EPIDEMIOLOGY AND MANAGEMENT OF LEAF BLOTCH OF TURMERIC CAUSED BY *TAPHRINA MACULANS* BUTLER

BY

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THESIS SUBMITTED TO THE
ACHARYA N.G. RANGA AGRICULTURAL UNIVERSITY
IN PARTIAL FULFILMENT OF THE REQUIREMENTS
FOR THE AWARD OF THE DEGREE OF

DOCTOR OF PHILOSOPHY

IN THE FACULTY OF AGRICULTURE

DEPARTMENT OF PLANT PATHOLOGY
AGRICULTURAL COLLEGE
ACHARYA N.G. RANGA AGRICULTURAL UNIVERSITY
BAPATLA – 522 101, A.P., INDIA

AUGUST, 2001

CERTIFICATE

Mr. J. KRISHNA PRASADJI has satisfactorily prosecuted the course of research and that the thesis entitled "EPIDEMIOLOGY AND MANAGEMENT OF LEAF BLOTCH OF TURMERIC CAUSED BY *TAPHRINA MACULANS* BUTLER" 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 : 13.08.2001

(DR. S. RAMA PANDU)

Major Advisor

CERTIFICATE

This is to certify that the thesis entitled "EPIDEMIOLOGY AND MANAGEMENT OF LEAF BLOTCH OF TURMERIC CAUSED BY *TAPHRINA MACULANS* BUTLER", submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy of the Acharya NG Ranga Agricultural University, Hyderabad, is a record of the bonafide research work carried out by **Mr. J. Krishna Prasadji** under my 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 or has been published. 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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ACKNOWLEDGMENT

I am extremely happy to express my gratitude to Dr. S. Rama Pandu, University Head, Department of Plant Pathology and Director, Agricultural Human Resources Development Project (World Bank) of the Acharya NG Ranga Agricultural University, Hyderabad for his affectionate and effective guidance during the course of the investigation and writing of the thesis.

I offer my sincere thanks to the members of my Advisory Committee, Dr. K.V.M. Krishna Murthy, Associate Professor, Plant Pathology and Dr. T.C. M. Naidu, Asst. Professor, Plant Physiology, Agricultural College, Bapatla and Dr. S. M. Zaheruddeen, Senior Scientist, Entomology for their valuable suggestions and encouragement.

This thesis has richly benefited from the expert counsel of Dr. K. Muralidharan, Principal Scientist and Head, Directorate of Rice Research, Hyderabad. I am indebted to him for kindly sparing a long time and enormous effort in the analysis of the epidemiological aspects and interpretation of results. I am grateful to him for teaching me the practice of epidemiology.

I am thankful to Dr. K. A. Balasubrahmaniam, Professor (Retd.), Dr. M. Subba Rao and Dr. C. Subba Reddy, Associate Professors, Department of Plant Pathology for their encouragement during my stay at Bapatla. I am also thankful to Dr. Bapuji Rao and Dr. Veeraraghavaiah, Associate Professors in Agronomy for their friendly support.

I gratefully acknowledge the help rendered by Dr. C. Subba Rao, Senior Scientist, (Retd.) in Statistics and Smt. B. Sailaja, Scientist, Computer Applications, DRR in the analysis of data. I am thankful to Dr. A. Satyanarayana, Professor, Computer Science of our University.

I am also thankful to Dr. Raman, Dr. Satyanarayana, Dr. C.S. Reddy, Dr. Krishna Veni, Dr. Laha and all other members of the Division of Plant Pathology and Dr. G. S. V. Prasad of Directorate of Rice Research for their help and encouragement.

I am fortunate to have the support of my colleagues at Agricultural Research Station, Kovvuru. I am extremely thankful to each one of them, especially to Sri. D. Madhava Rao and Sri. B.V.K. Bhagawan.

I wish to sincerely acknowledge the help and support provided by my friends Dr. Reddy, Dr. C.A. Raju, Dr. Shiva Raju, Dr. Chandrasekhar and Dr. Sridhar of the Central Tobacco Research Institute, Rajahmundry.

I am grateful to my University, the Acharya NG Ranga Agricultural University for granting me an opportunity with study leave to pursue Ph.D degree.

I thank Smt. Aparna for her good type work of my thesis.

(J. KRISHNA PRASADJI)

DECLARATION

I, **J. KRISHNA PRASADJI** hereby declare that the thesis entitled "EPIDEMIOLOGY AND MANAGEMENT OF LEAF BLOTCH OF TURMERIC CAUSED BY *TAPHRINA MACULANS* BUTLER" submitted to Acharya NG Ranga Agricultural University for the degree of DOCTOR OF PHILOSOPHY in Agriculture is the result of the original research work done by me. It is further declared that the thesis or part thereof has not been published earlier in any manner.

Date : 13.08.2001

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ABSTRACT

Name of the student	:	J. KRISHNA PRASADJI
Title of the thesis	:	EPIDEMIOLOGY AND MANAGEMENT OF LEAF BLOTCH OF TURMERIC CAUSED BY *TAPHRINA MACULANS* BUTLER.
Degree to which it is submitted	:	DOCTOR OF PHILOSOPHY
Faculty	:	AGRICULTURE
Discipline	:	PLANT PATHOLOGY
Major Advisor	:	DR. S. RAMA PANDU
University	:	ACHARYA NG RANGA AGRICULTURAL UNIVERSITY
Year of submission	:	2001.

The investigations on the epidemiology and management of leaf blotch disease of turmeric caused by *Taphrina maculans* were conducted at the Agricultural Research Station, Kovvur, West Godavari District, Andhra Pradesh during 1998-99, 1999-2000.

The pathogen, *Taphrina maculans* Butler was isolated on PDA having a pH of 4.5 at an incubation temperature of 20° C by suspending the infected leaf bits from inside of the lid of Petri dish. The optimum incubation temperature was found to be 15 - 20° C and the optimum pH of the culture medium was around 5.5 for the growth of *T. maculans* in culture. There was no difference in the SDS-PAGE protein profiles of the isolates of *T. maculans* from the three turmeric growing tracts of Andhra Pradesh.

Taphrina maculans was found to have many avenues to perpetuate from one season to the other. Seed rhizomes, leaf debris and soil from infected fields of the previous year, were established to harbour the pathogen. *Canna* spp. was found to serve as other host of *T. maculans* and hence suspected to serve as primary source of inoculum.

The incubation period (p), latent period (l) and infectious period (i) in *C. longa* - *T. maculans* pathosystem were 19 - 25 days, 5 - 6 days and 24 - 25 days, respectively, in six susceptible turmeric cultivars.

The groups of turmeric cultivars susceptible to *Taphrina maculans* and *Colletotrichum capsici* were separated based on differences in visually perceivable characters, Euclidean D^2 technique, curcumin content and gel electrophoresis.

Planting turmeric late was found to reduce leaf blotch disease severity and yield in turmeric. Decrease in plant density was also found to decrease leaf blotch disease severity and yield. A plant population density of 49383 plants ha^{-1} at 45 x 45 cm spacing was found to reduce leaf blotch disease severity without significant reduction in yield.

Propiconazole (0.1%), bitertenol (0.1%) and chlorothalonil (0.2%) were found to be highly effective in controlling leaf blotch disease and increasing yield. Micronutrients, zinc sulphate (0.2%) and ferrous sulphate (0.2%) were also found to reduce leaf blotch severity and increase yield. Propiconazole gave the best economic return (C/B ratio 8.15). Raising barrier crops around plots of susceptible turmeric cultivar resulted in an increase in leaf blotch severity.

Four weather variables namely, maximum temperature, minimum temperature, rainfall and relative humidity (0800 h) were found to influence leaf blotch severity in turmeric by stepwise linear regression analysis. However, the best fit predictive models were found to predict disease severity only partially.

The temporal analysis of disease progress revealed that the leaf blotch disease increases at a very slow rate with the maximum inflection point at the middle of the epidemic 50 - 60 days after onset of disease. Logistic model fitted well in describing the disease progress. The doubling time of disease calculated using the rate parameter of logistic model was found to be 30 - 33 days and did not differ among six cultivars. The area under disease progress curves (AUDPC) also did not differ among the six cultivars.

Random sampling method was found suitable for studying spatial pattern of temporal changes in leaf blotch disease in turmeric. The indices of dispersion, Lloyd's index of patchiness (LIP) and Morisita's index (I δ) indicated an aggregated pattern in the initial stages of epidemic (up to 109 DAP) and random pattern at the later stages of epidemic (from 140 DAP).

ABBREVIATIONS

The expansion of the abbreviations used in this thesis are given below.

C = Celsius
c = clumping size
CD = Critical difference
cm = Centimeter
CV = Co-efficient of variation
DAP = Days after planting
d Sm = desi Seimen
Fig = Figure
g = gram
ha = hectare
ICS = Index of clumping size
Kg = Kilogram
l = litre
LIP = Lloyd's index of patchiness
m = metre
ml = millilitre
PDA = potato dextrose agar
PDI = per cent disease index
Sem = Standard error of mean
t = tonnes
µl = microlitre
µm = micrometer
VM = variance to mean ratio

INTRODUCTION

CHAPTER I

INTRODUCTION

Turmeric (*Curcuma longa* L.) is an important herbaceous spice plant widely distributed in India, China, Indonesia, Siam, Malay Archipelago and Northern Australia. Turmeric occupies a very important place in the day-to-day life of Indians. The yellow powder of turmeric is indispensable in Hindu religious ceremonies because its colour symbolises prosperity. It has been used since the Vedic period owing to its unique colour, flavour and medicinal properties. Turmeric finds an important position in Ayurveda as an aromatic, stimulant, tonic, carminative and anti-helminthic. The essential oil of turmeric is widely known for its antiseptic properties. Turmeric is also used in treating gallstones and related complaints (Pruthi, 1997; Shah, 1997). Torrents of modern day cosmetic preparations also claim to contain turmeric as a major ingredient.

The reports about turmeric having anti-fungal properties suggest scope for a possible exploitation of it in the integrated management of plant diseases. *In vitro* inhibition of *Rhizoctonia solani* by essential oils derived from turmeric at 2000 ppm was observed (Kishore *et al.* 1998). Turmeric plant extracts were also shown to have antifungal activity against the rice seed-borne pathogens like *Pyricularia oryzae*, *Rhizoctonia solani* and *Gibberella fujikurai* (Miah *et al.* 1990).

India is the world leader in turmeric with a production of 4,87,400 tonnes annually from a cultivated area of 1,24,600 hectares. Andhra Pradesh ranks first in India with an area of 48,000 hectares and produces more than one-half of the nation's

production (2,73,000 tonnes) (CMIE Report, 1999). More than 90% is consumed in the domestic markets and only 5-8% is exported. In Andhra Pradesh, turmeric is grown in all the three regions of the state namely Andhra, Rayalaseema and Telangana. Karimnagar, Nizamabad, Guntur, Adilabad, Cuddapah, Warangal, Kurnool, Krishna and West Godavari are the chief turmeric growing districts in the state.

Turmeric, in the context of World Trade Organisation (WTO), was the centre of a recent controversy. The controversy arose because of the grant of a patent in the United States (# US 5,401,504, which is commonly referred to as the turmeric patent) regarding its use in wound healing. The patent, however, was revoked upon India's challenge. This instance shows the paramount importance of turmeric plant in India. The scope for utilization of the turmeric plant products is likely to expand. This plant cultivated commercially over a large area, therefore, should be tended to with a very scientific approach. The crop production and crop protection in the cultivation of turmeric should be in tune with the WTO requirements.

The turmeric crop suffers from infection by four pathogens namely *Pythium* spp. (Rao, 1995) and *Fusarium solani* (Rattaiah, 1987) causing rhizome rots; *Colletotrichum capsici* causing leaf spot (McRae, 1917); and *Taphrina maculans* causing leaf blotch (Butler, 1911). The leaf blotch disease caused by *T. maculans* was earlier mentioned as leaf spot (Butler, 1911 and 1918; Upadhyay and Pavgi, 1966; Ahmed and Kulkarni, 1968a). Apart from the isolated work on etiology and perpetuation of the pathogenic fungus (Pavgi and Upadhyay, 1964; Upadhyay and Pavgi, 1967a; Ahmed and Kulkarni, 1968a; Kulkarni and Ahmed, 1968), much of the later work was centered either on screening varieties to the disease (Reddy *et al.* 1963; Philip and Nair, 1981; Rao *et al.*

1992) or on management with fungicide application (Nirwan *et. al.* 1974 and Srivastava and Gupta, 1977). Leaf blotch epidemics occur regularly and cause considerable damage. There has been no systematic and comprehensive study of the *Curcuma longa–Taphrina maculans* pathosystem. Even the few reports on the perpetuation of the pathogen (Upadhyay and Pavgi, 1967a; Ahmed and Kulkarni, 1968b) contradicted each other. There is no information on the efficacy of the new generation of fungicides in the management of the disease. The impact of modified cultural practices adopted for harvesting high yields in turmeric on the leaf blotch pathogen and development of epidemic has not yet been investigated.

Taphrina maculans causes leaf blotch in all the turmeric-growing areas in the tropical region. A related species of *T. maculans*, that is, *Taphrina deformans* is often encountered in peach-growing areas in the temperate regions. There is a paucity of information on disease development in peach leaf curl disease also. Epidemiological understanding on the onset of disease and the dynamic changes that lead to spread and spatial pattern of the disease in plant populations help to find a better and more efficient management strategy. This assumes greater importance as the turmeric product must be pesticide-free to meet stringent export quality standards. Although some basic studies have been made in understanding the temporal changes in the disease development, the temporal changes in the spatial pattern is least understood. For such a study, the *Curcuma longa-Taphrina maculans* pathosystem is an ideal model system with a reasonably extended period of epidemic of over 4-months duration. It was, therefore, felt important to investigate on different epidemiological and management aspects in this pathosystem.

The present research was conducted at the Agricultural Research Station, Kovvur, West Godavari district, Andhra Pradesh with the following objectives:

1. To study the differences among isolates of *Taphrina maculans*, if any, from different turmeric growing tracts of Andhra Pradesh.
2. To study the survival and perpetuation of the pathogen and to establish the primary source of inoculum.
3. To determine the incubation period (ρ), latent period (λ) and infectious period (i) in *C. longa - T. maculans* pathosystem.
4. To estimate the apparent rate of infection (r) of the disease during the crop season.
5. To find out the relationship between weather factors and disease.
6. To analyse changes in temporal and spatio-temporal progress of the leaf blotch disease in turmeric.
7. To understand and document the differences between the *Taphrina* leaf blotch susceptible cultivars and *Colletotrichum* leaf spot susceptible cultivars.
8. To evaluate the efficacy of certain management practices like cultural operations, application of fungicides and micronutrients and raising barrier crops on disease severity and yield in leaf blotch susceptible turmeric cultivar.

The research results of this study are presented and discussed in this thesis with the help of statistically analyzed data and illustrations.

REVIEW OF LITERATURE

CHAPTER II

REVIEW OF LITERATURE

The literature on leaf blotch disease of turmeric caused by *Taphrina maculans* is mostly of Indian origin and is very scanty. The available literature on this disease as also the relevant literature on diseases caused by other species of *Taphrina* on several other crops is reviewed in this chapter.

2.1 THE DISEASE

2.1.1 Occurrence

The earliest mention of the leaf blotch (leaf spot) caused by *Taphrina maculans* was by Butler (1911) and later by Mc Rae (1917). Butler reported the occurrence of the disease mostly from North India, while Mc Rae reported from South India.

2.1.2 Symptoms

Butler (1918) and Mundkar (1949) described the symptoms of the disease in detail. The disease appears as spots on both surfaces of leaf in great numbers. The spots are generally more on the upper surface. They begin as a pale yellow discolouration not sharply defined, which soon becomes dirty yellow, then deepens to the colour of old gold or sometimes nearly to a bay shade. The spots are small, usually measure 1 to 2 mm in diameter and coalesce freely. They are not in any way limited by the veins and do not produce any local distortion of the leaf.

2.1.3 The Pathogen

Taphrina maculans Butler like other species of *Taphrina* is a dimorphic fungus (Romano, 1966), which is filamentous in the host and yeast-like (conidial form) in culture.

2.1.3.1 Life Cycle of the Pathogen

Butler (1918) and Kulkarni and Ahmed (1968) gave a detailed account of the life cycle of *Taphrina maculans.* The first formed hyphae of the fungus after infection are found embedded in the cuticle, which covers the epidermis of the leaf. They extend and grow in the intercellular spaces between the cuticle and epidermis aggregating at the angles of the cells. Flat bands of hyphae from the cuticular mycelium pass downwards between the epidermal cells, spread along the inner walls of epidermis and continue to grow through the vertical walls of hypodermis and reach the walls in contact with the spongy parenchyma of the leaf. The fungus sends star-like and globular haustoria from the hyphae for its nourishment (Pavgi and Upadhyay, 1967). The mycelium fills up cavities of epidermal cells, becomes aggregated and forms short, slightly thick, club shaped or bottle shaped ascogenous cells forming parenchymatous masses each consisting of a layer of 2 - 3 cells. Asci are formed from the top layer of these ascogenous cells. The asci forming cells elongate and exert pressure due to which epidermal cell wall and cuticle are broken. The asci are usually produced in rows in erect fashion on the host tissue. As a rule the ascogenous cells mature at different periods so that the ripe asci are found in small groups. The fully developed asci are hyaline, cylindrical, flattened at the top and measure 20 to 30μ in length and 6.5 to 10 μ in breadth. The asci usually contain eight ascospores but up to 16 or more spores or

conidia are also found. The spores are hyaline, unicellular, ovoid or almost oblong and measure 4 to 6.5 μ in length and 2 to 2.5 μ in breadth. In *T. maculans* dikaryosis involves fusion between sister nuclei. The fungus has a short diploid phase restricted to the ascogenous cells and production of ascospores and a prolonged haploid phase representing production of blastospores. Dikaryosis in the blastospores is brought about through division of the single haploid nucleus (Chiplonkar, 1969).

2.1.3.2 Isolation

The earliest attempts at culturing *Taphrina* species were encountered with difficulty. According to Mix (1924), Brefeld and Sadebeck in the late 19th century brought *Taphrina johansonii* and allied species of *Taphrina* into conidial form on nutrient solutions. Pierce (1900) could bring *T. deformans* into culture on extract of malt and beer solutions. Klebahn (1924) reported on the isolation of *T. tosquinetii* in solid medium.

Brefeld (1891), though made no mention of the media used, reported that *Taphrina johansonii* would bud readily in nutrient solutions. Pierce (1900) grew *Exoascus deformans* in various nutrient solutions such as extract of malt, sterilized beer, solutions of various sugars etc., in yeast form, but could not induce the yeast cells to filamentous germination.

The most extensive work on isolation of species of *Taphrina* was by Mix (1924) and Martin (1925). Although successful isolation of *T.deformans* (*Exoascus deformans*) in the form of pale, pink and yeast-like colonies was reported by them on a variety of media like potato sucrose, potato maltose, pea malt and peach leaf agars, stem corn meal, steamed rice, plugs of sweet potato, carrot and beet and on bean pods, the fungus

grew best on cleared potato dextrose agar having a pH of 4.5. Jankowska (1929) found PDA to be the best medium for isolation of *T. sadebeckii*.

Mix (1924) obtained cultures of *T. deformans* with scrapings of mature asci and spores in drops of water by pouring dilution plates on potato dextrose agar. Martin (1925) reported a novel method of isolating this pathogen in which the infected portions with mature asci were fastened to the cover of the Petri dish so that the asci could eject their spores down on to the clear potato dextrose agar.

The successful isolation of other species of *Taphrina* provided useful leads for the isolation of *T. maculans* from infected leaves of turmeric. Pavgi and Upadhyay (1964) reported the successful isolation of *T. maculans* from fresh mature leaf lesions collected from the fields. Fresh leaf material with mature lesions was soaked in water for 30 minutes, frozen for 48 hours and incubated at room temperature for 24 hours. Later, small leaf pieces bearing the lesions were suspended from the inside of the lid of a Petri dish containing nutrient medium. This enabled the ascospores that were expelled forcibly from the asci to germinate on the nutrient agar medium and develop into small colonies. More than one half of the number of single ascospore isolates obtained by Pavgi and Upadhyay (1964) were bright salmon red coloured type while the rest showed a dull, creamy white, and glistening viscid or thick and sticky type of growth.

The stage of development of spot was found to have a bearing on the success of isolation (Ahmed and Kulkarni, 1968a). The initial stage of spot development showing pale yellowish flecks, the middle stage expressing yellow necrotic areas and the final stage showing fully developed, chocolate brown coloured spots with golden yellow halo were assessed for their ascus forming ability after taking thin hand sections and

incubating them in moist chamber. Sections from the initial and middle stages of spot development were found to produce profuse asci in 24 hours while those from the final stage of spot development were found to produce only very few asci 5-6 days after incubation. Thus it was concluded that the leaf material from initial and middle stages of spot development is ideal for isolating the pathogen. Ahmed and Kulkarni (1968a) also demonstrated three methods of isolation of *T. maculans*. In the first method conidial and ascospore suspension, obtained by incubating thin hand sections of leaf spot at the initial stages of spot development in sterilized acidified water in cavity slides, was streaked on potato dextrose agar (pH 4.5) and turmeric leaf decoction agar (pH 4.5). In the second method, originally described by Martin (1925) for isolation of other species of *Taphrina,* leaf pieces with initial stages of infection were stuck to the lid of the Petri dish. In the third method, originally explained by Mix (1924) washings from infected leaves with final stages of spot development were streaked out on plates with acidified potato dextrose agar. In all the methods the incubation temperature was maintained at 20° C.

2.1.3.3 Conditions for Growth of *Taphrina* in Culture

Mix (1924) found that cultures of *Exoascus deformans* (*Taphrina deformans),* kept in laboratory were found to be devitalized at temperatures prevailing in early summer. This indicated a rather low maximum temperature requirement for growth of the fungus. The minimum temperature for growth was found to be well below 10° C and optimum in the neighbourhood of 20° C and maximum ranged between 25-30° C. He also demonstrated that *T. deformans* has a wide range of tolerance to acid and alkaline conditions when grown on potato dextrose agar, and the limits of growth was beyond

the range of indicators employed (pH 3.3 to pH 9.75). The optimum concentration for growth was around pH 5.0. A pronounced shift was observed in the direction of increased acidity when *T. deformans* was grown on potato dextrose broth with initial pH ranging from 3.9 to 8.7 (Mix, 1924).

2.1.3.4 Pathogenicity

Pathogenicity of the pure culture of *T. maculans* was reported by Pavgi and Upadhyay (1964). They observed the first visible symptoms of the disease four days after inoculation. Ahmed and Kulkarni (1968a) reported pathogenicity of both ascospores and conidia produced from infected leaf tissue and culture. They observed development of spots on plants 25 days after inoculation with ascospores and conidia derived from infected tissue and 45 days after inoculation with conidia obtained from culture.

2.1.4 The Host

Turmeric, (*Curcuma longa* L. and *C. aromatica* L.) is affected by two foliar pathogens, *Colletotrichum capsici* causing leaf spot and *Taphrina maculans* causing leaf blotch.

2.1.4.1 Host Resistance in Genotypes

Reddy (1957) made a detailed study of turmeric genotypes for their reaction to the two foliar diseases. He reported that the two foliar diseases are mutually exclusive and that any given genotype is susceptible to only one of the pathogens and remains invariably resistant to the other. Reddy *et al* (1963) reported that the turmeric accessions exhibited varying degrees of resistance but no single variety was infected by or resistant to both the pathogens.

Sarma and Dakshinamurthy (1962) studied the host resistance against leaf spot diseases and their intensities on certain varieties of turmeric. They found that long duration varieties of *C. longa* were found to be susceptible to *Colletotrichum capsici* and medium duration types were attacked by *Taphrina maculans*. The early duration *C. aromatica* types were found to be highly resistant to both pathogens.

Philip and Nair (1981) screened 19 promising turmeric types for their reaction to pests and diseases. They observed that all the 19 types were susceptible to *C. capsici* leaf spot. These types were also found susceptible to simultaneous incidence of leaf spot and leaf blotch. These screening results were in contrast to the earlier findings (Reddy, 1957, Reddy *et al.*1963; Sarma and Dakshinamurthy, 1962).

Nambiar *et.al.* (1977) reported that some turmeric types belonging to *Curcuma longa* and *C. aromatica* were susceptible to *Taphrina maculans* and that the disease was more intense in the types of *C. longa.*

Several turmeric clones with good agronomic traits have been reported to be resistant to *Taphrina* leaf blotch (Upadhyay and Pavgi, 1967b; Maurya, 1990; Rao *et.al.*1992 and Chandra *et al.*1996).

2.1.4.2 Genetic Divergence

Kendall (1980) suggests the simple Euclidean D^2 distance statistic to measure the distance between two objects.

The turmeric cultivars of *Curcuma aromatica* and *C. longa* were grouped into four clusters based on generalized distance D^2 statistic (Nambiar, 1979). According to him cultivars belonging to *C. aromatica* maintain a separate identity from the cultivars of *C. longa.*

Using Metroglyph analysis, Chandra *et al.* (1997) found that PCT 13 and Lakadong formed solitary groups and were genetically distant from other genotypes. The greatest number of genotypes with moderate yield potential was clustered into two groups. Turmeric land races of the North East region of India were found clustered in to low to moderate yield groups, while the genotypes from South India were scattered among different complexes, ranging from moderate to high yield producers.

Rao (2000) grouped 54 turmeric accessions into six clusters based on Mahalanobis' D^2 values calculated with nineteen characters. One cluster comprised mostly of long duration cultivars; another cluster comprised mostly of intermediary duration cultivars; and the rest comprised mostly of the short duration cultivars.

Types belonging to *C. aromatica* were in a separate cluster along with a few other short duration types.

2.1.4.3 Age of Host Leaves

Upadhyay and Pavgi (1967c) observed that turmeric leaves become susceptible to infection two weeks after unrolling. The susceptibility of turmeric leaves was maintained for about a month and then gradually it declined with advancing age. There was a reduction in the susceptibility of leaves with maturity but the leaves did not attain total resistance or immunity.

2.2 HOST RANGE

Taphrina maculans is known to infect turmeric and several other plant genera and species including *Curcuma amada, C. angustifolia, Zingiber casumunar, Zingiber zerumbet* and *Hedychium* (Butler, 1918) and *Zingiber meioga* (Mix, 1949).

2.3 VARIABILITY IN *T. maculans*

Only a cursory mention of a possible differential pathogenicity of the isolate relative to the naturally occurring infection by *T. maculans* on *C. amada* was made (Upadhyay and Pavgi, 1967b). No other researcher suspected variability in *T. maculans* or other *Taphrina* species so far.

2.4 SURVIVAL AND PRIMARY SOURCE OF INOCULUM

Diverse opinions were expressed on the period and place of survival of *Taphrina* sp. Large, thick walled, shining atypical spores from the centre of old cultures of *E. deformans* were designated by Mix (1924) to be the resting cells. Martin (1925) also similarly designated such spores in *T. johnsonii*, *T. communis*, *T. mirabilis*, *T. coryli*, *T. deformans* and *T. coerulescens*. Mix (1924) found that desiccated cells could survive for 140 days at 30° C while spores from fresh cultures could not survive even for a few days at 30° C. The thermal death point of desiccated cells was found to be as high as 100° C for 10 minutes and that desiccated cells survived 10 minutes' exposure to 95° C. Mix concluded that the level of resistance possessed by the conidia of *E. deformans* was perhaps sufficient to account for the continued survival of the organism from one year to the next year. Presence of a similar potentiality in *T. maculans* was demonstrated by Upadhyay and Pavgi (1967a). Fresh cultures of the fungus were found devitalized at 44° C in 12 hours, at 42° C in 48 hours and at 40° C in 72 hours, whereas the desiccated blastospores were devitalized by one hour exposure to 90° C and 12 hours exposure to 60° C.

In field conditions *T. maculans* was reported to persist during summer by means of ascogenous cells on leaf debris or as desiccated ascospores and blastospores in soil

and amongst fallen leaves (Upadhyay and Pavgi, 1967a). *T. maculans* was also shown to survive above and below (up to 10 cm) the soil surface until September and in laboratory at room temperature until February next year *i.e.* one full year after harvest of the crop. The blastospores washed down from the infection spots colonize the soil surface. The mycelia and conidia produced are liable to desiccation with the start of summer season and become highly resistant to high temperatures.

Ahmed and Kulkarni (1968b) conducted detailed studies on the perpetuation of *T. maculans* in field conditions. They found that plant debris, rhizomes of the previously infected crop or soil from turmeric fields did not serve as primary source of inoculum. Hence they concluded that the primary source of infection should be spores or conidia which remain viable wherever they fall and initiate infection when humid conditions and cool weather prevail.

Many species of *Taphrina viz. T. tosquineti, T. belulina* and *T. borealis* were reported to survive in the form a perennial mycelium (Johanson, 1897). The spores of *T. deformans,* which are lodged on young twigs and scales, have been proved to perpetuate, overcome the summer and serve as primary source of inoculum (Pierce, 1900 and Mix 1935). Spores of *T. populina* were reported to hibernate on the outside of buds (Schneider and Sutra, 1969). *T. pruni* was reported to over winter in shoots (Holzer, 1991). *Taphrina* was shown to survive in lenticel bark of peach by using bark washing, direct or indirect impression plating techniques and scanning electron microscopy (Buck *et al.*1998). Lenticels were found to support greater fungal population than smooth (non-lenticel) bark surfaces.

2.5 TIME OF DISEASE OCCURRENCE

Upadhyay and Pavgi (1967c) studied the first incidence of *Taphrina maculans.* The primary infection that occurred on the lower most leaves as early as late July matured by late August and fresh infections continued up to November in north India. In the Deccan plateau, however, outbreak of the disease was usually during September - October and fresh infections continued up to December (Ahmed and Kulkarni, 1968b).

2.6 BIOCHEMICAL CHANGES

Agarwal *et al.* (1982) reported higher levels of phenols, O-dihydric phenols, IAA, reducing sugars and lower contents of chlorophyll ., starch, total sugars, non-reducing sugars and proteins in *Taphrina* infected leaves of turmeric than in healthy leaves. The activity of peroxidase, amylase and invertase enzymes increased with disease development while that of IAA oxidase and polyphenol oxidase decreased.

Drakina and Kelfeli (1967) observed that fungal indoles and host phenols were formed 72 hours after contact by the pathogens, namely *T. epiphylla* and *T. sadebeckii* with their hosts, *Alnus incana* and *A. glutinosa,* respectively. *T. deformans* hyphae growing in the intercellular spaces caused a partial dissolution of the leaf cell walls by secretion of polysaccharide - degrading enzymes including cellulase (Bassi *et al.* 1984).

2.7 WEATHER FACTORS

The early appearance and severity of *T. maculans* depended on the concentration of inoculum in the soil and was further enhanced by favourable weather. Upadhyay and Pavgi (1967c) reported that the moist weather with intermittent rains ideally provided the condition for the release of spore inoculum from over-summering ascostromata. The day temperature prevalent in north India during August - September (25-30° C)

accommodated the optimal temperature for ascospore discharge and subsequent growth of the pathogen. They also observed that a shift in the start of monsoon rains caused a corresponding change in the time of infection. Ahmed and Kulkarni (1968b) observed that the disease incidence increased with any decrease in mean temperature from September to December. Such increase in disease occurred when mean daily temperature was around 25° C in September and around 20° C in December. Relative humidity during this period ranged from 77-90%.

Studies of weather conditions influencing infection by other species of *Taphrina* on their respective hosts have also been reported. A delay in disease incidence was reported in *T. deformans* due to unusual weather conditions at the time of its normal occurrence (Mix, 1935). A temperature of 20-25° C was found optimum for the development of ascospores from mycelium in infected leaf tissue of cherry infected by *T. weisneri* (Iida *et al.*1977).

Based on the length of the sensitivity period and dependency on rainfall and temperature, a prediction model was developed for *T. deformans* on peach (Safran and Levy, 1995).

Once a prediction model is built linear regression analysis is conducted to assess the agreement between the prediction and observed values at each date (Timmer and Zitko, 1996).

2.8 SECONDARY SPREAD

Upadhyay and Pavgi (1966) demonstrated secondary infection under controlled conditions. The ascospores discharged from the successively maturing asci grow into eight-spored microcolonies, and infect fresh leaves without any intervening dormancy.

They observed that secondary infection was more dangerous than primary, causing profuse spotting all over the leaves.

Ahmed and Kulkarni (1968b) felt that secondary spread of the disease appeared to be caused by ascospores and conidia that were forcibly discharged by the exposed asci. Ascospore dispersal in *T. weisneri,* infecting cherry began in April, reached a maximum in mid-April and finished before late-May (Iida *et al.*1977).

2.9 MANAGEMENT OF THE DISEASE

Butler (1918) proposed that spraying with Bordeaux mixture should be effective in checking the spread of *Taphrina maculans* infection in turmeric. Zineb at 0.1% concentration controlled *T. maculans* on turmeric and was found superior to copper compounds and antibiotics (Nirwan *et al.*1974). Srivastava and Gupta (1977) found that Dithane Z 78 (0.2%) gave the best control of *T. maculans* infection in turmeric followed by Blitox-50, Bavistin and Cuman-L. *In vitro* inhibition of *T. maculans* by Aureofungin at 2.5 µg/ml was reported (Tirumalachar, *et al.*1969).

Extensive studies on fungicidal control of diseases caused by other *Taphrina* spp. have been reported. The earliest report on fungicidal control of diseases caused by *Taphrina* spp. was on *T. deformaus*. Pierce (1900) achieved 95 - 98% disease control of peach leaf curl by a single fungicidal spray. English (1958) could achieve excellent control of peach leaf curl caused by *T. deformans* with fall applications of ziram and ferbam. Ogawa *et al.* (1977) reported that ziram (2 lb/100 gallons) and copper oxychloride (4 lb/100 gallons) were very effective in controlling the peach leaf curl disease. Addition of biofilm or oil did not enhance the efficacy of fungicides. Fungicides, Orthorix (calcium polysulphide) and Microcop (tribasic copper sulphate)

were found to give complete control of *T. deformans* on peach and nectarines in California (McCain, 1983).

Copper fungicides, *viz.*, Bordeaux mixture, copper oxychloride and cupric hydroxide were found equally effective in controlling severe leaf curl (*T. deformans)* in New Zealand when applied at late dormant and bud movement stages. Captafol at 100 g a.i./100 l also gave excellent control. At 200 g a.i./ha/100 l, captafol was found superior to copper fungicides (Tate and Mespel 1985). In field trials in Argentina, Montero *et.al.* (1989) found that copper oxychloride, tribasic copper sulphate, dithianon, captan and diclone gave good control of leaf curl of nectarines caused by *T. deformans.* Sulphur and a colloidal formulation with metallic copper were found ineffective. No phytotoxic effect was observed with any of these fungicides.

In Himachal Pradesh, India, a single application of carbendazim (0.05%) at the bud swell stage or guazatine (0.15%) at pink bud stage gave the lowest incidence of peach leaf curl. Other fungicides like mancozeb, dithianon, dodine, bitertenol, myclobutanil, triforine, clotriafol, penconazole along with captan, and resorcinol were also found to reduce leaf curl infection (Thakur *et.al.*1991).

Demethylation inhibitor (DMI) fungicides, flusilazole, prochloraz, myclobutanil and propiconazole were found generally ineffective, except at high rates in reducing the leaf curl disease of nectarines. Protectant fungicides like chlorothalonil plus cupric hydroxide applied at 85% leaf fall or captafol, chlorothalonil or cupric hydroxide applied at leaf bud movement or chlorothalonil applied at blossom, reduced disease to 0-3% (Tate *et.al.*1991).

