGV 86699.

4.1.7 Defoliation

At 4 WAI, per cent defoliation was highest (100) at 25°C in TMV2 followed by ICGV 86590. No defoliation at 15°C and highest defoliation was observed in all the cultivars. Significant differences were also noticed among the cultivars in all the temperatures except at 15°C (Table 4).

4.1.8 Disease severity

Maximum disease (9) score was observed 6 WAI in TMV2 at 25°C . Lowest disease was recorded in ICGV 86699 in all the temperatures. Significant differences were observed among the cultivars at all the temperatures (Table 5). The disease score was highest at 25°C and lowest at 15°C in all the three cultivars (Plate 1). TMV2 and ICGA 86699 showed highest (5, 7 and 9) and lowest (2, 3 and 3) disease score respectively at 15, 20 and 25°C.

4.2 Effect of humidity on the components of resistance to LLS

4.2.1 Incubation period

The IP was longer at low humidity treatments (4, 8 and 12 h) (Table 6), but shortest at 16 h treatment in all the three cvs while the IP's at 20 and 24 h treatments

Table 5: Effect of temperature on disease score 4WAI.

	Disease score			
Temperature^{0}C*	15	20	25	Cvar.mean**
Cultivar				
TMV2(S)	5	7	9	4.2
ICGV86590(MR)	4	4.33	5	2.66
ICGV86699(R)	2	3	3	1.6
Treat.mean	3.66	4.77	5.66	

	SEM	CD(0.05)
Cultivar	0.0385	0.1112
Temperature	0.0497	0.1435
Interaction	0.0861	0.2486
CV%	5.3	

All the figures are average of three replications

* = No significant amount of disease was observed at 30 and 35^{0}C

** = Mean of five temperatures (15, 20, 25, 30, 35)

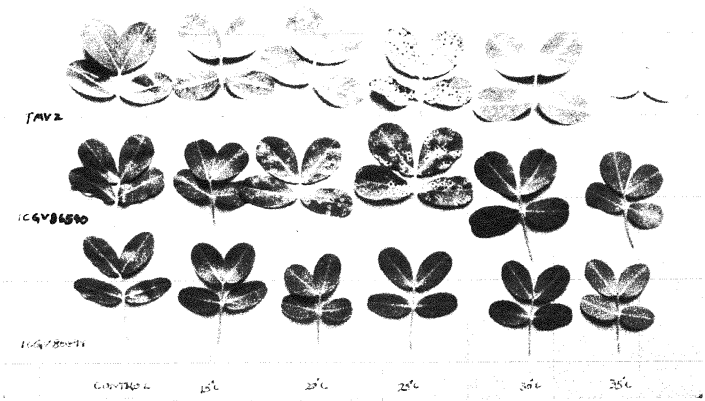


Plate 1 : Effect of temperature on the components of resistance to late leaf spot in groundnut.

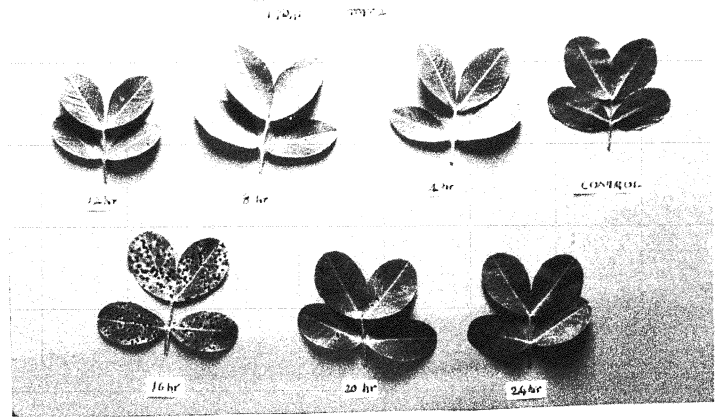


Plate 2 : Effect of humidity on the components of resistance to late leaf spot in TMV2.

were on par with the 16 h treatment in TMV2 and ICGV 86590. IP was shortest (7 days) at 16h in TMV2 among all the treatments. In ICGV 86699, IP was shortest (11 days) at 16 h treatment and no IP was noticed at 4 h. ICGV 86699 showed lowest IP than TMV2 and ICGV 86590 in all the treatments.

4.2.2 Latent period (LS 1 and LS 50)

No sporulation was observed in ICGV 86699 in any of the treatments while it was nil in 4h treatment in TMV2 and ICGV 86590. Significant differences were observed in all the treatments in TMV2 and ICGV 86590 (Table 6). Both LS 1 and LS 50 were shortest at 16 h treatment and longest at 8 h treatment in all the cultivars. In TMV2 no significant difference was found for LS 1 and LS 50 between 16 h and 20 h treatments.

4.2.3 Sporulation index

Sporulation index reached maximum (9) in TMV2 for 16 h and 20 h treatments at 3 WAI (Table 6). SI was highest (9) in TMV2 and lowest (1) in ICGV in ICGV 86699 in all the treatments. Maximum SI (9) was recorded at 16 h treatment followed by 20, 24, 12 and 8 h treatments in the same order in TMV2 and ICGV 86590. At 4 h treatment there was no sporulation in all the three cultivars. In ICGV 86699, sporulation was observed sparsely (1.33) at 16 h treatment only.

4.2.4 Lesion number

In all the cvs, maximum and minimum number of lesions per leaf were observed at 16 h and 4 h treatments, respectively 3 WAI (Table 7). Lesion number was highest (143.2) at 16 h treatment in TMV2 and nil at 4 h treatmnet in ICGV 86699. The number of lesions per leaf was highest in TMV2 and lowest in ICGV 86699, in all the treatments. Significant differences were noticed among cultivars in lesion number at 16 h treatments.

Table 6 : Effect of humidity on incubation period, latent period and sporulation index.

Humidity duration	Incubation period[a]							Latent period- LS1[b]						
	4	8	12	16	20	24	Cv.mean	4	8	12	16	20	24	Cv.mean
Cultivar														
TMV2(S)	17	12.33	8.33	7	7	7.67	9.94	_	28	17	12.33	12.33	14.67	16.86
ICGV86590(MR)	18	15	11.67	7.33	7.67	8	13.53	_	29.33	15.67	14.33	18	19	16
ICGV86699(R)	_	18.67	19.33	11	13.67	14.33	12.78	_	_	_	_	_	_	0
Treat.mean	11.66	15.33	13.11	8.22	9.44	10.11		_	19	10.89	8.89	10.11	11.22	
	SEM	CD(0.05)						SEM	CD(0.05)					
Cultivar	0.124	0.356						0.154	0.441					
Humidity	0.176	0.504						0.218	0.624					
Interaction	0.304	0.873						0.377	1.081					
CV%	4.7							6.6						

Humidity duration	Latent period- LS50[c]							Sporulation index[d]						
	4	8	12	16	20	24	Cv.mean	4	8	12	16	20	24	Cv.mean
Cultivar														
TMV2(S)	_	36	19.33	14.33	14.33	16	19.99	1	7	7.78	9	9	8	6.96
ICGV86590(MR)	_	38	22	18	22.33	23	20.05	1	6	6.33	7.73	7	6.67	6.94
ICGV86699(R)	_	_	_	_	_	_	0	1	1	1	1.33	1	1	1.05
Treat.mean	_	24.66	13.78	9.76	12.22	13		1	4.59	5.03	6.02	5.66	5.22	
	SEM	CD(0.05)						SEM	CD(0.05)					
Cultivar	0.064	0.184						0.033	0.094					
Humidity	0.091	0.26						0.046	0.133					
Interaction	0.157	0.451						0.08	0.231					
CV%	2.2							2.9						

All the figures are average of three replications

a = Incubation period as number of days from inoculation to the appearence of first lesion

b = Latent period as number of days inoculation to the appearence of first sporulating lesion

c = Latent period as number of days inoculation to the appearence of 50% lesions sporulating

d = Sporulation index on 1-9 scale 2 WAI, when maximum amount of sporulation was observed in susceptible cultivar- TMV2

S = Susceptible cultivar MR = Moderately resistant cultivar R = Resistant cultivar

4.2.5 Lesion diameter

Lesion size significantly varied between the cultivars in all the treatments. Lesion diameter was the highest (4.89 mm) at 20 h treatment in TMV2 and no lesions were observed at 4 h treatment in ICGV 86699 TMV2 and ICGV 86699 showed highest and lowest lesion diameter respectively for all the treatments (Table7). The difference in size of the lesions were not significant in 20 and 24 h wetness treatments in TMV2 and among 16, 20 and 24 h wetness treatments in ICGV 86590.

4.2.6 Per cent necrotic area

There were no significant differences in PNA in 4, 8, 12 and 24 h wetness treatments for all the cultivars and also between the cvs 5 WAI (Table 7). The maximum PNA (25, 13.33 and 3) was recorded in 16 h treatment in TMV2, ICGV 86590 and ICGV 86699 respectively. The PNA was highest (25) in TMV2 at 16 h and lowest (0) in ICGV 86699 at 4 h treatment. PNA was not recorded in ICGV 86699 in 4 h wetness treatment.

4.2.7 Defoliation

At 6 WAI, maximum defoliation (100) was observed in TMV2 and ICGV 86590 at 16 h wetness treatment followed by 20 and 24 h treatments. No defoliation was observed TMV2 and ICGV 86590 at 4, 8 and 12 h wetness treatments. There were no significant differences between TMV2 and ICGV 86590 in all the treatments (Table 7).

4.2.8 Disease severity

Maximum disease score (9) was recorded on TMV2 6 WAI in 16 h wetness treatment followed by 20, 24, 12, 8 and 4 h wetnes treatments. Significant differences were also observed among cultivars in all the treatments (Plates 2, 3 and

Table 7 . Effect of humidity on lesion number, lesion diameter, per cent necrotic area and defoliation

Humidity	Lesion number per leaf [a]							Lesion diameter [b]						
duration	4	8	12	16	20	24	Cv.mean	4	8	12	16	20	24	Cv.mean
Cultivar														
TMV2(S)	2.8	3	10	143.2	55.3	16	37.9	1	2.88	4	4.7	4.89	4.88	3.72
ICGV86590(MR)	0.8	1.2	8	110.3	22.2	13.3	25.6	0.5	3	3.33	4.13	4.05	4	3.8
ICGV86699(R)	0	2	2	16.8	6.2	4	4	0	0.67	1.11	2	1.85	1.33	1.17
Treat.mean	1.2	2.06	3.6	90.1	27.9	10.5		0.5	2.19	2.81	3.61	3.61	3.41	

	Lesion number per leaf [a]		Lesion diameter [b]	
	SEM	CD(0.05)	SEM	CD(0.05)
Cultivar	3.82	10.97	0.022	0.622
Humidity	5.41	15.51	0.031	0.088
Interaction	9.37	26.87	0.053	0.152
CV%	72.1		3.2	

Humidity	Per cent necrotic area [c]							Defoliation [d]						
duration	4	8	12	16	20	24	Cv.mean	4	8	12	16	20	24	Cv.mean
Cultivar														
TMV2(S)	0.5	0.83	1.83	25	24.7	4.67	9.5	0	0	0	100	41.7	12.5	13.1
ICGV86590(MR)	0.5	0.42	1.67	13.33	11.33	4.83	5.35	0	0	0	100	41.7	29.2	23.5
ICGV86699(R)	0	0.5	0.5	3	0.83	0.5	0.91	0	0	0	0	0	0	0
Treat.mean	0.37	0.58	1.33	13.78	12.1	3.33		0	0	0	66.67	27.8	13.9	

	Per cent necrotic area [c]		Defoliation [d]	
	SEM	CD(0.05)	SEM	CD(0.05)
Cultivar	0.692	1.985	3.5	10.03
Humidity	0.979	2.807	4.94	14.8
Interaction	1.695	4.362	8.56	24.56
CV%	55.9		95.6	

All the figures are average of three replications

a = Number of lesions per leaf 3WAI, when the increase in the lesion number was first stopped in susceptible cultivar-TMV2

b = Lesion diameter (mm) 6WAI, just before 100% defoliation was observed in the susceptible cultivar

c = Percentage necrotic area 5WAI, just before 100% defoliation was observed in the susceptible cultivar

d = Defoliation 6WAI, when 100% defoliation was first observed in the susceptible cultivar

Cvar.mean = Cultivar mean

Table 8 :Effect of humidity on disease score 6 WAI.