Brunelli *et al.* (1992) reported that the traditional control methods with the first treatment at the end of leaf fall using copper fungicides and the second one at the end of the winter using ziram gave the best control of peach leaf curl in Italy. Ponti *et al.* (1993) found that dodine (as Styllit Flo) at 60 -80 g a.i.ha^{-1} gave good control of peach lister (*T. deformans)* on nectarines. In the Kashmir valley, India, Mehdi *et al.* (1994) evaluated carbendazim, thiophanate methyl, mancozeb, captan, bitertenol, triadimefon and tridemorph in a field experiment for the control of *T. deformans* on peachs. Disease was reduced by 90% with captan, 87.9% with mancozeb, 73% with bitertenol and 71.6% with tridemorph. The most effective combination of chemical sprays to control leaf curl of peach in Korea Republic was lime sulphur applied in middle to late March followed by thalonil (chlorothalonil) spray, one week later (Choi *et al.*1998).

Ko *et al.* (1998) reported from Taiwan that for growth suppression of *T. deformans,* chlorothalonil was most effective (EC 50 at 0.42 ppm) followed by thiram (EC 50 at 3.16 ppm), diathianon (EC 50 at 5.21 ppm), flusilazole (EC 50 at 43.04 ppm) and prochloraz (EC 50 at 43.04 ppm). In the field trials diathianon and chlorothalonil were the most effective. Fungicide spray at 5-10 days before bud break was required for an effective control of peach leaf curl disease.

Schwabe and Williams (1999) conducted field trials in South Africa to test the efficacy of copper fungicides for the control of peach leaf curl and found that copper oxychloride (as Cupravit), copper hydroxide (as copper Nordox), copper hydroxide (as Kocide) and copper oxide (as Oleo-Nordox) were effective in controlling leaf curl. Two applications of fungicides resulted in better control than a single application. The

application dosage of 250 g l^{-1} was found too low for satisfactory leaf curl control under high pressure.

2.10 TEMPORAL AND SPATIAL SPREAD OF DISEASE AND EPIDEMIC ANALYSIS

Till-date, there is no systematic study on the temporal and spatial spread in *Taphrina maculans* infection in turmeric, or in any other related species as seen from a literature search. Therefore, important literature connected with this study as documented in other pathosystems is reviewed briefly hereunder.

2.10.1 Disease Progress Curves

As early as 1936, Fracker studied epidemics of white pine blister rust by describing disease progress curves with the logistic model. Unfortunately, his study had no impact on the development of botanical epidemiology. Barratt (1945) published an abstract that emphasized the importance of determining disease level at several times, calculating rates of increase, and comparing rates among treatments. Gaumann (1946) provided a conceptual basis for modeling disease progress by describing the epidemic or an infection chain and examining the process of an epidemic. He did not, however, provide a quantitative methodology for examining epidemics. In a landmark paper, Large (1952) clearly showed the importance of constructing disease progress curves for understanding epidemics and resulting yield loss. The period up to about 1960 can be considered the early, formative phase of epidemic analysis in which the quantitative contributions had little impact on phytopathology. Van der Plank (1960, 1963) brought this phase to a close and simultaneously initiated the quantitative phase of epidemic

analysis. Through the force of his arguments and clear description of the methodology, Van der Plank inspired many epidemiologists and set the stage for the extensive studies.

An epidemic can be defined as a change in disease (incidence or severity) in a host population over time and space (Kranz, 1974b). The fundamental way of depicting a plant disease epidemic is to plot disease level at several times or distances. The plot of disease versus time, the *disease progress curve,* summarizes the interaction of pathogen, host, and environment in disease development (Van der Plank, 1963; Kranz, 1974a, 1978). Whether an investigator is interested in understanding an epidemic process or merely wishes to compare two or more epidemics, disease progress curves must be prepared, quantified, and analyzed.

Quantification of disease progress curves, as well as other biological phenomena, is done with the use of mathematical or statistical models (Madden, 1980; Rouse, 1985). The value of a model is its "potential for bringing out relationships which are not obvious from the data alone" (Pruitt *et al.* 1979). There are many types of models that can be used to describe a disease progress curve, but typically the one chosen is *nonlinear in the parameters.* In fact, even the simplest useable model of an epidemic, the exponential, is nonlinear. It is, therefore, imperative that phytopathologists interested in describing and comparing epidemics have a solid understanding of the nonlinear model. (Madden and Campbell, 1990).

2.10.2 Models of Disease Progression

Dynamic processes are defined by their rate of change with time. If y represents disease severity or incidence, then an epidemic can be defined in terms of dy/dt, the change in y with infinitesimal change in time (t). The term dy/dt represents the *absolute*

rate of disease increase or absolute growth rate. Quantification of epidemics is done by expressing *dy/dt* as a function of *y, t,* or possibly other variables. There are several models which contain only the variable *y* and from two to four parameters. These models are very important from an historical perspective and for the widespread use some of them currently have in epidemiology and other growth-related fields. The most common of these so-called biological models have been reviewed (Sandland and McGilchrist, 1979).

It may not be possible to evaluate each disease progress curve (Madden and Campbell 1990). If valuable data on disease intensity over time are collected, investigators should always plot *y vs t* and also *dy/dt vs t*. Even when no model is assumed, these plots give valuable information on disease dynamics, which may eventually be related to host, environment, and pathogen interactions (Madden and Campbell 1990). If no model appears reasonable, then empirical measures such as area under the disease progress curve should be used for comparisons (Madden, 1983; Campbell, 1986; Waggoner, 1986; Berger, 1988; Campbell and Madden, 1990).

2.10.3 Variable Host

In many pathosystems, disease intensity increases over a relatively short period of time or when there is little change in host size (area or number). Maize dwarf mosaic of maize (Madden *et al.*1987a) and late blight of potato (Fry *et al.*1983) are two examples. In other pathosystems the host changes in number or size throughout the duration of the epidemic and treating host's tissue as a fixed quantity can be misleading. Rust of coffee (Kushalappa and Ludwig, 1982) is one example of the situation. The proportion of leaves infected can decline during some periods even though disease

level in absolute numbers (say, number of pustules) actually increases. Host size (e.g., number of leaves) increasing faster than the pathogen increase is responsible for this phenomenon. Another example is leaf spot of alfalfa, which occurs on a host that is growing rapidly throughout the epidemic, with new tissue produced continuously (Thal and Campbell, 1986). A factor, which complicates analysis of disease progression, is the chlorosis and abscission of diseased leaves. With addition of new, non-diseased tissue and the removal of some diseased tissue, disease severity (expressed as a proportion) can decline during certain periods, even though the amount of diseased tissue increases. Van der Plank (1963) corrected *r* for host growth in empirical manner in his original treatise. More recently, Kushalappa and Ludwig (1982) further considered the problem. However, neither of those methods proposed for host growth is entirely satisfactory (Campbell and Madden, 1990).

2.10.4 Quantification of Disease Progress Curve

In general, there are several arbitrary stages to the quantification of disease progress curves. The initial stage involves the calculation of absolute (and relative) rates, and the plotting of these rates, as well as *y* and transformation of *y*. The second stage involves the fitting of inflexible models to the data and an appraisal of the goodness of fit. Flexible models could then be fitted to the data when a single inflexible model is not acceptable or when none of the inflexible models fit. The third stage involves considering special features of the pathosystem that are not directly incorporated in the models. Aspects such as a growing host, variable environment (and hence variable *r),* disease aggregation, and the interaction of two or more diseases may be deemed of enough importance to consider quantifying. At this stage, disease progress

also can be assessed in terms of latent, infectious, and post-infectious disease intensity. Many investigators will limit their activity to the first two stages for two reasons. First, the mathematics are much more difficult for the latter stage. Second, very extensive data sets (both in number of times and variables [e.g., disease and host size in absolute area]) often are necessary to reasonably estimate parameters and evaluate goodness of fit. Other approaches to analyzing disease progress curves are discussed by Campbell and Madden (1990).

2.10.5 Disease Components

The models and procedures discussed so far have been based on a single disease variable y. This is most convenient for routine data analysis because most disease progress curves represent total *observed* disease at several times. A more realistic theoretical approach is to consider *latent, infectious,* and *removed* disease intensity and derive models in which the absolute rate of total disease increase is proportional to infectious (e.g., sporulating) disease level (Jeger, 1982, 1990; Berger, 1989).

2.10.6 Spatial Distribution of Disease in Fields

The models discussed in previous sections, for the most part, were developed without consideration of the spatial pattern of the organisms. The standard assumption is that the organism (= disease or pathogen) is uniformly or randomly distributed in space (Rouse, 1985). It is now well established that diseases are distributed non-randomly in fields (e.g., Campbell and Noe, 1985; Madden, 1989) and it may be of value to incorporate this information into disease progress models. Foliar diseases often spread in space from one or more foci as they increase over time. Root diseases often

occur in discrete foci due to occurrence of aggregated inoculum in soil. Models incorporating spread and temporal increase, have been discussed by Jeger (1990).

2.10.6.1 Spatial Aspects of Plant Disease Epidemics

The question may legitimately be asked whether paucity of information on spatial pattern is due to an intrinsically greater importance or to the relative ease with which temporal change in pathosystems can be studied experimentally or analysed mathematically (Jeger, 1990). For purely practical reasons there is inevitably a trade-off between temporal and spatial scales of resolution in plant disease epidemiology. It is now common practice to make sequential assessments of disease in time to characterize the pattern of epidemic progress. Recommendations can be made on the number of assessments and their frequency. One question often asked is what sort of (spatial) sampling can practicably be undertaken to enable a given frequency of (temporal) assessment. And yet, although the theory of sampling from populations in space is well advanced, it is sampling in time that commonly determines procedures for the measurement of an epidemic (Jeger, 1990 and Campbell and Madden, 1990).

2.10.6.2 Host Plant Populations

The spatial patterns of host populations are directly related to the host's status as an agricultural crop or natural population. There is a clear difference, for example, between an annual cereal crop and a relatively unmanaged forest, even where the latter may be exploited economically. Patterns of host plant populations, whether uniform, random or aggregated [alternative definitions for these terms are given by Madden (1989)] have been interpreted in terms of underlying biotic processes such as competition, interactive processes, and heterogeneity of the environment (Gill, 1975).

2.10.6.3 Pathogen Populations

A considerable effort has been directed towards analysis of the spatial patterns of soilborne pathogens at a single time (Gilligan, 1988). By contrast with airborne pathogens causing foliar diseases, the relatively static nature of soil-borne populations makes a single-time analysis biologically relevant and computationally simple. Most work has been done with nematodes or fungal propagules, either resting spores or sclerotia. Selected formulae for techniques used in spatial analysis and applicable also to the examples relating to disease patterns are given in Table 1.

Most disease assessments are host-based in that the host plant or plant organ is the unit of assessment. Thus, the spatial patterns of disease depend in part on the spatial patterns of the host plants. In agricultural crops, especially those planted in rows or as lattices, these patterns should not be ignored; these types of planting offer distinct advantages in that plant-by-plant assessment is possible. For certain techniques of analysis, the regular geometry of planting means that location cannot be isolated from the assessment of disease at that location and thus use of frequency distribution techniques may mean a loss of information in examining spatial pattern (Nicot *et al.*1984).

As noted with the nematode examples, techniques other than the use of frequency distributions can be used to examine spatial pattern. Indices such as Morisita's indices of dispersion and clumping (Table 1) give information not only on the degree of departure from randomness but also estimate the size of the aggregated units, based on using quadrat techniques. Mihail and Alcorn (1987) used the indices to determine the level of aggregation in spatial pattern of *Macrophomina phaseolina* in Arizona fields.

Table 1: Examples of analyses of spatial patterns of disease (plants or plant parts) using a range of techniques (Jeger, 1990)

Authors/pathogen	Disease	Assessment	Technique
Boivin and Sauriol *(1984)/ Botrytis squamosa*	Leaf blight (onion)	Lesions/designated leaf	Mean crowding, Iwao's patchiness (regression)
Campbell and Pennypacker *(1980)/Rhizoctonia solani*	Hypocotyl rot (common bean)	Number of infected plants/quadrat, number of lesions/quadrat	Variance-to-mean ratio, frequency distribution
Dhanvantari and Dirks *(1987)/Erwinia carotovora* ssp. *carotovora*	Stem rot (tomato)	Disease status (+/-) (plant-by- plant)	Doublet analysis, run (chain) analysis
Gitaitis *et al.(1978) /Pseudomonas alboprecipitans*	Bacterial leaf blight (sweet corn)	Disease status (+/ -) (plant-by- plant)	Doublet analysis
Madden *et al.*(1982)/virus	Maize dwarf mosaic	Disease status (+/-) (plant-by- plant)	Doublet analysis, runs analysis
Marois and Adams *(1985)/ Sclerotinia minor*	Lettuce drop	Diseased plants/quadrat	Frequency distributions, Lloyds index of mean patchiness
Martin *et al.(1983)/ Rhizoctonia solani*	Brown patch (turf grass)	Foliar blight severity/quadrat	Variance-to-mean ratio, frequency distributions
Schuh *et al.(1986)/ Peronosclerospora sorghi*	Sorghum downy mildew	Disease incidence/ segment (Im)	Morisita's indices of dispersion (clump size)
Shaw and Royle *(1987)/ Septoria nodorum*	Glume and leaf blotch of wheat	Lesion numbers/designated leaf	Frequency distributions, spatial autocorrelation
Shew *et al.(1984)/Sclerotium rolfsii*	Southern stem rot	Lesions/quadrat	Frequency distribution, spatial autocorrelation
Thal and Campbell *(1986)/ Leptosphaerulina briosiana*	Alfalfa leaf spot	Quadrat disease severity	Frequency distribution, variance to-mean ratio, Morisita's index of dispersion

Spatial autocorrelation analysis, a technique, which takes into account location of sampling quadrats, confirmed that inoculum density was similar in quadrats of close proximity. This information was used to assess sampling strategies to determine population density. Schuh *et al.*(1986) used Morisita's indices to look at spatial patterns

of oospores of *Peronosclerospora sorghi,* causing downy mildew of sorghum, and estimated characteristic sizes of clumps at different hierarchical levels. Subsequently (Schuh *et al.*1988) these patterns and clump sizes were related to spatial patterns observed for the diseased plants. This relationship between spatial pattern of inoculum and that of disease is a critical but relatively little studied aspect of epidemiology, often only described in qualitative terms [e.g. Bloomberg (1985) for forest nursery diseases].

2.10.6.4 Spatial Component Estimation of Plant Disease Epidemics

Epidemiologists have now come to agree that spatial component of plant disease epidemics is as important as the temporal component (Campbell and Noe, 1985 and Madden, 1988). Many have attempted to make spatial and temporal analysis in soil-borne diseases. The most frequently used method for assessing the aggregation of fungal pathogens and soil-borne inoculum is the point pattern analysis, which entails determining the mean, variance and some times the frequency distribution of diseased plants (Campbell and Noe, 1985). The other methods used for the analysis of spatial pattern *viz.*, nearest neighbour, Greig Smith's method, spatial autocorrelation analysis, and two dimensional spectral analysis that take into account the location of each sample or quadrat (Nicot *et al.*1984).

Autocorrelation test statistics like the "I statistic" (coefficient of spatial autocorrelation) were employed to measure aggregation (Nicot *et al.*1984).

Campbell and Noe (1985) described four major approaches to the characterization of spatial pattern based on quadrat count data

i) The population may be mapped for easy visual interpretation of data

ii) Using goodness of fit techniques, discrete probability distributions may be fit to the data and conclusions drawn concerning the randomness or non-randomness of spatial pattern

iii) The degree of pattern departure from randomness may be measured by several indices of dispersion such as variance to mean ratio or Morisita's index of dispersion; and

iv) Techniques such as spectral analysis, lag analysis, and auto-correlation analysis may be used to more carefully characterize spatial patterns, by using the location of each quadrat.

Gitaitis *et al.* (1978) demonstrated a nonrandom aggregation of bacterial blight infected corn plants using doublet analysis. Madden *et al.* (1982) evaluated tests for randomness of maize dwarf mosaic virus infected plants. The ordinary run analysis, original doublet analysis and corrected doublet analysis were evaluated and found that ordinary runs was the best for determining the randomness of infected plants.

Studies of the propagule pattern of several soil borne fungi revealed non-random patterns (Adams, 1981; Taylor *et al.*1981 and Stanghellini *et al.*1982). Mihail and Alcorn (1987) determined the spatial pattern of the microsclerotia of *Macrophomina phaseolina* using mean to variance ratio and Morisita's index of dispersion. They found the microsclerotial pattern in soil to be aggregated. Based on Moran's I, a statistic of spatial autocorrelation they also found significant positive autocorrelation among quadrats in all the blocks which indicated that similar levels of inoculum density tended to occur in quadrates of close proximity.

In a study of the analysis of epidemics for spatial pattern of virus diseased tobacco plants, Madden *et al.*(1987b) determined the aggregation of virus diseased plants by dividing fields into contiguous quadrats and using point pattern (variance to mean ratio and Lloyd's patchiness) and spatial auto correlation analyses. Spatial distribution of diseased plants was neither solely clustered nor random but changed with time during the epidemics. All indices of aggregation indicated a random pattern at the beginning of the epidemics. Patchiness increased to a maximum and then declined throughout the reminder of the epidemics. First order autocorrelations increased throughout most epidemics eventually indicating clustering. Autocorrelations declined at the end of the epidemics when mean disease intensity approached 100% incidence. Ordinary runs analysis also indicated that percentage of rows of clustered pattern increased during most of the epidemics.

Schuh *et al.*(1988) found Morisita's index of dispersion to be similar for both oospores of *Peronosclerospora sorghi* and infected sorghum plants and suggested the use of susceptible plants to assess spatial patterns of oospores of the fungus to assess spatial patterns instead of direct sampling by soil cores. Thal and Campbell (1986) analyzed the spatial pattern of alfalfa leaf spot by using variance to mean ratio, Morisita's index and Taylor's b and found that alfalfa leaf spots occur randomly or with slight regularity or with slight aggregation when a single quadrat is the measured unit. When individual stems in a quadrat were analyzed the disease is generally aggregated. Aggregation is not unexpected since alfalfa is a genetically heterogeneous crop and plants within a cultivar may vary in level of resistance. Secondly, the spores of *Leptosphaerulina briosiana* are relatively large and are covered with a mucilaginous

matrix for adhesion to surfaces. These spore characters may contribute to a greater degree of autoinfection than alloinfection and thus contribute to aggregation, at the plant level. The lack of aggregation at quadrat level may be due to the averaging effect of the plant-to-plant aggregation and the spread of inoculum over time. The degree of aggregation appeared to increase as leaf spot severity increased in the field.

MATERIALS AND METHODS

CHAPTER III

MATERIALS AND METHODS

The materials used and the methods followed in this investigation are described under the following three major categories of experiments: Laboratory experiments, Pot culture experiments and Field experiments. All these experiments were carried out during Kharif or wet seasons during 1998-99; 1999-2000 and 2000-2001 at the Agricultural Research Station, Kovvur, West Godavari district, Andhra Pradesh.

The Agricultural Research Station, Kovvur, the site of investigation, is located at 17° 00' N latitude; 81° 43' E longitude and 15.66 m above mean sea level altitude. This site receives an annual rainfall of 110 cm from South-West monsoon (June to September), North-East monsoon (October to November) and summer showers. The alluvial soil at the experiment site is endowed with good drainage with pH ranging from 7.5 - 7.9 and EC 0.34 dSm^{-1}. Besides rainfall, irrigation is made with ground water tapped through filter points. The pH of irrigation water is 7.2 and EC is 1.66 dSm^{-1}.

3.1 LABORATORY EXPERIMENTS

3.1.1 Glassware

The glassware used in the present investigation is of Borosil and Vensil make.

3.1.2 Cleaning and Sterilization of Glassware

The glassware was thoroughly washed with washing soda and rinsed with tap water. The cleaned glassware either was filled with cleaning solution (sulphuric acid, potassium dichromate and distilled water) or was immersed in cleaning solution

depending on its size and kept over night and finally cleaned with tap water and rinsed with distilled water. The cleaned glassware was sterilized at 160° C for 1 hour in a hot air oven.

3.1.3 Chemicals and Water

Analytical grade chemicals manufactured by M/s. Himedia and M/s. Qualigens were used. Glass distilled water was used in all the laboratory experiments.

3.1.4 Media

For isolation of the fungus, *Taphrina maculans* Butler and for studying its growth requirements, the following culture media were used:

Potato Dextrose Agar (PDA)

Peeled potatoes	200 g
Dextrose	20 g
Agar	20 g
Distilled water	1000 ml

Nutrient Agar (NA)

Peptone	10 g
Beef extract	3 g
Agar	20 g
Distilled water	1000 ml

Oat Meal Agar (OMA)

Oats	30 g
Agar	20 g
Distilled water	1000 ml

Turmeric Leaf Extract Agar

Mature leaves of susceptible turmeric cultivar CLI 317	200 g
Agar	20 g
Distilled water	1000 ml

To the media used for isolation of the pathogen, drops of lactic acid and 0.1 N NaOH were added to adjust the pH depending on the need.

3.1.4.1 Sterilization of Media

The nutrient media were sterilized by autoclaving at 121.6° C and 15 PSI for 15 minutes.

3.1.5 Isolation of the Pathogen

3.1.5.1 Isolation from Freshly Infected Leaves

Three methods as described below were followed for isolation of the pathogen from infected leaves.

i) Fresh leaf bits with initial, middle and final stages of spot development were collected, surface sterilized with 0.1% mercuric chloride for 30 seconds, washed in sterilized distilled water and separately placed directly on the culture media (pH 4 to 8.0) PDA, OMA, NA and turmeric leaf extract agar under aseptic conditions. The Petri plates were incubated at 20° C and observed for the appearance of the colonies of the pathogen for ten days.

ii) Fresh leaf bits with initial, middle and advanced stages of spot development were surface sterilized, washed in sterilized distilled water and separately suspended from the inside of the lid of Petri dishes (Martin, 1925) containing culture media with pH ranging

between 4.0 to 8.0.The Petri plates were incubated at 20° C and observed for the appearance of the colonies of the pathogen for ten days (Plate 1).

iii) Very thin hand sections of the fresh leaf bits with initial and middle stages of spot development were cut after surface sterilization of the leaf bits. The thin hand sections were placed one each in the cavity of sterilized cavity slides. The cavities were filled with acidified (pH 4.5) sterile water and the slides were placed in moist chambers and incubated at 20°C and observed daily for the production of ascospores and blastospores. When the spores were produced, a few drops of the spore suspension were poured on to culture media and incubated at 20° C (Ahmed and Kulkarni, 1968a). The Petri plates were observed for appearance of the colonies of the pathogen for ten days.

3.1.5.2 Isolation from Mature Spots on Infected Leaves and from Preserved (at Room Temperature) Infected Leaves Collected in the Previous Year

Infected and dried leaves collected from the field were cut into small bits of 1 to 2 cm^2. The leaf bits were surface sterilized using 0.1% mercuric chloride, washed in sterile water and suspended from the inside of the lid of a Petri plate containing culture media with pH ranging from 4 to 8.0. The Petri plates were incubated at 20° C and observed for ten days.

Isolation from leaf pieces from dried infected leaves collected from the previous year's crop were pretreated (Pavgi and Upadhyay, 1964), by soaking in water for one hour and frozen for 48 hours. These frozen leaf pieces were later incubated in water for 24 hours at room temperature 32 to 36° C. The leaf bits were then surface sterilized using 0.1% mercuric chloride and washed with sterile distilled water and were stuck to the inside of the lid of Petri plates containing culture media with pH ranging

between 4 and 8. The Petri plates were incubated at 20°C and observed for colonies of the pathogen for ten days.

3.1.5.3 Isolation from Seed Rhizomes

Seed rhizomes from seed lots were randomly collected and washed in acidified (pH 4.5) sterile water. The washings were then plated on culture media with pH ranging between 4 and 8.0. The Petri plates were incubated at 20° C and observed for the colonies of the pathogen for ten days.

3.1.5.4 Isolation from Soil Samples in Infected Fields

Soil samples up to a depth of 5 cm were collected from several fields where infection on crop was observed in the previous year. One gram of soil from each sample was mixed in 100 ml of sterilized water and further dilution was made up to 10^4 ml^{-1}. The diluted soil suspensions were plated on to culture media with pH ranging between 4 and 8.0. The Petri plates were incubated at 20° C and observed for the colonies of the pathogen for ten days.

3.1.5.5 Identification of the Pathogen

The pathogen isolated on culture media was identified with the help of description made by Pavgi and Upadhyay (1964) and Kulkarni and Ahmed (1968).

3.1.5.6 Cultural Characteristics and Spore Measurements

The cultural characteristics of the fungus were studied by growing the fungus on culture media. Blastospore (conidial) measurements were made using a microscope with stage and ocular micrometers. Fifty conidia were measured and the mean was taken for statistical analysis. The identity of the fungus was confirmed by following the equation of John (1970):

$\mu = \overline{\chi} \pm t\ 0.05\ SE$

$SE = \sigma / \sqrt{\eta}$

Where

σ = Standard deviation

μ = Population mean

$\overline{\chi}$ = Sample mean

η = Number of spores observed

t = Table t value (P=0.05)

SE = Standard error.

Ascospores and blastospores liberated on to the microscope slides from infected spots on leaves were also measured following this method.

3.1.5.7 Effect of Temperature and pH on Growth of *T. maculans* on Culture Media

Potato dextrose agar (PDA) with differentially adjusted pH 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0 and 8.0 was poured into sterilized Petri plates. The pH of the medium was adjusted by adding lactic acid for the acidic range and N/10 NaOH for the alkaline range. A 3 mm disc of the fungus from a ten-days old culture was inoculated on the medium in each Petri plate. The inoculated plates were incubated at four-temperature regimes viz., 10, 15, 20 and 25° C using separate incubators. A set of 3 plates for each level of pH was maintained at each temperature regime. Observations on radial growth of the colony were recorded on 10 and 20 days after plating. Radial growth was measured on two axes of the colony perpendicular to each other and the mean was calculated.

3.1.6 Survival Period in Infected Leaves and Rhizomes

From infected leaves collected in previous year's crop during March isolations were attempted at monthly intervals from April to December using PDA as per the method described by Pavgi and Upadhayay (1964).

3.1.7 Determination of Latent and Infectious Periods

Leaves with very initial spots that have just completed the incubation period as indicated by the very pale yellow colour of spots were collected from six turmeric cultivars viz., CLI 317, KTS 1, KTS 4, PCT 13, CLI 315 and CLI 385. Leaf bits showing such spots were cut (1 cm^2) with healthy leaf area around them. The cut leaf bits were disinfected with 0.1% mercuric chloride for 30 seconds and were washed with acidified sterile water (pH 4.5) for three times. The leaf bits were stuck to the inside of the lid of Petri dish. The bottom plate of the Petri dish was lined with sterilized moist blotter papers over which a sterilized microscope slide was kept for collecting spores, if any, discharged from the spots. The slides were observed under a microscope daily for the presence of distinctive colonies of ascospores and/or conidia. Infectious period was determined by the number of days from the first to the last date of observing distinct colonies of ascospores and/or blastospores.

3.1.8 Estimation of Curcumin Content in Rhizomes

Curcumin content in the rhizomes of turmeric cultivars was estimated by following the method of Manjunath *et al.* (1991).

Fresh turmeric rhizomes were cooked in water @ one litre water for 1 kg of rhizomes. Sodium bicarbonate at 10 g l^{-1} water was added to the cooking medium and cooking was continued till no water remained in the container. The cooked rhizomes

were sun dried and slightly polished mechanically by rotating in a metallic vessel to remove adhering particles.

The cured rhizomes were powdered in a mixer and sieved through 60 microns sieve. Finely ground turmeric powder sample weighing 0.2 g was extracted by refluxing over a water-cooled condenser with 40 ml of distilled alcohol for 2 hours and 30 minutes. The extract was transferred to a volumetric flask (100 ml) and made to100 ml by adding alcohol. This extract was filtered and an aliquot of 5 ml was transferred to another volumetric flask and once again made up to 100 ml by adding alcohol. This diluted extract was mixed well and its absorbance was measured at 425 nm against alcohol blank with the help of Spectronic 20. Using the absorbance value of a standard solution of curcumin (0.00025g/100ml gives an absorbance of 0.42) the curcumin percentage was calculated with the following formula.

$$\text{Curcumin content (per cent by weight)} = \frac{0.00025 \times \text{absorbance of sample} \times 100 \times 100}{0.42 \times \text{wt. of sample} \times 5}$$

The pH of the alcohol used for the extraction was maintained below 6.5.

3.1.9 SDS – PAGE Analysis of Proteins of Isolates of *Taphrina maculans*

The infected turmeric leaf samples from three turmeric-growing tracts in Andhra Pradesh viz., coastal area (Kovvur), Telangana (Jagtial) and Rayalseema (Anantarajupet) were collected. The pathogen was isolated from the leaf samples of the three tracts on PDA and the three isolates were maintained on PDA slants. The soluble protein profiles of the three isolates of *T. maculans* were made on SDS-PAGE (Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis) by the method of Laemmli (1970).

Stock Solutions

1. Acrylamide solution

   Acrylamide - 29 g and

   N'- N'- Methylene bis acrylamide - 1 g in 100 ml distilled water.

2. *Tris*-glycine electrode buffer pH 8.8

   Tris - 15.15 g

   Glycine - 72 g

   Sodium dodecyl sulphate (SDS) - 5 g was dissolved in distilled water and made up to a volume of 5000 ml.

3. *Tris*-chloride buffer pH 8.8

   Tris - 18.1g was dissolved in 90 ml of distilled water and pH adjusted to 8.8 with hydrochloric acid (HCl) and made up to 100 ml.

4. *Tris*-chloride buffer pH 6.8

   Tris 12.1 g was dissolved in 90 ml of distilled water and pH was adjusted to 6.8 with hydrochloric acid (HCl) and made up to 100 ml.

5. 25% Sodium dodecyl sulphate (SDS).

6. Sample loading buffer

   Tris chloride buffer pH 6.8 - 10 ml

   Glycerol -10 ml

   SDS - 2 ml

   2 - Mercaptoethonol - 5 ml

   1% Bromophenol blue - 0.1 ml

   Volume of the buffer was made up to 50 ml with distilled water.

7. Ammonium persulphate - 0.1 g ml^{-1}

8. Staining solution

Coomassie brilliant blue R 250	- 0.1 g
Methonol	- 40 ml
Acetic acid	- 10 ml
Distilled water	- 50 ml

9. Destaining solution

Methonol	- 50 ml
Glacial acetic acid	- 75 ml
Distilled water	- 875 ml

The following gel solutions were prepared with the stock solutions.

1. Resolving gel solution (12 %)

Polyacrylamide solution	- 10 ml
Tris-chloride buffer pH 8.8	- 6.3 ml
Distilled water	- 8.2 ml
2% SDS	- 250 µl
Ammonium persulphate	- 250 µl
TEMED	- 15 µl

2. Stacking gel solution (3%)

Acrylamide solution	- 1.3 ml
Tris chloride buffer pH 6.8	- 1 ml
Distilled water	- 5.5 ml

2% SDS	- 90 µ l
Ammonium persulphate	- 90 µ l
TEMED	- 10 µ l

Protein Extraction

The three isolates were grown on PDA fortified with L-aspargine for ten days at 20° C. Five hundred milligrams of fungal material from ten day old cultures was homogenized with 1 ml of phosphate buffer (0.1 M pH 7.0) and the homogenate was centrifuged at 13000 rpm for ten minutes. The supernatant was used as protein source after adjusting the protein concentration 100 µg /40 µ l (Lowry *et al.* 1951).

Preparation of Sample for Loading on the Gel

The protein extract and sample loading buffer were mixed in 1:1 ratio and kept in a boiling water bath for three minutes and kept for cooling.

Preparation of Gel

Clean and dry glass plates were assembled properly. Resolving gel solution was degassed and ammonium persulphate solution was added and mixed gently. The gel solution was poured between the glass plates leaving space for stacking gel. A layer of distilled water was added on the top of the gel.

After the gel was polymerized, water layer was removed and degassed stacking gel solution was poured and comb was inserted. After polymerization of the stacking gel, the comb was removed and the wells were washed with water.

Loading on the Gel

The cooled prepared protein samples (40 µl each) were loaded in the wells on the gel. The protein marker of a medium range molecular weight was also loaded on the

gel after treating it similarly as the sample. Protein molecular weight markers (range 3 to 43 kda) were obtained from Genei, Bangalore. One well was loaded with bromophenol blue.

Electrophoretic Run

The glass plates unit with the gel was placed in the electrophoretic unit. The upper and lower tanks of the units were filled with electrode buffer and electrophoresis was carried out at 1 mAmp $well^{-1}$ for 12 hours.

After the electrophoretic run was completed the gel was carefully removed and kept in staining solution for 6 hours. After proper staining the gel was transferred to destaining solution and kept in it till the gel background was clear.

3.1.10 Isoenzyme Analysis of Susceptible and Resistant Turmeric Cultivars to Leaf Blotch

Isoenzyme analysis of leaf blotch susceptible and leaf spot susceptible turmeric cultivars was carried out by native polycarylamide gel electrophoresis in anionic system (Davis, 1964).

Stock Solutions

1. Acrylamide stock solution

Acrylamide	- 29 g
N' N' - methylene *Bis*-acrylamide	- 1 g

Dissolved in distilled water and made up to 100 ml and stored in amber coloured bottle.

2. *Tris* - glycine electrode buffer pH 8.3

Tris	- 6 g
Glycine	- 28.8 g

Distilled water - 1000 ml

The electrode buffer stock was diluted in 1:9 ratio with distilled water for use.

3. *Tris*-chloride buffer pH 8.8

HCl 1 N - 48 ml

Tris - 36.6 g

Distilled water - 100 ml

pH was adjusted to 8.8

4. *Tris*-chloride buffer pH 6.8

HCl 1 N - 48 ml

Tris - 5.98 g

Distilled water - 100 ml

pH was adjusted to 6.8

5. Ammonium persulphate - 0.1 g ml^{-1} prepared fresh.

6. Bromophenol solution

Bromophenol blue - 25 mg made up to 10 ml with *tris*-chloride buffer pH 6.8.

7. Extraction buffer

Tris-HCl buffer 0.1 M pH 8.0 containing 0.1% each of ascorbic acid, L cysteine hydrochloride and 17% sucrose.

The following gel solutions were prepared with the stock solutions:

1. Resolving gel solution (8%):

Tris-chloride, pH 8.8 - 5 ml

Acrylamide - 5.3 ml

Distilled water - 9.3 ml

Ammonium persulphate - 150 µ l

TEMED - 10 µ l

2. Stacking gel solution:

Tris-chloride, pH 6.8 - 1.0 ml

Acrylamide - 1.3 ml

Distilled water - 5.5 ml

Ammonium persulphate - 150 µ l

TEMED - 10 µ l

Extraction of the Enzymes from Leaf Tissue

One gram of leaf material from the third leaf after sprouting before it unfurled (Indian Institute of Spices Research, Annual Report, 1995-96) was homogenized with 5 ml of extraction buffer and filtered through four layers of muslin cloth. The filtrate was centrifuged at 18000 rpm for 15 minutes at 4° C. The supernatant was used as enzyme source after adjusting the protein concentration at 50 µ g/ 40µl (Lowry *et al.*, 1951).

Preparation of gel and electrophoretic run were done as described for SDS-PAGE analysis of protein from isolates of *T. maculans,* except that the electrophoretic run was done at 4° C in a refrigerator.

Electrophoretic run was completed with two gels and the gels were carefully removed. One gel was stained for esterase activity and the other for polyphenol oxidase activity. For esterase activity the gel was stained at room temperature using 1.3 mM α-napththyl acetate, 0.17 mM acetone and 2.79 mM fast blue RR salt in 0.2M phosphate buffer pH 6.0 (Wetter and Dyck, 1983). For polyphenol oxidase activity, the gel was stained in 0.1% *p*-phenylenediamine in 0.1 M potassium phosphate buffer (pH 7.0) for

30 minutes followed by 10 mM catechol in the same buffer (Sadasivam and Manikam, 1992). After proper staining, the gels were washed in distilled water.