Humidity duration	Disease score						
Cultivar	4	8	12	16	20	24	Cv.mean
TMV2(S)	2	2	4 33	9	7 67	6	5 16
ICGV86590(MR)	1	2	3	9	5 67	3	3 94
ICGV86699(R)	1	2	2	3	2	2	2
Treat.mean	1.33	2	3 11	7	5 11	3	
	SEM	CD(0.05)					
Cultivar	0 147	0.4216					
Humidity	0 208	0 596					
Interaction	0.36	1.033					
CV%	17.9						

All the figures are average of three replications

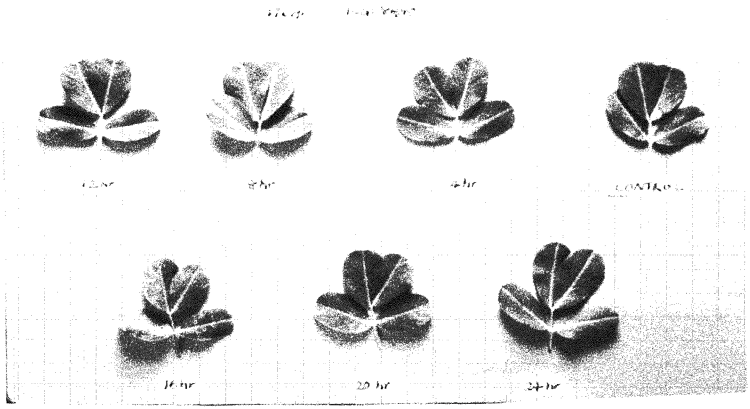


Plate 3 : Effect of humidity on the components of resistance to late leaf spot in ICGV86590.

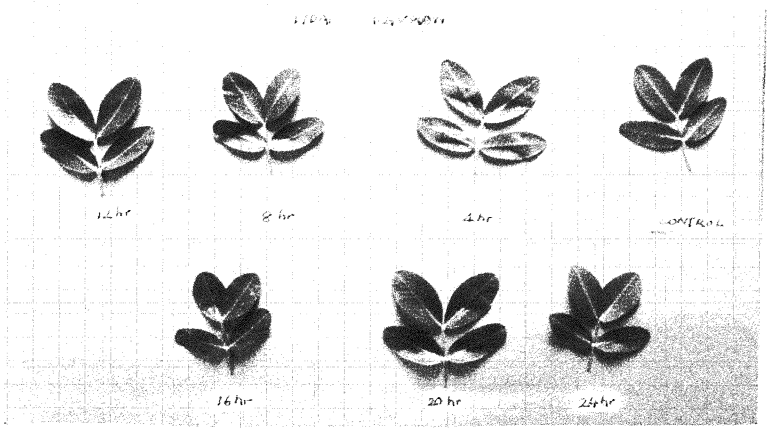


Plate 4 : Effect of humidity on the components of resistance to late leaf spot in ICGV86699.

4). There was no significant difference between 12 and 24 h wetness treatments in ICGV 86590. TMV2 and ICGV 86699 showed maximum and minimum disease score respectively in all the treatments (Table 8).

4.3 Effect of inoculum concentration on the components of resistance to LLS

4.3.1 Incubation period

The differences between the concentrations were non-significant in all the cultivars. IP was shortest (8 days) in TMV2 and longest (12.33-13 days) in ICGV 86699 in all the concentrations. No significant differences in IP between TMV2 and ICGV 86590 but the two cultivars differed significantly with ICGV 86699 (Table 9).

4.3.2 Latent period (LS1 and LS 50)

No sporulation was recorded in ICGV 86699 in all the concentrations. The differences between TMV2 and ICGV 86590 were significant, whereas the differences between the concentrations in each cultivar were non-significant (Table 9). LS1 (13.67-14.33 days) and LS 50 (15-15.33 days) were shortest in TMV2 in all the concentrations.

4.3.3 Sporulation index

Cultivar differences were significant in all the concentrations 3 WAI. SI was maximum in TMV2 (8.33-9) followed by ICGV 86590 (3.33-3.83) in all the concentrations (Table 9). No sporulation was recorded in ICGV 86699 in any of the concentrations tested. Maximum SI (9) was recorded in 25000, 20000 and 15000 conidia ml^{-1} in TMV2. No significant differences were observed in ICGV 86590 among the concentrations.

Table 9 : Effect of inoculum concentration on Incubation period, latent period and sporulation index.

	Incubation period (IP)[a]								
Inoc. Conc*	25000	20000	15000	10000	7500	5000	2500	1000	Cv. mean
Cultivar									
TMV2(S)	8	8	8	8	8	8	8	8	8
ICGV86590(MR)	7.67	8.33	8.33	8.67	8.67	8.67	8.67	8.67	8.48
ICGV86699(R)	12.33	12.33	12.67	13	13	12.67	12.67	12.67	12.67
Treat.mean	9.33	9.55	9.66	9.89	9.77	9.78	10	9.78	10.44
	SEM	CD(0.05)							
Cultivar	0.108	0.306							
Inoc. Conc	0.176	0.5							
Interaction	0.304	0.86							
CV%	5.5								

	Latent period - (LS1)[c]								
Inoc. Conc*	25000	20000	15000	10000	7500	5000	2500	1000	Cv.mean
Cultivar									
TMV2(S)	13.67	13.7	13.67	14	14	14	14.33	14.33	14
ICGV86590(MR)	14.33	14.63	14.67	15	15	15	15.33	15.33	14.91
ICGV86699(R)	–	–	–	–	–	–	–	–	–
Treat.mean	9.33	9.46	9.44	9.77	9.66	9.66	9.88	9.88	
	SEM	CD(0.05)							
Cultivar	0.072	0.208							
Inoc. Conc	0.118	0.34							
Interaction	0.204	0.76							
CV%	2.5								

	Latent period - (LS50)[c]								
Inoc. Conc*	25000	20000	15000	10000	7500	5000	2500	1000	Cv. mean
Cultivar									
TMV2(S)	15	15	15	15.33	15	15	15.33	15	15.08
ICGV86590(MR)	19	19	19	19	19.33	19.33	19	19.33	19.12
ICGV86699(R)	–	–	–	–	–	–	–	–	–
Treat. mean	11.33	11.33	11.33	11.77	11.44	11.44	11.44	11.94	
	SEM	CD(0.05)							
Cultivar	0.059	0.17							
Inoc. Conc	0.096	0.277							
Interaction	0.167	0.48							
CV%	1.7								

	Sporulation index (SI)[d]								
Inoc. Conc*	25000	20000	15000	10000	7500	5000	2500	1000	Cv.mean
Cultivar									
TMV2(S)	9	9	9	8.67	7.8	7.89	8	8.33	8.46
ICGV86590(MR)	3.73	3.73	3.83	3.83	3.33	3.33	3.42	3.44	3.58
ICGV86699(R)	1	1	1	1	1	1	1	1	1
Treat. mean	4.58	4.58	4.61	4.5	4.04	4.07	4.14	4.14	
	SEM	CD(0.05)							
Cultivar	0.602	0.018							
Inoc. Conc	0.101	0.286							
Interaction	0.174	0.496							
CV%	7.1								

All the figures are average of three replications

a = Incubation period as number of days from inoculation to the appearence of first lesion

b = Latent period as number of days inoculation to the appearence of first sporulating lesion

c = Latent period as number of days inoculation to the appearence of 50% lesions sporulating

d = Sporulation index on 1-9 scale 3 WAI, when maximum amount of sporulation was observed in susceptible cultivar- TMV2

S = Susceptible cultivar MR = Moderately resistant cultivar R = Resistant cultivar

* = Inoculum concentration as number of conidia per ml

4.3.4 Lesion number

Maximum number of lesions per leaf were observed at in 25000 and 20000 conidia ml^{-1} in all the cultivars with no significant differences between the two treatments. In TMV2 (7.7-22) and ICGV 86590 (3.3-11.2) lesion number was minimum in 1000 - 7500 conidia ml^{-1} concentrations and significant differences were not noticed both among the treatments and among the cultivars. Lesion number was highest in TMV2 (7.7-125.3) and lowest in ICGV 86699 (0.5-20.7) at all the concentrations. Cultivar differences were significant at 25000 and 20000 conidia ml^{-1} concentrations but no significant differences were observed in 7500, 5000, 2500 and 1000 conidia ml^{-1} concentrations. In 15000 and 10000 conidia ml^{-1} treatments, no significant differences were observed between TMV2 and ICGV 86590 but both differed significantly with ICGV 86699 (Table 10).

4.3.5 Lesion diameter

Lesion diameter was highest (5.88-7 mm) in TMV2 and lowest (0.67-1.5 mm) in ICGV 86699 in all the concentrations. The differences between the cultivars were significant in all the concentrations. Lesion diameter was maximum in 25000, 20000, 15000 and 10000 conidia ml^{-1} concentrations with no significant differences among them in all the cultivars. In 7500, 5000, 2500 and 1000 conidia ml^{-1} treatments, lesion diameter was minimum in all the cultivars with no significant differences among the treatments(Table 10).

4.3.6 Per cent necrotic area

PNA was maximum (3.5-27.5) in TMV2 and minimum (0.5-3) in ICGV 86699 in all the treatments 5 WAI (Table 10). In TMV2 and ICGV 86590, PNA was maximum in 25000, 20000 and 15000 conidia ml^{-1} treatments with no significant differences among them. In ICGV 86699, no significant differences

Table 10 : Effect of inoculum concentration on lesion number, lesion diameter, per cent necrotic area and defoliation

	Lesion number per leaf [a]									Lesion diameter [b]								
Inoc. Conc*	25000	20000	15000	10000	7500	5000	2500	1000	Cv. mean	25000	20000	15000	10000	7500	5000	2500	1000	Cv.mean
Cultivar																		
TMV2(S)	125.3	122	41.2	31	22	15.2	10.8	7.7	46.9	6.83	7	6.93	7	6	6	5.88	5.88	6.42
ICGV86590(MR)	70.5	73.2	30	26.7	11.2	8.3	8.2	3.3	28.92	4.8	4.62	4.86	4.8	4	4	3.88	4	4.37
ICGV86699(R)	20.7	22.7	10.2	4	2.7	2.2	1.8	0.5	8.1	1.5	1.5	1.5	1.22	0.9	0.88	0.67	0.83	1.12
Treat.mean	72.16	72.6	27.1	20.56	11.95	8.6	6.9	3.8		4.38	4.37	4.43	4.34	3.63	3.63	3.48	3.57	
	SEM	CD(0.05)								SEM	CD(0.05)							
Cultivar	2.43	6.9								0.0312	0.0886							
Inoc. Conc	3.96	11.27								0.0509	0.1447							
Interaction	6.86	19.51								0.0881	0.2506							
CV%	43.3									3.9								

	Per cent necrotic area [c]									Defoliation [d]								
Inoc. Conc*	25000	20000	15000	10000	7500	5000	2500	1000	Cv. mean	25000	20000	15000	10000	7500	5000	2500	1000	Cv.mean
Cultivar																		
TMV2(S)	25	27.5	20.83	16.67	14.17	12.5	6.5	3.5	15.83	100	87.5	50	20.8	0	0	0	0	32.3
ICGV86590(MR)	17.5	16.67	14.17	9.17	3.67	4.17	2.5	1.5	8.66	70.8	75	50.2	33.3	0	0	0	0	28.66
ICGV86699(R)	3	3	2.67	1.67	0.75	0.67	0.5	0.5	1.59	0	0	0	0	0	0	0	0	0
Treat. mean	15.17	15.72	12.56	9.17	6.2	5.75	3.2	1.83		56.9	54.2	33.4	18.03	0	0	0	0	
	SEM	CD(0.05)								SEM	CD(0.05)							
Cultivar	1.083	3.08								2.35	6.68							
Inoc. Conc	1.769	5.029								3.84	10.91							
Interaction	3.063	8.71								6.64	18.89							
	61.3									56.2								

All the figures are average of three replications

a = Number of lesions per leaf 3WAI, when the increase in the lesion number was first stopped in susceptible cultivar-TMV2

b = Lesion diameter 6WAI, just before 100% defoliation was observed in susceptible cultivar-TMV2

c = Percentage necrotic area 5WAI, just before 100% defoliation was observed in susceptible cultivar-TMV2

d = Defoliation 6WAI, when 100% defoliation was observed in susceptible cultivar-TMV2

S = Susceptible cultivar MR = Moderately resistant cultivar R = Resistant cultivar

* = Inoculum concentration as number of conidia per ml

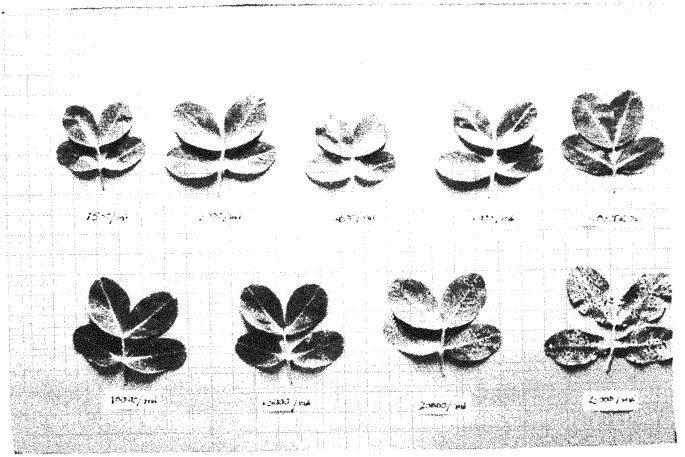


Plate 5 : Effect of inoculum concentration on the components of resistance to late leaf spo in TMV2.