3.2 POT CULTURE EXPERIMENTS

Pot culture experiments were conducted in cement pots of 60 cm diameter and 50 cm height. The pots were filled with soil and the soil was sterilized with formalin (4%) @ 100 ml m^{-3}. To prevent external contamination to the pot culture experiments, four bamboo posts of 2.5 m tall were erected on all four sides close to each cement pot. A polythene sheet of 500 gauge was firmly skirted around the four bamboo posts from the brim of the pots to the top of the bamboo posts. In the centre of the cement pot another bamboo post was erected which stood taller than the outside posts. A polythene top cover was placed over the middle bamboo so that it over-lapped the polythene sheet wall all around (Plate 1). This arrangement was made to facilitate escape of water vapour and to prevent contamination from outside. The pots were irrigated carefully and ensuring no outside contamination took place.

3.2.1 Pathogenicity Test

Two sets of turmeric plants of susceptible cultivar, CLI-317 raised from disinfected seed rhizomes and maintained in isolation in cement pots were inoculated, one set with a conidial suspension (6 x 10^4 conidia ml^{-1}) obtained by incubating cross sections of leaf with initial stage of infection in acidified water and the other set with conidial suspension (7 x 10^4 conidia ml^{-1}) obtained from pure culture of *Taphrina maculans*. Inoculation was done by sprinkling the spore suspension on the lower leaves of four months old turmeric plants.

Plate 1. Arrangement used to ensure isolation of test pot culture from external contaminants

3.2.2 Determination of Incubation Period

Seed rhizomes of turmeric cultivars CLI 317, KTS 1, KTS 4, PCT 13, CLI 315 and CLI 385 were disinfected with 0.1% mercuric chloride for 2 minutes and then washed four times in sterile water. In each cement pot, four disinfected seed rhizomes were sown in the first week of August, 1998 and July, 1999.

For all sowings, three pots for each turmeric line were maintained. The bottom five leaves of each plant of the six turmeric lines in pots were inoculated by sprinkling the conidial suspension harvested from thin hand sections of leaf with initial and middle stage of spot development (Ahmed and Kulkarni, 1968a)that were placed in acidified water in cavity slides for 5 to 7 days. In 1998-99 inoculation was done with spore suspension having 6 x 10^4 conidia ml^{-1} on 6-12-1998, while in 1999-2000 with a suspension having 6 x 10^4 conidia ml^{-1} on 5-10-1999. An additional set of three pots sown with disinfected seed rhizomes of CLI 317 turmeric line was also maintained. The plants in this set of pots were inoculated on 10-1-1999 with conidia (7 x 10^4 conidia ml^{-1} harvested from pure culture of *T. maculans* grown on PDA.

Regular observations were made for the appearance of the first perceptible symptoms i.e. small speck like white to pale yellow spots. The dates on which the first perceptible symptom appeared in each pot on all turmeric lines were recorded. The number of days from the date of inoculation to the date on which the first perceptible symptom appeared was counted as the incubation period.

3.2.3. Primary Source of Inoculum

Disinfected seed rhizomes were planted @ four per cement pot on 4-8-1998 and the sowings were repeated on 6-7-1999. The following possible sources of inoculum were evaluated:

i) infected leaf debris collected from fields where infection occurred during the previous year was placed on the surface of soil in pots.

ii) surface soil collected from the field where the pervious year's crop was infected was mixed with the surface soil in pots

iii) untreated seed rhizomes were planted in place of disinfected seed rhizomes

Pots sown with disinfected rhizomes served as check. For all treatments four replications were maintained. The plants in the pots were regularly observed for the appearance of infection.

3.2.3.1. Search for Other Hosts

The entire weed flora in the field and other plants in the vicinity of turmeric fields were carefully observed for the presence of symptoms similar to that caused by *T. maculans* on turmeric. Observations of spores in all suspected spots on other hosts were made using a research microscope. Isolations from the suspected *Taphrina* infections on other hosts were also attempted on PDA.

3.3 FIELD EXPERIMENTS

3.3.1 Disease Assessment

For assessment of diseases severity the whole infected plants were scored on a scale of 1-9 (Rao and Rao, 1987). The different ratings in the scale proportional to the severity of disease on the plant are as follows:

Rating	Extent of disease infection on plant
1	No infection (no spot)
2	2 - 3 small spots
3	5 - 10 spots
4	10 - 15 spots
5	50% leaf area covered with spots
6	60% leaf area covered with spots
7	75% leaf area covered with spots
8	90% leaf area covered with spots
9	Plants dried up completely due to severe infection.

Percent disease index (PDI) was calculated as per the following formula (Wheeler, 1969).

$$PDI = \frac{\text{Numerical scores of all plants}}{\text{Number of plants scored x Maximum score}} \times 100$$

3.3.2 Seed Material

Seed material of the turmeric cultivars available at Agricultural Research Station, Kovvur was used in all the experiments. Healthy rhizomes with sound buds were selected for experimental purpose. One of the widely grown susceptible cultivar CLI 317 and two other susceptible selections of Agricultural Research Station, Kovvur viz. KTS 1 and KTS 4 were employed in most of the field studies. Other susceptible cultivars, PCT 13, CLI 315 and CLI 385 were also used.

3.3.3. Spacing and Plant Population

The rhizomes were planted adopting a spacing of 45 cm between rows and 22.5 cm within the rows, maintaining a plant population of 98,765 plants ha^{-1}

3.3.4 Fertilizers

Nitrogen, phosphorous and potassium were applied @ 150 : 60 : 75 kg/ha in the form of urea, single super phosphate and muriate of potash respectively. The entire quantity of phosphorous was applied in one single basal dose. Nitrogen and potassium were applied at 40, 80 and 120 days after planting in three equal splits by pocketing.

3.3.5 Irrigation

A total of 16 to 18 irrigations were given by flooding method at approximately 10-days interval.

3.3.6 Cultural Operations

As a pre-emergence weedicide, Butachlor was applied @ 4 l/ha by mixing in sand along with the first irrigation after planting rhizomes. Additionally, two hand weedings at 40 and 80 days after planting were done to check the weeds.

3.3.7 Monitoring Air-borne Spores of *T. maculans*

Wind vane spore traps were locally fabricated. Two spore traps were installed in an area of 500 m^2. Microscope slides were coated on one side with a mixture of three parts of vaseline and one part of wax. On the other side of the slide markings were drawn dividing the slide area into four equal parts, to facilitate easy counting of spores. The slides were placed in the spore traps in the slit provided for them with the vaseline - wax coated side facing the outer side. The height of the spore trap was adjusted to 30 cm above the crop canopy throughout the crop growth period from August to February. Every day, the slides were removed and a fresh set of slides was placed both in the morning and in the evening. The slides were scanned for the presence of ascospores or conidia of *T. maculans* under a microscope.

3.3.8 Weather and the Disease

Weather parameters viz., rainfall (Rf), number of rainy days (Rd), maximum temperature (max), minimum temperature (min), relative humidity at 0800 hours (RH08) and relative humidity at 1400 hours (RH14), were recorded.

For estimating the per cent disease index, regular and periodic observations of disease ratings were recorded on a blotch susceptible turmeric cultivar, namely CLI 317. The first observation was recorded at 75% germination of crop, i.e., approximately 30 days after planting. The total number of observations to be made for calculating disease severity and the interval between two observations were determined by using the equation: df = n-k-1

where, df = degrees of freedom, n = number of observations to be made, and k = number of weather parameters studied. After estimation on the number of observations needed, the interval between observations was calculated by dividing crop duration by the number of estimated observations.

In the present study, the crop duration of the test cultivars was 8 months, i.e., 240 days of which 30 days were needed for crop sprouting. The number of weather parameters (k) was six, and the degrees of freedom were taken as a statistically accepted 14. Therefore as per the above equation, 14 = n-6-1, or n = 21.

The interval of observation $= \frac{210}{21} = 10$ days.

Accordingly observations were recorded on disease ratings at ten day interval, starting from 3-8-1998 in 1998-99, and from 26-7-1999 in 1999-2000, and PDI were calculated for each of the observations.

Regression analysis was performed as per the methods described by Snedecor and Cochran, (1967).

3.3.9 Reaction of Turmeric Cultivars to Natural Infection of *T. maculans*

Sixty-seven turmeric cultivars were planted in plots measuring 3.15 x 0.9 m in 1999-2000 and 2000-2001. The cultivars were observed for natural incidence of leaf blotch disease. Disease ratings were recorded at 180 days after planting and PDI was calculated for each cultivar.

3.3.10 Dates of Planting and Disease Severity

Taphrina maculans leaf blotch susceptible cultivar, CLI 317 was planted at fortnightly interval beginning from the first fortnight of May, in 1999 and 2000 in plots measuring 3.15 x 1.8 m. The experiment was replicated four times. The observations were recorded on disease ratings and PDI was calculated. The yield of rhizomes was recorded for each date of planting.

3.3.11 Plant Density and Disease Severity

T. maculans leaf blotch susceptible turmeric cultivar CLI 317 was planted at three wider spacings, besides the spacing normally adopted by farmers, in plots measuring 3.15 x 1.8 m.

The spacing followed and the plant populations were,

Spacing	Plant population/ha
1. 45 x 45 cm	49,383
2. 90 x 45 cm	24,691
3. 90 x 22.5 cm	49,383
4. 45 x 22.5 cm (check)	98,675

The experiment was conducted in a randomized block design with five replications, during 1999-2000 and 2000-2001. Observations on disease severity and rhizome yield were recorded.

3.3.12. Non-host Barrier Crops and Disease Severity

The experiment was laid out in a field where no turmeric crop was grown in the previous seasons to ensure the field to be free from any infected leaf debris. Leaf blotch susceptible turmeric cultivar, CLI 317 was planted in plots of 3.6 x 3.6 m. The non-host barrier crop was planted around the susceptible turmeric cultivar in a strip of 1.2 m on all the four sides. The non-host barrier crop treatments were:

1. Elephant foot yam (*Amorphophallus*) variety : Gajendra (Plate 3).
2. Taro (*Colocasia*) vareity KCS 2 (Plate 4).
3. Leaf blotch resistant turmeric cultivar KTS 8 (Plate 5).

Plots without any non-host barrier crop (Plate 2) served as control. The experiment was laid out in a randomized block design with seven replications in 1998-99 and 1999-2000.

3.3.13 Disease Management with Fungicides

A field experiment was conducted during 1998-99 and 1999-2000 to test the efficacy of fungicides. Leaf blotch susceptible turmeric cultivar CLI 317 was planted in plots measuring 3.6 x 3.6 m in a randomized block design with four replications. The fungicide treatments were:

1. Bitertenol (Baycor, Bayer India Ltd.) 0.1%
2. Chlorothalonil (Kavach, Novartis India Ltd.) 0.2%
3. Propiconazole (Tilt, Novartis India Ltd.) 0.1%

Plate 2. Leaf blotch susceptible turmeric cultivar planted without any cover of barrier crop

Plate 3. Leaf blotch susceptible turmeric cultivar planted with a cover provided by a barrier crop of *Amorphophallus*

Plate 4. Leaf blotch susceptible turmeric cultivar planted with cover provided by a barrier crop of *Colocasia*

Plate 5. Leaf blotch susceptible turmeric cultivar planted with a cover provided by barrier crop of leaf blotch resistant turmeric cultivar

4. Kitazin (Kitazin, Pesticides India Ltd.) 0.2%

5. Tridemorph (Calixin, BASF India Ltd.) 0.1%

6. Copper oxchloride (Blitox, Rallis India, Ltd.) 0.25%

7. Control

An adhesive (Indtran) was mixed @ 3 ml/10 litres in the spray fluid. The first round of fungicide sprays was given with the observation of earliest disease symptoms on lower leaves nearer to soil. Subsequent two rounds of sprays were given at 25 days interval. Observations on disease ratings for calculating per cent disease index and yield were recorded at 180 days after planting.

3.3.14 Disease Management with Micronutrients

A field experiment was conducted during 1998-99 and 1999-2000 to find out whether micronutrients have any effect on leaf blotch disease. Leaf blotch susceptible turmeric cultivar CLI 317 was planted in plots measuring 3.6 x 3.6 m in a randomized block design with four replications. The micronutrient treatments were:

1. Zinc sulphate 0.2%

2. Ferrous sulphate 0.2%

3. Magnesium sulphate 0.2%

4. Control

An adhesive (Indtran) was mixed @ 3 ml/10 litres in the spray fluid. With micronutrients, first spray (@750 l/ha) was given one month before the on-set of disease, i.e., in the mid-August (based on previous observation or experience) and subsequent two sprays were given at 15 days interval. Observations on disease ratings for calculating per cent disease index and yield were recorded at 180 days after planting.

3.3.15. Variability Among Genotypes Susceptible to *T. maculans* Leaf Blotch and Those Susceptible to *C. capsici* Leaf Spot

To study the morphological divergence among leaf blotch susceptible, leaf spot susceptible genotypes, and genotypes resistant to the two foliar diseases, 67 turmeric cultivars were planted at 3.15 x 0.9 m with two replications. The 67 genotypes were qualitatively assessed for their reaction to the two foliar diseases, i.e., *Taphrina* leaf blotch and *Colletotrichum* leaf spot.

The diversity among the 67 turmeric genotypes was estimated using Euclidean distance (Kendall, 1980). Thirteen morphological characters were used in estimating the distance. The Euclidean distance (D^2) was calculated by the formula:

$$D^2(A, B) = \sum_{i=1}^{p} (x_iA - x_iB)$$

Where A and B are objects, x is the variable from i to p i.e., 1 to 13

D^2 (A,B)= D^2 between objects A and B

Based on Euclidean ^{2} distance the 67 genotypes were grouped into clusters following Ward's minimum variance dendrogram.

For finding the characters that significantly contributed to the variability between leaf blotch susceptible and leaf spot susceptible genotypes, individual discriminant function and multiple discriminant function analyses were also done with the thirteen characters.

Discriminant function analysis was also performed to find the significant contributing character to the variability between the genotypes susceptible to the two foliar diseases and the resistant genotypes following the method described by Snedecor and Cochran (1967).

Observations on vegetative growth characters like plant height, number of tillers, leaf length and leaf breadth, and rhizome characters like number of mother rhizomes/plant, number of finger rhizomes/plant, average finger rhizome weight, average mother rhizome weight, length of finger rhizome, girth of finger rhizome, length/girth ratio, number of nodes/finger rhizome and internodal length on finger rhizome were recorded.

Curcumin was estimated in a few representative cultivars belonging to both the groups. Isoenzyme analysis by native gel electrophoresis was done with six *Taphrina* leaf blotch susceptible and four *Colletotrichum* leaf spot susceptible cultivars. The enzyme activity of esterase and polyphenol oxidase was studied.

3.3.16 Plant Characters

3.3.16.1 Vegetative Growth Characters

Number of Tillers

The number of tillers in five clumps in each plot was counted and the mean number of tillers per clump was calculated.

Height of the Plant

The height of the main shoot from the base of the plant to the base of the last unfolded leaf sheath for five plants per plot was measured and the mean plant height was calculated.

Length of Leaf

Length of leaf from the base of the lamina up to the tip was measured for all the leaves in the main shoot of five plants per plot and the mean leaf length was calculated.

Breadth of Leaf

For all the leaves in the main shoot of five plants per plot, the breadth was measured at the broadest point and the mean leaf breadth was calculated.

3.3.16.2 Rhizome Characters at Harvest

Number of Mother Rhizomes per Plant

The total number of mother rhizomes for five plants per plot was counted and the mean number of mother rhizomes per plant was calculated.

Number of Finger Rhizomes per Plant

The total number of finger rhizomes for five plants per plot was counted and the mean number of fingers per plant was calculated.

Average Weight of Mother Rhizome

The total number of mother rhizomes for five plants per plot was weighed and the mean weight of mother rhizome was calculated.

Average Weight of Finger Rhizome

The total number of finger rhizomes for five plants per plot was weighed and the mean finger weight was calculated.

Length of Finger Rhizome

The length of each finger rhizome in five plants per plot was measured and the mean length of finger rhizome was calculated.

Girth of Finger Rhizome

The girth of each finger rhizome in five plants per plot was measured and the mean girth of finger rhizome was calculated.

Length/Girth Ratio of Finger Rhizome

The mean length of finger rhizome was divided by its mean girth to calculate the length/girth ratio.

Number of Nodes per Finger Rhizome

The number of nodes in 25% of randomly selected finger rhizomes in each plant for five plants per plot was counted and the mean number of nodes per finger rhizome was calculated.

Internodal Length

The mean length of finger rhizome was divided by its mean number of nodes to calculate the internodal length.

Yield

The yield of fresh rhizomes in the plots at harvest was recorded in kilograms and was expressed as tonnes/ha.

3.3.17 Statistical Analysis of Data

The experimental data were statistically analyzed following the standard procedures (Sendecor and Cochran, 1967; Panse and Sukhatme, 1978; and Gomez and Gomez, 1976).

3.4 DISEASE GROWTH

For any disease symptoms to occur, three parameters namely a vulnerable host, a virulent pathogen and a favourable environment must occur together. Under such conditions, a pathogen not only completes its life-cycle, but also undergoes repeated generations. Obviously, to complete generations, pathogens require not only time but also space to grow. So the disease growth is both a temporal (pertaining to time) and a

spatial (relating to space or area to grow) process. Usually, it is observed that a disease grows in three phases, an initial lag phase, a rapid growth and then stagnation. The time to complete each phase depends on various factors such as favourable weather conditions, inoculum, field sanitation etc. The initial lag phase depends on inoculum load and if the load is weak, this phase may be prolonged. The rapid growth occurs when there is a favourable environmental condition. This phase may be sudden and develop faster. The last stage occurs when no host tissue is available and the disease stagnates. So, all the three phases combined together form the disease growth or progress. The disease progress can be plotted on the graph paper. The form is usually a sigmoid curve or a prolonged English alphabet "S". To analyze such curves, in this study various models were tried.

3.4.1 Fitting Disease Progress Curves

Basically, different types of growth models are widely tried in plant epidemiology. Only three, namely Exponential, Logistic, and Gompertz models were used in this study to analyze *Curcuma longa* - *Taphrina maculans* pathosystem. In the following equations, variables "r" denote rate at which the disease progresses or disease growth rate, "y_t" is the disease proportion range from 0 to 1.0 (0-100%) at time t and Y_o is the initial disease proportion from 0 to 1.0 and "t" is the time.

Exponential Model

The simplest model for disease progression is exponential model. The rationale behind this model is the disease progress at a particular time is proportional to the disease already present. The model can be written as:

$Yt = Yo * \exp(rt)$ (Eq.)

But the drawback of the model is that the disease may not grow indefinitely.

Logistic Model

One of the most popular and important model is the logistic model. The model is based on the fact that the absolute rate of disease increase is proportional to both the level of the disease present and the level of healthy tissue or plant material available. The logistic model was proposed for the population growth and a form of this model is given by:

$y_t = 1/(1+\exp(-(\ln(y_o(1-y_o)+rt))$ (Eq.)

Gompertz Model

This model is based on the assumption that the healthy tissues lose the fighting ability to be free from disease as the disease progresses. The mathematical model for this model is given by:

$Yt = \exp[\ln(y_o * exp(rt))]$ (Eq.)

This model is almost similar to the logistic model but with more of asymmetry. This is because of the fact the model shows an inflection (change) when 37% of total growth is achieved. On the other hand, logistic model shows an inflection only at half-way (50%). This explains the difference between the two models.

Basically, Exponential, Logistic and Gompertz models were used in this work. To fit a particular model, firstly the actual disease incidence values were transformed. These calculations were done using Microsoft Excel. Then, using the transformed values as the dependent values and the time (in days after first observation) as independent values the model was fit using simple regression analysis. To interpret the standard regression analysis, first the values of coefficient of determination (R^2) were

considered. This gives the proportion of the variation in the data accounted for by the regression model. The other fact considered is the Mean Square Errors and the graph of the standardized residuals versus predicted values. In the graph or plot of residuals against the predicted values, if a random scatter of points observed, it is taken to indicate an appropriate fit of the chosen models. Comparison of disease progress curves was made between years of study.

3.4.2 Estimation of Apparent Rate of Infection

The apparent rate of infection of *Taphrina maculans* during the crop period was estimated in three blotch susceptible turmeric cultivars viz., CLI 317, KTS 1 and KTS 4. The three turmeric cultivars were sown each in an area of 300 m^2 (20 x 15 m).

The per cent disease index from disease progress data was used to calculate the apparent rate of infection (r) of the disease in the six cultivars by employing the formula suggested by Van der Plank (1963).

$$r = \frac{1}{t_2 - t_1}\{\log\ x_2(1-x_1)/x_1(1-x_2)\}$$

Where t_1 and t_2 are time intervals when x_1 and x_2 are the per cent disease indices in fraction.

3.4.3 Area Under Disease Progress Curves (AUDPC)

The area under disease progress curves (AUDPC) also provides a quantitative measure of epidemic development and intensity that is not dependent on a particular curve shape or mathematical form. The AUDPC can be calculated using the midpoint method as given by Wilcoxson *et. al.* (1975).

$$\text{AUDPC} = \sum_{i=1}^{n-1} [y_i + y_{i-1})/2](t_{i-1}-t_i)$$

where n is the number of disease assessments, y is the disease intensity, t is time, i and i + 1 represent observations from 1 to n. The AUDPC is standardized by dividing the total length of the assessment period. This enables direct comparisons among epidemics of different lengths in the two years of study.

3.4.4 Spatial Pattern and Temporal Changes Associated with Disease Incidence and Progress

The spatial pattern of disease incidence and later temporal changes were studied in a turmeric cultivar CLI 317 susceptible to leaf blotch during 1999-2000. The crop was planted in a plot measuring1050 m^2 (35 x 30 m) on 24-6-1999. In the field, 13 quadrats (2 x 2 m size) were marked with bamboo stakes. Quadrats were placed (Figure 1) in such a way to enable regrouping later these quadrats to satisfy horizontal, vertical, diagonal and random sampling methods.

In each quadrat, disease assessment was made four times at an interval of 30 days in two plants. The sampled plants were sacrificed by removing for data recording. The proportion of the diseased or infected area was recorded in each plant. For this purpose the total leaf area and the infected leaf area were mapped with appropriate labels to indicate the sample number, quadrat number and the time of observation on to a Gateway tracing sheet in the field itself to avoid leaf rolling. Later in the laboratory, using a transparent graph sheet, the exact area of entire leaf and infected leaf area in each plant sample was measured in mm^2. From this, the proportion of infected area was computed in each plant by dividing the infected leaf area by total leaf area.

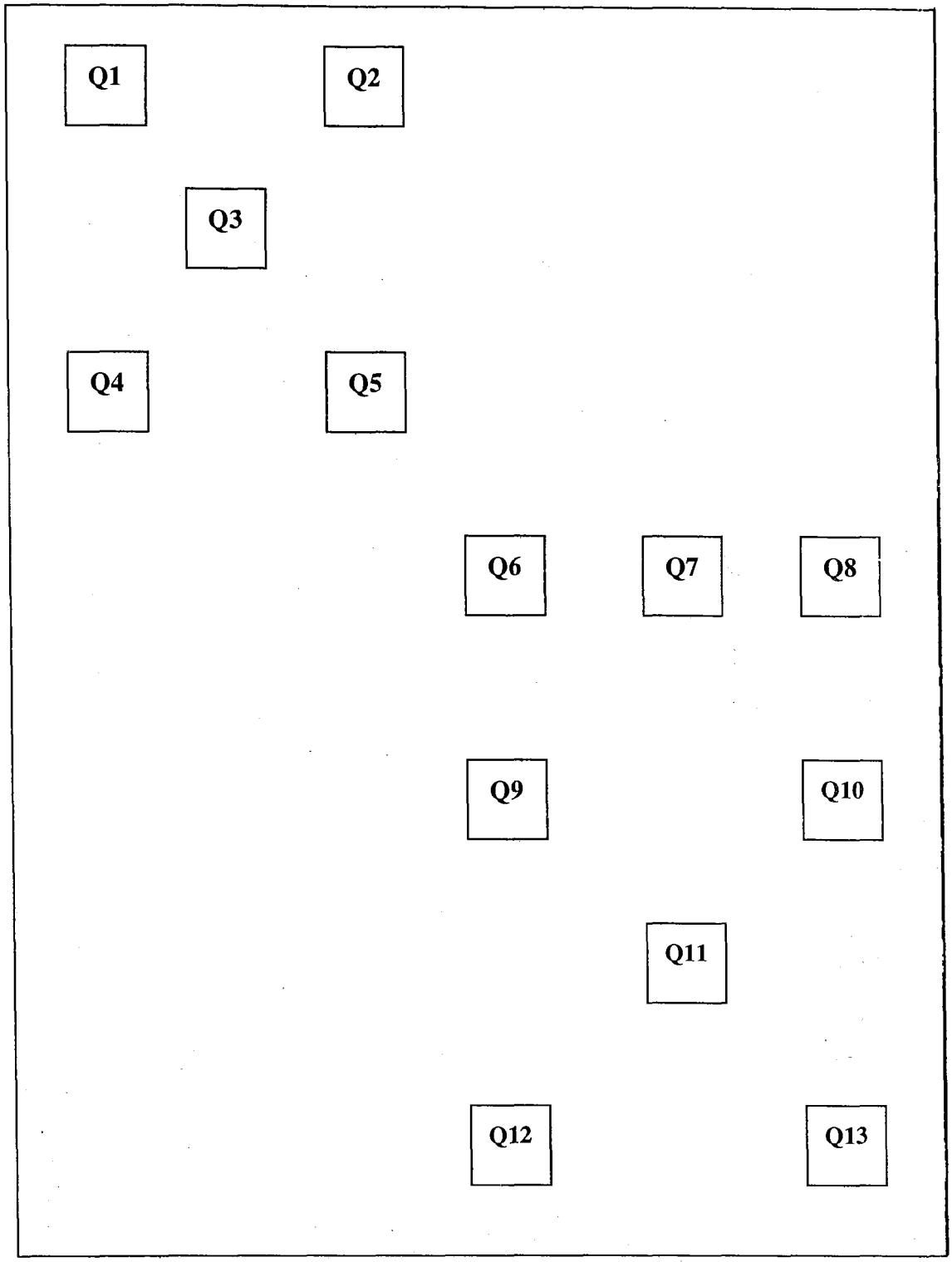

Figure 1. Diagrammatic representation of location of quadrats (Q-2m^2) in the field from which samples on proportion of leaf blotch disease on two plants, was estimated by measuring infected and healthy leaf area (mm^2) to study temporal changes in spatial pattern of occurrence

(continued...)

Vertical sampling:

Block 1-Q1 and Q4;
Block 2-Q2 and Q5;
Block 3-Q9 and Q12; and
Block 4-Q10 and Q13

Diagonal sampling:

Block 1-Q1 and Q5;
Block 2-Q2 and Q4;
Block 3-Q9 and Q13; and
Block 4-Q10 and Q12

Horizontal sampling:

Block 1-Q1 and Q2;
Block 2-Q4 and Q5;
Block 3-Q6 and Q8;
Block 4-Q9 and Q10; and
Block 5-Q12 and Q13

Random sampling:

Block 1-Q1;
Block 2-Q2;
Block 3-Q3;
Block 4-Q4;
Block 5-Q5;
Block 6-Q6;
Block 7-Q7;

Block 8-Q8;
Block 9-Q9;
Block 10-Q10;
Block 11-Q11;
Block 12-Q12; and
Block 13-Q13

To test the type of sampling method to be followed for understanding the spatial pattern of disease incidence and progress over time, the data from 13 quadrats were regrouped into horizontal, vertical, diagonal, and random sampling blocks as illustrated in Figure 1.

As indices of dispersion or aggregation provide a measure of the degree of spatial aggregation in a population, several dispersion measures were calculated for data from randomly located sampling quadrats or from rearranged sampling designs.

After the samples in the quadrats were regrouped to satisfy different sampling methods, the proportion of disease (infected blotch area/total area) was analyzed by ANOVA in hierarchial classification (Kempthrone, 1952).

The variance-to-mean ratio (VM) was calculated using data from quadrats by dividing the sample variance by the sample mean:

$$VM = s^2 / \bar{x}$$

Where s^2 = sample variance and x = sample mean. For regular spatial pattern, this is expected to be <1, for a random pattern =1, and for an aggregate pattern >1. Variation of VM is used as an index of aggregation (Upton and Fingleton, 1985).

$$ICS = VM - 1 \quad (C = \text{clumping size})$$

Using VM (or ICS or index of clump size) as an index of aggregation, performed formal statistical test for aggregation as:

$$C = (n-1) VM$$

Computed χ^2 value with n-1 degrees of freedom under null hypothesis of randomness. If the index value for C greatly exceeds the table value of χ^2, the highly aggregated pattern of disease is confirmed.

Lloyd's index of patchiness (Lloyd, 1967) was calculated to find the relative degree of aggregation as:

$$LIP = \frac{\bar{x} + s^2/\bar{x} - 1}{\bar{x}}$$

where $\bar{x} + s^2/\bar{x} - 1$ is defined as Lloyd's index of mean crowding (m).

Another index of dispersion, I_δ proposed by Morisita (1964) was calculated as:

$$I_\delta = \frac{n[\sum x\,(x-1)]}{\sum x\,(\sum x - 1)} \quad \text{or,}$$

$$I_\delta = \frac{n[\sum (x^2) - \sum x]}{(\sum x)^2 - \sum x} \quad \text{or,}$$

where x represents the proportion of disease in each sample quadrat.

The departure of a value of I_δ from 1 (= random) is judged to be significant *($P<0.05$)* when

$$I_\delta^* = I_\delta\,(\sum x - 1) + n - \sum x$$

If outside the appropriate 0.05 and 0.95 significance levels of Chi square for n-1 degrees of freedom, $I_\delta^* > 1$ table value means significant aggregation.

RESULTS

CHAPTER IV

RESULTS

4.1 THE DISEASE

4.1.1 Symptoms

The disease was observed in all the turmeric cultivars studied. These cultivars belonged to the intermediary group with growth duration of eight months. Symptoms of the disease were observed first on the lower most leaves during the last week of September in 1998-99 and during the last week of August in 1999-2000 and 2000-01. First symptoms appeared as dot sized (1-2 mm diameter) pale yellow specks on the portion of lamina nearer to the petiole. The colour of these pale yellow specks turned to dirty yellow and finally to orange brown. The specks or spots coalesced freely as they increased in size giving the leaf a blotched appearance (Plates 6,7 and 8). Blotch appears as irregular discolouration due to pustules produced by the pathogen following infection. The infection progressed from the bottom leaves to the top leaves in a successive fashion. Late in the season, a month before harvest, infection was found to extend into the petiolar canal also (Plate 9).

4.1.2 Histopathology

Thin hand cross sections of leaf through infection spots were taken using a razor blade. They were incubated in acidified (pH 4.5) water in cavity slides at 20° C for 48 hours, stained with cotton blue and observed under a microscope. A series of groups of naked asci with ascospores were observed between the cuticle and epidermis. Mycelium embedded in cuticle and in the intercellular spaces of epidermis was also observed

Plate 6. The earliest symptom in leaf blotch disease development. In this early stage, yellow specks (1-2 mm) initially arise at the basal portion of the leaf near petiolar region and spread to the entire leaf

Plate 7. The middle stage in leaf blotch disease development. In this stage, the yellow specks grow in size and develop small rusting centers

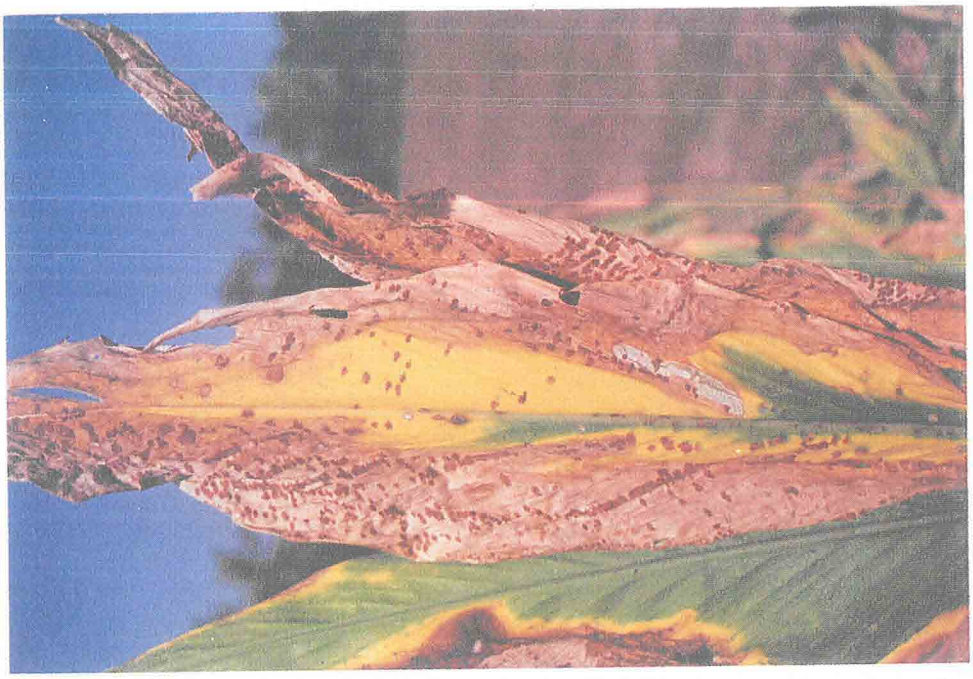

Plate 8. The final stage in leaf blotch disease development. In this stage the yellow specks and spots develop further and coalesce, turn to chocolate brown colour and give the leaf a blotched appearance

Plate 9. Late in the season infection extends into the petiolar canal

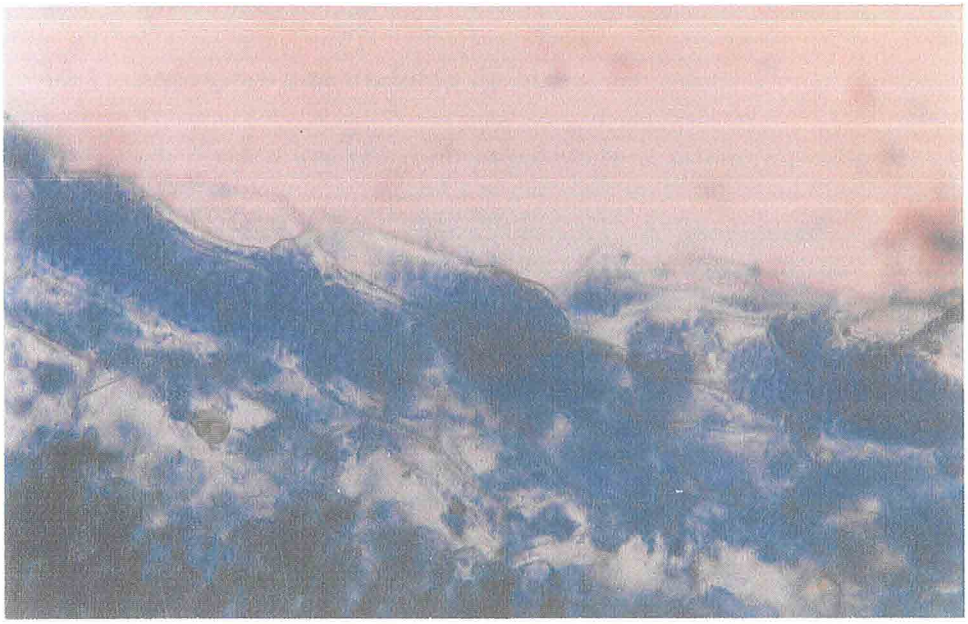

Plate 10. Transverse section of infected turmeric leaf showing groups of asci in sub-cuticular region with mycelium embedded in epidermal walls

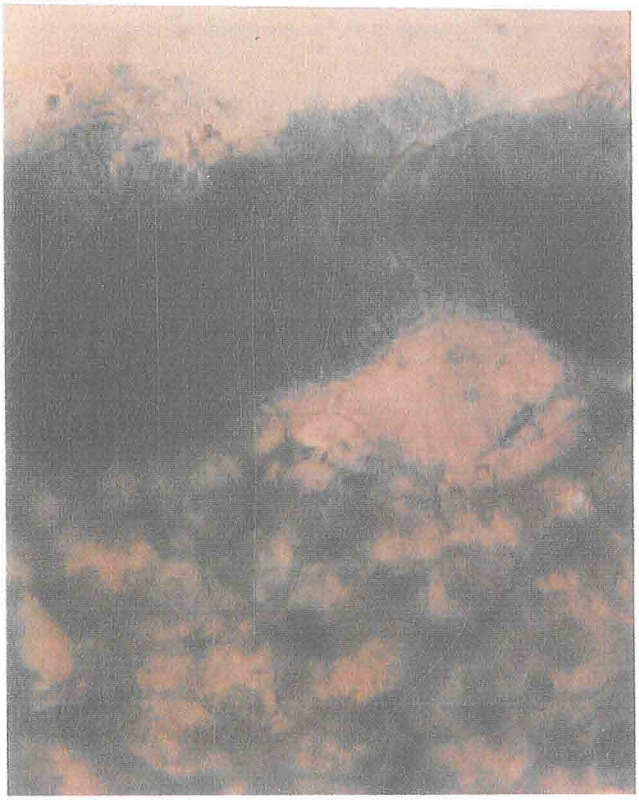

Plate 11. Transverse section of infected turmeric leaf showing mature asci releasing ascospores

(Plate 10). Ascospores and blastospores were observed being liberated from asci (Plate 11).

4.2 THE PATHOGEN

4.2.1 Ascospore and Budding Colonies

Disinfected leaf bits with initial stage of disease development were stuck to the inside of the lid of a Petri dish. Clean and sterilized microscope slides smeared with cotton blue lactophenol were kept on a moist blotter paper in the bottom plate of a Petri plate for collecting the expelled ascospores from the spots. The slides were observed daily under a microscope. Octosporus ascospore colonies were observed on the slides (Plate 12). On slides without cotton blue lactophenol, the spores were found budding into colonies of blastospores or conidia (Plate 13).

4.2.2 Isolation of the Pathogen

White and pink colonies of the fungus were obtained when fresh turmeric leaf bits with initial or middle stages of spot development were stuck to the inside of the lid of a Petri dish containing PDA (pH 4.5) after 8-10 days of incubation (Plate 14). With other methods of isolation from leaves, such as direct placement of infected leaf bits and streaking of spore suspension on PDA, pink colonies of the pathogen were isolated. However, in these two methods, other microbial contaminants grew faster than the pathogen. The pathogen was sub-cultured on PDA (Plate 16) and maintained on PDA slants in mineral oil.

Potato dextrose agar (pH 4.5) was found to be the suitable growth medium to isolate the pathogen than nutrient agar (NA), oatmeal agar (OMA) and turmeric leaf decoction agar.

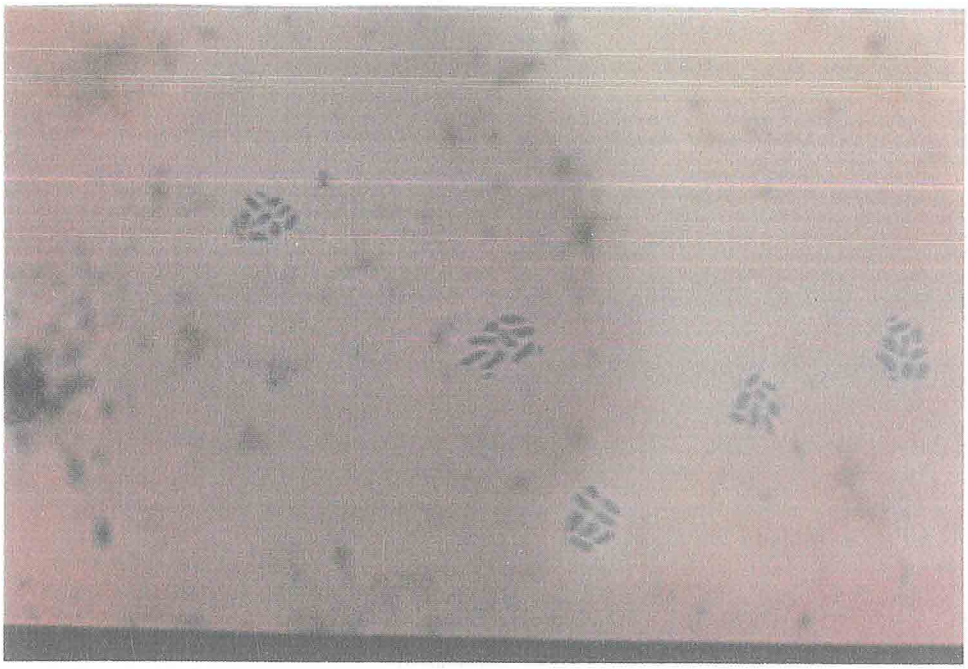

Plate 12. Octosporus ascorpore colonies of *Taphrina maculans* - Ascospores are stained with cotton blue

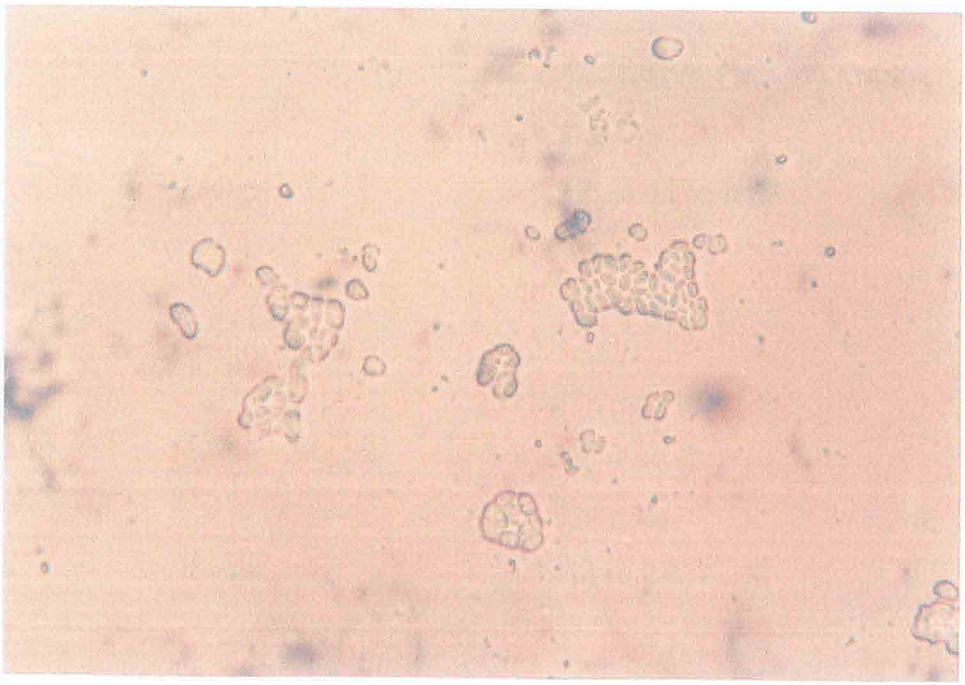

Plate 13. Ascospores (unstained) in a film of water on slides budding into colonies of conidia or blastopores within 1-2 h after discharge

4.2.3 Identification of the Pathogen

The fungus in culture was identified based on the morphological descriptions made by earlier researchers. The fungus in culture was yeast like. No filamentous growth of the fungus was observed in the culture. The individual cells, that is, conidia of the culture were elliptical to ovoid (Plate 15).

The length and breadth of fifty conidia from culture were measured using a microscope with stage and ocular micrometers. Similar measurements were also made on fifty conidia obtained from naturally infected spots on leaves. To compare conidial measurements with earlier reports, they were statistically tested with the formula:

Measurement of population mean (μ) = $\bar{x} \pm t\ 0.05\ SE$

Conidia from natural infection spots on leaves

Length:

Population mean (μ) = 4.58 + 1.96 (0.136)
= 4.846 μ m
= 4.58 - 1.96 (0.136)
= 4.313 μ m

Range = 4.313 to 4.846 μ m

Breadth:

Population mean (μ) = 2.28 + 1.96 (0.08103)
= 2.439 μ m
= 2.28 - 1.96 (0.08103)
= 2.121 μ m

Range = 2.121 to 2.439 μ m

The dimensions of the conidia reported by Butler (1918) and Kulkarni and Ahmed(1968)were; length : 4 to 6.5 μ m and breadth : 2 to 2.8 μ m. The range of measurements of conidia observed in this study fall within the above ranges.

Conidia from culture medium (PDA)

Length:

Population mean (μ) = 5.98 + 1.96 (0.179)
= 6.33 μ m

Population mean (μ) = 5.98 - 1.96 (0.1796)
= 5.63 μ m

Range = 5.63 to 6.33 μ m

Breadth:

Population mean (μ) = 2.98 + 1.96 (0.10875)
= 3.193 μ m

Population mean (μ) = 2.98 - 1.96 (0.10875)
= 2.767 μ m

Range = 2.767 to 3.193 μ m

The dimensions of conidia from pure culture as reported by Kulkarni and Ahmed (1968) were; length : 6 to 7 μ m and breadth : 2.5 to 4 μ m. The measurements made in this study fall within this range.

4.2.4 Growth of *T. maculans* in Culture as Influenced by Temperature and pH

The pathogen, *T. maculans* was inoculated on Petri dishes containing PDA that were differentially adjusted to pH ranging from 4.0 to 8.0. These inoculated plates were incubated at different temperatures viz., 10, 15, 20 and 25° C. The radial growth of the fungal colony in Petri dishes was measured at 10 and 20 days after incubation.

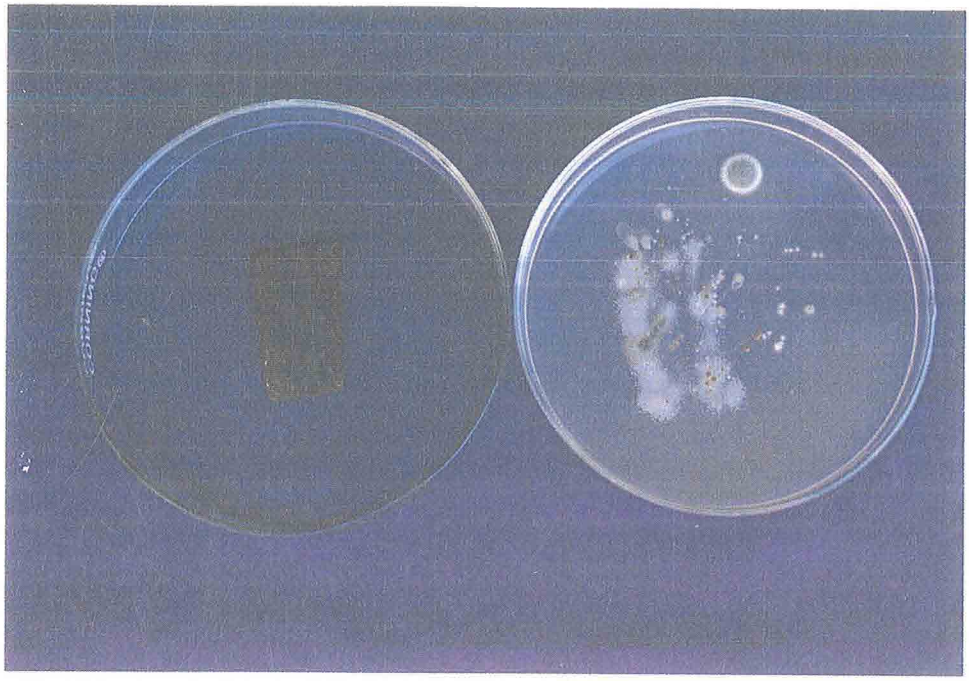

Plate 14. Pink and white-viscid colonies of *T. maculans* isolated on PDA (bottom dish). Infected leaf piece stuck to the inside of the lid of Petri plate is also shown

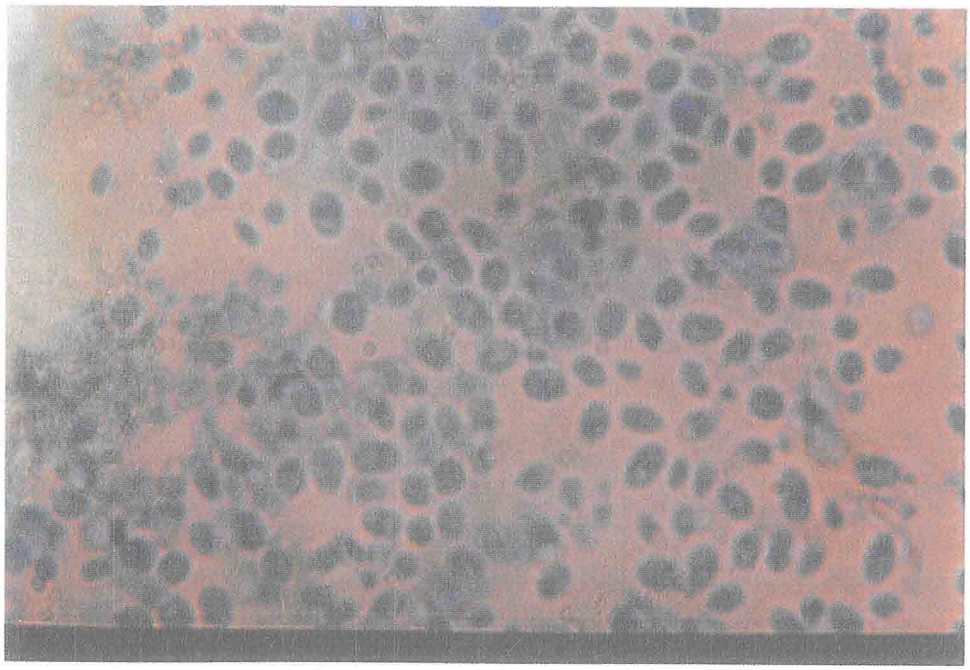

Plate 15. Conidia of *T. maculans* from pure culture grown on PDA - stained with cotton blue

Effect of pH

Relatively the growth of fungal colony was more at 20 days of incubation of inoculated Petri plates than at 10 days of incubation at all pH and temperature levels studied. At 10 days of incubation of inoculated plates, the maximum radial growth of the fungus was observed at pH ranging from 5.5 to 7.0 (Table 2). The least radial growth was recorded at pH 4.0 while at alkaline pH of 8.0, the radial growth was significantly less than the maximum recorded at pH 5.5. At 20 days after incubation a similar trend in radial growth of fungal colony was observed, the maximum radial growth being recorded between pH 5.5 and 7.0. Maximum reduction in radial growth was observed at pH 4.0, followed by pH 8.0.

Table 2: Main effects of different pH of potato dextrose agar (PDA) medium and incubation temperatures on the radial growth of *Taphrina maculans*

Parameter	Radial growth (mm)	
	10 days after inoculation	20 days after inoculation
pH		
4.0	9.50	15.38
4.5	12.83	17.33
5.0	12.88	17.13
5.5	13.58	17.71
6.0	13.21	18.13
6.5	13.13	17.83
7.0	13.21	17.54
8.0	12.38	16.58
SEm	0.321	0.27
CD (0.05)	0.89	0.76
Temperature (^oC)		
10	10.35	13.33
15	15.08	21.46
20	15.63	21.56
25	9.25	12.46
SEm	0.227	0.194
CD (0.05)	0.63	0.54

Effect of Temperature

In general, the growth of fungal colony was more at 20 days of incubation. Significant maximum radial growth of the fungus was recorded 15 and 20° C at both 10 and 20 days of incubation. The radial growth was reduced at incubation temperatures of 10° C and 25° C. This reduction was more pronounced at 20 days of incubation (Table 2).

There were no significant differences in the radial growth of the fungus due to interaction of temperature and pH over the levels studied (Table 3).

Table 3: Interaction of pH and temperature on the radial growth of *Taphrina maculans* on PDA

pH	10 days after inoculation Temperature °C				20 days after inoculation Temperature °C			
	10	15	20	25	10	15	20	25
4.0	8.50	11.17	10.50	7.83	11.33	19.00	20.00	11.17
4.5	10.50	15.33	16.67	8.83	13.83	21.50	21.50	12.50
5.0	10.50	15.50	16.67	8.83	13.83	20.50	21.50	12.67
5.5	11.17	16.50	17.00	9.67	14.17	21.67	22.17	12.83
6.0	11.17	15.83	16.33	9.50	14.33	22.83	22.83	12.50
6.5	10.83	15.33	16.50	9.83	14.00	22.33	21.83	13.17
7.0	10.33	16.17	16.17	10.17	13.00	22.67	22.00	12.50
8.0	9.83	14.83	15.17	9.67	12.17	21.17	20.67	12.33
SEm				0.642				0.549
C.D (0.05)		NS				NS		

4.2.5 Pathogenicity Test with Pure Culture Isolate of *Taphrina maculans*

Pathogenicity of the pure culture of *T. maculans* was established in pot culture on susceptible turmeric cultivar CLI 317. On four months old plants in three pots, inoculation was done by smearing the lower leaves with spore suspension (7 x 10^4 conidia ml^{-1}) harvested from the pure culture of *T. maculans*. The first clearly visible symptoms of the disease on inoculated leaves were observed on 28, 29 and 31 days after

inoculation in the first, second and third pots respectively. The average period for expression of disease symptoms on plant was 29 days after inoculation.

4.2.6 Variability among Isolates of *Taphrina maculans* Obtained from Three Turmeric Growing Tracts of Andhra Pradesh.

To determine if any variability exists among the isolates of *T. maculans* from the three major turmeric-growing tracts of Andhra Pradesh, infected leaf samples with very initial stage of infection were collected from Coastal, Telangana and Rayalaseema regions of Andhra Pradesh. The culture of *T. maculans* from the samples of each region was isolated and the isolates were subjected to SDS PAGE to find out the differences, if any, in their protein profiles.

The isolates of *T. maculans* from the three major turmeric growing tracts exhibited identical protein profiles on SDS PAGE. There was no perceptible difference in the protein bands among the three isolates (Plate 17).

4.3 PATHOGEN SURVIVAL AND PRIMARY SOURCE OF INOCULUM

4.3.1 Retrieval of *T. maculans in vitro*

From leaves, seed rhizomes and soils collected during previous year from infected fields, retrieval of *T. maculans in vitro* was attempted. From pre-treated infected leaves of previous year's crop, only a few colonies of *T. maculans* were obtained in isolations attempted during April to October in 1999-2000 and April to September in 2000-01 (Table 4). Beyond October in 1999-2000 and September in 2000-01 the fungus could not be isolated from preserved infected leaves.

From seed rhizomes of previous year's infected crop, the fungus could be isolated up to August by plating the washings of the rhizomes on PDA in Petri dishes.

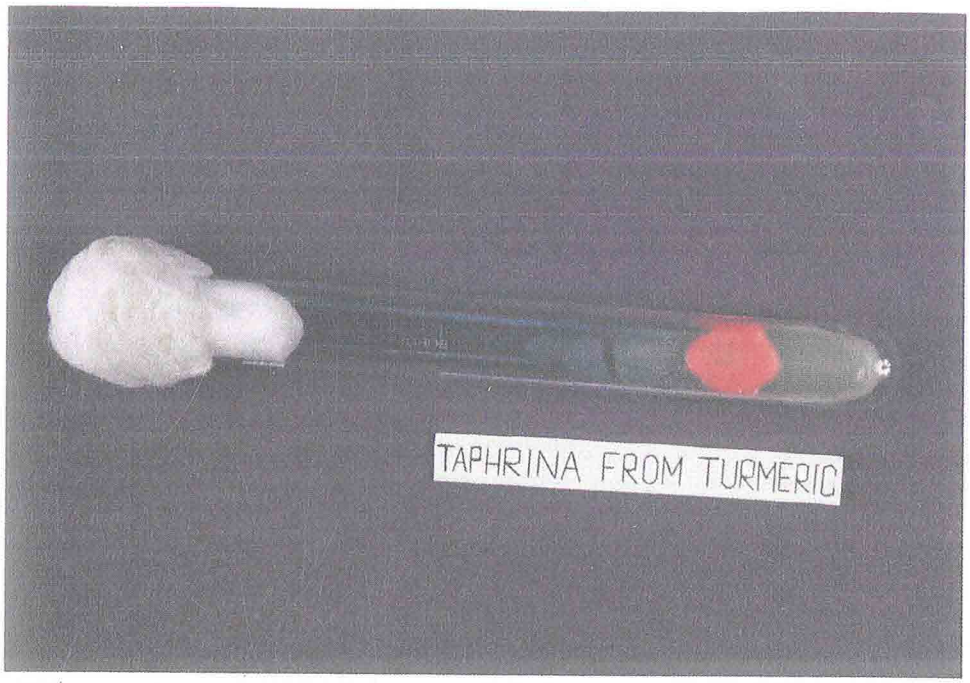

Plate 16. Pure culture of *T. maculans* maintained in PDA slants

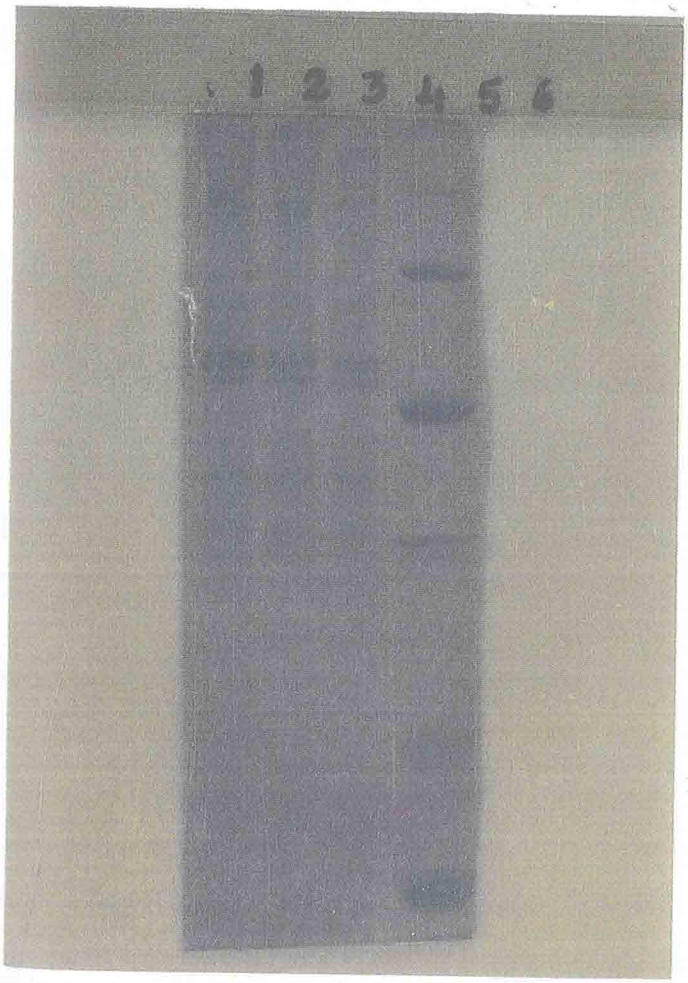

Plate 17. SDS-PAGE protein profiles of the conidial extracts of the isolates of *T. maculans* from different turmeric growing tracts of Andhra Pradesh. 1. Kovvur (Andhra) 2. Jagtial (Telengana) and 3. Anantharajupeta (Rayalaseema) regions 4. Protein marker

Table 4: Survival of *T. macuians** in infected leaves of previous year's crop at room temperature, soil samples and infected rhizomes

Month	Year	Number of attempts				Year	Number of attempts			
		1	2	3	4		1	2	3	4
Preserved infected leaves										
April	1999	+	+	+	+	2000	+	+	-	+
May	1999	+	+	-	+	2000	+	+	+	-
June	1999	+	+	+	-	2000	+	+	+	+
July	1999	+	+	+	+	2000	+	+	+	+
August	1999	+	-	-	+	2000	+	-	+	+
September	1999	+	+	-	-	2000	-	-	+	-
October	1999	-	+	-	-	2000	-	-	-	-
November	1999	-	-	-	-	2000	-	-	-	-
December	1999	-	-	-	-	2000	-	-	-	-
Preserved soil samples										
April	1999	-	-	-	-	2000	-	-	-	-
May	1999	-	-	-	-	2000	-	-	-	-
June	1999	-	-	-	-	2000	-	-	-	-
July	1999	-	-	-	-	2000	-	-	-	-
August	1999	-	-	-	-	2000	-	-	-	-
September	1999	-	-	-	-	2000	-	-	-	-
October	1999	-	-	-	-	2000	-	-	-	-
November	1999	-	-	-	-	2000	-	-	-	-
December	1999	-	-	-	-	2000	-	-	-	-
Preserved seed rhizomes										
April	1999	+	+	+	+	2000	+	+	+	+
May	1999	+	+	+	+	2000	+	+	+	+
June	1999	+	+	+	+	2000	+	+	+	+
July	1999	+	+	+	+	2000	+	+	+	+
August	1999	+	+	+	+	2000	+	+	+	+

* Based on isolation in PDA culture medium (*in vitro*)

\+ Viable (fungus could be isolated)

\- Not viable (fungus could not be isolated)

The fungus could not be isolated from soil collected from previous year's infected fields in any of the dilutions tried.

4.3.2 Testing Viability of Pathogen *in vivo* in Pot Culture

Pot culture experiment was conducted in 1998-99 using infected leaf debris, seed rhizomes and soil samples collected from infected fields during the previous year and preserved at room temperature to identify possible sources of inoculum. The experiment was repeated in 1999-2000 with similar collections made during the previous year.

Symptoms were first observed on the lower most leaves in plants that sprouted from untreated (not disinfected) rhizomes in all the pots, 119 days after planting during 1998-99 and 83 days after planting during 1999-2000. Symptoms were observed on plants in all the pots in which leaf debris from previous year's crop was mixed in soil 130 days after planting during 1998-99 and 92 days after planting during 1999-2000.

In one out of four pots during 1998-99 and two out of four pots in 1999-2000 in which soil from previous year's infected field was mixed, symptoms on plants were observed 134 days after planting during 1998-99 and 94 days after planting during 1999-2000. No disease symptom was observed on the plants in the pots in which disinfected seed rhizomes were planted.

4.3.3 Search for Other Hosts of *Taphrina maculans* that Serve as Primary Sources of Inoculum

The weed flora in fields and plants along the bunds in the vicinity of turmeric fields were carefully observed for the presence of symptoms resembling those caused by *Taphrina*. The lower leaves of *Canna* plants in turmeric fields and on bunds were found

to exhibit symptoms similar to those caused by *Taphrina maculans* in turmeric (Plate18). The infected leaves were collected and the pathogen was isolated on PDA (pH 4.5) (Plate 19). Based on the morphological characters the pathogen was identified as *T. maculans.*

The conidial suspension (6 x 10^4 conidia ml^{-1}) from the pure culture was tested on healthy plants of *Canna* and turmeric (CLI 317) in pot culture. The inoculated turmeric plants showed leaf blotch symptoms 33 days after inoculation, while the inoculated *Canna* plants showed symptoms 38 days after inoculation. Thus pathogenicity test also confirmed the identity of the pathogen isolated from *Canna sp* as *Taphrina maculans.*

4.4 INCUBATION (*P*), LATENT (*p*) AND INFECTIOUS (*i*) PERIODS

4.4.1 Incubation Period (*P*)

Incubation period (*P*) of *Taphrina maculans* in six leaf blotch susceptible turmeric cultivars viz., CLI 317, CLI 315, CLI 385, KTS 1, KTS 4 and PCT 13 was determined in pot culture experiments during 1998-99 and 1999-2000.

Inoculation of the plants was done by smearing the lower leaves of plants with spore suspension harvested from thin hand sections of infected leaf bits incubated in cavity slides filled with acidified water. Inoculation was made with a spore suspension having a spore concentration of 6 to 9 x 10^4 ml^{-1}.

The incubation period on CLI 317 estimated in the pathogenicity test with spores derived from pure culture was used for comparison with the incubation period recorded with spore suspension harvested from thin hand sections of infected leaf bits incubated in cavity slides filled with acidified water.

Plate 18. Leaf of *Canna* sp showing infection by *T. maculans*

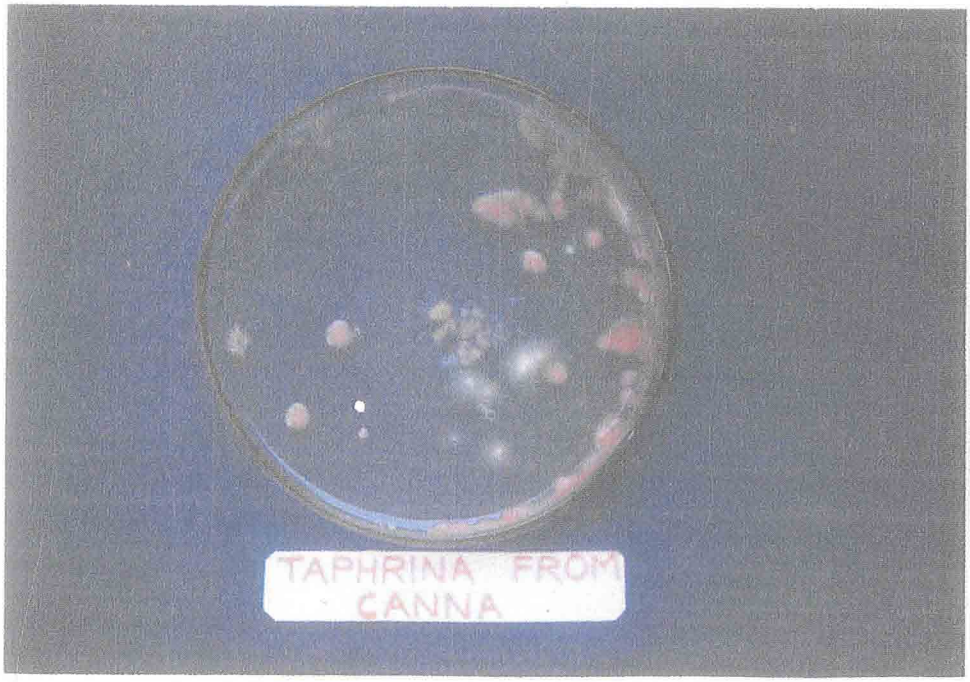

Plate 19. Pink colonies of *T. maculans* isolated from infected leaves of *Canna* sp.

The incubation period in the six susceptible turmeric cultivars was found to vary from 20 to 24 days during 1998-99. The incubation period in CLI 317 was significantly longer (24 days) than that in all the other cultivars. The incubation period in other cultivars ranged between 20 and 22 days (Table 5). During 1999-2000 also, the incubation period of the fungus varied among the six susceptible cultivars from 19 to 26 days, the longest incubation period being recorded again in the cultivar CLI 317. The incubation period in other cultivars ranged between 19 and 22 days. The pooled analysis for the two years shows that the incubation period varied significantly among the six cultivars. The incubation period ranged between 20 and 25 days with the shortest incubation period in KTS 4 and the longest in CLI 317.

The incubation period with conidia harvested from pure culture of *T. maculans* on the cultivar CLI 317 in the pathogenicity test was found to be 29 days.

Table 5: Incubation period (*P*) in *C. longa* - *Taphrina maculans* pathosystem

Cultivar	Incubation period (days)		
	1998-99	1999-2000	Pooled
KTS 1	20.67	21.33	21.00
KTS 4	20.67	19.33	20.00
CLI 315	20.33	21.00	20.67
CLI 385	21.00	20.67	20.83
PCT 13	22.33	22.33	22.33
CLI 317	24.00	26.33	25.17
SEm	0.59	0.45	0.435
CD (0.05)	1.82	1.39	0.91

4.4.2 Latent Period (*p*)

Leaf bits showing the most initial symptom i.e., on the first day of appearance of perceptible symptom were collected from six susceptible turmeric cultivars for studying the latent period (*p*) and infectious period (*i*) in the laboratory. The study was made for

one year during 2000-01. The latent period (5 to 6 days) did not vary significantly among the six turmeric cultivars (Table 6).

Table 6: Latent period and infectious period of *Taphrina maculans* in six susceptible cultivars of turmeric.

Cultivar	Latent period (days)	Infectious period (days)
CLI 317	5.33	24.50
KTS 1	5.83	24.50
KTS 4	5.50	24.17
PCT 13	5.17	24.33
CLI 315	5.17	24.67
CLI 385	5.33	25.17
SEm	0.46	1.21
CD (0.05)	NS	NS

4.4.3 Infectious Period (*i*)

Infectious period (24 to 25 days) also did not vary significantly among the six turmeric cultivars. Compared to short latent period there was nearly 5-fold extension in the infectious period (Table 6).

4.5 MONITORING OF *T. MACULANS* SPORES WITH SPORE TRAPS

The air-borne spores of *T. maculans* were monitored at crop canopy height using locally fabricated wind vane spore traps during turmeric growth season from August to February. The conidia of *T. maculans* were not observed before the first incidence of disease. No conidia were observed trapped until the time the disease developed in the top three leaves. Counting of conidia could not be done, as the conidia trapped were too numerous when the apical leaves were infected.

4.6 GENOTYPE REACTION

The reaction of 67 genotypes was studied during 1999-2000 and 2000-01 under field incidence conditions. Out of the 67 genotypes studied, 36 genotypes were found

susceptible to *Taphrina* leaf blotch disease and 25 were found susceptible to *Colletotrichum* leaf spot. In all only six genotypes were found resistant to both the foliar diseases.

The per cent disease index (PDI) for leaf blotch disease on 36 susceptible turmeric genotypes was recorded at 180 days after planting. The disease index varied from 63 to 76% during 1999-2000. Maximum PDI was recorded in KTS 4, while the minimum was observed in CLI 325. During 2000-01, the disease index ranged between 67 (ST 491) and 79% (PCT 11). The mean disease index for the two years ranged between 66.11% (CLI 325) and 76.11% (PCT 11). The average disease index over all the cultivars for the two years was 71.53% (Table 7). The leaf blotch disease susceptible genotypes belonged to short and medium duration types.

The 25 cultivars susceptible to *Colletotrichum* leaf spot were Kasturi, CA 70, CA 90, CA Sompeta, CA Shillong, CA 17-1, Alleppey, KTS 3, KTS 7, KTS 8, Ethamukkala, GL Puram, Roma, Tallapalem, Daghi, Meghalaya, CLI 38, TC 4, CC 94-01, ST 365, 15 b, Duggirala, CLL 326, CLL 327 and CLL 328.

The six genotypes resistant to both the foliar diseases were CLS 24, PTS 38, PTS 19, CA 146/4, CLI 362 and CA 92-2.