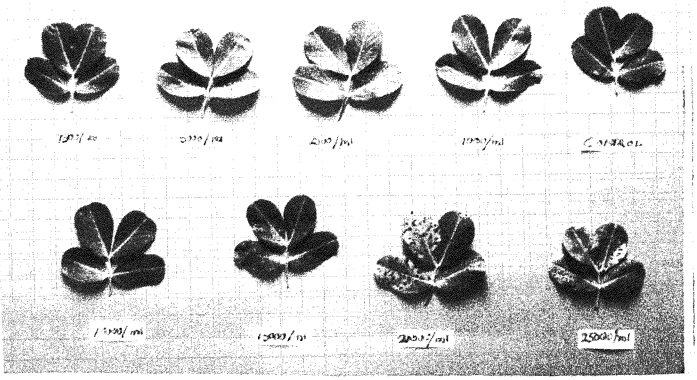


Plate 6 : Effect of inoculum concentration on the components of resistance to late leaf spo in ICGV 86590.

were observed among the treatments and significantly lower (0.5-3) PNA was observed than in TMV2 (3.5-27.5) and ICGV 86590 (1.5-17.5) in all the treatments. In all the cultivars, PNA gradually decreased with the decreasing inoculum concentration.

4.3.7 Defoliation

No defoliation was recorded in ICGV 86699 in all the treatments. TMV2 and ICGV 86590 also did not show any defoliation in 7500, 5000, 2500 and 1000 conidia ml^{-1} treatments (Table 10). Defoliation was maximum and on par in 25000 (100) and 20000 (87.5) conidia ml^{-1} treatments followed by 15000 and 10000 conidia ml^{-1} treatments in TMV2. Similar trend was observed in ICGV 86590 also. The differences between TMV2 and ICGV 86590 were not significant in all the treatments.

4.3.8 Disease severity

Disease score reached maximum (9) in TMV2 6 WAI in 25000 and 20000 conidia ml^{-1} treatments (Table 11). Disease score was highest and on par in 25000 and 20000 conidia ml^{-1} treatments in all the cultivars. Lowest disease score (3) was recorded in 7500, 5000, 2500 and 1000 conidia ml^{-1} treatments in TMV2 and ICGV 86590 with no significant differences among the treatments and between the cultivars. Significant differences were seen between TMV2 and ICGV 86590 in 25000, 20000, 15000 and 10000 conidia ml^{-1}. ICGV 86699 showed lowest disease (2-3) in all the treatments (Plates 5,6 and 7).

4.4 Effect of the age of the plant on the components of resistance to LLS

4.4.1 Incubation period

IP was shortest (7.67-9.33 days) in TMV2 and longest (10-10.33 days) in ICGV 86699 in all the treatments (Table 12). In TMV2 and ICGV 86590 IP was

Table 11 : Effect of inoculum concentration on disease score

	Disease score [a]								
Inoc. Conc*	25000	20000	15000	10000	7500	5000	2500	1000	Cv. mean
Cultivar									
TMV2(S)	9	9	8	5.66	3	3	3	3	5.45
ICGV86590(MR)	7	6.67	6	5	3	3	3	3	4.58
ICGV86699(R)	3	3	2	2	2	2	2	2	2
Treat.mean	6.33	6.22	5.33	4.22	2.67	2.67	2.67	2.67	

	SEM	CD(0.05)
Cultivar	0.0417	0.1185
Inoc. Conc	0.068	0.194
Interaction	0.1179	0.3351
CV%	5.1	

All the figures are average of three replications

a = Disease score 6 WAI

S = Susceptible cultivar MR = Moderately resistant cultivar R = Resistant cultivar

* = Inoculum concentration as number of conidia per ml

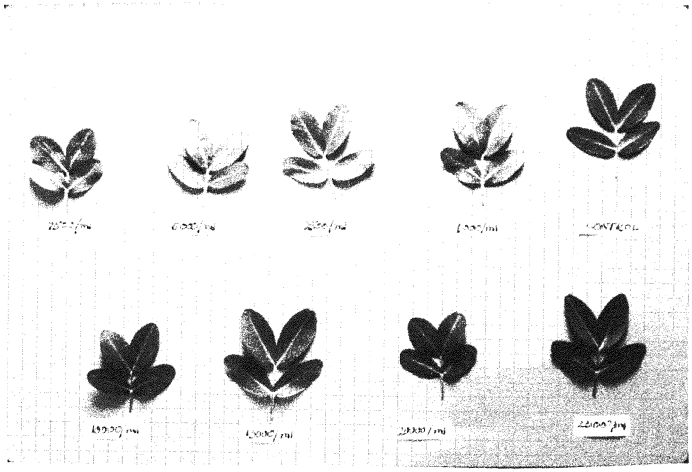


Plate 7 : Effect of inoculum concentration on the components of resistance to late leaf spot in ICGV 86699.

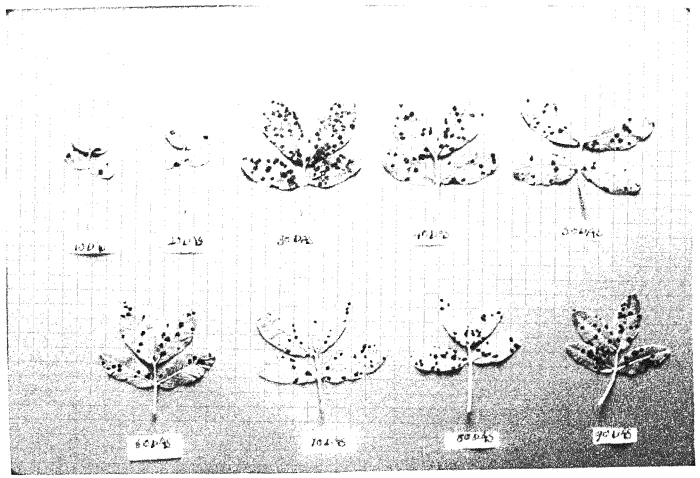


Plate 8 : Effect of age of the plant on the components of resistance to late leaf spot in TMV2.

shortest and on par in 10, 20, 30, 40 and 50 DAS treatments and longest and on par in 60, 70, 80 and 90 DAS treatments. Significant differences between TMV2 and ICGV 86590 were noticed in all the treatments. TMV2 was differing significantly with ICGV 86699 in all the treatments whereas ICGV 86590 was on par with ICGV 86699 in 60, 70, 80 and 90 DAS treatments.

4.4.2 Latent period (LS1 and LS 50)

Both LS1 and LS 50 were shortest (14-15.33 and 15.67-16.67 days respectively) in TMV2 and longest (14.67-15.33 and 19-21 days respectively) in ICGV 86590. In ICGV 86699, no sporulation was recorded in all the treatments (Table 12). In TMV2 and ICGV 86590, both LS1 and LS 50 were shortest and on par in 10, 20, 30, 40 and 50 DAS treatments. Cultivar differences between TMV2 and ICGV 86590 were significant in all the treatments for both LS1 and LS 50.

4.4.3 Sporulation index

SI was maximum (9) in TMV2 and no sporulation was recorded in ICGV 86699 in all the treatments 3 WAI (Table 12). In TMV2 and ICGV 86590, SI was higher and on par in 20, 30, 40 and 50 DAS treatments and lower and on par in 10, 60, 70, 80 and 90 DAS treatments. Cultivar differences for SI were significant in all the treatments with highest in TMV2 and lowest in ICGV 86699.

4.4.4 Lesion number

Lesion number per leaf was highest (6.33-52.5) in TMV2 and lowest (1.83-11.87) in ICGV 86699 in all the treatments 3 WAI (Table 13). In TMV2 and ICGV 86590, number of lesions per leaf was maximum and on par in 40 and 50 DAS treatments and minimum in 10 DAS treatments. In ICGV 86699, the number of lesions per leaf was significantly lower at 10 DAS treatment than all the other

Table 12 : Effect of age of the plant on incubation period, latent period and sporulation index.

	Incubation period (IP)[a]										Latent peroid - (LS1)[c]									
Age(DAS)	10	20	30	40	50	60	70	80	90	Cv. mean	10	20	30	40	50	60	70	80	90	Cv.mean
Cultivar																				
TMV2(S)	7.67	7.67	7.67	8	8	9	9	9.33	9.33	8.71	14	14	14	14	14.33	15	15	15	15.33	14.56
ICGV86590(MR)	8.63	8.67	8.33	9	9	10	9.67	10	10	9.25	14.67	14.67	14.67	14.67	15	16	16	16.33	16.33	15.37
ICGV86699(R)	10	10	10	10	10	10	10	10.33	10.33	10.07	–	–	–	–	–	–	–	–	–	–
Treat.mean	8.76	8.86	8.67	9	9	9.67	9.5	9.89	10		9.56	9.56	9.56	9.56	9.77	10.33	10.33	10.33	10.56	10.66
	SEM	CD(0.05)									SEM	CD(0.05)								
Cultivar	0.068	0.192									0.0786	0.2253								
Age	0.117	0.332									0.1361	0.3903								
Interaction	0.203	0.575									0.2357	0.58								
CV%	3.8										2.7									

	Latent period - (LS50)[c]										Sporulation index (SI)[d]									
Age(DAS)	10	20	30	40	50	60	70	80	90	Cv. mean	10	20	30	40	50	60	70	80	90	Cv.mean
Cultivar																				
TMV2(S)	15.67	16	16	15.67	16	16.67	16.67	16.33	16.67	16.18	8	9	9	9	9	8.33	8	8	8	8.48
ICGV86590(MR)	19	19.33	19	19	19.33	19.67	20.67	21	21	19.44	5.67	6.67	7	7	6.67	5.67	5.33	5.33	5.33	6.07
ICGV86699(R)	–	–	–	–	–	–	–	–	–	–	1	1	1	1	1	1	1	1	1	1
Treat. mean	11.55	11.78	11.66	11.56	11.78	12.11	12.45	12.44	12.44	12.56	4.89	5.44	5.67	5.67	5.56	5	4.77	4.77	4.77	
	SEM	CD(0.05)									SEM	CD(0.05)								
Cultivar	0.074	0.213									0.524	0.149								
Age	0.128	0.368									0.091	0.257								
Interaction	0.222	0.637									0.157	0.446								
CV%	2.1										5.3									

All the figures are average of three replications

a = Incubation period as number of days from inoculation to the appearence of first lesion

b = Latent period as number of days inoculation to the appearence of first sporulating lesion

c = Latent period as number of days inoculation to the appearence of 50% lesions sporulating

d = Sporulation index on 1-9 scale 3 WAI, when maximum amount of sporulation was observed in susceptible cultivar- TMV2

S = Susceptible cultivar MR = Moderately resistant cultivar R = Resistant cultivar

DAS = Days after inoculation

treatments which are on par. Cultivar differences were significant in all the treatments except in 10 DAS treatment.

4.4.5 Lesion diameter

Lesion diameter was significantly lower (0.5-1.67) in ICGV 86699 in all the treatments than TMV2 (3-5) and ICGV 86590 (3-4) 4 WAI (Table 13). In all the cultivars lesion diameter was highest and on par in 20, 30, 4-0 and 50 DAS treatments and lowest and on par in 10, 60, 70, 80 and 90 DAS treatments. Cultivar differences were significant in 20, 30, 40 and 50 DAS with maximum lesion diameter (4.67-5) in TMV2.

4.4.6 Per cent necrotic area

PNA was highest (9.33-27.48) in TMV2 and lowest (0.5) in ICGV 86699 in all the treatments 4 WAI (Table 13). In ICGV 86699, PNA in all the treatments were on par and significantly lower than TMV2 and ICGV 86590. PNA was maximum and on par in 20, 30, 40 and 50 DAS treatments in TMV2 and ICGV 86590. Cultivar differences between TMV2 and ICGV 86590 were significant in all the treatments except in 70, 80 and 90 DAS treatments.

4.4.7 Defoliation

No defoliation was recorded in all the treatments in ICGV 86699 and in 60, 70, 80 and 90 DAS treatments in TMV2 and ICGV 86590 at 4 WAI (Table 13). Significant differences between TMV2 and ICGV 86590 were observed only at 20 DAS treatments. In TMV2, maximum defoliation (40.4 and 33.33) was observed in 20 and 30 DAS treatments and minimum defoliation (8.3) was observed 10 DAS treatment. In ICGV 86590, no significant differences were observed among 10, 20, 30, 40 and 50 DAS treatments (8.3-29.2).

Table 13 : Effect of age of the plant on lesion number, lesion diameter, per cent necrotic area and defoliation

	Lesion number per leaf [a]									
Age(DAS)	10	20	30	40	50	60	70	80	90	Cv.mean
Cultivar										
TMV2(S)	6.33	28.17	32.5	52.5	46.83	34.5	27.17	28.83	24.5	31.25
ICGV86590(MR)	4	20.5	20	33.83	29.83	25.67	19	18.5	14.83	22.35
ICGV86699(R)	1.83	11.67	11.5	9.37	11.33	9.5	10	9.33	6.83	9.06
Treat.mean	4.06	20.18	21.33	32	29.33	23.22	18.72	18.86	15.39	

	SEM	CD(0.05)
Cultivar	0.924	2.62
Age	1.601	4.538
Interaction	2.772	7.86
CV%	22.8	

	Lesion diameter [b]									
Age(DAS)	10	20	30	40	50	60	70	80	90	Cv.mean
Cultivar										
TMV2(S)	3	4.67	5	5	4.56	3.17	3.33	3	3	3.85
ICGV86590(MR)	3	3.67	3.67	4	4	3	3	2.83	3	3.33
ICGV86699(R)	0.5	1.17	1.17	1.67	1.17	0.83	0.67	0.67	0.5	0.87
Treat.mean	2.17	3.22	3.28	3.39	3.28	2.5	2.4	2.17	2.17	

	SEM	CD(0.05)
Cultivar	0.0595	0.1688
Age	0.1031	0.2923
Interaction	0.1786	0.5064
CV%	11.4	

	Per cent necrotic area [c]									
Age(DAS)	10	20	30	40	50	60	70	80	90	Cv.mean
Cultivar										
TMV2(S)	10.17	27.48	23.75	24.17	19.17	17	8.67	9.5	9.33	16.58
ICGV86590(MR)	4.33	13..	14	14.83	11	11	6.33	8.17	7.33	10.03
ICGV86699(R)	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Treat. mean	5	13.77	12.75	14.17	10.89	9.78	5.17	6.05	5.72	

	SEM	CD(0.05)
Cultivar	0.456	1.295
Age	0.79	2.244
Interaction	1.369	3.886
CV%	25.6	

	Defoliation [d]									
Age(DAS)	10	20	30	40	50	60	70	80	90	Cv.mean
Cultivar										
TMV2(S)	8.3	40.4	33.33	20.8	20.8	0	0	0	0	13.6
ICGV86590(MR)	8.3	26.8	29.2	12.5	8.3	0	0	0	0	9.44
ICGV86699(R)	0	0	0	0	0	0	0	0	0	0
Treat. mean	11.1	22.4	17.4	11.1	9.7	0	0	0	0	

	SEM	CD(0.05)
Cultivar	2.75	7.8
Age	4.77	13.51
Interaction	8.26	23.41
CV%	280.8	

All the figures are average of three replications

a = Number of lesions per leaf 3WAI, when the increase in the lesion number was first stopped in susceptible cultivar-TMV2

b = Lesion diameter 4WAI

c = Percentage necrotic area 4WAI

d = Defoliation 4WAI

S = Susceptible cultivar MR = Moderately resistant cultivar R = Resistant cultivar

DAS = Days after sowing

4.4.8 Disease severity

In TMV2 and ICGV 86590, disease score was highest and on par in 20, 30, 40 and 50 DAS treatments 4 WAI (Table 14). In ICGV 86699, disease score was significantly lower (1-2.33) than TMV2 (3-5.67) and ICGV 86590 (3-4.67) in all the treatments (Plates 8, 9 and 10). Differences between TMV2 and ICGV 86590 were significant in 20, 30, 40, 50 and 60 DAS treatments with highest disease score in TMV2.

4.5 Surface studies on the inoculated leaves

Leaves from TMV2 and ICGV 86590 and ICGV 86699 which show differential reaction to LLS (susceptible, moderately resistant and resistant respectively) were inoculated with conidial suspension of LLS pathogen and incubated at 25°C in incubator. Conidia began to germinate within 6-8 h after inoculation irrespective of the cultivar. After eight hours, there were significant differences in the percentage of germination of conidia between the cultivars. At eight hours after inoculation (HAI), more than 50 per cent conidia germinated in TMV2 and ICGV 86590. But in ICGV 86699 it took 24 h for 50 per cent conidial germination. Conidia usually germinated from the terminal cells, although other cells occasionally produced germ tubes. At 8 HAI only a few conidia showed two germ tubes in TMV2 and ICGV 86590, but by 32 HAI, most of the conidia produced two or even more germ tubes (Plate 11 and 12). The average number of germ tube s per conidium were comparitively less in ICGV 86699 than in TMV2 and ICGV 85690. The germ tube branching was observed rarely in all the cvs. Germ tube branching was first sseen in TMV2 and ICGV 86590 at 48 HAI while in ICGV 86699, it was observed at 72 HAI.

Table 14 : Effect age of the plant on disease score.

	Disease score [a]									
Age(DAS)	10	20	30	40	50	60	70	80	90	Cv. mean
Cultivar										
TMV2(S)	3	5.67	5.67	5.67	5	4.33	3	3	3	4.26
ICGV86590(MR)	3	4.67	4.67	4.67	4.67	3.67	3	3.43	3	3.86
ICGV86699(R)	1	1.33	2.33	2.33	2	2	2	1	1	1.78
Treat.mean	2.33	3.89	4.22	4.22	3.89	3.33	2.66	3.44	2.1	

	SEM	CD(0.05)
Cultivar	0.0932	0.2643
Age	0.161	0.458
Interaction	0.28	0.793
CV%	14.1	

All the figures are average of three replications
a = Disease score 4 WAI
S = Susceptible cultivar MR = Moderately resistant cultivar R = Resistant cultivar
DAS = Days after sowing
Cvar.mean = Cultivar mean

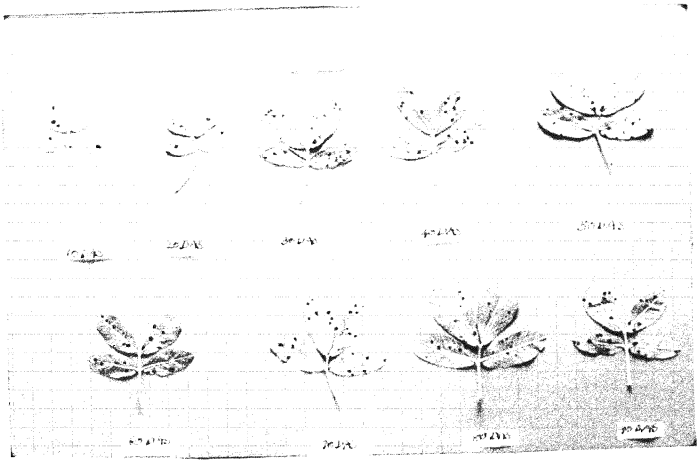


Plate 9 : Effect of age of the plant on the components of resistance to late leaf spot in ICGV86590.

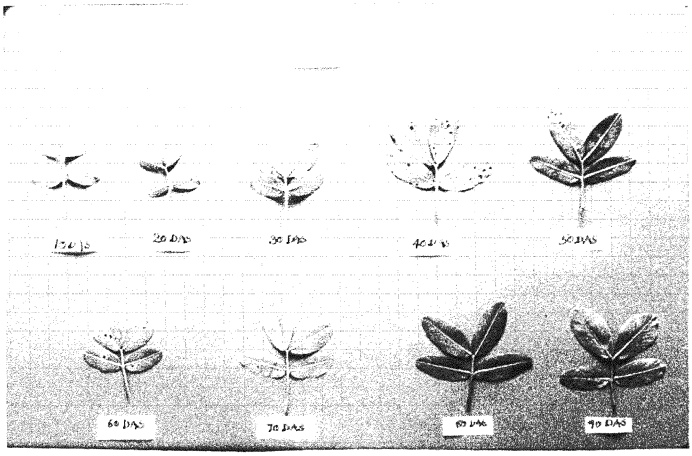


Plate 10 : Effect of age of the plant on the components of resistance to late leaf spot in ICGV86699.

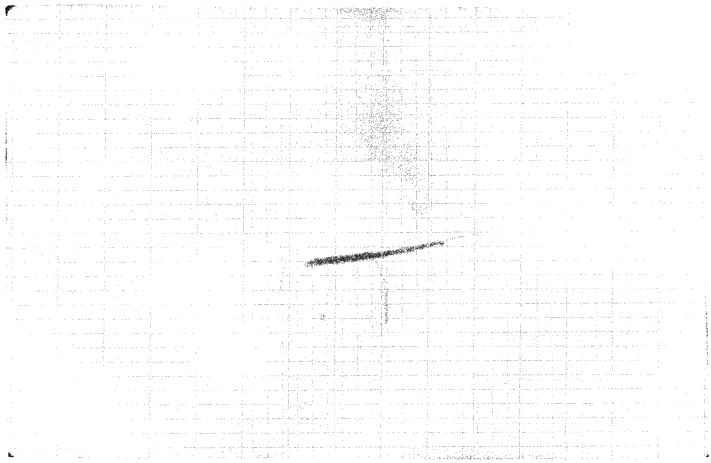


Plate 11 : Conidia with three germtubes on the leaf surface of ICGV 86699 (200X).

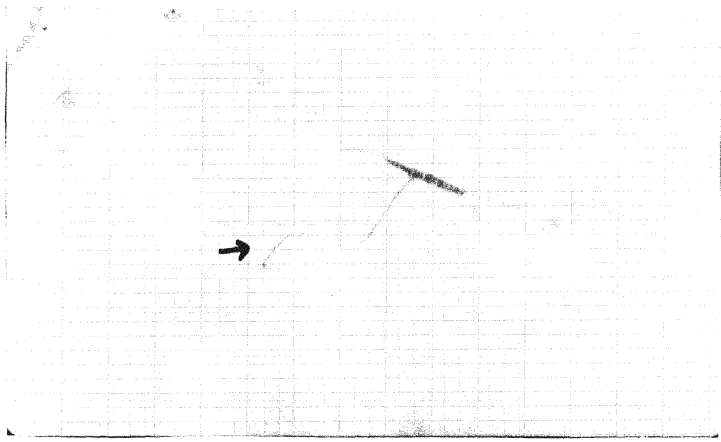


Plate 12 : Conidia with four germtubes and one germtube passing over the stomata on the leaf surface of ICGV 86590 (200X).

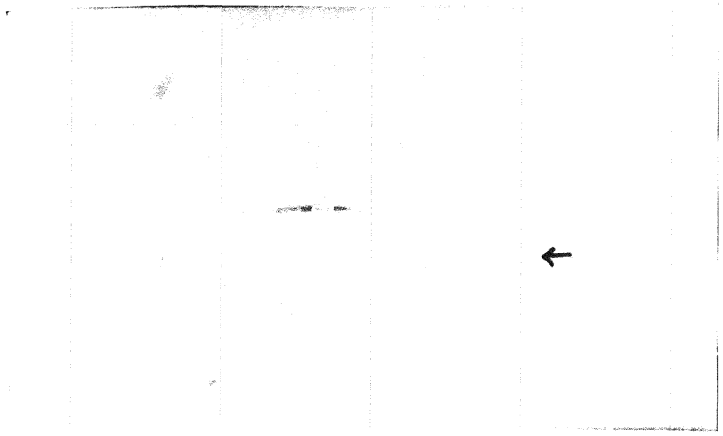


Plate 13 : Conidia with two germtubes and a germtube pointing towards stomata on the leaf surface of TMV2 (55 HAI) (200X).

Plate 14 : Penetration of germtube through stomata in TMV2 (72 HAI) (400X).

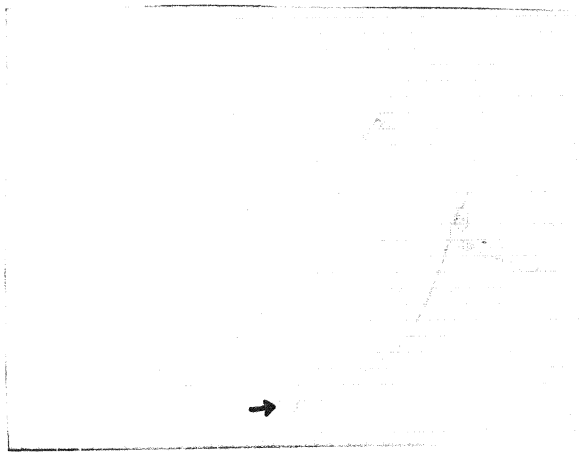


Plate 15 : Penetration of germtube through stomata in ICGV 86590 (72 HAI) (200X).