Table 7: Percent disease intensity of *Taphrina maculans* leaf blotch in susceptible turmeric cultivars

Cultivars	1999	2000	Mean
CLI 325	63.33	68.89	66.11
ST 760	64.44	70.00	67.22
PCT 3	67.78	67.78	67.78
CLI 369	65.55	71.11	68.33
PCT 13	68.89	67.78	68.34
CLI 317	70.00	67.78	68.89
ST 491	71.11	66.67	68.89
Ranga	70.00	67.78	68.89
CLI 370	66.67	72.22	69.45
BDJR 1183	66.67	72.22	69.45
BDJR 1192	67.78	71.11	69.45
PTS 16	67.78	72.22	70.00
CLI 196/4	66.67	73.33	70.00
CLI 322	71.11	68.89	70.00
PTS 7	73.33	67.78	70.56
PCT 14	71.11	72.22	71.67
CLI 361	68.89	74.44	71.67
CLI 385	70.00	73.33	71.67
PCT 10	73.33	71.11	72.22
CLI 315	70.00	74.44	72.22
CLI 390	70.00	74.44	72.22
PCT 15	71.11	74.44	72.78
PCT 16	71.11	74.44	72.78
CLI 335	71.11	74.44	72.78
Phubani Local	68.89	76.67	72.78
PCT 18	72.22	74.44	73.33
CLI 330	68.89	77.78	73.34
BDJR 1082	72.22	75.55	73.89
KTS 2	70.00	77.78	73.89
CLI 124/6	71.11	76.67	73.89
CLI 316	71.11	76.67	73.89
KTS 1	72.22	76.67	74.45
CLI 195/5	72.22	77.78	75.00
CLI 225/5	73.33	76.67	75.00
KTS 4	75.55	76.67	76.11
PCT 11	73.33	78.89	76.11
Overall mean			71.53
Standard error			0.43

4.6.1 Differences in Leaf Blotch Susceptible and Leaf Spot Susceptible Turmeric Genotypes

4.6.1.1 Site of Infection and Disease Progress

In the genotypes susceptible to leaf blotch, *Taphrina* incidence was first noticed on mature and old turmeric leaves. Infection was observed to start in the leaves at the bottom of plants and the disease progressed upwards to the progressively maturing leaves. In genotypes susceptible to leaf spot, infection caused by *Colletotrichum capsici* appeared first on the young leaves located at the top of the plants and the disease progressed downwards. Thus, while the site of infection and progress in *Curcuma–Taphrina* pathosystem was bottom-up, it was top-down in *Curcuma–Colletotrichum* pathosystem.

The genotypes susceptible to *Taphrina* and *Colletotrichum* exhibited differences in leaf colour and rhizome character. The leaves of leaf blotch susceptible genotypes showed dark green colour, while the leaves of leaf spot susceptible genotypes were pale green (Plate 20). The finger rhizomes of leaf blotch susceptible genotypes were relatively bolder than those of leaf spot susceptible genotypes. Also the finger rhizomes of leaf blotch susceptible genotypes were dirty brown in colour but with glistening skin while those of leaf spot susceptible genotypes were pale white to light yellow in colour without any glistening appearance (Plate 21). The inner core of the finger rhizomes of leaf blotch susceptible genotypes was deep orange in colour, while that of leaf spot susceptible genotypes was light yellow.

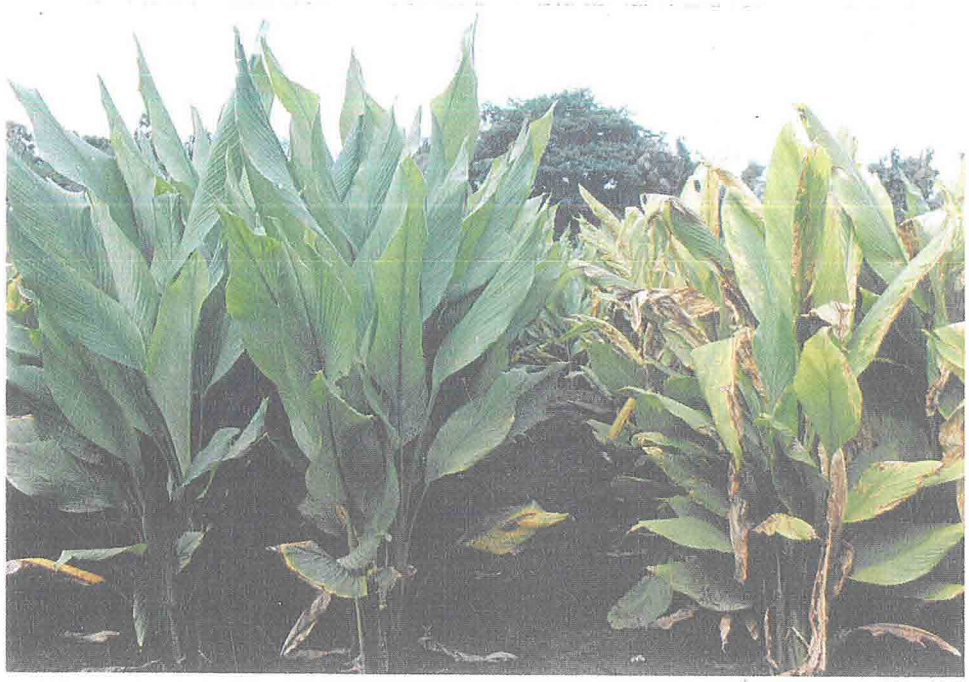

Plate 20. Turmeric cultivars susceptible to *Taphrina*_leaf blotch (on the left) and *Colletotrichum* leaf spot (on the right)

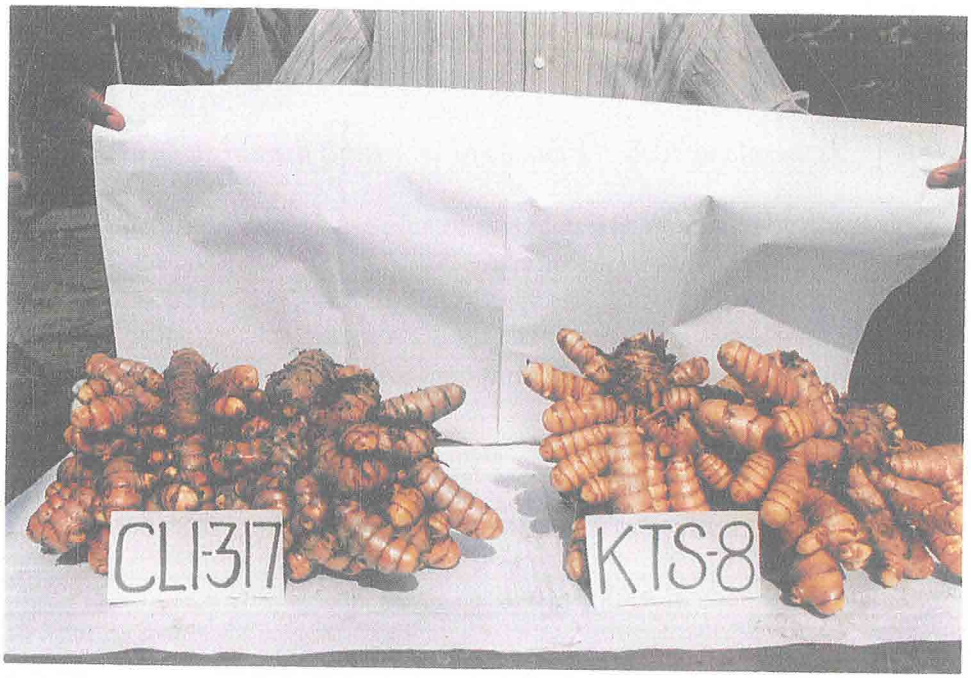

Plate 21. Rhizomes from turmeric cultivars susceptible to *Taphrina* leaf blotch (CL1 317) and *Colletotrichum* leaf spot (KTS 8)

4.6.1.2 Biometrical/Morphological Divergence in Relation to Qualitative Assessment of Reaction to Two Foliar Diseases in Turmeric

The qualitative assessment of divergence in relation to the two foliar diseases in turmeric was followed by standardized Euclidean[2] technique for 13 morphological characters.

Standardized Euclidean D^2 Distance

In order to assess the diversity present among 67 genotypes, Euclidean (D^2) distance statistic was calculated. The entries were grouped into four clusters based on Euclidean distance values (Figure 2). The genotypes belonging to the same cluster had an average smaller Euclidean distance values than those belonging to different clusters. The details on the distribution of different genotypes in clusters are shown in Table 8. Out of the four clusters formed, cluster I was the largest with 35 entries, followed by cluster III with 17 genotypes, cluster II with 11 genotypes and cluster IV with only 4 genotypes.

The average intra- and inter-cluster distances are shown in Figure 3. Intra-cluster distance values ranged from 3.57 in cluster I to 5.07 in cluster IV.

The maximum inter-cluster distance was observed between cluster III and cluster IV (8.44), while the minimum was between cluster I and cluster II (4.65). Among the four clusters, cluster IV maintained the maximum distance from the other three clusters.

The cluster I with the lowest intra-cluster distance comprised 34 entries that were qualitatively assessed for their susceptible reaction to *Taphrina* leaf blotch disease. One leaf blotch susceptible entry each was found in each of the clusters III and IV. The *Colletotrichum* leaf spot susceptible entries (25) were distributed in all the four clusters.

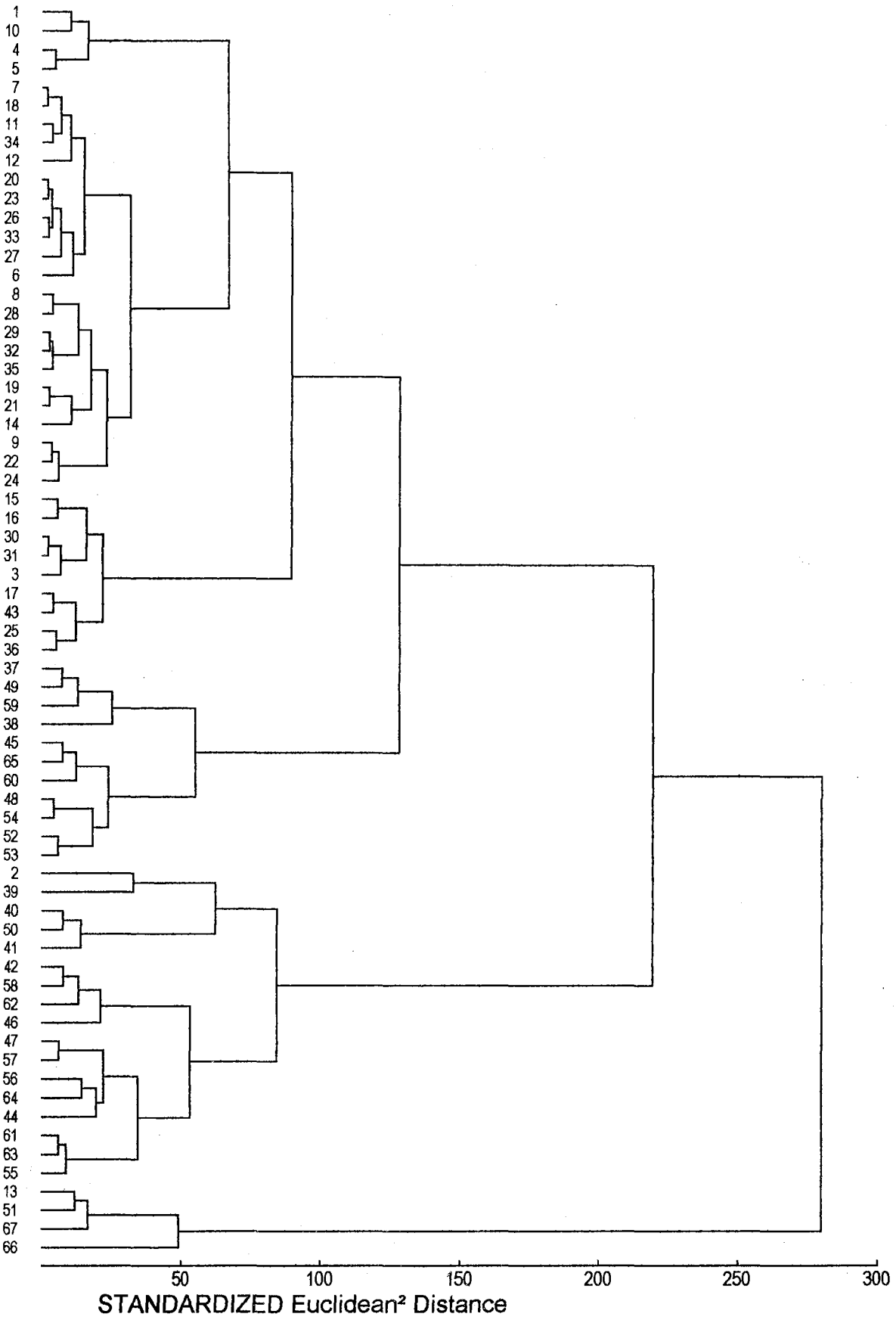

Figure 2. Dendrogram showing grouping of turmeric genotypes into clusters

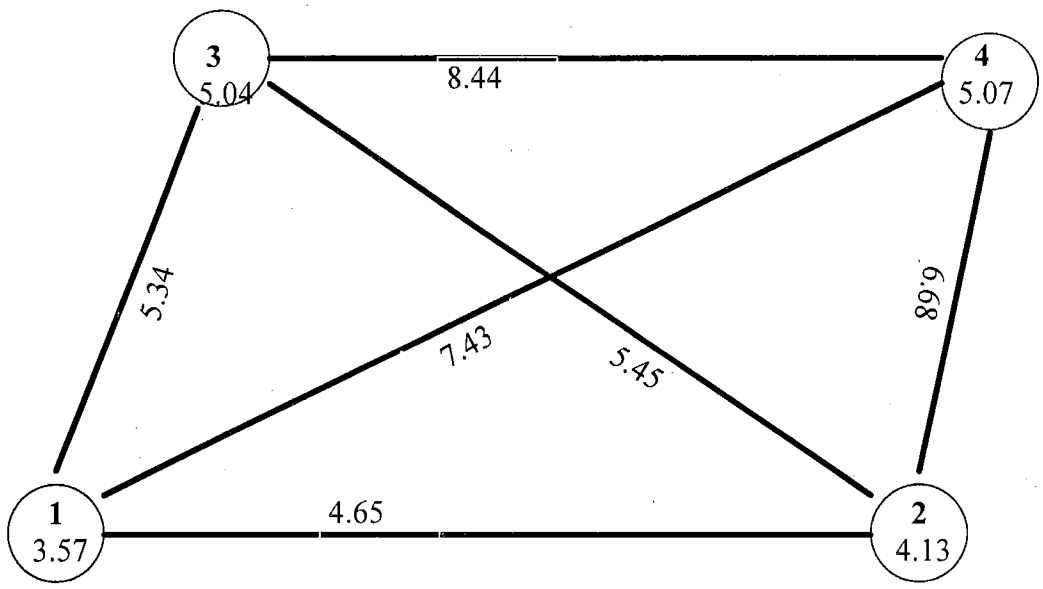

Figure 3. Diagram showing inter- and intra-cluster Euclidean distance

The distribution of leaf spot susceptible entries was 10 in cluster II, 14 in cluster III and one entry each in clusters I and IV. The six entries that were assessed as resistant to both the foliar diseases were found distributed in clusters II, III and IV.

Table 8: Distribution of genotypes of turmeric in different clusters

Cluster	Number	Genotypes (names)
I	35	KTS2*, PCT 15*, CLI 330*, CLI 361*, CLI 225/5*, ST 491*, PCT 18*, Ranga*, PTS 16*, KTS 1*, CLI 124/6*, CLI 385*, CLI 335*, CLI 390*, CLI 370*, PCT 3*, PCT 13*, PCT 14*, CLI 317*, CLI 369*, Phubani local*, KTS 4*, PCT 16*, PCT 11*, CLI 315*, CLI 196/4*, BDJR 1082*, BDJR 1183*, PTS 7*, CLI 325*, CLI 316*, ST 760*, CA 90, CLI 322*, BDJR 1992*.
II	11	KTS 7, Duggirala, Ethamukkala, KTS 8, Kasturi, CLS 24R, Tallapalem, KTS 3, CLL 328, CLL 326, CLL 327.
III	17	CLI 195/5*, Daghi, CLI 38, Meghalaya, CA 70, CA Sompeta, Alleppey, PTS 38R, CA 17-1, CA Shillong, TC 4, ST 365, CA 146/4R, GL Puram, Roma, PTS 19R, CC 94-01.
IV	4	PCT 10*, 15 b, CLI 362R, CA 92-2R.

*Leaf blotch susceptible; RResistant to both leaf spot and blotch and no symbol = Leaf spot susceptible

Table 9: Cluster mean for 13 morphological characters in *Curcuma* genotypes

Morphological character	Cluster I	Cluster II	Cluster III	Cluster IV
Number of genotypes	35	11	17	4
Plant height (cm)	45.460	48.826	42.694	69.813
Tillers/plant	2.358	1.845	2.408	1.497
Leaf length (cm)	50.809	57.872	48.063	77.150
Leaf width (cm)	16.250	17.162	16.041	20.116
Fingers/plant (no.)	25.611	19.255	20.265	24.900
Mothers/plant (no.)	1.901	1.818	2.188	2.250
Finger weight (g)	19.729	20.710	13.696	18.392
Mother weight (g)	43.169	50.464	38.069	71.419
Finger length (cm)	7.240	7.684	7.063	7.750
Finger girth (cm)	7.966	6.900	6.040	7.344
Length/girth ratio	0.910	1.128	1.172	1.060
Nodes/finger (no.)	7.049	7.523	7.065	6.700
Internodal length (cm)	1.029	1.024	1.006	1.164

The mean values of clusters for the thirteen morphological characters are presented in Table 9. Considerable differences between the clusters were detected in all the morphological characters studied. Plant height (69.81 cm), leaf length (77.15 cm), leaf width (20.12 cm), number of mother rhizomes/plant (2.25), weight of mother rhizome (71.42 g), finger length (7.75 cm) and inter nodal length (1.164 cm) were highest in genotypes grouped under cluster IV. In the most uniform cluster, i.e., cluster I, the genotypes recorded the highest girth of finger rhizome (7.97 cm) and the lowest length/girth ratio of finger rhizomes (0.91). Cluster II recorded the highest weight of finger rhizome (20.17 g). The genotypes in the cluster III recorded the highest number of tillers (2.41) and the highest length/girth ratio (1.17).

Contribution of Characters to Variability

Both individual discriminant function and multiple discriminant function analyses between genotypes susceptible or resistant to leaf blotch or leaf spot were performed.

Blotch Vs Spot

The *t*-values and means for each character of blotch susceptible and spot susceptible groups of genotypes are presented in Table 10. In the individual discriminant function analysis only leaf length and length/girth ratio differed between the leaf blotch susceptible and leaf spot susceptible groups of genotypes. The blotch susceptible group recorded a mean length of 51.17 cm in comparison to 54.70 cm in spot susceptible group. The length/girth ratio was 0.99 in leaf blotch susceptible group, while it was 1.05 in leaf spot susceptible group. In the multiple discriminant function analysis, leaf length, weight of finger rhizome and length of finger rhizome differed significantly (Table 10).

Table 10: Individual and multiple discriminant function analysis between *Taphrina* leaf blotch and *Celletotrichum* leaf spot susceptible genotype groups

Character	*t*-value		Mean	
	Individual	Multiple	Blotch susceptible genotypes	Spot susceptible genotypes
Plant height (cm)	1.043	1.299	46.34	47.92
Tillers/plant	1.091	0.474	2.33	2.15
Leaf length (cm)	2.293*	2.438*	51.17	54.70
Leaf width (cm)	0.741	0.949	16.50	16.71
Fingers/plant (no.)	0.543	0.869	23.60	22.89
Mothers/plant (no.)	1.390	1.555	2.07	1.93
Finger weight (g)	0.955	2.172*	17.87	18.70
Mother weight (g)	1.647	0.698	42.85	47.38
Finger length (cm)	0.986	2.070*	7.24	7.37
Finger girth (cm)	1.633	1.552	7.42	7.10
Length/girth ratio	2.294*	1.006	0.99	1.05
Nodes/finger (no.)	0.688	1.545	7.05	7.14
Internodal length (cm)	0.125	1.625	1.03	0.130

* Significant at 0.05

Blotch and Spot Susceptible Genotypes Vs Resistant Genotypes

The *t*-values and means for each character of susceptible group of genotypes and resistant group of genotypes are presented in Table 11. In the individual discriminant function analysis only one character namely number of mother rhizomes plant^{-1} differed significantly between the susceptible and resistant groups of genotypes. The resistant group of genotypes recorded significantly lower number of mother rhizomes (1.65) than the susceptible group (2.01). This was further confirmed by the significant values obtained for this character in multiple discriminant function analysis. Additionally, in the multiple discriminant function analysis plant height and leaf length also significantly contributed to the variation between the two groups.

Table 11: Individual and multiple discriminant function analysis between groups of of genotypes susceptible or resistant to foliar diseases

Morphological character	*t*-value		Mean	
	Individual	Multiple	Susceptible to foliar diseases	Resistant to foliar diseases
Plant height (cm)	1.046	2.633**	46.98	44.44
Tillers/plant	0.754	0.850	2.25	2.05
Leaf length (cm)	0.971	2.543**	52.62	55.17
Leaf width (cm)	0.167	-	16.58	16.51
Fingers/plant (no.)	0.733	-	23.31	21.77
Mothers/plant (no.)	2.319*	2.383*	2.01	1.65
Finger weight (g)	0.551	1.019	18.21	18.99
Mother weight (g)	0.132	-	44.71	45.30
Finger length (cm)	0.242	-	7.29	7.35
Finger girth (cm)	0.758	1.306	7.29	7.04
Length/girth ratio	1.069	-	1.02	1.07
Nodes/finger (no.)	1.210	0.946	7.09	7.33
Internodal length (cm)	0.948	-	1.03	1.00

* Significant at 0.05 ; ** Significant at 0.01

4.6.1.3 Curcumin Content in Turmeric Cultivars Susceptible to Leaf Blotch and Leaf Spot

To study the variability in curcumin content between turmeric cultivars susceptible to *Taphrina* leaf blotch or *Colletotrichum* leaf spot, six cultivars under each group were selected and the curcumin content was estimated from cured rhizomes as described in Materials and Methods.

Curcumin

The curcumin content in the cured rhizomes of cultivars susceptible to leaf blotch (Fig. 4) was much higher than that in cured rhizomes of leaf spot susceptible (Fig. 5) cultivars. The curcumin content varied in the cultivars susceptible to leaf blotch from 3.12 to 4.91% and from 1.89 to 2.38% in cultivars susceptible to leaf spot. The mean curcumin content in the cured rhizomes of cultivars susceptible to leaf blotch was 4.06% and in the cured rhizomes of cultivars susceptible to leaf spot it was 2.17%.

4.6.1.4 Variability in Isoenzyme Pattern between the Cultivars Susceptible to *Taphrina* Leaf Blotch and Cultivars Susceptible to *Colletotrichum* Leaf Spot

Polyacrylamide native gel electrophoresis was performed to distinguish isoenzymes in the leaf extracts of six leaf blotch susceptible cultivars and four leaf spot susceptible cultivars. The six leaf blotch susceptible cultivars were CLI 317, KTS 1, KTS 4, CLI 315, CLI 385 and PCT 13. The four leaf spot susceptible cultivars were Kasturi, KTS 3, CLI 325 and KTS 8.

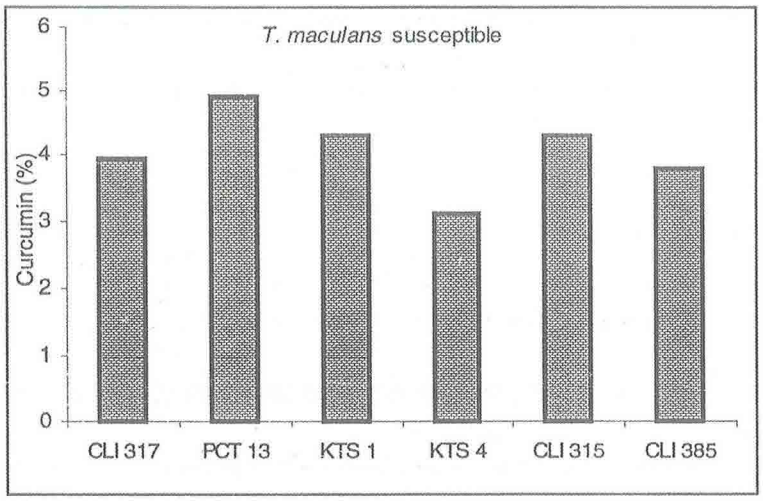

Figure 4: Curcumin content in rhizomes of plants from six cultivars damaged by leaf blotch disease

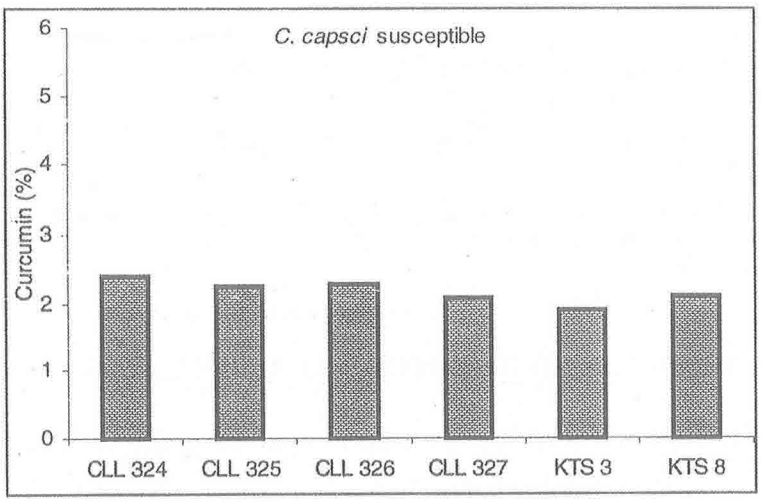

Figure 5: Curcumin content in rhizomes of plants from six cultivars damaged by leaf spot disease

Polyphenol Oxidase Isoenzyme Pattern

The polyphenol oxidase isoenzyme pattern (Plate 22) was similar in all the turmeric cultivars with a perceptible band of the same relative mobility (rm) except in Kasturi. Kasturi, which represents *Curcuma aromatica* showed an additional band of a higher relative mobility.

Esterase Isoenzyme Pattern

The esterase isoenzyme pattern (Plate 23) showed differences among the turmeric cultivars. All the leaf blotch susceptible cultivars except PCT 13 (10) showed the same number of bands and the matching bands had the identical relative mobility (rm). PCT 13 showed the highest number of bands (seven) of which only three bands matched with bands in the other leaf blotch susceptible cultivars.

The leaf spot susceptible turmeric cultivars KTS 3, CLI 325 and KTS 8 showed an identical pattern with both the number and relative mobility of bands matching. The other leaf spot susceptible cultivar, Kasturi (1) showed a distinctly different isoenzyme pattern. Of its two dark bands, one had a similar relative mobility as one of the bands in leaf spot susceptible cultivars and the other matched with one of the bands in leaf blotch susceptible cultivars. These two bands, however, matched two bands of PCT 13 with corresponding relative mobility.

4.7 PLANTING TIME AND LEAF BLOTCH DISEASE SEVERITY

To find out if the change in time of planting will have an effect on the leaf blotch severity and mother and finger rhizome yield in turmeric, an experiment was conducted by planting leaf blotch susceptible cultivar (CLI 317) on eight different dates at fortnightly interval starting from the first fortnight of May. The experiment was

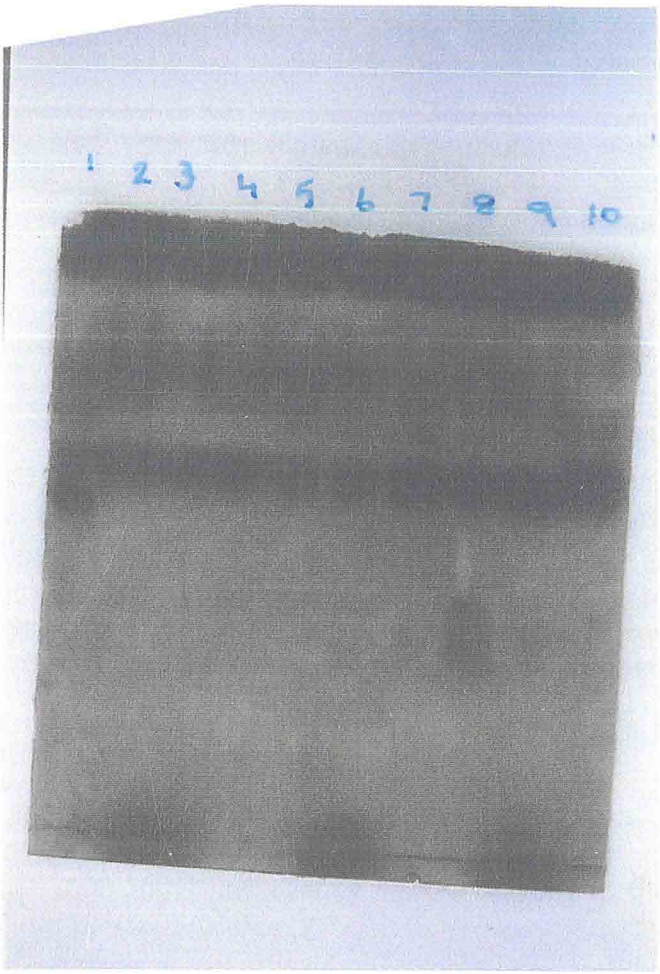

Plate 22 Polyphenol oxidase isoenzyme pattern in turmeric cultivars susceptible to *Colletotrichum* leaf spot (1 to 4) and *Taphrina* leaf blotch (5 to 10): 1.Kasturi, 2.KTS 3, 3.CLL 325, 4.KTS 8, 5.CLI 317, 6.KTS 1, 7.KTS 4, 8.CLI 315, 9.CLI 385, 10.PCT 13

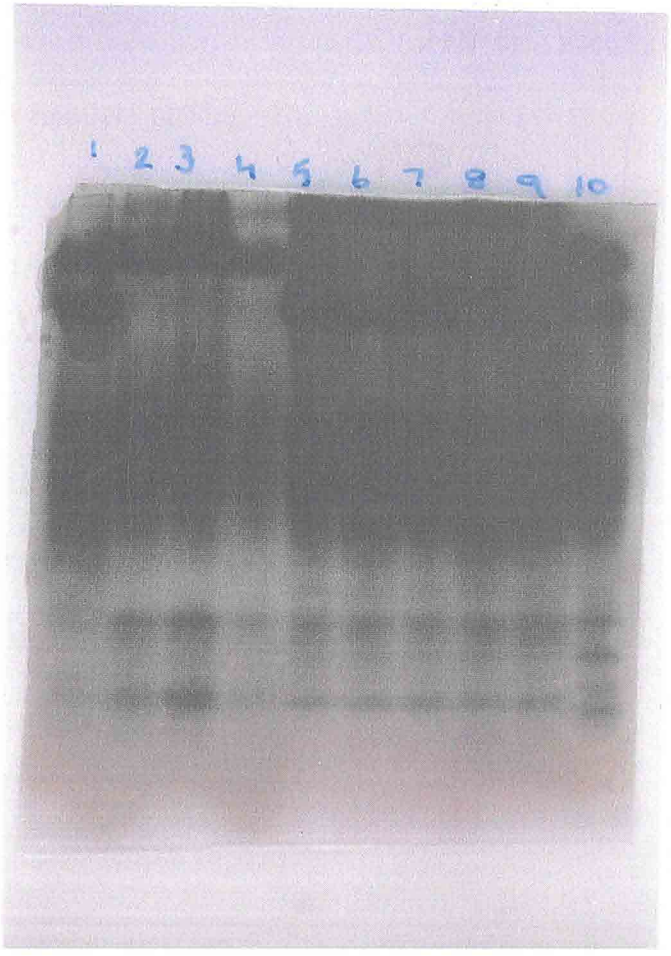

Plate 23 Esterase isoenzyme pattern in turmeric cultivars *Colletotrichum* leaf spot (1 to 4) and *Taphrina* leaf blotch (5 to 10): 1.Kasturi, 2.KTS 3, 3.CLL 325, 4.KTS 8, 5.CLI 317, 6.KTS 1, 7.KTS 4, 8.CLI 315, 9.CLI 385, 10.PCT 13

replicated four times and conducted in 1999-2000 and 2000-01. The two plantings in May were early while the three plantings, one made in the second fortnight of July and two in August were late compared to the normal time of planting followed in the region. The turmeric crop is normally planted in the region from the first fortnight of June to the end of first fortnight of July.

The leaf blotch severity (PDI) and yield recorded in both the years are presented in Table 12. The trend in leaf blotch disease incidence and severity was more or less similar during 1999-2000 and 2000-2001. The minimum level was 39% and the maximum was about 64%. In general, maximum disease severity was observed in the early planted crop i.e., planted in the first fortnight of May. In each successive planting, the leaf blotch severity (PDI) was significantly less in both the years. However, during 2000-2001, the level of disease severity in the first three plantings did not show any statistically significant differences.

The pooled data also showed a reduction in mean PDI with each successive delayed planting beginning the first fortnight of May. The mean disease severity in the two fortnightly plantings made in May did not differ significantly from one another, but showed significant differences with later plantings (Table 12).

In general, mother rhizome and finger rhizome yields decreased with delay in planting from the first fortnight of May to the second fortnight of August. In 1999-2000, the maximum mother, finger and total rhizome yields were recorded in the earliest planting made in the first fortnight of May. There was a steady decline in yields in the subsequent plantings except for minor increase in June first fortnight planting, which however was not significant.

During 2000-01, the yield of mother rhizomes decreased with delay in planting time upto the first fortnight of June, showed an increase in the next two plantings and declined steadily in the subsequent plantings. The finger and total rhizome yields also showed a decrease in yield with delay in plantings although there were fluctuations in the plantings made upto the second fortnight of June.

The pooled data also showed a general decline in the mother, finger and total rhizome yields with delay in planting from the second fortnight of May with minor and non-significant fluctuations. However, the finger and total rhizome yields in the planting of first fortnight of May was significantly lower than that recorded in the second fortnight planting of May (Table 13).

Table 12: Leaf blotch severity (PDI) in susceptible turmeric cultivar (CLI 317) planted at different dates

Date of planting*	Early /normal /late	Per cent disease index (PDI)		
		1999-2000	2000-01	Pooled
May I	Early	65.50 (82.78)	62.52 (78.61)	64.01 (80.69)
May II	Early	62.29 (79.72)	63.18 (79.63)	63.23 (79.67)
June I	Normal	58.92 (73.33)	62.40 (78.52)	60.66 (75.93)
June II	Normal	54.25 (65.83)	61.01 (76.48)	57.63 (71.16)
July I	Normal	49.65 (58.05)	49.04 (57.03)	49.35 (57.54)
July II	Late	45.00 (50.00)	45.53 (50.92)	45.26 (50.46)
August I	Late	40.04 (41.39)	41.81 (44.44)	40.92 (42.91)
August II	Late	38.90 (39.44)	38.58 (38.89)	38.74 (39.16)
S.Em		0.917	0.655	0.796
CD (0.05)		2.70	1.93	1.61

* I = first fortnight; II = second fortnight; Figures in parentheses are pre-transformed values

Table 13 : Mother (MR) and finger (FR) rhizome yield (t ha $^{-1}$) in susceptible turmeric cultivar (CLI 317) planted at different dates

Date of planting*	Early /normal /late	1999-2000			2000-01			Pooled		
		MR	FR	Total	MR	FR	Total	MR	FR	Total
May I	Early	6.46	18.79	25.25	3.92	20.50	24.22	5.19	19.64	24.83
May II	Early	5.23	16.20	21.43	3.77	28.79	32.56	4.50	22.50	27.00
June I	Normal	5.34	18.00	23.34	3.62	23.32	26.94	4.48	20.66	25.14
June II	Normal	4.37	14.47	18.83	4.38	28.00	32.38	4.37	21.23	25.60
July I	Normal	3.15	14.84	17.99	5.31	22.70	28.02	4.23	18.77	23.00
July II	Late	3.62	12.81	16.46	3.52	16.81	20.82	3.58	14.81	18.64
August I	Late	2.27	10.48	12.75	3.28	14.17	17.45	2.77	12.32	15.10
August II	Late	0.73	3.25	3.98	1.29	5.95	7.24	1.01	4.60	5.61
S.Em		0.409	0.994	1.232	0.21	0.663	0.724	0.325	0.845	1.010
CD (0.05)		1.20	2.93	3.62	0.62	1.95	2.13	0.66	1.70	2.04

* I = first fortnight; II = second fortnight

4.8 PLANT DENSITY AND DISEASE SEVERITY

Population density as adjusted by spacing between rows and plants in relation to leaf blotch severity was investigated. At three levels of population density and four spacing adjustments leaf blotch severity was recorded on susceptible turmeric cultivar CLI 317 at an interval of 30 days beginning from 90 days after planting.