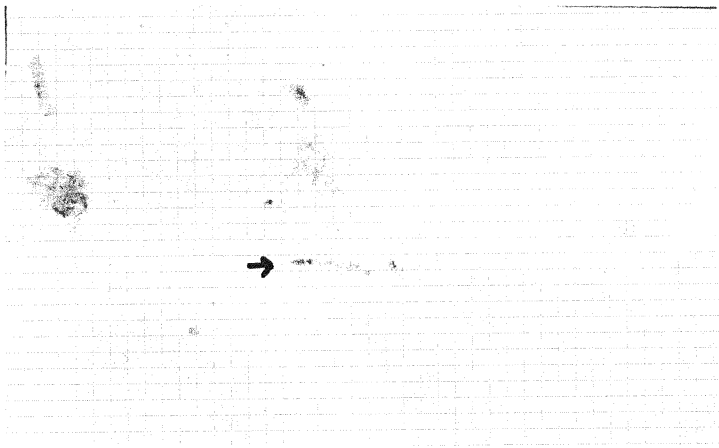


Plate 16 : Conidia lost stainability and became transparent in ICGV 86699 (200X).

Plate 17 : Penetration of germtube through stomata in ICGV 86699 (78 HAI) (400X).

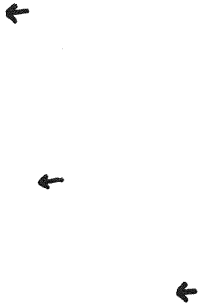


Plate 18 : Discolouration and shrinkage of cells surrounding the infection site in TMV2 (168 HAI) (200X).

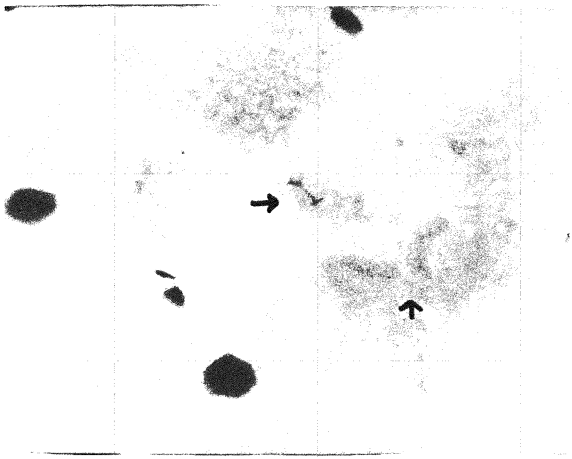


Plate 19 : Discolouration and shrinkage of cells surrounding the infection site in ICGV 86590 (168 HAI) (200X).

Plate 20 : Discolouration and shrinkage of cells surrounding the infection site in ICGV 86699 (191 HAI) (200X).

On TMV2 and ICGV 86590, which are susceptible and moderately resistant to LLS respectively, majority of germ tubes began to show attraction towards the open stomata(Plate13). Usually only one germ tube or its branch from a conidium resulted in penetration. Occasionally a germ tube grew besides or even over the stoma without entering (Plate 12). No competition between the germ tubes was observed and only one was found to enter through the stoma. Some germ tubes also showed directional growth towards the epidermal radial cell walls. But no penetrations were observed.

In TMV2 and ICGV 86590, penetration of the germ tube through stomata was first observed 72 HAI (Plates 14 and 15) and more number of penetrations were observed 80 HAI. On the resistant cultivar ICGV 86699, the germ tubes of most of the conidia apparently were not attracted towards the stomata. Most of the conidia eventually lost their stainability and became transparent (Plate 16). Eventhough infection was first observed 78 HAI (Plate 17), the frequency of penetrations was very less and further delayed. The distal end of the germ tube enlarged to form an irregularly shaped "appressorium-like" structure over the stomatal pore.

Macroscopic symptoms were first observed on TMV2 and ICGV 86590 at 72 h after penetration (168 HAI). When observed under microscope, the minute chlorotic spots included guard cells and some surrounding cells. The affected cells were discoloured and the shrinkage of the protoplasm was more obvious (Plates 18 and 19). In ICGV 86699, the first symptom was seen at 112 h after penetration (191 HAI) and showed the same pattern as the other two cultivars (Plate 20).

DISCUSSION

CHAPTER V

DISCUSSION

In the present investigations, TMV2, ICGV 86590 and ICGV 86699 remained susceptible, moderately resistant and resistant in all the experiments TMV2 is the most susceptible variety with the shortest incubation period, latent period and with the highest sporulation index, lesion count, per cent necrotic area, lesion diameter, defoliation and disease score over the range of temperatures, leaf wetness periods, inoculum concentrations and different ages of the plants tested followed by ICGV 86590. ICGV 86699 is the most resistant cultivar with all the components of resistance highly restricted The susceptibility of TMV2 is due to shortened incubation period, latent period, increased number of lesions, size of the lesions which results in increased per cent necrotic area, defoliation, finally leading to increased disease severity. The resistance in ICGV 86699 is associated with smaller lesions, longer latent period and reduced sporulation which finally resulted in reduced disease severity.

In some experiments, the differences between the cultivars found to be non-significant at certain treatments for incubation period and number of lesions per leaf. But these two components were not very useful in differentiating the resistance of the genotypes. Chiteka *et al.*, (1988a) reported that incubation period was not a criteria for isolating resistant genotypes but latent period, amount of sporulation and lesion size were useful. Number of lesions per leaf is also of limited usefulness because it is highly influenced by environmental factors (Subrahmanyam *et al* , 1982, Walls *et al.*, 1985 and Chiteka *et al.*, 1988a) But the differences for all the other components of resistance were highly significant between the cultivars. Genotypic differences were found to be significant for latent period (Chiteka *et al.*, 1988a), per cent necrotic area

(Subrahmanyam *et al*, 1982, Iroume and Knauft, 1987 and Chiteka *et al*, 1988a), lesion diameter (Subrahmanyam *et al*, 1982, Walls *et al.*, 1985 and Chiteka *et al*, 1988a), sporulation index and disease score (Nevill, 1981, Subrahmanyam *et al.*, 1982, Walls *et al*, 1985 and Chiteka *et al*, 1988a) There was a positive correlation between per cent defoliation, lesion diameter and sporulation (Subrahmanyam *et al*, 1982) and between lesion size, latent period and amount of sporulation and all with per cent necrotic area (Chiteka *et al*, 1988b) were reported

Success in efforts to develop high yielding cultivars with better resistance to late leaf spot depends on identification of resistance that is expressed even when environment favours disease increase (Shew *et al.* 1988) The present investigations were carried out to determine the temperature, duration of high humidity, concentration of inoculum and age of the plant that favour infection of groundnut by *Phaeoisariopsis personata* and to determine the factors favourable for post infection development of the pathogen to determine if temperature, relative humidity, inoculum concentration or age of the plant affect the expression of resistance to the LLS pathogen in peanut The results obtained during the course of investigation are discussed in this chapter

5.1 EFFECT OF TEMPERATURE ON THE COMPONENTS OF RESISTANCE

The results indicate that the incubation period of LLS pathogen was the shortest at 25°C followed by at 20 and 15°C in all the three cultivars But differences between 20 and 25°C were slight No significant amount of disease was observed at 30 and 35°C and even the plants died subsequently This is because of gradual decline and sharp inhibition of conidial germination (Sommartya and Beute, 1986) and complete inhibition of infection and disease development (Shew *et al.*,1988) at higher temperatures (>28°C) and also because of the continuous growing of the

inoculated plants at their respective treatment temperatures in the incubators till the end of the experiment which is highly unfavourable for the growth of the plant In the present investigation, severity of disease was maximum at 25°C in all the three cultivars However, the effect of temperature on *Phaeoisariopsis personata* infection and disease progression was highest between 15 and 20°C and slight between 20 and 25°C The nearly complete inhibition of leaf spot infection and development on all the cultivars at 30 and 35°C was unexpected, because plants in the field are nearly always exposed to daily maximum temperatures of 30 - 35°C Conidia must survive exposures to high temperatures and low humidity that occur between deposition and favourable infection periods The minimum period of exposure to high temperature that irreversibly inhibits the post infection development of leaf spot is not known, but lesions on leaves that were transferred from 32°C to lower temperatures at the end of the experiments (after 28 days exposure to 32°C) did not resume development within two weeks of transfer (Shew *et al* 1988) In controlled environment studies, temperature >30°C during periods of high relative humidity had a greater effect on reducing spore production than did the recommended rate of chlorothalonil applied at 7-10 day intervals (Labrinos and Nutter Jr , 1993)

Shew *et al* (1988) expected leaf spot severity to be the highest when temperatures near 24°C occur during long leaf wetness periods as the infections probably occur at night or in early morning when leaves are wet and temperatures are cool Post infection development probably proceeds rapidly at warm temperatures, slows during the hottest part of the day, and resumes in the evening as temperatures decrease

Incubation period was the shortest at 25°C and the longest at 15°C in all the cultivars However, there was no significant difference between 20 and 25°C

(Fig.3.1). No significant difference was found between TMV2 and ICGV 86590 for IP at 25°C. But there were highly significant differences for latent period which is the most important component of resistance (Nevill, 1980). Chiteka *et al.* (1988a) opined that incubation period did not appear to be a useful component for isolating resistant genotypes. But they found highly significant differences for latent period.

Latent period (LS1 and LS 50) was the shortest at 25°C and the longest at 15°C (Figs. 3.2 and 3.3). The longest latent periods occurred at the lowest temperatures and shortest periods occurred between 20°C and 30°C (Wadia and Butter, 1994). Nevill (1981) observed a latent period of 14.6 days for *Phaeoisariopsis personata* at 25°C on detached leaves of TMV2. Similar result (13.3 days) was obtained in the present investigations on TMV2 at 25°C on whole plant. There was no sporulation in resistant ICGV 86699 at all the temperature.

The number of lesions per leaf at 15°C in all the cultivars was not significantly differed with that at 20 and 25°C in all the cultivars, but, the severity of disease was low at 15°C because the lesions were generally small and poorly developed. Lesions at 13°C were generally small and poorly developed and included many hypersensitive specks, although the lesion number was large, disease severity was low (Butler *et al.* 1994).

In all the cultivars at all the temperatures tested, the increase in number of lesions continued for 20 or even more days. The increase in number of lesions per leaf stopped 3 WAI in TMV2 at all the temperatures and at 20 and 25°C in ICGV 86599. The increase was observed upto 6 WAI at 15°C in ICGV 86590 and at all the temperatures in ICGV 86699. The number of lesions per leaf were highest in TMV2 at 3 WAI followed by ICGV 86590 and ICGV 86699 at all the temperatures (Fig 3.5). Lannou and Blizoua Bi (1989) concluded that infection process continues for

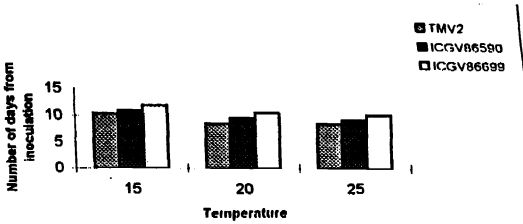


Figure 3.1 : Effect of temperature on incubation period

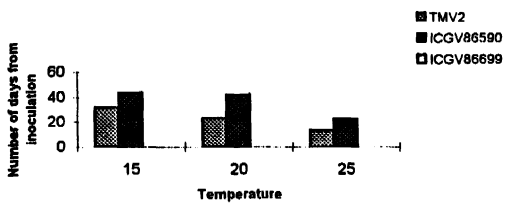


Figure 3.2 : Effect of temperature on latent period - LS1

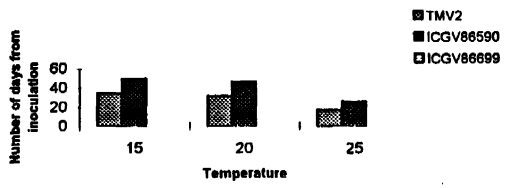


Figure 3.3 : Effect of temperature on latent perid - LS50

more than six days. Butler *et al.* (1994) observed continuation of infection process as long as 10 days. Alderman and Beute (1986) observed a continuing increasing in the number of lesions for 20 days after inoculation with *Cercospora arachidicola*. The stoppage of increase in number of lesions was earliest at 25°C in all the cultivars because of the rapid progression of disease. As the disease progresses, the lesion size will increase and often two or more lesions coalesce to give an appearance of single lesion. So, the increase in number of lesions apparently stopped and even started decreasing in TMV2 and ICGV 86590 3 WAI. But in ICGV 86699, the resistance is associated with appearance of small non-sporulating lesions, hence the increase in number of lesions was noticed even 6 WAI.