During 1999-2000, there were no significant differences in PDI in different population density levels at 90 days after planting. Later at 120, 150 and 180 days after planting, population densities significantly influenced PDI. Disease severity was low at a population density of 24691 plants ha^{-1} (90 x 45 cm), (52 PDI at 180 DAP), while it was high at population densities of 49383 (45 x 45 cm) and 98765 (45 x 22.5 cm) plants ha^{-1} (73 to 76 PDI at 180 DAP) (Table 14a).

During 2000-2001, there were significant differences in the leaf blotch severity at different population densities on all dates of observations. The maximum disease was observed at a population density of 98765 plants ha^{-1}; the level of disease recorded being about 81 PDI at 180 days after planting. At both plant densities of 24691 (90 x 45 cm) and 49383 (90 x 22.5 cm) plants ha^{-1}, the disease level was relatively low on all dates of observation (Table 14b).

In the pooled analysis, the trend observed in the individual years was confirmed. In general at a population density of 98765 plants ha^{-1}, the disease severity was comparatively higher, while at a population density of 24691 (90 x 45 cm) the leaf blotch severity was relatively less (Table 14c). Though the plant population density was same at 49383 plants ha^{-1}, wider inter tow spacing and narrow intra row spacing of 90 x 22.5 cm recorded less disease severity than 45 x 45 cm spacing.

Table 14a: Leaf blotch severity (PDI) in susceptible turmeric cultivar (CLI 317) planted at different plant population densities, 1999-2000

Plant population density ha $^{-1}$	Spacing (cm)	90DAP	120DAP	150DAP	180DAP
98765	45 x 22.5	24.92 (17.77)	39.48 (40.44)	45.38 (50.66)	60.43 (75.55)
24691	90 x 45	22.84 (15.11)	32.22 (28.44)	37.65 (37.33)	46.15 (52.00)
49383	90 x 22.5	25.21 (18.22)	38.97 (39.55)	40.26 (41.78)	50.00 (58.16)
49383	45 x 45	25.53 (18.66)	39.23 (40.00)	44.36 (48.88)	58.93 (73.33)
S.Em		0.846	0.526	0.68	1.251
CD (0.05)		NS	1.62	2.1	3.85

DAP = Days after planting; Parentheses - pre-transformed values; NS : Not Significant

Table 14b: Leaf blotch severity (PDI) in susceptible turmeric cultivar (CLI 317) planted at different plant population densities, 2000-2001

Plant population density ha $^{-1}$	Spacing (cm)	90DAP	120DAP	150DAP	180DAP
98765	45 x 22.5	27.27 (21.04)	37.03 (36.30)	55.92 (68.59)	64.52 (81.48)
24691	90 x 45	23.91 (16.44)	30.31 (25.48)	45.82 (51.43)	49.73 (58.22)
49383	90 x 22.5	24.58 (17.33)	32.32 (28.59)	46.91 (53.33)	50.69 (59.85)
49383	45 x 45	25.91 (19.11)	34.72 (32.44)	51.56 (61.33)	53.75 (65.04)
S.Em		0.429	0.426	0.534	0.494
CD (0.05)		1.32	1.31	1.64	1.52

DAP = Days after planting; Figures in parentheses are pre-transformed values

Table 14c: Leaf blotch severity (PDI) in susceptible turmeric cultivar (CLI 317) planted at different plant population densities, pooled for 1999-2000 and 2000-2001

Plant population density ha $^{-1}$	Spacing (cm)	90DAP	120DAP	150DAP	180DAP
98765	45 x 22.5	26.09 (19.40)	38.26 (38.37)	50.65 (59.63)	62.48 (78.52)
24691	90 x 45	23.38 (15.78)	31.27 (26.96)	41.73 (44.38)	47.94 (55.11)
49383	90 x 22.5	24.90 (17.77)	35.64 (34.07)	43.59 (47.55)	50.34 (59.26)
49383	45 x 45	25.72 (18.99)	36.97 (36.22)	47.96 (55.11)	56.34 (69.18)
S.Em		0.67	0.479	0.611	0.951
CD (0.05)		1.38	0.99	1.26	1.96

DAP = Days after planting; Figures in parentheses are pre-transformed values

AUPDC as Influenced by Plant Population Density

Area under disease progress curve (AUDPC) values for leaf blotch disease progress at different plant population densities were also calculated. The highest AUDPC values were recorded at a plant population density of 98765 plants ha^{-1}. The lowest AUDPC values were recorded at 24691 plants ha^{-1}. There were significant differences in the three plant population density levels. The maximum and minimum AUDPC values were relatively higher during 2000-2001 when compared to 1999-2000 (Table 15).

Table 15: Area under diseases progress curve (AUDPC) values of leaf blotch disease in susceptible turmeric cultivar (CLI 317) planted at different plant population densities

Plant population density ha^{-1}	Spacing (cm) l	1999-2000	2000-01	Pooled
98765	45 x 22.5	4946.25	5390.30	5168.28
24691	90 x 45	4157.17	4462.34	4309.76
49383	90 x 22.5	4771.79	4603.77	4687.78
49383	45 x 45	4921.15	4946.13	4933.64
S.Em		59.95	37.82	50.11
CD (0.05)		184.73	116.52	103.42

Vegetative Characters as Influenced by Plant Population Density

There were significant differences among the treatments in respect of plant height and number of tillers under the influence of epidemics of *T. maculans* during the two years of study. The maximum plant height was recorded at a population density of 98765 plants ha^{-1}, but the least number of tillers were recorded in this treatment during both the years. The minimum plant height and maximum number of tillers were recorded in both the years at a population density of 24691 plants ha^{-1} (Table 16a).

Table 16a: Changes in plant height and tillers in susceptible turmeric cultivar (CLI 317) infected by *T. maculans* at different population densities

Plant density ha $^{-1}$	Spacing	Plant height (cm)			Number of tillers		
		1999-2000	2000-01	Pooled	1999-2000	2000-01	Pooled
98765	45 x 22.5	61.76	43.89	52.82	3.44	3.48	3.46
24691	90 x 45	52.68	38.04	45.36	7.56	5.00	6.28
49383	90 x 22.5	55.12	42.00	48.26	4.52	3.84	4.18
49383	45 x 45	56.40	40.59	48.50	5.40	3.72	4.56
S.Em		0.874	0.859	0.866	0.232	0.255	0.244
CD (0.05)		2.69	2.65	1.79	0.72	0.79	0.50

PDI at 150 DAP was presented in tables 14a 14b 14c
Vegetative characters recorded at 150 days after planting

Leaf length and leaf width differed significantly at different plant population density levels only during 1999-2000. While the leaf length was significantly longer at 98765 plants ha^{-1}, the leaf width was significantly wider at a plant population density of 24691 plants ha^{-1} (Table 16b). Pooled analysis of the data showed significant differences in the leaf length and leaf width at different plant population densities. The trend was similar to that observed during 1999-2000. The disease severity (PDI) at 150 days after planting (DAP) did not appear to have any relation with vegetative characters at different population densities.

Table 16b: Changes in leaf length and leaf width in susceptible turmeric cultivar (CLI 317) infected by *T. maculans* at different plant population densities

Plant population density ha $^{-1}$	Spacing	Leaf length (cm)			Leaf width (cm)		
		1999-2000	2000-01	Pooled	1999-2000	2000-01	Pooled
98765	45 x 22.5	70.64	56.06	63.35	15.66	14.18	14.92
24691	90 x 45	63.80	52.52	58.16	18.46	15.00	16.73
49383	90 x 22.5	63.88	54.20	59.04	16.60	14.66	15.63
49383	45 x 45	68.32	53.88	61.10	17.08	14.84	15.96
S.Em		0.922	0.806	0.866	0.230	0.197	0.215
CD (0.05)		1.30	NS	1.79	0.71	NS	0.44

Vegetative characters recorded at 150 days after planting; NS : Not Significant
PDI at 150 DAP was presented in tables 14a, 14b, 14c

The maximum significant rhizome yield (mother, finger and total rhizome yields) was recorded at a plant population density of 98765 plants ha^{-1}. The mother, finger and total rhizome yield were much less at a plant population density of 24691 plants ha^{-1} in both the years of study. In general, the total rhizome yields harvested during 1999-2000 in different treatments were relatively higher compared to the harvests made during 2000-2001 (Table 17).

Table 17: Mother (MR) and finger (FR) rhizome yield (t ha^{-1}) in susceptible turmeric cultivar (CLI 317) planted at different plant population densities

Plant population density ha^{-1}	Spacing (cm)	1999-2000			2000-01			Pooled		
		MR	FR	Total	MR	FR	Total	MR	FR	Total
98765	45 x 22.5	11.98	45.16	57.14	8.45	28.40	36.84	10.21	36.78	46.99
24691	90 x 45	6.48	27.81	34.29	5.27	16.46	21.73	5.88	22.14	28.01
49383	90 x 22.5	7.63	30.81	38.44	5.25	23.87	29.11	6.44	27.34	33.78
49383	45 x 45	10.54	43.23	53.77	7.47	27.73	35.20	9.00	35.48	44.49
S.Em		0.615	0.687	1.183	0.468	1.695	2.010	0.546	1.346	1.647
CD (0.05)		1.89	2.67	3.64	1.44	5.22	6.18	1.13	2.78	3.40

PDI at 180 DAP was presented in tables 14a, 14b, 14c

4.9 MANAGEMENT OF *Taphrina* LEAF BLOTCH IN TURMERIC

4.9.1 Management of *Taphrina* Leaf Blotch of Turmeric with Fungicides

An experiment to evaluate fungicides for their efficiency in the management of *T.maculans* leaf blotch was conducted during 1998-99 and 1999-2000 with seven treatments and four replications.

All the fungicide treatments were found to significantly reduce disease severity (PDI) during the two years compared to control (Table 18). Propiconazole (0.1%) and bitertenol (0.1%) during 1998-99 and propiconazole (0.1%), bitertenol (0.1%) and chlorothalonil (0.2%) during 1999-2000 were on a par and found significantly superior

to other fungicidal treatments in reducing disease severity. The mean disease severity in pooled data ranged between 35 PDI in propiconazole treated plots and 73 PDI in control plots (Plate 24). Phytotoxic symptom in the form of slight scorching of margins of lamina at the leaf tip was observed in plots sprayed with copper oxychloride (0.25%).

In general, all the fungicide treatments were found to significantly increase the yield of rhizomes (Table 19). The highest yield was obtained with propiconazole (0.1%) which was on a par with the yield recorded in bitertenol (0.1%), and chlorothalonil (0.2%) treated plots in the two years studied. The yield obtained in kitazin (0.2%) and copper oxychloride (0.25%) treated plots did not differ significantly with each other in both the years. The tridemorph (0.1%) treated plots recorded rhizome yield on a par with the highest yield recorded in propiconazole (0.1%) treated plots during 1998-99, but it was significantly lower than the yield obtained with the three best treatments during 1999-2000. The mean total yields of rhizomes in the pooled data showed no significant differences among propiconazole, bitertenol and chlorothalonil treatments but were significantly superior to others. These three treatments increased the mean total yield of rhizomes by 44 to 53% over the yield in control plots.

Table 18: Leaf blotch severity (PDI) in susceptible turmeric cultivar (CLI 317) following application of fungicides

Treatments	Per cent disease index (PDI)		
	1998-99	1999-2000	Pooled
Bitertenol (0.1%)	32.86 (29.44)	40.52 (42.22)	36.69 (35.83)
Chlorothalonil (0.2%)	35.42 (33.61)	41.27 (43.52)	38.34 (38.56)
Propiconazole (0.1%)	31.41 (27.22)	40.41 (42.04)	35.91 (34.63)
Kitazin (0.2%)	43.40 (47.22)	53.03 (63.30)	48.22 (55.51)
Tridemorph (0.1%)	35.76 (34.16)	48.20 (55.25)	41.98 (44.86)
Copper oxychloride (0.25%)	39.51 (40.55)	50.98 (60.28)	45.24 (50.42)
Control	52.36 (62.50)	66.34 (83.89)	59.35 (73.19)
S.Em	1.48	1.35	1.48
CD (0.05)	4.41	4.02	2.88

Figures in parentheses are pre-transformed values

Plate 24. Fungicide in the control of leaf blotch in turmeric. On the left treatment with propiconazole and on the right, blotch damaged check

Table 19: Mother (MR) and finger (FR) rhizome yield (t ha^{-1}) in susceptible turmeric cultivar (CLI 317) following application of fungicides

Treatments	1998-99			1999-2000			Pooled		
	MR	FR	Total	MR	FR	Total	MR	FR	Total
Bitertenol (0.1%)	7.53	37.62	45.14	9.07	34.63	43.69	8.30	36.12	44.42
Chlorothalonil (0.2%)	7.14	37.62	44.75	9.74	33.76	43.50	8.44	35.69	44.13
Propiconazole (0.1%)	7.33	41.28	48.61	9.65	35.50	45.14	8.49	38.39	46.87
Kitazin (0.2%)	7.14	32.41	39.54	8.11	27.30	35.40	7.62	29.85	37.47
Tridemorph (0.1%)	6.94	36.65	43.60	8.01	29.51	37.52	7.47	33.08	40.56
Copper oxychloride (0.25%)	5.79	30.29	36.08	8.68	26.81	35.49	7.23	28.55	35.78
Control	5.79	24.11	29.90	8.20	23.05	31.25	6.99	23.58	30.57
S.Em	0.224	1.74	1.85	0.435	0.848	0.916	0.346	1.369	1.46
CD (0.05)	0.67	5.17	5.50	1.29	2.52	2.72	0.70	2.78	2.96

4.9.1.1 Estimates on Yield Loss Caused by Leaf Blotch in Turmeric Using Data from Fungicide trial

In the experiment on the management of leaf blotch disease, all applied fungicides brought about a change in disease severity along with a change in rhizome yield depending on their efficacy. The yield loss due to leaf blotch disease in susceptible turmeric cultivar (CLI 317) was estimated using data on PDI and rhizome yield. For this purpose a new database was created by pooling all the treatment observations from all replications on the two parameters viz., PDI and yield, recorded in both years under this study. The angular transformed values of PDI did not improve the model and hence, the original values only were used in the estimation of the yield loss.

Regression analysis was performed and a linear regression model constructed as given below:

$$y = 54.621^{*} - 0.308^{**}x$$

where y = yield; x = disease severity(PDI); * = significant at P= *0.01* and ** = significant at P= 0.05. The coefficient of determination was highly significant (R^2 = 0.599) and the mean square error was less (MSE = 4.62). This linear model (Fig. 6) predicted 308 kg ha ^{1} loss in rhizome yield for every one per cent disease index (PDI).

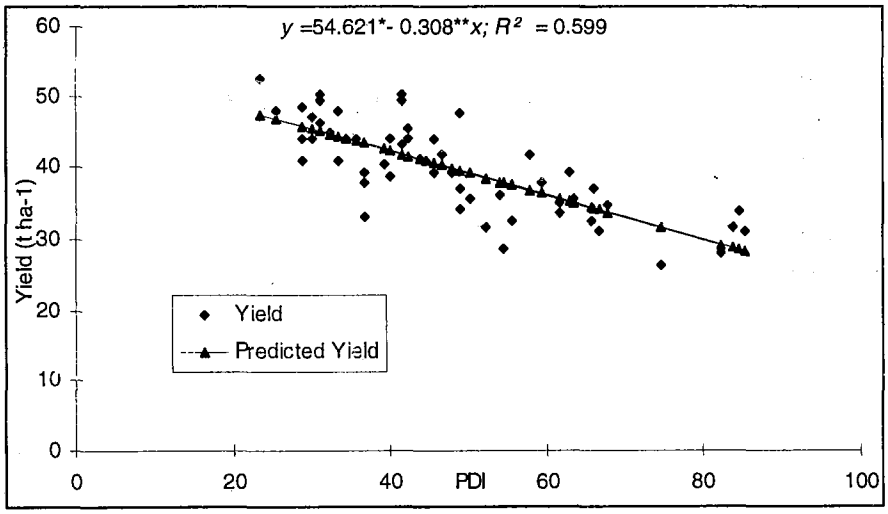

Figure 6: Relationship between the rhizome yield in turmeric and per cent disease index (PDI) caused by *Taphrina maculans*

4.9.2 Effect of Micronutrients on *T .maculans* Leaf Blotch Severity

To find out whether the micronutrients have any effect on the leaf blotch disease and yield of turmeric, an experiment was conducted with three foliar micronutrient treatments during 1998-99 and 1999-2000. The experiment was replicated four times. The observations on disease severity (PDI) and yield of fresh rhizomes are presented in Tables 20 and 21.

The PDI recorded in micronutrient treatments such as zinc sulpuhate (0.2%) and ferrous sulphate (0.2%) were on a par with one another and significantly lower than that recorded in the micronutrient treatment magnesium sulphate (0.2%) and control. Both control plot and plot treated with magnesium sulphate did not differ significantly in both the years. The pooled data also showed a similar trend. The mean leaf blotch disease severity ranged between 55 and 70 PDI (Table 20).

The yield of rhizomes in all the micronutrient treatments was significantly higher than in the control in 1998-99. The significantly highest yield of rhizomes (44 t ha^{-1}) was obtained with zinc sulphate (0.2%). The total yield obtained in ferrous sulphate and magnesium sulphate treatments did not differ significantly. During 1999-2000, only zinc sulphate gave significantly higher yield than control. However, it did not differ significantly with the yield obtained in ferrous sulphate treatment. The pooled data also showed that the highest mean yield (39.40 t ha^{-1}) was obtained with zinc sulphate (Table 21).

The micronutrient treatments, zinc sulphate and ferrous sulphate recorded significantly less disease severity with significant increase in yield over control.

Table 20: Leaf blotch severity (PDI) in susceptible turmeric cultivar (CLI 317) following application of micronutrients

Treatments	Per cent disease index (PDI)		
	1998-99	1999-2000	Pooled
Zinc sulphate (0.2%)	40.52(42.22)	55.02(67.03)	47.77(54.63)
Ferrous sulphate (0.2%)	42.13(44.99)	59.04(73.33)	50.58(59.16)
Magnesium sulphate (0.2%)	47.41(54.16)	63.53(83.85)	55.47(67.08)
Control	49.02(56.97)	66.54(83.85)	57.68(70.41)
S.Em	1.08	1.61	1.369
CD (0.05)	3.45	5.14	2.88

Figures in parentheses are pre-transformed values

Table 21: Mother (MR) and finger (FR) rhizome yield (t ha^{-1}) in susceptible turmeric cultivar (CLI 317) following application of micronutrients

Treatments	1998-99			1999-2000			Pooled		
	MR	FR	Total	MR	FR	Total	MR	FR	Total
Zinc sulphate (0.2%)	6.94	37.04	43.98	8.20	26.62	34.82	7.57	31.83	39.40
Ferrous sulphate (0.2%)	5.79	31.83	37.62	7.91	25.27	33.18	6.85	28.55	35.40
Magnesium sulphate (0.2%)	5.40	30.48	35.88	7.23	22.67	29.90	6.32	26.57	32.89
Control	5.63	24.80	30.43	7.70	23.25	30.95	6.67	24.03	30.70
S.Em	0.201	1.271	1.227	0.28	0.964	1.05	0.241	1.104	1.14
CD (0.05)	0.64	4.06	3.93	NS	3.09	3.37	0.51	2.32	2.40

NS : Not significant

4.9.3 Economics of Fungicide and Micronutrient Treatments

The economics for each of the fungicide and micronutrient treatments was calculated based on mean yield from the pooled analysis and presented in Table 22. All the fungicide and micronutrient treatments were economically beneficial relative to the control. The cost : benefit (C:B) ratio calculated revealed that propiconazole gave the best economic returns among fungicides. The return realized with Kitazin was the lowest. Among micronutrients, zinc sulphate (0.2%) gave the best C:B ratio. Magnesium sulphate was the least beneficial micronutrient studied.

The loss in yield in susceptible turmeric cultivar (CLI 317) due to *Taphrina* infection was as high as 35% relative to the best treatment i.e., propiconazole (0.1%). The additional yield obtained with propiconazole was the highest and at the market prices (1999), the additional monetary benefit was Rs. 34,845 ha^{-1}. The benefit derived was around Rs.8/- for every additional rupee invested on disease management with propiconazole. The other fungicide treatments also gave economical returns.

4.9.4 Effect of Barrier Crops on Leaf Blotch in Turmeric

The possibility of managing the leaf blotch disease by growing non host barrier crops all around the plots of leaf blotch susceptible turmeric cultivar was studied during 1998-99 and 1999-2000 with three barrier crop treatments and control replicated seven times. The barrier crop treatments were:

1. leaf blotch resistant turmeric cultivar (KTS 8);
2. elephant foot yam (*Amorphophallus*); and
3. taro (*Colocasia*).

Leaf blotch susceptible turmeric cultivar (CLI 317) without any barrier crop raised around it served as check.

Table 22: Comparative economics of fungicides and micronutrients used for the management of *Taphrina* leaf blotch of turmeric

Fungicide	Yield of fresh rhizomes (t ha^{-1})*	Yield of cured rhizomes (t ha^{-1})	Increase in yield of cured rhizomes over control (t ha^{-1})	Returns for the increased yield (Rs.)	Input cost + Labour charge (Rs.)	Net Returns (Rs.)	C.B. Ratio
Bitertenol (0.1%)	44.42	8.844	2.770	33240	4950	28290	5.72
Chlorothalonil (0.2%)	44.13	8.826	2.712	32554	5175	27369	5.29
Propiconazole (0.1%)	46.87	9.374	3.260	39120	4275	34845	8.15
Kitazin (0.2%)	37.47	6.694	0.580	6960	3600	3360	0.93
Tridemorph (0.1%)	40.56	8.112	1.998	23976	3038	20758	6.83
Copper oxych-loride (0.25%)	35.78	7.156	1.042	12504	2700	9804	3.63
Control	30.57	6.114	-	-	-	-	-
Micronutrients							
Zinc sulphate (0.2%)	39.40	7.880	1.740	20880	2475	18405	7.44
Ferrous sulphate (0.2%)	35.40	7.080	0.940	11280	2250	9030	4.01
Magnesium sulphate (0.2%)	32.89	6.578	0.438	5256	2250	3006	1.34
Control	30.70	6.140	-	-	-	-	-

* = Mean for 1998-99 and 1999-2000

Cost of cured rhizomes (1999-2000) = Rs.12000/= at Duggirala Market.

Number of sprays: Three sprays of 750 litres spray fluid each.

Cost of fungicides per kg or l = Bitertenol= Rs.1700.00; Chlorothalonil = Rs.900.00; Propiconazole = Rs.1400.00; Kitazin =Rs.550.00; Tridemorph = Rs.850; Copper oxychloride = Rs.250.00; Zinc sulphate = Rs.300.00; Ferrous sulphate = Rs.250.00 and Magnesium sulphate = Rs.250.00

The observations on disease severity (PDI) and yield of fresh rhizomes are presented in Tables 23 and 24. The barrier crops *Colocasia* and leaf blotch resistant turmeric cultivars increased disease severity significantly in the centrally located leaf blotch susceptible turmeric crop than in the crop that had no barrier. The plot surrounded by *Amorphophallus* in the two years of experimentation was very similar to control plots with no barrier crop in disease severity (Table 23). In both the years under study, the maximum yield in mother, finger and total rhizomes was recorded in control plots without any barrier. All treatments with barrier crops recorded significantly lower rhizome yields (Table 24).

Table 23: Leaf blotch severity (PDI) in susceptible turmeric cultivar (CLI 317) with barrier crops grown on all sides

Treatments	Per cent disease index (PDI)		
	1998-99	1999-2000	Pooled
No barrier crop	39.83 (41.11)	54.01 (65.39)	46.92 (53.25)
Amorphophallus	38.95 (39.52)	55.63 (68.04)	47.29 (53.78)
Colocasia	55.22 (67.30)	62.67 (78.73)	58.94 (73.01)
Resistant cultivar (KTS 8)	53.13 (63.97)	60.07 (75.02)	56.60 (69.49)
S.Em	1.297	0.837	1.092
CD (0.05)	3.85	2.49	2.22

Figures in parentheses are pre-transformed values

Table 24: Mother (MR) and finger (FR) rhizome yield (t ha^{-1}) in susceptible turmeric cultivar (CLI 317) with barrier crops grown on all sides

Treatments	1998-99			1999-2000			Pooled		
	MR	FR	Total	MR	FR	Total	MR	FR	Total
No barrier crop	7.61	49.27	56.88	10.86	44.31	55.17	9.23	46.79	56.02
Amorphophallus	4.85	28.88	33.73	10.20	39.02	49.22	7.52	33.95	41.48
Colocasia	5.29	29.87	35.16	9.37	34.23	43.60	7.33	32.05	39.38
Resistant cultivar (KTS 8)	5.29	28.55	33.84	10.41	37.92	48.34	7.85	33.25	41.09
S.Em	0.339	1.357	1.557	0.421	1.492	1.791	0.382	1.426	1.678
CD (0.05)	1.01	4.03	4.63	NS	4.43	5.32	0.78	2.90	3.41

NS : Not significant

4.10. INFLUENCE OF WEATHER ON *Curcuma longa-Taphrina maculans* PATHOSYSTEM

The per cent disease index was recorded at periodical interval of 10 days from the first appearance of *Taphrina maculans* on turmeric cultivar CLI 317. The data on the weather parameters such as maximum temperature (max), minimum temperature (min), rainfall (Rf), rainy days (Rd), relative humidity (RH08) and relative humidity (RH14) were recorded daily during the entire period of experimentation. This experiment was repeated for 3 years. Except for the rainfall, and rainy days, the averages of 10 days interval corresponding to the period of observation when PDI was recorded were calculated for use in statistical analysis. In case of rainfall and rainy days data, cumulative totals were calculated. The data on weather parameters and PDI recorded in the three years are presented in Tables 25,27 and 29.

Stepwise multiple regression analysis was performed using the following prediction equation:

$$y = b_o + b_1x_1 + b_2x_2 + b_3x_3 \ldots\ldots\ldots\ldots + b_nx_n$$

where y = percent disease index, b_o = intercept, $b_1, b_2 \ldots\ldots b_n$ = regression coefficient, and x_1, x_2, $\ldots\ldots\ldots\ldots\ldots\ldots x_n$ = independent variables. The goodness of fit of multiple regression models was evaluated by the coefficients of determination (R^2). For each year's data, simple linear regressions were also performed to find relation between PDI and chosen weather parameters.

4.10.1 Relationship of Weather with Disease Index 1998-99

The variation in the weather parameters during 1998-99 is shown in Table 25 and Figure 7. The correlation matrix and the regression equations for 1998-99 are presented in Table 26. Of the six weather variables, rainfall, rainy days, and relative humidity at 0800 and 1400h were negatively correlated with PDI ($P \leq 0.01$). Only maximum temperature showed a positive correlation ($P \leq 0.05$) with PDI. There was no significant correlation between minimum temperature and PDI. There were correlations among the weather variables like that of rainfall with rainy days and relative humidity, as well as maximum temperature with minimum temperature during 1998-99.

Stepwise regression yielded five distinct equations for prediction of PDI that were significant ($P \leq 0.01$), with R^2 values ranging from 0.898 to 0.847; these R^2 values decreased marginally with dropping of each independent parameter used in the stepwise regression. The best-fit equation was Eq. 5, using maximum and minimum temperature as independent variables to predict PDI. The partial regression coefficients were also highly significant ($P \leq 0.01$). Simple linear regression with either of the variables also produced significant ($P \leq 0.05$) equations for prediction of PDI, but R^2 values were much lower and explained only 14 to 30% variability in the observed PDI.

Table25: Data on weather parameters and per cent disease index recorded during 1998-99

Date	Days after planting	x_1 Max	x_2 Min	x_3 Rf	x_4 Rd	x_5 RH08h	x_6 RH14h	y PDI
2-Nov	107	32.41	25.18	101.8	4	93.55	72.70	13.77
12-Nov	118	32.55	25.45	16.4	1	90.50	70.60	19.55
22-Nov	128	31.05	23.30	88.2	3	89.70	70.00	26.22
2-Dec	138	32.20	22.15	28.8	2	89.50	68.00	28.44
12-Dec	148	30.75	19.25	0	0	88.30	64.90	35.11
22-Dec	158	30.35	18.40	0	0	84.60	63.50	38.22
2-Jan	168	29.86	18.18	0	0	86.70	65.00	42.66
12-Jan	179	29.85	18.80	0	0	87.30	65.20	48.00
22-Jan	189	30.25	18.90	0	0	84.40	66.10	58.66
2-Feb	199	31.95	19.23	0	0	85.73	65.90	64.00
12-Feb	210	33.30	20.95	0	0	87.40	63.70	69.77
22-Feb	220	35.00	20.60	0	0	79.10	61.90	72.00
2-Mar	230	36.25	21.94	0	0	83.60	63.40	73.33
12-Mar	238	36.60	23.15	0	0	85.40	64.90	74.66
Mean		32.31	21.11	16.8	0.71	86.84	66.13	47.46
Standard Error		0.60	0.66	9.17	0.35	0.94	0.83	5.69
Minimum		29.85	18.18	0	0	79.10	61.90	13.77
Maximum		36.60	25.45	101.8	4	93.55	72.70	74.66

Max = maximum temperature; Min = minimum temperature; Rf = rainfall; Rd = rainy day; RH08h = relative humidity at 0800h; RH14h = relative humidity at 1400h; PDI = per cent disease index

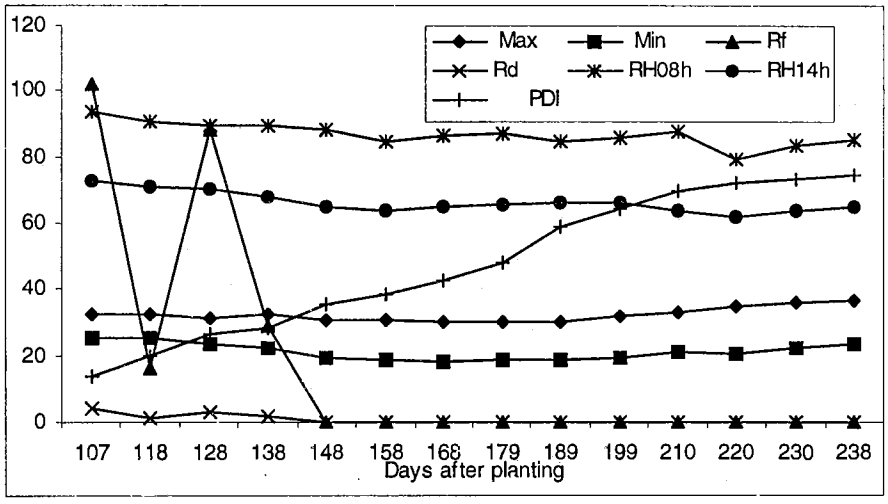

Figure 7. Changes in meteorological conditions over time from 2-11-1998 to 12-3-99; 14 observations on 10-day mean values

Table 26: Correlation matrix, stepwise regression and linear regression analyses of weather parameters on per cent disease index in *Taphrina maculans–Curcuma longa* pathosystem 1998-99

Correlation matrix:

Paramerter	x_1 Max	x_2 Min	x_3 Rf	x_4 Rd	x_5 RH08h	x_6 RH14h
x_1	*1.0000*					
x_2	*0.4862*	*1.0000*				
x_3	*-0.0998*	*0.6434*	*1.0000*			
x_4	*-0.0867*	*0.6874*	*0.9774*	*1.0000*		
x_5	*-0.3363*	*0.5357*	*0.6829*	*0.7316*	*1.0000*	
x_6	*-0.2291*	*0.6989*	*0.8229*	*0.8627*	*0.8644*	*1.0000*
y (PDI)	*0.5498**	*-0.3777ns*	*-0.6643***	*-0.7202***	*-0.8026***	*-0.7987***

Stepwise regression:

$y = -264.18 + 9.867^{**} x_1 - 7.97 x_2 + 0.502 x_3 - 18.11 x_4 - 0.533 x_5 + 3.15 x_6$ *Eq. 1*

($R^2 = 0.898$; Adj $R^2 = 0.811$; $F = 10.29^{**}$)

$y = -295.96 + 10.20^{**} x_1 - 8.06^{*} x_2 + 0.524 x_3 - 18.71 x_4 + 2.85 x_6$ *Eq. 2*

($R^2 = 0.896$; Adj $R^2 = 0.832$; $F = 13.86^{**}$)

$y = -93.66 + 7.805^{**} x_1 - 5.10^{*} x_2 + 0.489 x_3 - 16.24 x_4$ *Eq. 3*

($R^2 = 0.884$; Adj $R^2 = 0.833$; $F = 17.20^{**}$)

$y = -90.20 + 8.05^{**} x_1 - 5.71^{*} x_2 - 3.06 x_4$ *Eq. 4*

($R^2 = 0.858$; Adj $R^2 = 0.815$; $F = 20.11^{**}$)

$y = -91.27 + 9.04^{**} x_1 - 7.27^{**} x_2$ *Eq. 5*

($R^2 = 0.847$; Adj $R^2 = 0.819$; $F = 30.47^{**}$)

Simple linear regression:

$y = -119.79 + 5.18^{*} x_1$ *Eq. 6*

($R^2 = 0.302$; Adj $R^2 = 0.244$; $F = 5.20^{**}$)

$y = 116.08 - 3.25 x_2$ *Eq. 7*

($R^2 = 0.142$; Adj $R^2 = 0.071$; $F = 1.99$)

4.10.2 Relationship of Weather with Disease Index 1999-2000

The variation in the weather parameters during 1999-2000 is shown in Table 27 and Figure 8. The correlation matrix and the regression equations for 1999-2000 are presented in Table 28. All the six weather variables were negatively correlated with PDI ($P \leq 0.01$). There was no significant correlation between minimum temperature and PDI. There were correlations among the weather variables like that of rainfall with rainy days and relative humidity, as well as maximum temperature with minimum temperature, and minimum temperature and relative humidity (RH14h) during 1999-2000.

Stepwise regression yielded four distinct equations for prediction of PDI that were significant ($P \leq 0.01$), with R^2 values ranging from 0.902 to 0.899; these R^2 values decreased marginally with dropping of each independent parameter used in the stepwise regression. The best-fit equation was Eq. 4, using maximum temperature, rainfall and relative humidity (RH08h) as independent variables to predict PDI. The partial regression coefficients for maximum temperature and relative humidity (RH08h) were also highly significant ($P \leq 0.01$). Simple linear regression with each of these three variables in the best fit equation also produced significant ($P \leq 0.01$) equations for prediction of PDI, but R^2 values were much lower and explained only 47 to 52% variability in the observed PDI.