The increase in size of the lesion with disease progression lead to increased per cent necrotic area. Per cent necrotic area was more at 20 and 25°C in all the cultivars but it was the highest at 25°C as some leaflets were already defoliated.

Defoliation was also highest at 25°C in all the cultivars followed by 20°C (Fig.3.8). No defoliation was observed at 15°C in all the cultivars. Sporulation index reached highest respective to the cultivar depending on its resistance behaviour at 25°C followed by 20 and 15°C (Fig 3.4). The disease score on 1-9 scale has given conformation of highest amount of disease at 25°C and lowest at 15° in all the cultivars (Fig.3.9).

Therefore it can be concluded that the severity and rapidity of disease progression was increasing from 15 to 25°C irrespective of the resistance nature of the host system. The effect of temperature on *Phaeoisariopsis personata* infection and disease progression was highest between 15 to 20°C and the lowest between 20 to 25°C. Under controlled environmental conditions, Shew *et al.* (1988) found that infection by LLS pathogen was optimum when exposed to 20°C and at least 12 hr/day

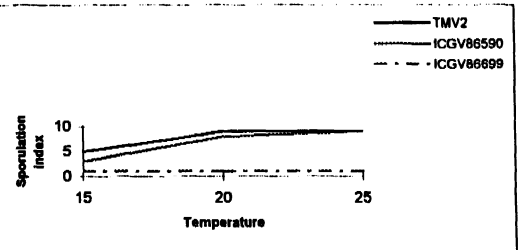


Figure 3.4 : Effect of temperature on sporulation index

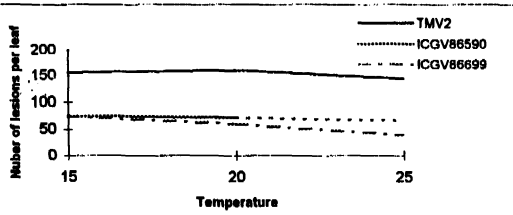


Figure 3.5 : Effect of temperature on lesion number per leaf

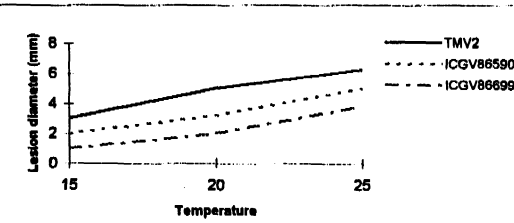


Figure 3.6 : Effect of temperature on lesion diameter

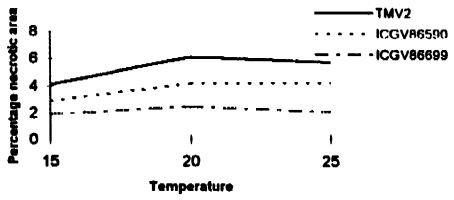


Figure 3.7 : Effect of temperature on per cent necrotic area

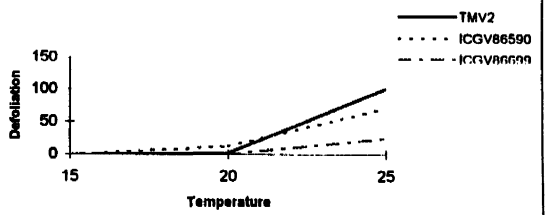


Figure 3.8 : Effect of temperature on defoliation

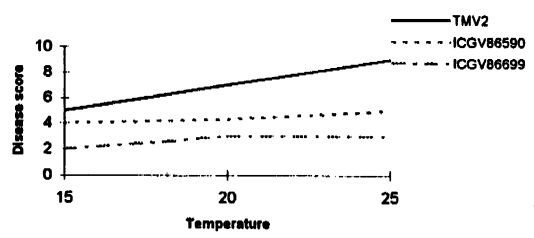


Figure 3.9 : Effect of temperature on disease score

duration of >93 per cent RH for six days. Similar results were reported with temperature optima close to 20°C and minimum and maximum of about eight and 34°C, respectively by Butler *et al.* (1994). In all the cultivars, there were no significant differences between 20 and 25°C for incubation period, lesion number, per cent necrotic area and sporulation index. But the differences were clear with the lengthened latent period, smaller lesion size, reduced defoliation and disease score at 20°C, compared to 25°C. Eventhough the per cent necrotic area in 20 and 25°C were on par 4 WAI, defoliation was more in 25°C at that point of time.

5.2 EFFECT OF HUMIDITY ON THE COMPONENTS OF RESISTANCE

High daily leaf wetness periods/relative humidity is very much favourable for the infection of the LLS pathogen. Incubation period was shortest in 16 h humidity treatment in all the cultivars (Fig.4.1). Eventhough the incubation period did not differ much between the high humidity treatments (16, 20 and 24 hr) for TMV2 and ICGV 86590 and in between the two cultivars, the differences in the other components of resistance play an important role in differentiating them. Chiteka *et al.* (1988a) opined that IP was not a useful component for isolating resistant genotypes for *P. personata*. There was no difference for spore germination, incubation period and number of lesions per leaf between Southern runner and Florunner, but Southern runner has higher LS 50, less sporulation index and lesion diameter (Watson *et al.*, 1997). Infection was delayed at low humidity treatments with longest IP at 4 h treatment in TMV2 and ICGV 86590. In ICGV 86699, no infection observed at 4 h treatment. The relative humidity rate appears to be a limiting factor on lesion development and influential for incubation period (Rapilley, 1983).

Latent period (LS1 and LS 50) was also shortest in 16 h treatment followed by 20, 24, 12 and 8 h treatments in TMV2 and ICGV 86590 (Figs.4.2 and 4.3). In

ICGV 86699, sporulation was greatly restricted at all the humidity treatments Alderman and Nutter (1994) reported that in controlled environmental experiments, a minimum of 4 hr relative humidity >95% per day was required for conidial production and the highest number of conidia were produced when lesions were subjected to daily periods of 16 or more hours of relative humidity >95%. In the present investigation, though symptoms were seen in 4 h treatment, no sporulation was recorded even in the most susceptible cultivar TMV2, but sporulation was observed in 8 h treatment. So, for conidial production, the minimum period of humidity saturation conditions was probably in between 4-8 h.

Maximum number of lesions per leaf were observed in 16 h humidity treatment in all the three cultivars (Fig.4.5). The differences in the number of lesions per leaf among 4, 8, 12, 20 and 24 h treatments were not significant but the severity of the disease at 20 and 24 h was more with higher lesion size and per cent necrotic area. The number of lesions resulting from a fixed amount of inoculum was several times greater when the leaves were exposed to long periods of humidity saturation alternating with short dry periods (Butler *et al.* 1994). Lannou and Blizoua Bi (1989) and Butler *et al.* (1994) reported that number of lesions increased if leaves were exposed to alternate wet and dry periods. Butler *et al.* (1994) also found maximum infection with daily wetness periods of 16 h. The results of the present investigation confirm this as the number of lesions were higher at 16h treatment than other treatments in all the cultivars. In contrary to this, Shew *et al.* (1988) found maximum lesion number with 24 h of RH per day (i.e., continuous wet period). Butler *et al.* (1994) explained this variation stating that, in high humidity chambers used by Shew *et al.* (1988), the RH varied between 93 and 99% and although leaves were wetted

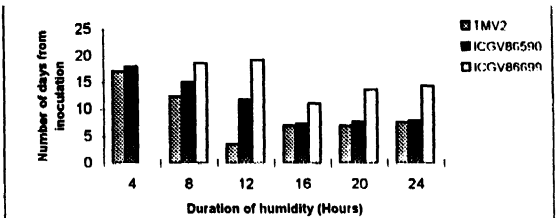


Fig 4.1 : Effect of humidity on incubation period

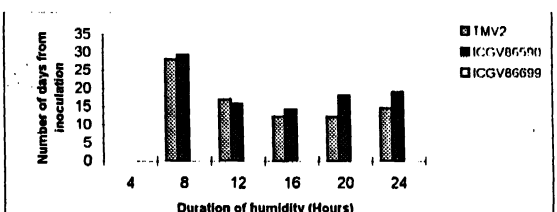


Fig 4.2 : Effect of humidity on latent period - LS1

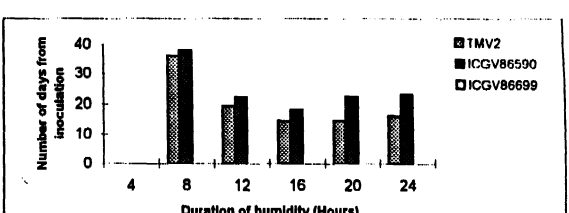


Fig 4.3 : Effect of humidity on latent period - LS50

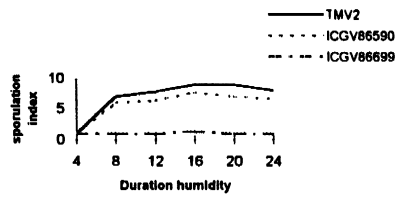


Fig 4.4 : Effect of humidity on sporulation index

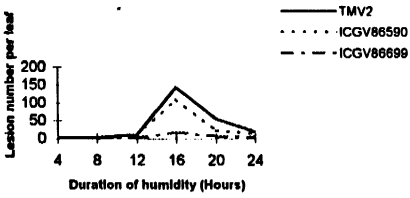


Fig 4.5 : Effect of humidity on lesion number per leaf

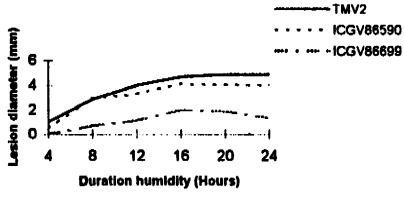


Fig 4.6 : Effect of humidity on leison diameter

each evening, they may have subsequently dried. Illumination in the growth cabinet would have increased evaporation from the leaves.

Hemingway (1954) proposed that enhanced penetration under interrupted wetting was due to hydrotropism which directed the germ tubes towards stomata. Cook (1981) observed germ tube growth towards stomata from the first day after incubation on detached leaves when condensation inside the petri dish dried during part of the day. With continuous condensation / leaf wetness germ tube growth was not directional. Similar results were reported with *Cercospora beticola* (Rathaiah, 1977), *C. musae* (Goos and Tschirch, 1963) and *C.medicaginis* (Baxter, 1956).

Alderman and Beute (1996) observed stoppage of growth of germ tube of *Cercospora arachidicola* with a relative humidity less than 65% during the dry period and resumed in subsequent wet periods. With relative humidity of 30 - 40%, growth will not resume with subsequent periods. But in the present study and in the studies of Butler *et al.*, 1994, where the relative humidity was 70 – 80 per cent during the dry periods, the lesion number was increased which reveals that germtube growth was continuing and directional towards stomata.

The reaction of all the cultivars with respect to their resistance to the infection by the LLS pathogen followed similar trends in all the humidity treatments. The infection and disease progression was reduced at low humidity treatments (4, 8 and 12 h), increased at high humidity treatments (16, 20 and 24 h) with maximum infection at 16 h treatment followed by 20, 24 h treatments. At 16 h treatment, all the components of resistance such as lesion number (Fig.4.5), lesion size (Fig.4.6), percentage necrotic area (Fig.4.7), defoliation (Fig.4.8), sporulation index (Fig.4.4) and disease score (Fig.4.9) were higher than in other treatments in all the cultivars. Eventhough per cent necrotic area in 16 and 20h humidity treatments were on par 5

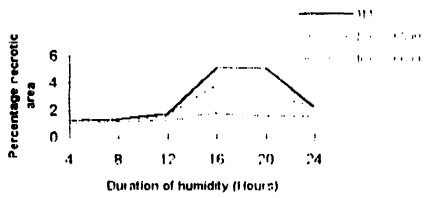


Fig 4.7 : Effect of humidity on per cent necrotic area

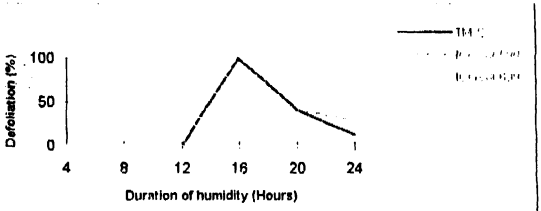


Fig 4.8 : Effect of humidity on defoliation

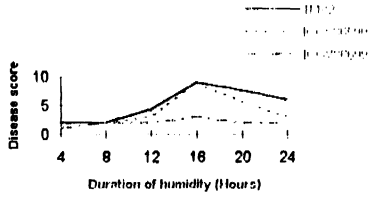


Fig 4.9 : Effect of humidity on disease score

WAI, percentage of defoliation was more in 16h treatment at that point of time. Therefore, it can be concluded that the severity and rapidity of disease progression was increasing from 4 to 16 h humidity duration irrespective of the resistance nature of the host system. The effect of humidity on *Phaeoiriopsis personata* infection and disease progression was highest between 4 to 16 h and slight between 16 to 24 h with maximum disease at 16 h in all the cultivars. Eventhough no significant differences observed for incubation period, sporulation index and lesion diameter between 16 and 20h treatments in TMV2 and ICGV 86590, the severity disease was more at 16h treatment with higher number of lesions per leaf, pre cent necrotic area and defoliation. This clearly indicates that intermittent wetness with 16h wet and 8h dry periods per day for six days is highly favourable for disease development.

5.3 EFFECT OF INOCULUM CONCENTRATION ON THE COMPONENTS OF RESISTANCE

Inoculum concentration has no effect on incubation period, latent period and sporulation index in all the three cultivars. However, the inoculum concentration has direct effect on the number of lesions per leaf, per cent necrotic area, lesion diameter, defoliation and disease score in all the three cultivars. Defoliation and lesion number were directly related to inoculum level (Nevill, 1981) and lesion number will increase with increasing spore concentrations (Shew and Beute, 1984). Similar results were obtained in the present investigation

Incubation periods in all the inoculum levels were on par in all the cultivars (Fig.5.1). No significant differences were observed between TMV2 and ICGV 86590 for incubation period but both differed significantly with ICGV 86699. Similarly no significant differences were observed between different concentrations

for latent period (both LS1 and LS 50) but the cultivar differences were highly significant in all the concentrations (Figs.5.2 and 5.3).

Sporulation index was also found to be not effected by inoculum concentration. No significant differences were found among concentrations in all the cultivars (Fig.5.4). Lesion number per leaf was highly influenced by inoculum concentration. Lesion counts were maximum and on par in higher concentrations (25000 and 20000 conidia per ml) and cultivar differences were highly significant (Fig.5.5). However differences for lesion count were non-significant among the concentrations and also in between cultivars in the lower concentrations (7500 - 1000 conidia per ml). Lower inoculum densities will lead to lower mean leaf spot counts (Cook, 1981) and with higher densities there was a little change in mean counts (Hassan and Beute, 1977). Non-significance of differences in lesion number per leaf at higher concentrations may be due to interference between spores or competition for infection sites, but it is also possible that lesions fused together and thereby produced an apparently lower number of lesions at highest concentration (Nevill, 1981).

Per cent necrotic area was also highest and on par at 25000 and 20000 conidia per ml and decreased gradually with the decreasing inoculum concentrations in all the cultivars (Fig.5.7). Higher per cent necrotic area at higher concentrations was due to increased lesion size (Fig.5.6) and increased number of lesions per leaf (Fig.5.5). Per cent necrotic area was highly correlated with lesion diameter, since the rating scale for per cent necrotic area is based on lesion count and lesion diameter (Chiteka *et al.* 1988b). They also found per cent necrotic area in field was highly and significantly correlated with incubation period and latent period (LS1).

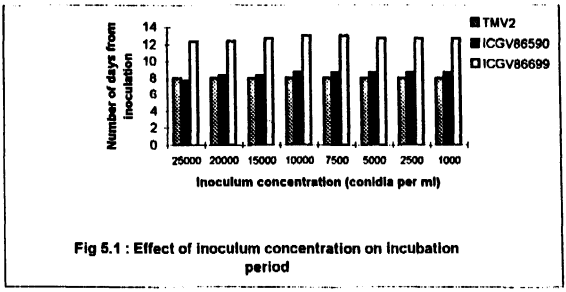


Fig 5.1 : Effect of inoculum concentration on incubation period

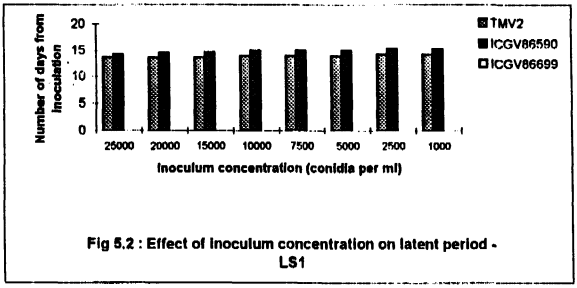


Fig 5.2 : Effect of inoculum concentration on latent period - LS1

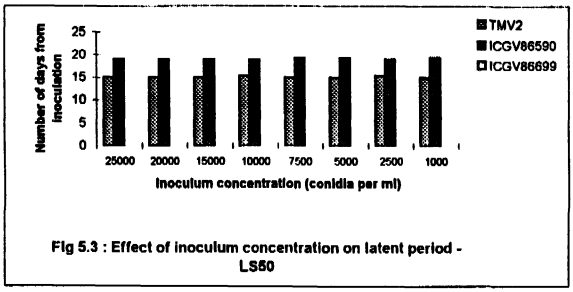


Fig 5.3 : Effect of inoculum concentration on latent period - LS50

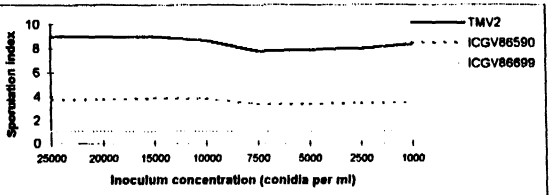


Fig 5.4 : Effect of inoculum concentration on sporulation index

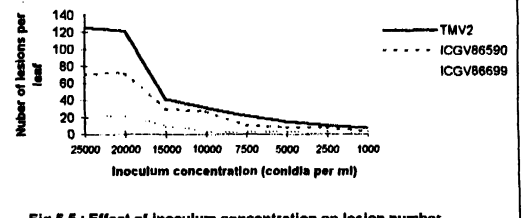


Fig 5.5 : Effect of inoculum concentration on lesion number per leaf

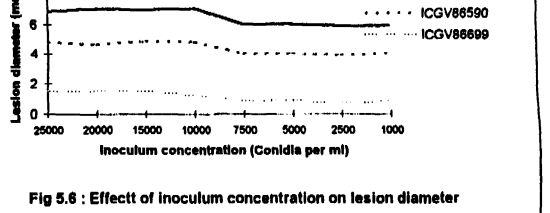


Fig 5.6 : Effectt of inoculum concentration on lesion diameter

In the present investigation although lesion diameter does not vary among lower concentrations (7500 - 1000 conidia per ml), per cent necrotic area was decreased gradually. This may be due to decrease in lesion number from 7500 through 1000 conidia per ml concentrations. Defoliation was maximum in higher concentrations (25000 and 20000 conidia per ml) in TMV2 and ICGV 86590 (Fig.5.8). No defoliation was recorded in ICGV 86699 in all the concentrations and in 7500 - 1000 conidia per ml concentrations in TMV2 and ICGV 86590. Maximum disease score / plant appearance score (PAS) was recorded in 25000 and 20000 conidia per ml concentrations and lowest and on par in 7500 - 1000 conidia per ml concentrations in all the cultivars (Fig.5.9).

Except for incubation period, latent period and sporulation index, all the other components of resistance decreased gradually with the decreasing inoculum concentrations with highest amount of disease in higher concentrations (25000 and 20000 conidia per ml) and lowest and on par in 7500 - 1000 conidia per ml concentrations in all the cultivars. Hence the optimum concentration to cause maximum amount of disease was 20000 conidia per ml as there was no further significant increase in the severity of disease with 25000 conidia per ml concentration.

5.4 EFFECT OF AGE OF THE PLANT ON THE COMPONENTS OF RESISTANCE

In susceptible cultivar - TMV2 and moderately resistant cultivar - ICGV 86590 significant differences were observed in between the different age treatments. But in ICGV 86699, no significant interaction between plant age and components of resistance was observed. Irrespective of the resistance reaction of the host system, disease severity was higher in 20 - 50 day old plants. The disease severity was highly reduced in 10, 80 and 90 day old plants in case of TMV2 and ICGV 86590.

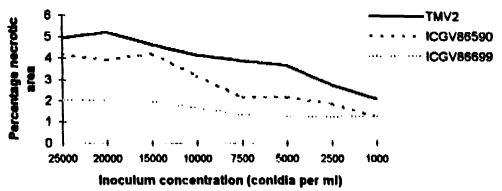


Fig 5.7 : Effect of inoculum concentration on per cent necrotic area

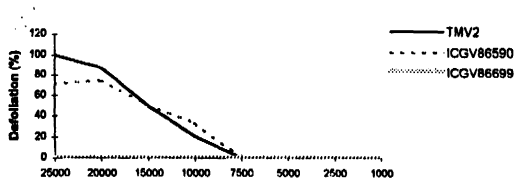


Fig 5.8 : Effect of inoculum concentration on defoliation

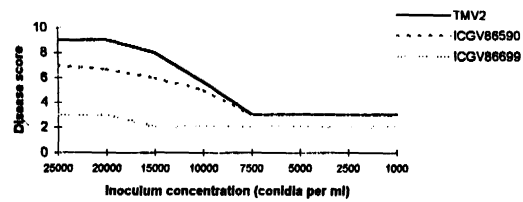


Fig 5.9 : Effect of inoculum concentration on disease score

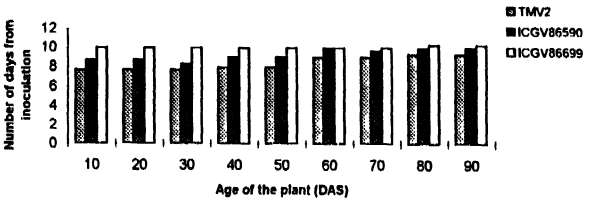


Fig 6.1 : Effect of age of the plant on incubation period

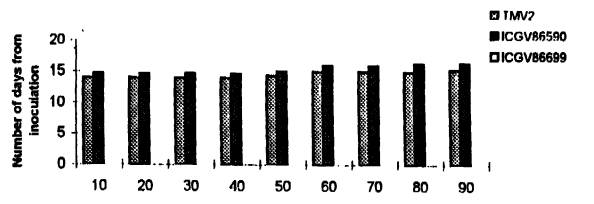


Fig 6.2 : Effect of age of the plant on latent period - LS1

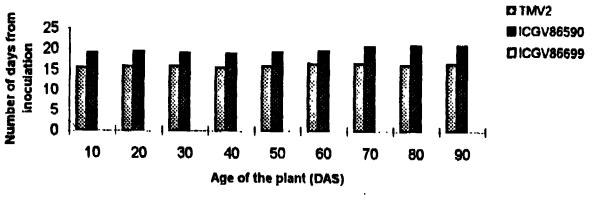


Fig 6.3 : Effect of age of the plant on latent period - LS60

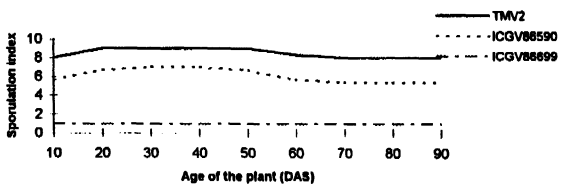


Fig 6.4 : Effect of age of the plant on sporulation index

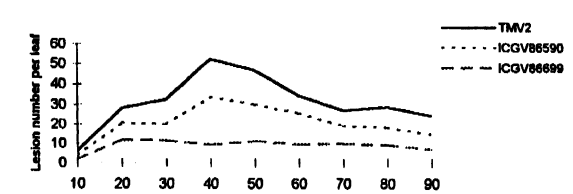


Fig 6.5 : Effect of age of the plant on lesion number per leaf

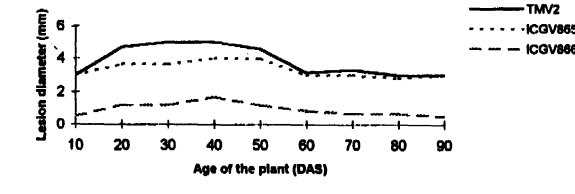


Fig 6.6 : Effect of age of the plant on lesion diameter

Incubation period was shortest and on par in 10 - 50 day old plants in TMV2 and ICGV 86590 (Fig.6.1). There was no interaction between incubation period and plant age in ICGV 86699. Similar results like that of IP were observed for latent period (both LS1 and LS 50) also in TMV2 and ICGV 86590 (Figs.6.2 and 6.3). Sporulation of lesions was highly reduced in all the ages in ICGV 86699. Sporulation index was highest and on par in 20 - 50 day old plants (Fig.6.4) in TMV2 and ICGV 86590. Subrahmanyam *et al.* (1982) reported that sporulation score was a consistent component for rating genotypes and they observed no interaction between sporulation index and plant age (30 and 50 DAS).