Table 27:Data on weather parameters and per cent disease index recorded during 1999-2000

Date	Days after planting	x_1 Max	x_2 Min	x_3 Rf	x_4 Rd	x_5 RH08h	x_6 RH14h	y PDI
5-Sep	63	32.50	25.45	73.4	5	92.09	74.00	16.66
15-Sep	74	33.20	26.30	30.6	2	91.40	73.90	16.66
25-Sep	84	35.85	26.70	10.6	3	89.20	70.90	20.00
5-Oct	94	32.25	26.10	78.4	5	91.90	76.70	28.88
15-Oct	104	32.65	25.50	73	5	88.10	74.30	34.44
25-Oct	114	33.55	26.50	46.1	3	85.80	71.20	40.00
5-Nov	124	32.77	24.91	32	3	85.70	70.30	51.11
15-Nov	135	32.85	22.60	0	0	84.00	62.90	51.11
25-Nov	145	32.05	21.75	0	0	89.00	65.30	58.88
5-Dec	155	31.60	20.80	0	0	87.60	66.50	60.00
15-Dec	165	30.65	19.35	0	0	84.90	64.20	62.22
25-Dec	175	30.30	19.35	0	0	88.00	65.50	63.33
5-Jan	185	30.95	19.27	0	0	85.00	68.45	67.77
Mean		32.40	23.43	26.47	2	87.90	69.55	43.93
Standard Error		0.40	0.83	8.75	0.59	0.76	1.23	5.22
Minimum		30.30	19.27	0	0	84.00	62.90	16.66
Maximum		35.85	26.70	78.4	5	92.09	76.70	67.77

Max = maximum temperature; Min = minimum temperature; Rf = rainfall; Rd = rainy day; RH08h = relative humidity at 0800h; RH14h = relative humidity at 1400h; PDI = per cent disease index

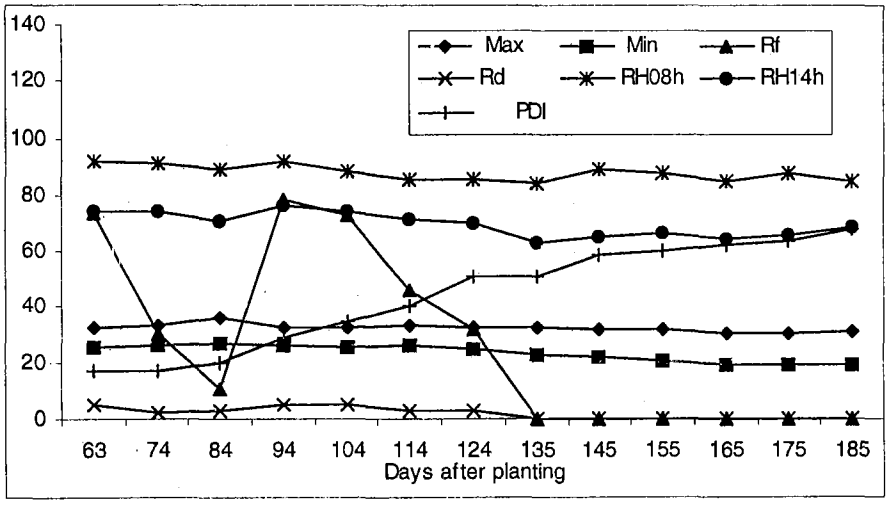

Figure 8. Changes in meteorological conditions over time from 5-7-1999 to 5-1-2000; 14 observations on 10-day mean values

Table 28: Correlation matrix, stepwise regression and linear regression analyses of weather parameters on per cent disease index in *Taphrina maculans–Curcuma longa* pathosystem 1999-2000

Correlation matrix:

Paramerter	x_1 Max	x_2 Min	x_3 Rf	x_4 Rd	x_5 RH08h	x_6 RH14h
x_1	*1.0000*					
x_2	*0.8184*	*1.0000*				
x_3	*0.2590*	*0.7252*	*1.0000*			
x_4	*0.4817*	*0.8288*	*0.9498*	*1.0000*		
x_5	*0.2384*	*0.5096*	*0.5663*	*0.5711*	*1.0000*	
x_6	*0.4107*	*0.7849*	*0.8833*	*0.9021*	*0.6917*	*1.0000*
y (PDI)	*-0.7142***	*-0.8876***	*-0.6888***	*-0.7834***	*-0.7224***	*-0.7884***

Stepwise regression:

$y = 531.748 - 6.571\ x_1 - 0.7822\ x_2 - 0.251\ x_3 + 1.969\ x_4 - 2.773^{**}\ x_5 - 0.146\ x_6$ *Eq. 1*
$(R^2 = 0.902;\ Adj\ R^2 = 0.805;\ F = 9.26^{**})$

$y = 523.844 - 6.406\ x_1 - 0.901\ x_2 - 0.247\ x_3 + 1.765\ x_4 - 2.824^{*}\ x_5$ *Eq. 2*
$(R^2 = 0.902;\ Adj.R^2 = 0.833;\ F = 12.95^{**})$

$y = 557.808 - 7.911^{**}\ x_1 - 0.3305\ x_3 + 2.427\ x_4 - 2.886^{**}\ x_5$ *Eq. 3*
$(R^2 = 0.902;\ Adj\ R^2 = 0.852;\ F = 18.35^{**})$

$y = 527.179 - 7.009^{**}\ x_1 - 0.188^{*}\ x_3 - 2.858^{**}\ x_5$ *Eq. 4*
$(R^2 = 0.899;\ Adj\ R^2 = 0.865\ ;\ F = 26.66^{**})$

Simple linear regression:

$y = 348.03 - 9.39^{**}\ x_1$ *Eq. 5*
$(R^2 = 0.510;\ Adj\ R^2 = 0.465;\ F = 11.45^{**})$

$y = 54.81 - 0.41^{**}\ x_3$ *Eq. 6*
$(R^2 = 0.474;\ Adj\ R^2 = 0.427,\ F = 9.93^{**})$

$y = 478.889 - 4.95^{**}\ x_5$ *Eq. 7*
$(R^2 = 0.522;\ Adj\ R^2 = 0.478;\ F = 12.00^{**})$

4.10.3 Relationship of Weather with Disease Index 2000-20001

The variation in the weather parameters during 2000-01 is shown in Table 29 and Figure 9. The correlation matrix and the regression equations for 2000-01 are presented in Table 30. Of the six weather variables, minimum temperature, rainfall, rainy days, and relative humidity at 0800 and 1400h were negatively correlated with PDI ($P \leq 0.01$). There was no significant correlation between maximum temperature and PDI. There were correlations among the weather variables like that of maximum temperature with minimum temperature, as well as rainfall with rainy days and relative humidity.

Stepwise regression yielded four distinct equations for prediction of PDI that were significant ($P \leq 0.01$), with R^2 values ranging from 0.921 to 0.903; these R^2 values decreased marginally with dropping of each independent parameter used in the stepwise regression. The best-fit equation was Eq. 4, using minimum temperature, rainfall and relative humidity (RH 08h) as independent variables to predict PDI. The partial regression coefficients for minimum temperature and rainfall were also highly significant ($P \leq 0.01$). Simple linear regression with either of the variables also produced significant ($P \leq 0.01$) equations for prediction of PDI, but R^2 values were much lower and explained only 59 to 69% variability in the observed PDI.

Table 29:Data on weather parameters and percent disease index recorded during 2000-01

Date	Days after planting	x_1 Max	x_2 Min	x_3 Rf	x_4 Rd	x_5 RH08h	x_6 RH14h	y PDI
30-Aug	68	29.45	24.50	139.1	9	89.90	86.70	16.67
10-Sep	79	33.17	24.37	47.8	4	88.75	79.92	27.22
20-Sep	89	35.70	25.05	10.2	1	87.60	76.50	39.89
30-Sep	99	34.80	24.35	33.2	2	90.00	79.00	46.11
10-Oct	109	35.50	25.00	17.2	2	89.50	76.60	57.22
20-Oct	119	35.70	23.90	10.4	2	89.00	76.40	62.22
30-Oct	129	34.40	23.60	2.2	0	87.40	67.80	63.89
10-Nov	140	34.27	23.40	1.6	0	89.00	73.00	66.11
20-Nov	150	33.75	20.30	0	0	87.50	70.60	70.56
30-Nov	160	33.00	19.55	0	0	85.20	69.00	71.67
10-Dec	170	31.70	19.85	0	0	83.70	68.00	74.44
20-Dec	180	30.50	16.80	0	0	81.60	65.20	76.67
30-Dec	190	29.75	17.45	0	0	80.90	71.20	79.44
10-Jan	211	30.68	19.68	0	0	86.36	70.91	81.11
20-Jan	221	28.55	19.55	0	0	86.60	68.50	83.89
Mean		32.73	21.82	17.45	1.33	86.87	73.29	61.14
Standard Error		0.63	0.74	9.43	0.63	0.74	1.50	5.22
Minimum		28.55	16.80	0	0	80.90	65.20	16.67
Maximum		35.70	25.05	139.1	9	90.00	86.70	83.89

Max = maximum temperature; Min = minimum temperature; Rf = rainfall; Rd = rainy day; RH08h = relative humidity at 0800h; RH14h = relative humidity at 1400h; PDI = per cent disease index

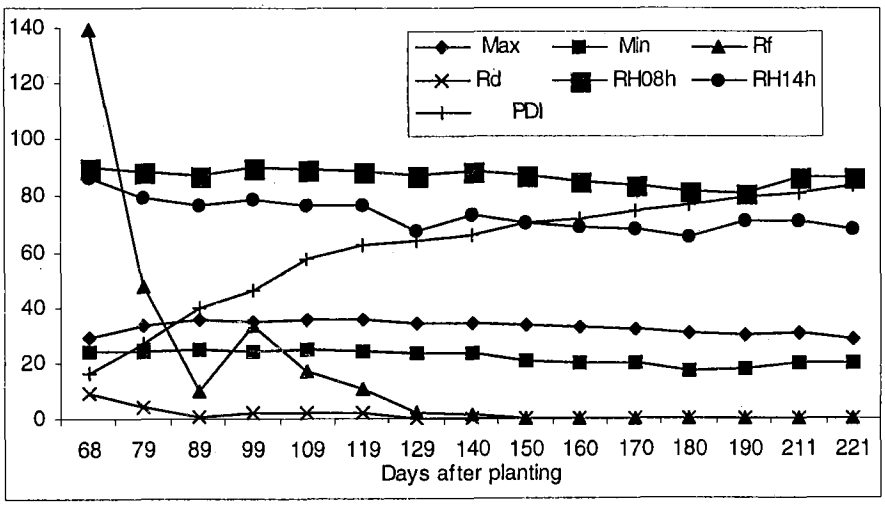

Figure 9. Changes in meteorological conditions over time from 29-8-2000 to 20-1-2001; 15 observations on 10-day mean values

Table 30: Correlation matrix, stepwise regression and linear regression analyses of weather parameters on per cent disease index in *Taphrina maculans – Curcuma longa* pathosystem 2000 – 01

Correlation matrix:

Paramerter	x_1 Max	x_2 Min	x_3 Rf	x_4 Rd	x_5 RH08h	x_6 RH14h
x_1	*1.0000*					
x_2	*0.6660*	*1.0000*				
x_3	*-0.2011*	*0.4711*	*1.0000*			
x_4	*-0.1095*	*0.5397*	*0.9825*	*1.0000*		
x_5	*0.5329*	*0.8922*	*0.4717*	*0.5216*	*1.0000*	
x_6	*0.2011*	*0.7472*	*0.8378*	*0.8832*	*0.7059*	*1.0000*
y (PDI)	*-0.2679*	*-0.7690***	*-0.8283***	*-0.8585***	*-0.6352**	*-0.8922***

Stepwise regression:

$$y = 23.82 - 1.936 x_1 - 3.628 x_2 - 0.434 x_3 + 1.121 x_4 + 2.717 x_5 - 0.683 x_6 \quad \text{Eq. 1}$$
$$(R^2 = 0.921 ; Adj\ R^2 = 0.862\ F = 15.59^{**})$$

$$y = 19.80 - 1.898 x_1 - 3.608 x_2 - 0.370^{*} x_3 + 2.677 x_5 - 0.599 x_6 \quad \text{Eq. 2}$$
$$(R^2 = 0.921; Adj\ R^2 = 0.877; F = 20.93^{**})$$

$$y = -4.190 - 2.071 x_1 - 3.956 x_2 - 0.437^{**} x_3 - 2.614 x_5 \quad \text{Eq. 3}$$
$$(R^2 = 0.917; Adj\ R^2 = 0.883\ F = 27.56^{**})$$

$$y = - 52.299 - 5.878^{**} x_2 - 0.346^{**} x_3 + 2.852 x_5 \quad \text{Eq. 4}$$
$$(R^2 = 0.903; Adj\ R^2 = 0.877\ F = 34.36^{**})$$

Simple linear regression:

$$y = 179.02 - 5.40^{**} x_2 \quad \text{Eq. 5}$$
$$(R^2 = 0.591; Adj\ R^2 = 0.560 ; F = 18.81^{**})$$

$$y = 16.14 - 0.458^{**} x_3 \quad \text{Eq. 6}$$
$$(R^2 = 0.686; Adj\ R^2 = 0.662 ; F = 28.40^{**})$$

4.10.4 Validation of Models on Weather and Disease Index Relationships

In each of the years studied, different best-fit regression models were identified. For at least two years each of the four weather variables namely, maximum temperature, minimum temperature, rainfall and relative humidity (RH08h) found a place in these models. Using the best-fit model identified in each year, disease index values were predicted for the other two years. The best-fit equation derived on the data for 1998-99 was used for predicting disease severity (PDI) for 1999-2000, and for 2000-01. Likewise the best fit for 1999-2000 was used to predict disease severity for 2000-01 and for 1998-99; and the best fit for 2000-01 was used to predict disease severity for 1998-99 and for 1999-2000. Both the predicted and actually observed data are presented in Table 31. The linear regression analyses were performed on the predicted and observed per cent disease index values to verify if these two are related. The linear regression also further established that whenever the variables, which repeated for two years in the best-fit equations occurred, there was a relationship (R^2–values ranging from 0.43 to 0.57) between the predicted and observed values on PDI. The observed R^2–values, in all the comparisons between predicted and actually recorded per cent disease index varied indicating a relationship in one year and no or poor relationship in the other year.

Table 31: Linear regression performed between predicted and observed PDI using the best-fit regression models*

Obs.	1999-2000		2000-01		1998-99		2000-01		1998-99		1999-2000	
No.	pred	obs	pred	obs	pred	obs	pred	obs	pred	obs	pred	obs
1	17.51	16.66	-3.16	16.67	13.09	13.77	37.19	16.67	30.63	13.77	34.82	16.66
2	17.66	16.66	31.42	27.22	37.06	19.55	31.75	27.22	50.24	19.55	42.84	16.66
3	38.71	20.00	49.34	39.89	36.22	26.22	24.45	39.89	35.47	26.22	41.21	20.00
4	10.52	28.88	46.30	46.11	40.02	28.44	19.52	46.11	62.45	28.44	28.71	28.88
5	18.50	34.44	47.90	57.22	59.08	35.11	19.09	57.22	86.16	35.11	23.3	34.44
6	19.37	40.00	57.71	62.22	72.47	38.22	20.41	62.22	80.62	38.22	20.28	40.00
7	23.88	51.11	48.13	63.89	69.90	42.66	35.65	63.89	87.90	42.66	34.27	51.11
8	41.39	51.11	48.41	66.11	68.25	48.00	32.10	66.11	85.91	48.00	54.21	51.11
9	40.34	58.88	66.25	70.56	73.74	58.66	40.34	70.56	77.11	58.66	73.46	58.88
10	43.18	60.00	64.92	71.67	58.02	64.00	52.18	71.67	78.96	64.00	75.06	60.00
11	45.13	62.22	50.99	74.44	43.57	69.77	65.58	74.44	73.60	69.77	75.89	62.22
12	41.97	63.33	62.31	76.67	55.60	72.00	80.00	76.67	52.01	72.00	84.72	63.33
13	48.43	67.77	50.81	79.44	15.02	73.33	87.26	79.44	56.95	73.33	76.64	67.77
14			42.73	81.11	33.97	74.66	65.12	81.11	54.97	74.66		
15			24.69	83.89			79.37	83.89				
R^2	*0.56*		*0.33*		*0.02*^{ns}		*0.43*		*0.08*^{ns}		*0.57*	

* The three best-fit equations in each year were used to predict PDI in the other two years. pred = predicted; obs = observed; R^2 values :^{ns}Not significant, rest significant

4.11 TEMPORAL ANALYSIS OF *T. maculans* EPIDEMICS ON *C. longa*

The amount of disease as percent disease index was assessed at several times, at an interval of about 10 days beginning with the appearance of the disease in the turmeric crop (Tables 32 and 33). The disease intensity as measured by PDI versus time was plotted to obtain disease progress curve. These disease progress curves that represent integration of all host, pathogen and environmental effects occurring during the epidemics in the 2-years under study are presented in Fig. 10 and 11. The length and overall duration of the epidemic of *T. maculans* on *C. longa* was 140 days during 1998-99 (Table 32 and Fig. 10). The time of onset of the epidemic was at 109 days after planting rhizomes in the field plots when leaf blotch disease symptoms were first observed. During the entire duration of the epidemic, only *progressive* phase of the epidemic was observed, that is, the PDI continued to increase in all six cultivars studied.

Table 32: Disease progress in *Curcuma longa* - *Taphrina maculans* pathosystem with time in different cultivars 1998-99.

Observation	DAP*	Per cent disease index (PDI)					
		CLI 317	KTS 1	KTS 4	PCT 13	CLI315	CLI 385
3-11-1998	118	14.22	14.22	15.11	12.88	13.77	13.77
13-11-1998	128	18.22	20.44	22.22	17.77	19.55	20.00
23-11-1998	138	21.33	25.77	27.55	22.66	26.22	27.55
3-12-1998	148	24.44	28.88	30.22	25.77	28.44	29.77
13-12-1998	158	31.55	36.44	33.77	32.44	35.11	36.00
23-12-1998	168	36.88	40.44	39.55	38.66	38.22	41.77
3-1-1999	179	40.00	43.11	44.88	40.88	42.66	45.33
13-1-1999	189	44.88	47.55	47.55	45.77	48.00	49.33
23-1-1999	199	54.66	56.00	58.22	56.88	58.66	59.11
3-2-1999	210	59.55	61.77	63.55	63.11	64.00	64.00
13-2-1999	220	65.33	65.77	70.22	68.88	69.77	68.88
23-2-1999	230	68.00	70.66	75.11	71.11	72.00	75.11
3-3-1999	238	69.33	72.00	76.44	72.88	73.33	76.88
13-3-1999	248	70.66	74.22	78.22	74.22	74.66	78.66

*Days after planting rhizomes on 8-7-1998; Date of first appearance of disease: 25-10-1998.

The maximum disease recorded in the epidemic ranged from 71 to 79 PDI. There were minor variations in the maximum level of disease recorded in the six turmeric cultivars. KTS 4 and CLI 385 showed a PDI of 78 to 79, while KTS 1, PCT 13 and CLI 315 showed 74 to 75 PDI. Among the six cultivars, CLI 317 recorded the lowest PDI (71).

The length of the epidemic or the overall duration of the epidemic of *T. maculans* on *C. longa* was about 132 days during 1999-2000 (Table 32a and Fig. 11). The time of onset of the epidemic was at 64 days after planting rhizomes in the field plots when leaf blotch disease symptoms were first observed. The maximum disease recorded in the epidemic ranged from 61 to 70 PDI. There were minor variations in the maximum level of disease recorded in the six turmeric cultivars. KTS 4 and CLI 315 showed a PDI of 68 to 70, while PCT 13, CLI 317 and KTS 1 showed 63 to 66 PDI. The lowest PDI of 61 was recorded in CLI 385.

Table 32a: Disease progress in *Curcuma longa* - *Taphrina maculans* pathosystem with time in different cultivars 1999-2000.

Observation	DAP*	Per cent disease index (PDI)					
		CLI 317	KTS 1	KTS 4	PCT 13	CLI 315	CLI 385
6-9-1999	74	12.22	14.44	14.44	16.66	16.66	11.11
16-9-1999	84	18.88	17.77	22.22	16.66	16.66	14.44
26-9-1999	94	25.55	26.66	24.44	16.66	20.00	22.22
6-10-1999	104	30.00	32.22	28.88	27.77	28.88	28.28
16-10-1999	114	34.44	32.22	37.77	35.55	34.44	32.22
26-10-1999	124	36.66	40.00	40.00	42.22	40.00	38.88
6-11-1999	135	46.66	51.11	50.00	51.11	51.11	48.88
16-11-1999	145	50.00	54.44	51.11	54.44	51.11	54.44
26-11-1999	155	53.33	56.66	65.00	56.66	58.88	55.55
6-12-1999	165	55.55	57.77	66.66	57.77	60.00	58.88
16-12-1999	175	57.77	61.11	68.88	60.00	62.22	58.88
26-12-1999	185	62.22	63.33	68.88	61.11	63.33	58.88
6-1-2000	196	64.44	65.55	70.00	63.33	67.77	61.11

*Days after planting rhizomes on 24-6-1999.
Date of first appearance of disease: 28-8-1999.

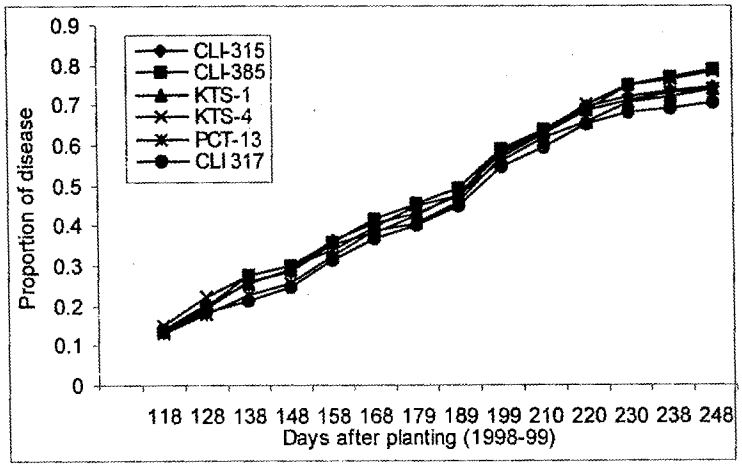

Figure 10: Disease progress curves in *Curcuma longa* - *Taphrina maculans* pathosystem during 1998-99 on six cultivars

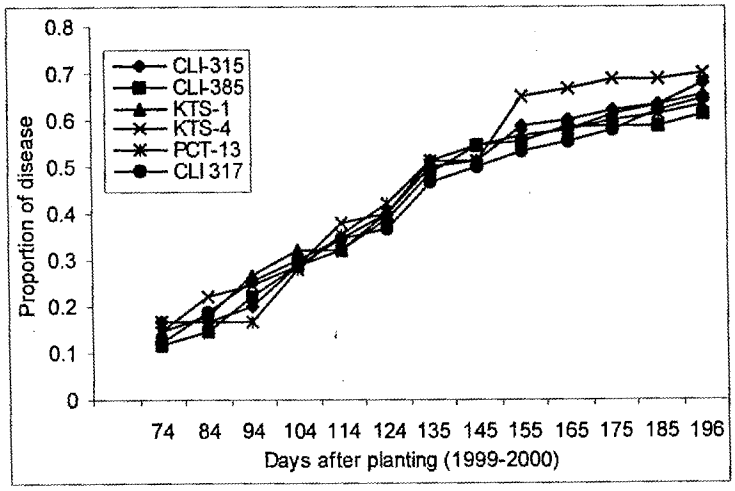

Figure 11: Disease progress curves in *Curcuma longa* - *Taphrina maculans* pathosystem during 1999-2000 on six cultivars

4.11.1 Analysing Disease Progress

As the dynamic processes of change in the amount of disease intensity in a population over time are defined by their rate of change with time, the small change in PDI (*dy*, change in PDI between two observations) with a small change in time (*dt*, time interval between the same two observations) was studied. The growth rate or *absolute rate* of disease increase (*dy/dt* versus *t*) was estimated for each time interval between observations. The graphs showing the absolute rate of disease growth in the two epidemics are presented in Figures 12 and 13.

During 1998-99, throughout the duration of the epidemic of *T. maculans* on *C. longa*, the leaf blotch disease continued to increase at a minimum absolute rate of 0.002 (Fig 12). The maximum absolute rate of disease increase was about 0.01 between 179 and 199 days after planting rhizomes in all the six cultivars studied. More or less the same trend in the absolute rate of disease increase over time was observed in all the cultivars.

During 1999-2000, throughout the duration of the epidemic of *T. maculans* on *C. longa*, the leaf blotch disease continued to increase at a minimum absolute rate of less than 0.002 (Fig 13). The maximum absolute rate of disease increase was about 0.01 between 114 and 135 days after planting rhizomes in all the six cultivars studied. The only exceptions were KTS 4 and PCT 13, which showed another maximum inflection point (0.014) between 135 and 155 days after planting rhizomes. Otherwise, more or less the same trend in the absolute rate of disease increase was observed in all the cultivars.

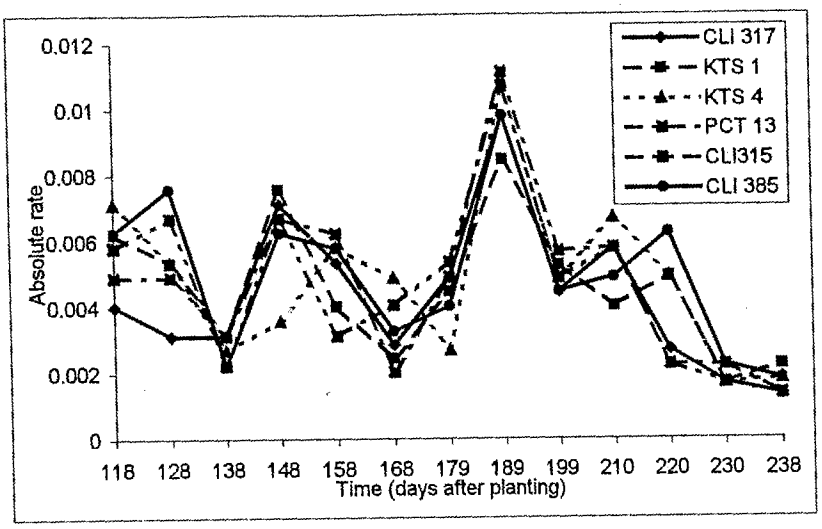

Figure 12: Estimated *dy/dt* versus *t* (absolute rate) in *Curcuma longa-Taphrina maculans* pathosystem during 1998-1999

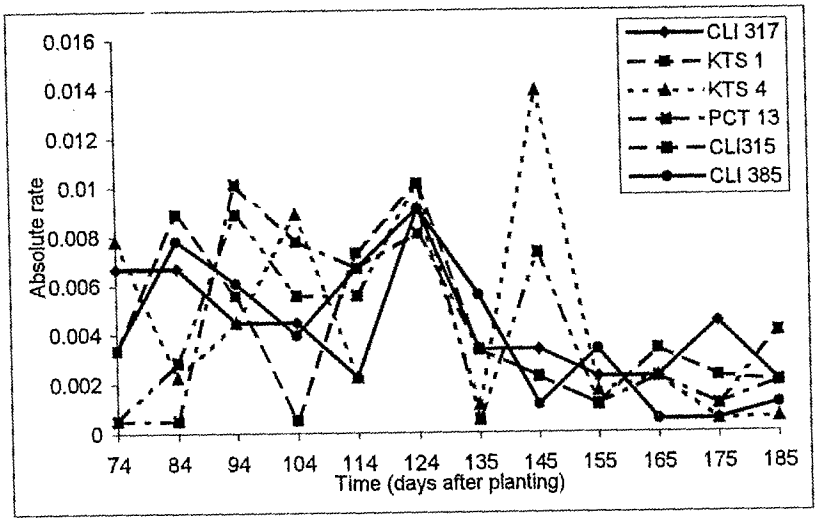

Figure 13: Estimated *dy/dt* versus *t* (absolute rate) in *Curcuma longa-Taphrina maculans* pathosystem during 1999-2000

4.11.2 Fitting Models to Analyse Disease Progress Data

One of the important aspects of temporal analysis of epidemics is the selection of appropriate model for describing disease progress data. Inspection of the disease progress curves (Fig. 10 and 11) showed a clear levelling of *y* (PDI) at high *t* (days after planting - DAP). Therefore, exponential model would not be appropriate. The curves are slightly *S* shaped, so monomolecular model would not be appropriate. Calculation of *dy/dt* confirmed that the maximum estimated *dy/dt* (0.01) was in the middle part of the epidemic, by about 189 DAP in 1998-99, and 124 DAP in 1999-2000 (Fig. 12 and 13) in all the six cultivars.

In biological interpretation, absolute rate of disease increase is directly proportional to the amount of inoculum. The *dy/dt* represents new lesions per day. A greater level of disease leads to greater disease increase. In turmeric field, the level of disease at several times was observed in the population and not on the absolute rate of increase. Therefore the logistic model was used as it is integrated to express *y* as a function of *t*. Van der Plank called the rate parameter for logistic model as "apparent rate of infection" because what was observed was the apparently diseased or symptomatic tissue. The apparent rate of infection estimated in the six cultivars for the two years are presented in Table 33. The apparent rate (*r- value*) was not constant. There were four peaks during 1998-99 and 5-6 peaks in 1999-2000. The maximum *inflection point* (0.017) was reached at 189 DAP during 1998-99 epidemic of *T. maculans* in turmeric crop, while the maximum *inflection point* (0.016) was at 124 DAP during 1999-2000 epidemic of *T. maculans*.

Table 33: Apparent rate of infection in *Curcuma longa-Taphrina maculans* pathosystem

DAP	CLI 317	KTS 1	KTS 4	PCT 13	CLI 315	CLI 385
			1998-99			
118	0.013	0.019	0.021	0.016	0.018	0.019
128	0.009	0.013	0.012	0.013	0.017	0.018
138	0.008	0.007	0.006	0.007	0.005	0.005
148	0.015	0.015	0.007	0.014	0.013	0.012
158	0.010	0.007	0.011	0.012	0.006	0.011
168	0.005	0.004	0.009	0.004	0.007	0.006
179	0.009	0.008	0.005	0.009	0.009	0.007
189	0.017	0.015	0.019	0.019	0.019	0.017
199	0.008	0.009	0.009	0.010	0.009	0.008
210	0.011	0.008	0.013	0.011	0.011	0.010
220	0.005	0.010	0.011	0.005	0.005	0.013
230	0.003	0.004	0.004	0.005	0.004	0.005
238	0.003	0.005	0.004	0.003	0.003	0.004
			1999-2000			
74	0.022	0.011	0.023	0.002	0.002	0.013
84	0.017	0.023	0.005	0.002	0.008	0.023
94	0.010	0.012	0.010	0.025	0.021	0.014
104	0.009	0.001	0.017	0.016	0.011	0.008
114	0.004	0.014	0.004	0.012	0.010	0.013
124	0.016	0.018	0.016	0.014	0.018	0.016
135	0.006	0.006	0.002	0.006	0.001	0.010
145	0.006	0.004	0.025	0.004	0.013	0.002
155	0.004	0.002	0.003	0.002	0.002	0.006
165	0.004	0.006	0.004	0.004	0.004	0.001
175	0.008	0.004	0.001	0.002	0.002	0.001
185	0.004	0.004	0.001	0.004	0.008	0.002

Although maximum r values were recorded at the beginning of epidemic (118 DAP) during 1998-99, and between 74 and 94 DAP during 1999-2000; they were not considered as the disease was at low level.

The linearized forms of logistic and Gompertz models were evaluated for goodness-of-fit to the entire set of disease progress data by using the procedure of regression analysis. The criteria for evaluating models are presented in Table 34. Logistic models fitted well with all the 12 data sets (six cultivars in two years). The coefficients of determination in these logistic models explained the variability in proportion of disease over time up to 99% in data sets during 1998-99, and 90 – 96% in data sets during 1999-2000.

Gompertz model appeared to provide a marginally better description in just four cases (with CLI 317, KTS 1 and CLI 385 in 1999-2000). It is not correct to compare directly R^2 values or mean square error when *different transformations* of y are used. The residual plot for the estimates provided by logistic or Gompertz models showed no distinct or undesirable pattern.

Since the logistic model satisfied most cases (eight in all) and is simpler than the Gompertz model, it was concluded that logistic model was the most appropriate model for describing the leaf blotch disease data.