Lesion number was significantly high and on par in 40 and 50 day old plants and lowest in 10 day old plants in TMV2 and ICGV 86590. ICGV 86699 showed no interaction between plant age and lesion number but lesion number in 10 day old plants was highly reduced (Fig.6.5). Subrahmanyam *et al.* (1982) reported high infection frequency in 50 day old plants than in 30 day old in TMV2. Lesion count was not a consistent measure of resistance because of its high environmental variations (Subrahmanyam *et al*,. 1982; Walls *et a.,l* 1985 and Chiteka *et al.*, 1988a).

Both lesion diameter (Fig.6.6) and PNA (Fig.6.7) were higher in 20 - 50 days old plants in all the cultivars. Eventhough lesion number was higher in 40 and 50 day old plants, lesion diameter and per cent necrotic area were on par with 20 and 30 day old plants in TMV2 and ICGV 86590. This may be due to comparatively smaller size of leaves in 20 and 30 day old plants. Subrahmanyam *et al.*, (1982) observed higher per cent necrotic area in 30 day old plants than in 50 day old plants in TMV2 in glasshouse. Shanta (1960) and Cook (1981) found that the youngest (4 week) and oldest (10 week) leaves of the plants were least susceptible to infection. However, Cook (1981) opined that this effect appears to be related to leaf size rather than leaf

age. Leaflets of expanded leaves increase in area with ascending position on the main axis of the plant until plants are about six week old. Leaflets of leaves opening after this time are comparable with each other in size when fully expanded.

Defoliation was also highest in 20-50 day old plants in TMV2 and ICGV 86590 (Fig.6.8). The percentage of defoliation was slightly higher in 20 and 30 day old plants eventhough not significantly differed with 40 and 50 day old plants. Disease score was also highest and on par in 20-50 day old plants (Fig.6.9).

The present studies revealed that the susceptible stage of the plant to the infection and disease development was 20-50 days in green house conditions. But, in field conditions this disease appears late in the season (40 - 45 DAS). In the present investigation under greenhouse conditions, the susceptibility of 20 and 30 day old plants can be more related to leaf size rather than leaf age. The smaller leaf size has lead to more per cent necrotic area and defolition even with less number of lesions per leaf compared to 40 and 50 day old plants.

Cultivar differences were significant for all the components of resistance in 20-50 day old plants. In all the cultivars, disease was highly restricted in 10 day old plants followed by higher ages (60-90 DAS). TMV2 was the most susceptible in 20-50 day ages and ICGV 86699 was most resistant in all the ages.

The amount and extent of infection depends on the age of the plant with various other factors (Miller, 1946). Ten-day old plants showed reduction in all the components of resistance except incubation period and latent period which are on par with 20-50 day old plants. But in 60-90 day old plants, all the components were significantly lower than 20-50 day old plants. In older plants, the accessibility was reduced by high foliar density and high leaf area index (Savary and Van Santen, 1992).

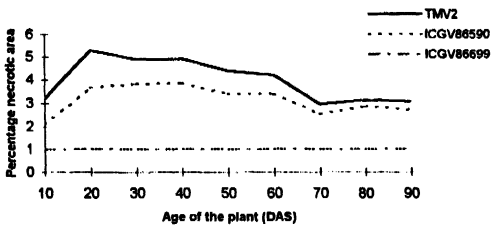


Fig 6.7 : Effect of age of the plant on per cent necrotic area

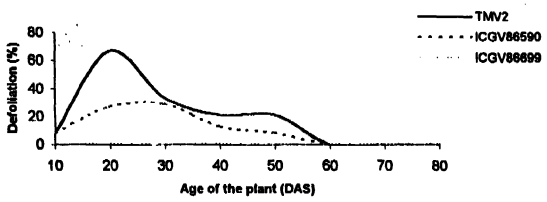


Fig 6.8 : Effect of age of the plant on defoliation

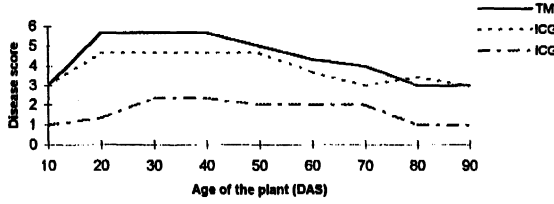


Fig 6.9 : Effect of age of the plant on disease score

It was suggested that, in the variation of susceptibility with leaf age and development stage of a groundnut cultivar, more factors involve than just leaf wettability. Infection efficiency decreases with increasing development and age of the plant as reported in several other host pathogen systems (Schein, 1965; Aust *et al.*, 1980 and Savary, 1987). Similar results were reported for latent period (Parlevliet, 1975 and Savary,1987) and sporulation intensity (Tomerlin *et al.*, 1983).

Inspite of the diversity among pathosystems, several authors (Popular, 1978; Zadoks and Schein, 1979 and Vanderplank, 1982) hve suggested generalizations to account for the variation in the pathological interactions between fungi and ageing plants. The most general hypothesis for a biotrophic pathogen is, perhaps, that the younger and healthier the host tissues, the easier their recognition and use as a convenient habitat for faster and more intense growth and multiplication. This has led Zadoks and Schein (1979) to suggest that partial resistance against biotrophic fungi would generally increase with age and development.

5.5 SURFACE STUDIES ON THE INOCULATED GROUNDNUT LEAVES

The surface studies of the susceptible (TMV2), moderately resistant (ICGV 86590) and resistant (ICGV 86699) cultivars after inoculation revealed the reaction of conidia on the leaves. No significant differences observed on the leaves of TMV2 and ICGV 86590. The pathogen reacted more or less similar on both these hosts. But in ICGV 86699, the difference in the reaction of conidia on the leaves was differing with the susceptible and moderately resistant cultivars.

The spores of *Phaeoisariopsis personata* germinated within 6-8 hours after inoculation (HAI) irrespective of the resistance. In TMV2 and ICGV 86590, more than 50 per cent of the conidia germinated within 8 HAI but in ICGV 86699, it took more than 20 hours. Eventhough the percentage germination of the conidia has no effect on the resistance, in ICGV 86699 most of the germinated conidia eventually lost their stainability, became transparent and disintegrated. No significant differences in germination of conidia were observed on leaves of various peanut cultivars (Cook 1981; Abdou, 1974). The number of germ tubes per conidia did not play any role in reduced infection of resistant cultivar because generally only one germ tube entered through the stomata.

The delayed and reduced number of penetrations through stomata and late appearance of symptoms in the resistant cultivar can be effectively explained by Mayee and Suryawanshi (1995) that penetration of germ tube through stomata will be followed by accumulation of intracellular hypha in the cells surrounding the infection site. The time required for accumulation of such hyphae was longer in resistant cultivar compared to the susceptible cultivar. This will subsequently lead to delay in the occlusion of the intercellular spaces, thickening of palisade cells and formation of conidiophores in the stroma.

The delayed and defective germination and penetration of conidia in resistant cultivar lead to reduced infection. However the resistance to LLS pathogen is of partial type (Nevill, 1981; McDonald, 1985) and is characterized by delayed incubation period, latent period, reduced sporulation, necrotic leaf area and less defoliation. In ICGV 86699, all the components of resistance play an important role in imparting partial resistance. The expression of resistance in ICGV 86699 was associated with appearance of many small, non-sporulating lesions. These characters appear to be the consequence of slow invasion of the leaf tissues by the pathogen.

The initial symptoms of LLS infection in susceptible cultivar were coagulation and shrinkage of protoplasm of the cells surrounding the infection site, disappearance of chloroplasts, swelling and destruction of nuclei and ultimately the cells became shrunken and filled with dark brown material. Kaur and Dhillon (1988) noted shrunken epidermal and mesophyll cells and damage to protoplast in LLS infection. The reduced number of infections in the resistant cultivar can be explained by the anatomical differences between the resistant and susceptible cultivars. In the susceptible cultivar the stomata will be wider than resistant cultivar and the slow rate of pathogen growth in the resistant tissues is due to the presence of thick palisade and spongy tissue (Bera *et al.*, 1997). Mayee and Suryawanshi (1995) found thickening of palisade cells and production of zone of smaller cells around the infection site which can be related to small size of spot and less production of mycelial mass resulting in low sporulation. Basra *et al.*, (1985) observed thicker epidermis, smaller stomata and compact palisade in resistant leaves which accounted for fewer penetrations in resistant cultivar. However Cook (1981) reported that variations in stomatal density and stomatal length were not related to resistance to infection. In the moderately resistant cultivar (ICGV 86590), the germination of conidia was similar to susceptible

cultivar (TMV2). There were not much difference observed between these two cultivars. All the components of resistance were delayed than susceptible cultivar but never restricted. But both these cultivars significantly differed with the resistant cultivar (ICGV 86699). The delayed and defective germination of conidia, reduced number of penetrations, slower rate of invasion of host cells in the resistant cultivar can be highly correlated with late appearance of symptoms.

The difference in the level of minerals (Sindhan and Parashar, 1996; Jagalan and Sindhan, 1988; and Mogle and Mayee, 1981), the histochemical changes that takes place after infection (Kaur and Dhillon, 1988), the anatomical differences (Bera *et al.*, 1997; Mayee and Suryawanshi, 1995; and Basra *et al.*, 1995) between the resistant and susceptible cultivars play an important role in imparting partial resistance along with the defective germination of the conidia, delayed and reduced number of penetrations/infections and slower rate of pathogen growth in resistant cultivar.

In conclusion, the studies on the effect of temperature, humidity, inoculum concentration and age of the plant provide the basis for understanding how these factors affect various components of resistance in the host system. The late appearance of symptoms which impart partial resistance in addition to the other components of resistance in the resistant cultivar can be related to defective germination and delayed penetration of the host tissue by the conidia of the pathogen from the present study. Though it is an established fact that the anatomical characters and the histochemical changes take place during post infection development in the resistant cultivar which result in resistance reaction, more concentrated and valiant effort by the future researchers on the exogenous and endogenous factors which play an important role in the spore germination and penetration of *Phaeoisariopsis*

personata on resistant cultivar can add on to the important results emerged out of the present study.

SUMMARY

CHAPTER VI

SUMMARY

Studies on the effect of temperature, humidity, inoculum concentration and age of the plant on the components of resistance to late leaf spot disease of groundnut were carried out. Histopathological investigations were also carried out to further quantify the components of resistance. All these studies were conducted at the International Crops Research Institute for Semi-Arid Tropics (ICRISAT), Patancheru-502 324, Andhra Pradesh, India. The results are summarized below.

In all the studies, TMV2 and ICGV 86699 were the most susceptible and resistant cultivars respectively. ICGV 86590 was found to be moderately resistant. Resistance in ICGV 86699 was expressed by prolonged incubation period, absence of sporulating lesions (i.e. no latent period), reduced lesion number, lesion size, sporulation index, per cent necrotic area, defoliation and disease score.

Over the range of temperatures studied, 25°C was the most favourable for infection and disease development in all the three cultivars. However, the effect of temperature on *Phaeoisariopsis personata* infection and disease development was the highest between 15 to 20°C and slight between 20 to 25°C. No significant amount of disease was observed at 30 and 35°C.

The maximum disease severity was observed in 16 h humidity treatment in all the three cultivars. The infection and disease development were reduced in low humidity treatments (4, 8 and 12 h) and higher in high humidity treatments (16,20 and 24 h) with the maximum in 16 h treatment. Although the lesion number did not vary much between low humidity treatments (9, 8 and 12 h) and 20, 24 h treatments, the differences were clear for all the other components of resistance.

The optimum concentration of inoculum to cause the highest amount of disease was 20000 conidia per ml as there was no difference in the severity of disease with further higher concentrations (25000 conidia ml^{-1}) in all the three cultivars. Concentration of inoculum has no effect on incubation period, latent period and sporulation index Disease severity was reduced in lower concentration treatments (7500 - 1000 conidia ml^{-1}) and no significant differences were observed among the treatments for all the components of resistance Cultivar differences were not significant for lesion number in all lower concentration treatments The higher concentrations (25000 and 20000 conidia ml^{-1}) significantly differed with immediate lower concentrations (15000 and 10000 conidia ml^{-1}) for lesion number, defoliation and disease score

Twenty to fifty day old plants were found to be the most susceptible to LLS in all the cultivars. However, the interaction between plant age and LLS infection and disease development was not significant in ICGV 86699. Cultivar differences were also significant for all the components of resistance in 20 - 50 day old plants Ten day old plants were found to be highly resistant followed by higher ages (60 - 90 DAS) in all the cultivars

Histopathological investigations revealed that defective germination, delayed penetration, slow invasion of host tissues lead to prolonged/lengthened incubation period and reduced severity of disease in resistant cultivar - ICGV 86699. The differences between TMV2 (susceptible) and ICGV 86590 (moderately resistant) were not large.

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