Table 34: Summary of linear regression statistics* used in evaluation of logistic and Gompertz models for appropriateness for describing leaf blotch disease progress in turmeric cultivars

	R^2	MSE	Intercept	Std. Dev. of intercept	Rate parameter	Std. Dev. of rate parameter
			1998-99			
CLI 317						
Logistic	0.987	0.047	-0.743	0.024	0.009	0.0003
Gompertz	0.990	0.027	0.052	0.014	0.006	0.0002
KTS 1						
Logistic	0.986	0.049	-0.678	0.025	0.009	0.0003
Gompertz	0.994	0.022	0.084	0.011	0.006	0.0001
KTS 4						
Logistic	0.990	0.045	-0.680	0.023	0.010	0.0003
Gompertz	0.985	0.037	0.073	0.019	0.007	0.0002
PCT13						
Logistic	0.985	0.054	-0.759	0.028	0.010	0.0004
Gompertz	0.989	0.032	0.039	0.016	0.007	0.0002
CLI 315						
Logistic	0.982	0.058	-0.703	0.029	0.010	0.0004
Gompertz	0.987	0.033	0.068	0.017	0.007	0.0002
CLI 385						
Logistic	0.988	0.049	-0.695	0.025	0.010	0.0003
Gompertz	0.992	0.028	0.067	0.014	0.007	0.0002
			1999-2000			
CLI 317						
Logistic	0.945	0.084	-0.679	0.044	0.008	0.0006
Gompertz	0.977	0.034	0.101	0.018	0.005	0.0003
KTS 1						
Logistic	0.936	0.093	-0.648	0.049	0.009	0.0007
Gompertz	0.962	0.046	0.116	0.024	0.007	0.0003
KTS 4						
Logistic	0.957	0.085	-0.662	0.045	0.010	0.0006
Gompertz	0.967	0.050	0.098	0.026	0.007	0.0004
PCT13						
Logistic	0.910	0.112	-0.674	0.059	0.009	0.0008
Gompertz	0.935	0.060	0.105	0.032	0.006	0.0004
CLI 315						
Logistic	0.951	0.085	-0.682	0.049	0.009	0.0006
Gompertz	0.969	0.044	0.094	0.023	0.006	0.0003
CLI 385						
Logistic	0.903	0.124	-0.736	0.065	0.009	0.0009
Gompertz	0.934	0.062	0.078	0.033	0.006	0.0005

*R^2 =coefficient of determination; MSE = mean square of error; Std. Dev. = standard deviation. Note: Only disease proportion values greater than 0, were used for the models

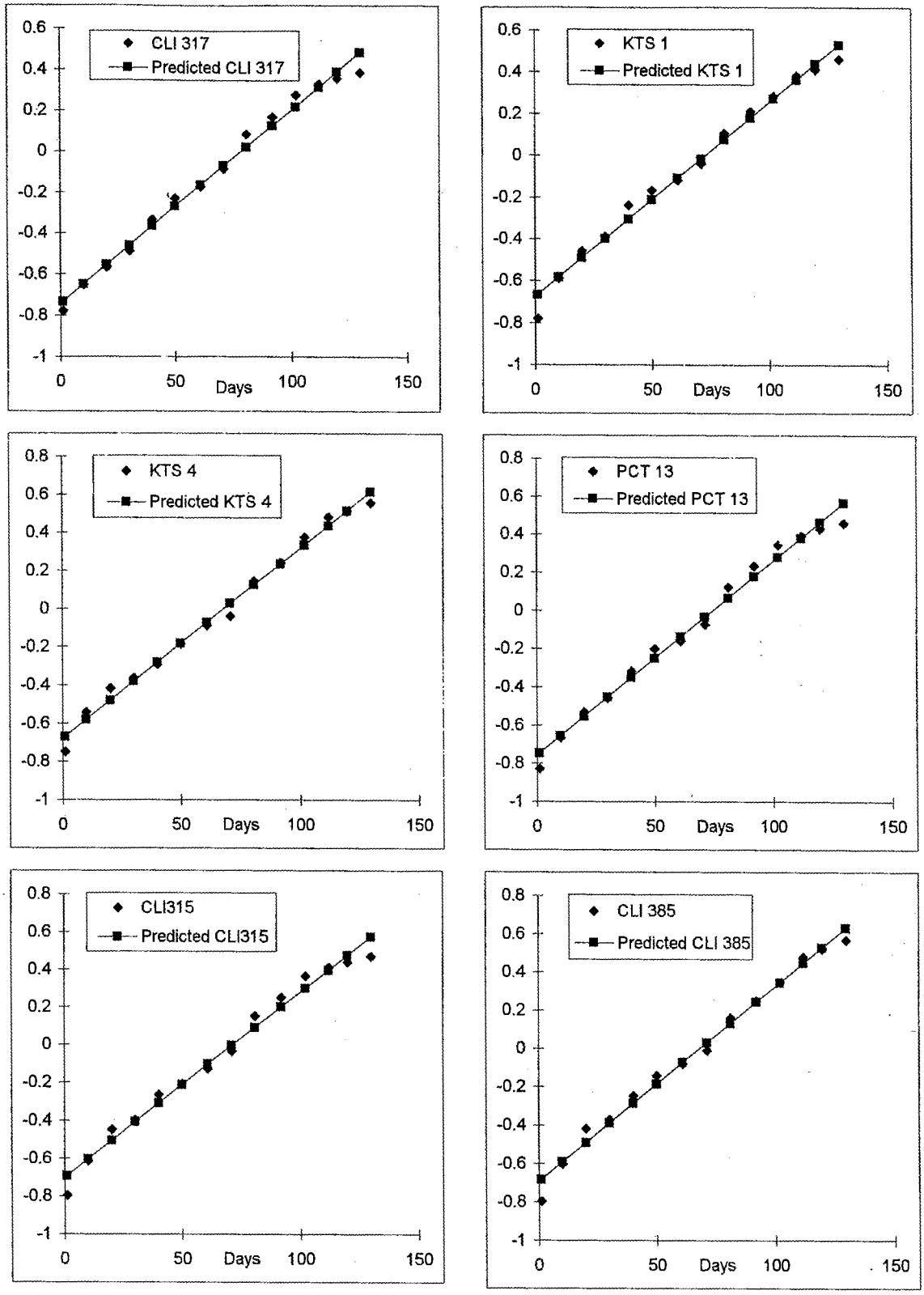

Figure 14: Line-fit plot on actual and predicted leaf blotch mean disease proportion values versus time in logistic models for turmeric cultivars during 1998-99

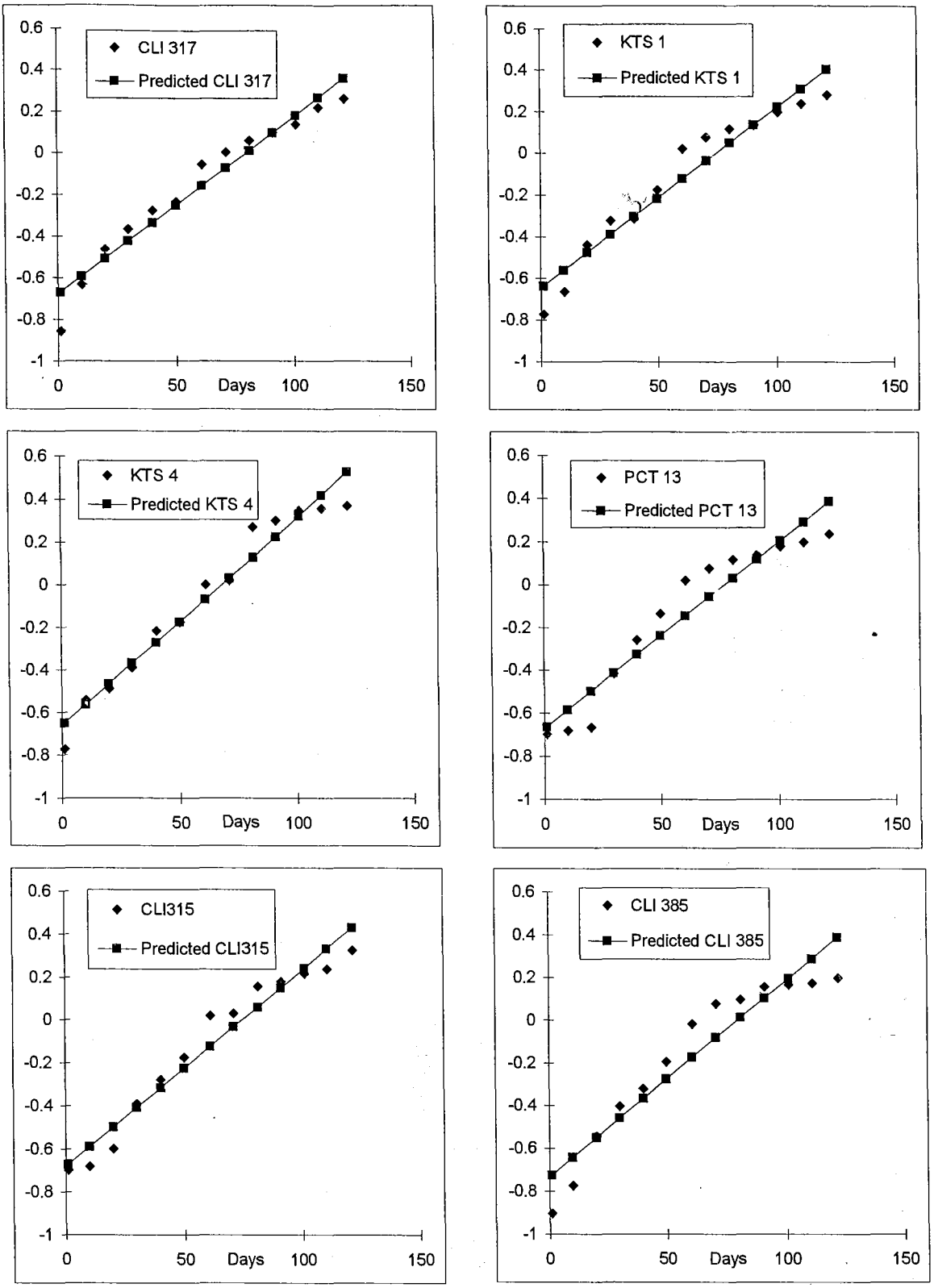

Figure 15: Line-fit plot on actual and predicted leaf blotch mean disease proportion values versu time in logistic models for turmeric cultivars during 1999-2000

4.11.3 Estimating Leaf Blotch Disease Doubling Time

As Campbell and Madden (1990) have suggested for the use of logistic model for such epidemics with no serious errors in understanding of making comparisons, where high R^2 values indicated good-fit, the logistic models were further analysed. In the plot of predicted and actual mean values in proportion of disease versus time (Figure 14 and 15), the predicted values were quite similar to the observed values. The rate parameter estimated for leaf blotch on a cultivar by linear regression was used to derive disease doubling time. The details on the calculated disease doubling time for the cultivars in epidemics during 1998-99 and 1999-2000 are presented in Figure 16.

The leaf blotch disease doubling time was more or less the same in all the cultivars and varied from 29 to 32 days in 1998-99, and from 32 to 35 days in 1999-2000. ANOVA showed no significant differences in the doubling time recorded either between cultivars or between mean data in the two years studied.

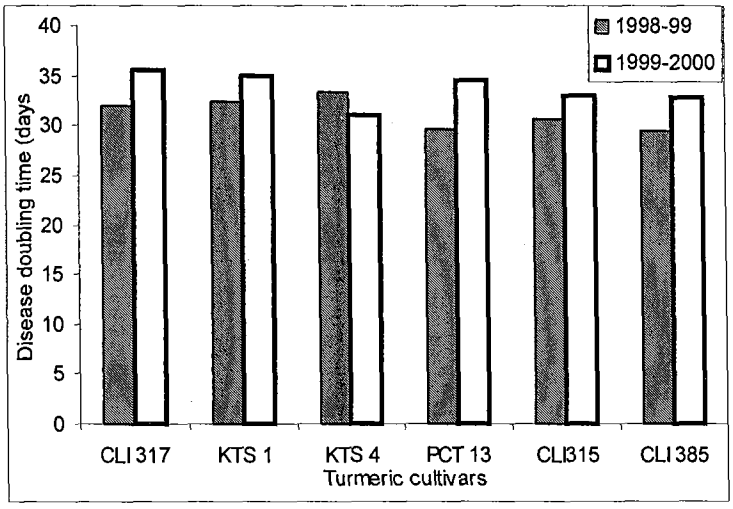

Figure 16: Disease doubling time in *Curcuma longa-Taphrina maculans* pathosystem (Estimated using logistic model fitted for disease progress)

4.11.4 Area Under Disease Progress Curve

To summarise a disease progress curve for comparative or analytical purposes, the area under the disease progress curve (AUDPC) was used as a descriptor for the epidemics on *Taphrina* in *C. longa*. As AUDPC incorporates the variation in time of disease onset and final level of disease attained, AUDPC values for the epidemics in the 2-years were estimated for each of the six cultivars (Fig. 17).

In the epidemic during 1998-99, the AUDPC values ranged from a minimum value of 5851 in CLI 317 to a maximum value of 6447 in CLI 385. The overall mean AUDPC value inclusive of all the six cultivars was 6210. In the epidemic during 1999-2000, the AUDPC values ranged from a minimum value of 5298 in CLI 385 to a maximum value of 5903 in KTS 4. The overall mean AUDPC value inclusive of all the six cultivars was 5512.

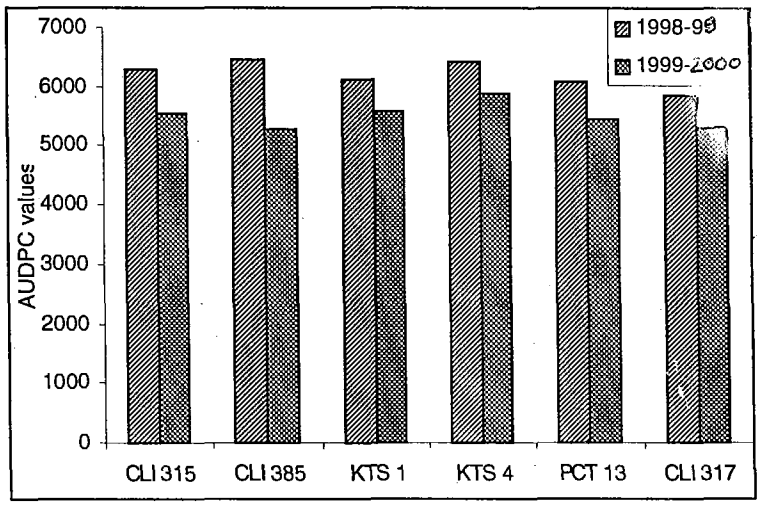

Figure 17: Area under disease progress curve (AUDPC) of *Taphrina maculans* in turmeric

4.12 SPATIAL ANALYSIS OF TEMPORAL CHANGES IN LEAF BLOTCH DISEASE EPIDEMIC ON TURMERIC

4.12.1 Evaluation of Sampling Methods

As spatial pattern or arrangement of disease entities relative to each other and to the architecture of the host provides quantitative information on population dynamics of the pathogen, a large plot (>1000 m^2) was planted with susceptible turmeric cultivar CLI 317. This plot was exposed to natural epidemic outbreak of *T. maculans*. The first symptom of the disease was observed in this field at 69 days after planting (on September 4, 1999). In the field, quadrats (2 m^2) were marked (Figure 1) as described in Materials and Methods to enable regrouping of the quadrats to satisfy vertical, diagonal, horizontal and random sampling methods. In each of the quadrats, assessment on the proportion of disease or infected area was made four times at an interval of 30 days in two plants to study the spatial and temporal changes in epidemic of leaf blotch. The leaf area (mm^2) was mapped as healthy and infected and dividing infected area by healthy area derived proportion of infected area. The proportion of disease in the first assessment was low. Therefore, to consider more precisely minute changes also, all values on all the proportion of disease were multiplied by 10^3 prior to analysis of the data. On all the dates of disease assessment in each of the sampling methods studied the proportions of leaf blotch disease between blocks were analysed by Hierarchal ANOVA. The temporal changes in the mean proportion of leaf blotch disease derived from 2-plants in each of the quadrats in different sampling methods are presented in Tables 35-38.

4.12.1.1Vertical Sampling Method

In vertical sampling method, data on 2-plants in each of the two quadrats in four blocks were used to calculate the mean proportion of leaf blotch disease (Table 35). From the first to second assessments during 79 to 109 days after planting turmeric, in all the blocks, there was a gradual and small increase in the proportion of disease. This increase in the mean proportion of leaf blotch disease was relatively less in the blocks 3 and 4 compared to others. However, there was a sudden increase in the proportion of disease in all the blocks from the second to third assessment during 109 to 140 days after planting. This increase in proportional disease from third to fourth assessment i.e., from 140 to 170 DAP is not spectacular. All blocks showed similar temporal changes in the proportion of leaf blotch disease. The *F*-value for each time of assessment showed no significant differences in the mean proportion of leaf blotch disease recorded for the four blocks.

Table 35: Spatio-temporal changes in the mean proportion[†] of leaf blotch disease in susceptible turmeric cultivar in blocks used to test vertical sampling method

Blocks	Quadrats	Days after planting			
		79	109	140	170
1	Q1 and Q4	0.0977	7.1918	750.14	870.65
2	Q2 and Q5	0.0344	6.4185	806.16	921.07
3	Q9 and Q12	0.0219	2.9832	597.75	53.97
4	Q10 and Q13	0.0437	2.4321	767.32	914.63
F-value		1.0349^{NS}	2.101^{NS}	1.1631^{NS}	1.2217^{NS}

[†] All values multiplied by 10^3 ; [NS] Not significant

4.12.1.2 Diagonal Sampling Method

In diagonal sampling method, data on 2-plants in each of the two quadrats in four blocks were used to calculate the mean proportion of leaf blotch disease (Table 36).

The results obtained are similar to those obtained with vertical sampling method. From the first to second assessments during 79 to 109 days after planting turmeric, in all the blocks, there was a gradual and small increase in the proportion of disease. This increase in the mean proportion of leaf blotch disease was relatively less in the blocks 3 and 4 compared to others. However, there was a sudden increase in the proportion of disease in all the blocks from the second to third assessment during 109 to 140 days after planting. All blocks showed similar temporal changes in the proportion of leaf blotch disease. The *F*-value for each time of assessment showed no significant differences in the mean proportion of disease recorded for the four blocks.

Table 36: Spatio-temporal changes in the mean proportion[†] of leaf blotch disease in susceptible turmeric cultivar in blocks used to test diagonal sampling method

Blocks	Quadrats	Date of assessment			
		79	109	140	170
1	Q1 and Q5	0.0890	5.6404	755.15	904.22
2	Q2 and Q4	0.0431	7.9696	801.15	887.51
3	Q9 and Q13	0.0115	3.0799	688.92	922.89
4	Q10 and Q12	0.0541	2.3354	676.15	945.71
F-value		0.9798 ^{NS}	2.1598 ^{NS}	0.6419 ^{NS}	0 ^{NS}

[†] All values multiplied by 10^3 ; ^{NS} Not significant

4.12.1.3 Horizontal Sampling Method

In horizontal sampling method, data on 2-plants in each of the two quadrats in five blocks were used to calculate the mean proportion of leaf blotch disease (Table 37). The results obtained are similar to those obtained with vertical and diagonal sampling methods. From the first to second assessments during 79 to 109 days after planting turmeric, in all the blocks, there was a gradual and small increase in the proportion of disease. This increase in the mean proportion of leaf blotch disease was relatively less in the blocks 3,4 and 5 compared to others. However, there was a sudden increase in the

proportion of disease in all the blocks from the second to third assessment during 109 to 140 days after planting. All blocks showed similar temporal changes in the proportion of leaf blotch disease. The *F*-value for each time of assessment showed no significant differences in the mean proportion of disease recorded for the five blocks.

Table 37: Spatio-temporal changes in the mean proportion[†] of leaf blotch disease in susceptible turmeric cultivar in blocks used to test horizontal sampling method

Blocks	Quadrats	Date of assessment			
		79	109	140	170
1	Q1 and Q2	0.0106	6.3171	703.23	882.7
2	Q4 and Q5	0.0026	7.2932	853.07	909.03
3	Q6 and Q8	0.0112	4.0774	578.32	954.59
4	Q9 and Q10	0.0042	3.0267	726.34	957.42
5	Q12 and Q13	0.0023	2.3886	638.73	911.18
F-value		0.9278 ^{NS}	1.7212 ^{NS}	1.0523 ^{NS}	1.2024 ^{NS}

[†] All values multiplied by 10^3 ; ^{NS} Not significant

4.12.1.4 Random Sampling Method

In random sampling method, data on 2-plants in all 13 quadrats, each taken as a block, were used to calculate the mean proportion of leaf blotch disease (Table 38). From the first to second assessments during 79 to 109 days after planting turmeric, in all the blocks, there was a gradual and small increase in the proportion of di[illegible]his increase in the mean proportion of leaf blotch disease was relatively less in the quadrats 7 to 13 compared to that recorded in the quadrats 1 to 6. However, there was a sudden increase in the proportion of disease in all the blocks from the second to third assessments during 109 to 140 days after planting. All blocks showed similar temporal changes in the proportion of leaf blotch disease. On the first date of assessment (79 DAP), the *F*-value showed highly significant ($P \leq 0.01$) differences between quadrats in the mean proportion of leaf blotch disease. On the second date of assessment (109

DAP) also, the *F*-value showed significant ($P \leq 0.05$) differences between quadrats in the mean proportion of leaf blotch disease. But, on later dates of assessment (140 and 170 DAP), the *F*-values were non-significant indicating no differences in the mean proportion of leaf blotch disease on turmeric crop.

Table 38: Spatio-temporal changes in the mean proportion[†] of leaf blotch disease in susceptible turmeric cultivar in blocks used to test random sampling method

Blocks/Quadrats	Days after planting			
	79	109	140	170
1	0.161	5.539	652.21	865.84
2	0.051	7.095	754.24	899.56
3	0.601	7.882	847.33	928.71
4	0.035	8.844	848.06	875.47
5	0.017	5.742	858.08	942.59
6	0.179	5.894	500.27	933.77
7	0.012	1.990	606.31	985.63
8	0.045	2.260	656.37	975.41
9	0.001	3.674	647.94	965.68
10	0.075	2.379	804.74	949.16
11	0.011	4.200	746.59	890.33
12	0.034	2.292	547.56	942.26
13	0.013	2.485	729.90	880.10
F-value	11.469**	3.245*	1.266 ^{NS}	2.045 ^{NS}

[†] All values multiplied by 10^3; ** Significant ($P \leq 0.01$); * Significant ($P \leq 0.05$);
^{NS} Not significant

4.12.1.5 Comparison of Sampling Methods

For the different sampling methods, the overall mean proportion of disease estimated during the epidemic of *T. maculans* on turmeric on the four dates of assessment is presented in Fig.18. In vertical sampling method, the overall mean proportion of disease increased from 0.05 on 79th day after planting to 4.76 on 109th DAP, to 730 on 140th DAP, and to 915 on 170th DAP. The rapid increase in the proportion of leaf blotch disease between 109 and 140 days after planting turmeric in the field was clearly evident. In diagonal sampling method also, the overall mean

proportion of disease increased from 0.05 on 79th day after planting to 4.76 on 109th DAP, to 730 on 140th DAP, and to 915 on 170th DAP. The rapid increase in the proportion of leaf blotch disease between 109 and 140 days after planting turmeric in the field was again clearly evident. Compared to other sampling methods, the lowest mean proportion of disease (0.006) was recorded on the first date of disease assessment (79 DAP) in the horizontal method of sampling. The overall mean proportion of disease increased to 4.62 on 109th DAP, to 700 on 140th DAP, and to 925 on 170th DAP. The rapid increase in the proportion of leaf blotch disease between 109 and 140 days after planting turmeric in the field was once again clearly evident.

Compared to other sampling methods, the highest mean proportion of disease was recorded on the first date of disease assessment (79 DAP) in the random sampling method. The overall mean proportion of disease increased from 0.10 on 79th day after planting to 4.64 on 109th DAP, to 708 on 140th DAP, and to 926 on 170th DAP. The rapid increase in the proportion of leaf blotch disease between 109 and 140 days after planting turmeric in the field was clearly evident.

The values for the mean proportion of disease recorded in random sampling method on 79th and 170th DAP were higher than those recorded in other sampling methods. On other dates of disease assessment (109 and 140 DAP), the values in vertical and diagonal sampling methods were higher than those recorded in random and horizontal sampling methods. However, these differences were only marginal.

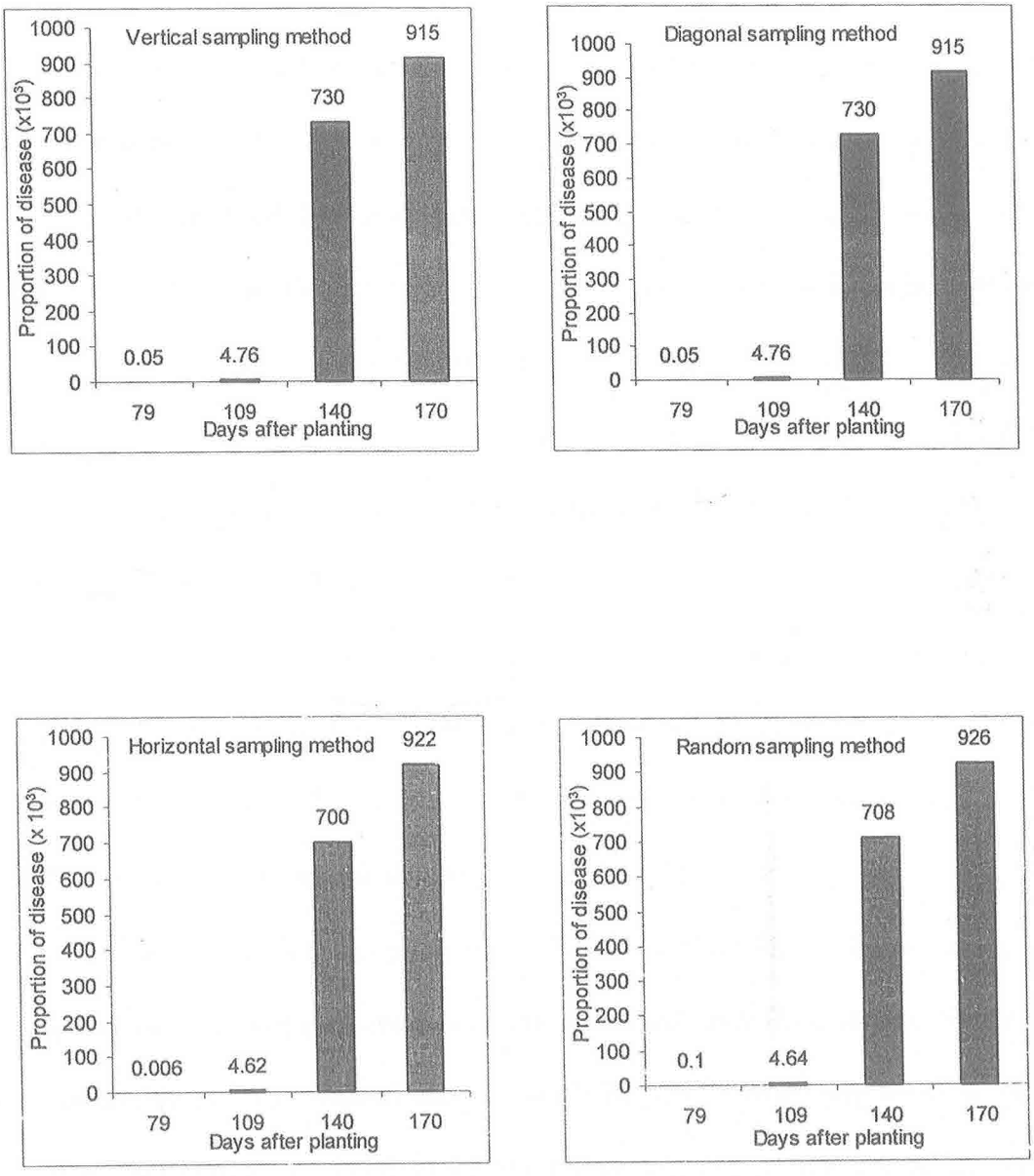

Figure 18: Spatio-temporal changes in the overall mean proportion (x 10^3) of leaf blotch disease in turmeric crop detected with different sampling methods

There were differences in the coefficients of variation (CV %) estimated by different sampling methods for the mean proportion of leaf blotch disease assessed on four different dates (Fig. 19). On the first date of disease assessment (79 DAP), the CV was very high; relatively lowest CV (71%) was recorded in random sampling method compared to other methods (CV 131 to 144%). On the second date of disease assessment (109 DAP), the CV was moderately high; again relatively lowest CV (40%) was recorded in random sampling method compared to other methods (CV 70 to 73%).

On the third date of disease assessment (140 DAP), the CV estimated for the overall mean proportion of disease in all the sampling methods remained between 20 to 29% only. On the fourth date of disease assessment (170 DAP), the CV was extremely low (4 to 7 %) and the lowest (CV 4.25%) was recorded in random sampling method.

4.12.2 Indices of Dispersion or Aggregation of Leaf Blotch Disease

4.12.2.1 Variance to Mean Ratio (VM)

As variance to mean ratio is a simple index of dispersion, using the data from regrouped quadrats into blocks, VM was calculated by dividing sample variance (s^2) by the sample mean (x) in various sampling methods. The temporal changes in VM as estimated in different methods are presented in Table 39.

In the vertical sampling method, VM was far below 1, indicating a regular spatial pattern during assessment of disease on all dates. Yet, certain differences were discernible between disease assessments. VM increased slightly between 79 and 109 DAP. In the diagonal, horizontal and random sampling methods, trend in the temporal changes in VM was also more or less similar to that observed in the vertical sampling method barring a few deviations in the intensity of the indices.

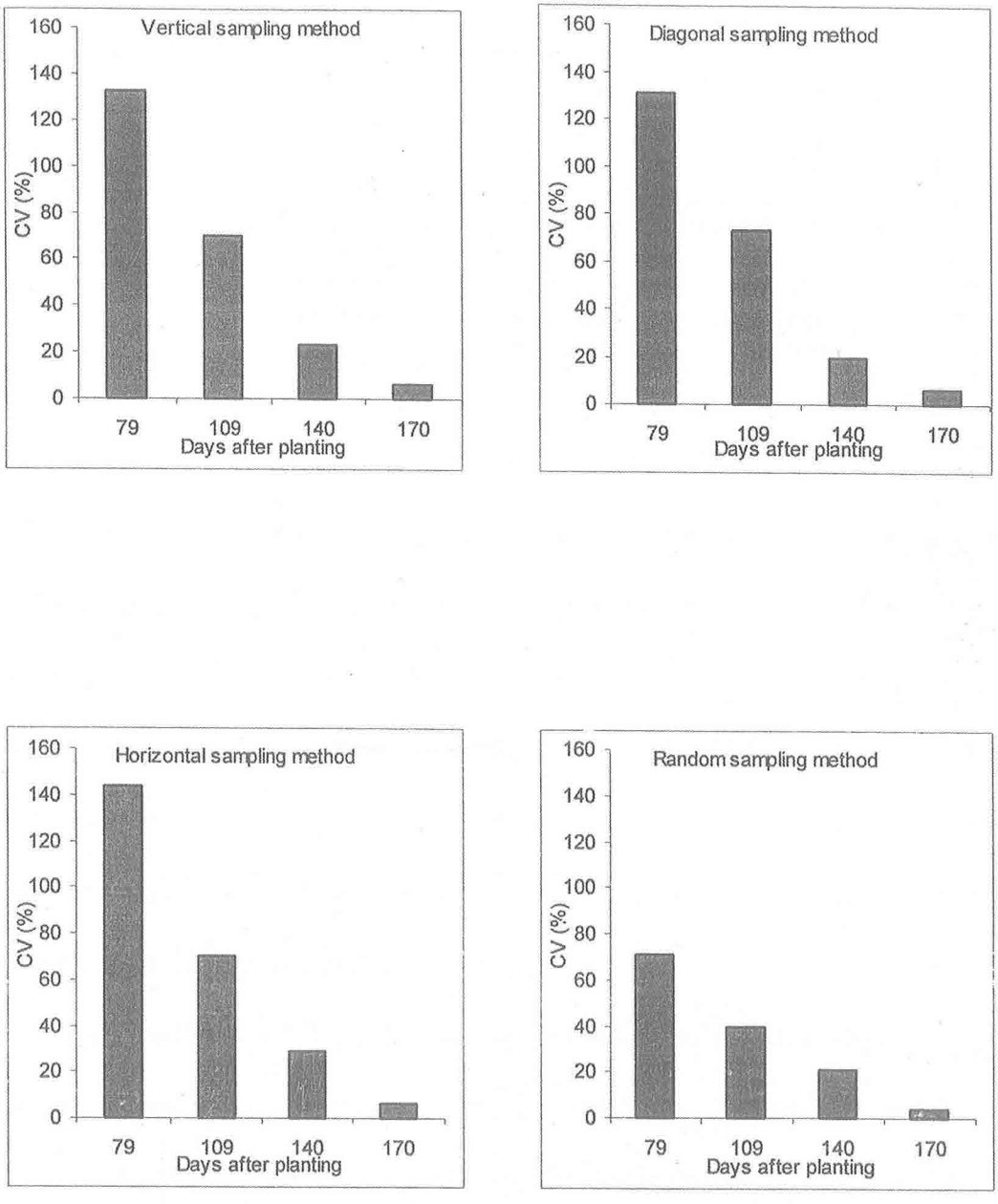

Figure 19: Spatio-temporal changes in the coefficient of variation (CV %) for the mean proportion (x 10^3) of leaf blotch disease in turmeric crop detected with different sampling methods

Table 39: Spatio-temporal changes in the variance-to-mean ratio (VM)[†] in the proportion of leaf blotch disease estimated for different sampling methods

DAP	Vertical	Diagonal	Horizontal	Random
	Original values on mean proportion of disease			
79	0.000020	0.000021	0.000003	0.000273
109	0.001208	0.001386	0.000965	0.001202
140	0.011450	0.004693	0.015249	0.019270
170	0.001283	0.000684	0.001122	0.001707
	Original values on mean proportion of disease x 10^3			
79	0.023	0.021	0.003	0.273
109	1.208	1.386	0.965	1.202
140	11.450	4.693	15.249	19.270
170	1.283	0.684	1.122	1.707
	Original values on mean proportion of disease x 10^6			
79	22.570	20.680	3.096	273.077
109	1208.222	1386.084	965.151	1202.189
140	11450.491	4692.668	15249.447	19269.654
170	1283.144	683.814	1121.803	1707.218

[†] $(s^2/\bar{x})$

When the low values on the mean proportion of leaf blotch disease values were multiplied by 10^3 or 10^6, it lead to a simple displacement of the decimal point without any change in the values. In the data analysis and VM estimates original values showed no aggregation on all dates of disease assessment. But when values multiplied by 10^3 were used to estimate VM, it suggested aggregation from 189 DAP and when values were multiplied by 10^6, VM estimates tended to show aggregation on all dates of assessment. This index was apparently not effective in discerning the spatial pattern.

4.12.2.2 Lloyd's Index of Patchiness (LIP)

As Lloyd's index of patchiness can be used to indicate the relative degree of aggregation, this index was also estimated for each date of assessment on leaf blotch disease in all the sampling methods. The original low values on the mean proportion of disease gave negative values. When these original values were multiplied by a constant (10^3), on the first date of assessment once again negative values were encountered for

Lloyd's index. Only when the original values on the mean proportion of disease were multiplied by a constant (10^3 or10^6), it was possible to derive the Lloyd's index for all dates of assessments, which are presented in Table 40.

Table 40: Spatio-temporal changes in the Lloyd's index of patchiness† based on the proportion of leaf blotch disease estimated for different sampling methods

DAP	Vertical	Diagonal	Horizontal	Random
	Original values on mean proportion of disease			
79	-20231.2	-20231.3	-161811	-10446.3
109	-208.989	-208.955	-215.213	-214.415
140	-0.35354	-0.3628	-0.40691	-0.38587
170	-0.0914	-0.09205	-0.08223	-0.07838
	Original values on mean proportion of disease x 10^3			
79	-18.776	-18.8143	-160.311	-6.59646
109	1.043777	1.081173	0.992458	1.043607
140	1.014309	1.005056	1.020358	1.025817
170	1.000309	0.999654	1.000132	1.000764
	Original values on mean proportion of disease x 10^6			
79	1.436427	1.398182	1.339125	3.843252
109	1.253810	1.291209	1.208664	1.259066
140	1.015677	1.006424	1.021785	1.027229
170	1.001401	1.000746	1.001214	1.001843

† LIP = $(\bar{x} + s^2/\bar{x}\text{-}1)/\bar{x}$

The index values derived after multiplication by a constant showed nearly similar values except for the first date of assessment where multiplication by 10^3 resulted in negative index values. The main reason was the extremely low proportions of disease recorded on 79 DAP.

As the multiplication of original values on the mean proportion of disease by a constant (10^6) satisfied Lloyd's index derivation fully, only these values were further studied. All the sampling methods more or less exhibited the same trend in the temporal changes in the Lloyd's index of patchiness. In the first date of disease assessment on 79 DAP, the Lloyd's index showed clear patchiness with values above 1. On the second date of assessment, the degree of patchiness drastically reduced and finally on the third

and fourth dates of disease assessment, this index value consistently remained close to 1 indicating a random spatial pattern of leaf blotch disease in the turmeric field.

4.12.2.3 Index of Clumping Size (ICS)

As index of clumping size, that is, a variation in the variance to mean ratio, is also used to discern the spatial pattern of disease, ICS was calculated for each date of assessment of leaf blotch disease in all the sampling methods and is presented in Table 41. Further clump size (C) was also estimated for each date of assessment of leaf blotch disease in all the sampling methods to perform a formal statistical test for aggregation. The significance was tested using chi-square distribution (χ^2) with n-1 degrees of freedom under the null hypothesis of randomness. The original low values on the mean proportion of leaf blotch disease gave negative ICS values.

Table 41: Spatio-temporal changes in the index of aggregation or index of clumping size (ICS) and clump size (C)[†] based on the proportion of leaf blotch disease estimated for different sampling methods

DAP	Vertical	Diagonal	Horizontal	Random	Vertical	Diagonal	Horizontal	Random
	Index of clumping size (ICS)				Clump size (C)			
	Original values on mean proportion of disease							
79	-1.0000	-1.0000	-1.0000	-0.9997	0.0001	0.0001	0.0000	0.0033
109	-0.9988	-0.9986	-0.9990	-0.9988	0.0036	0.0042	0.0039	0.0144
140	-0.9885	-0.9953	-0.9848	-0.9807	0.0344	0.0141	0.0610	0.2312
170	-0.9987	-0.9993	-0.9989	-0.9983	0.0038	0.0021	0.0045	0.0205
	Original values on mean proportion of disease x 10^3							
79	-0.977	-0.979	-0.997	-0.727	0.07	0.06	0.012	3.277
109	0.208	0.386	-0.035	0.202	3.63	4.16	3.86	14.43
140	10.450	3.693	14.249	18.270	34.35[a]	14.08[a]	60.10[a]	231.24[a]
170	0.283	-0.316	0.122	0.707	3.85	2.05	4.49	20.49
	Original values on mean proportion of disease x 10^6							
79	21.57	19.68	2.10	272.08	67.71[a]	62.04[a]	12.38[a]	3276.93[a]
109	1207.22	1385.08	964.15	1201.19	3624.67[a]	4158.25[a]	3860.61[a]	14426.27[a]
140	11449.49	4691.67	15248.45	19268.65	34351.47[a]	14078.0[a]	60997.79[a]	231235.85[a]
170	1282.14	682.81	1120.80	1706.22	3849.43[a]	2051.44[a]	4487.21[a]	20486.62[a]

[†]ICS = VM-1; C = n-1(VM) ; [a]significant ($P \leq 0.01$)

When the original values on the mean proportion of disease were multiplied by a constant (10^3), on the first date of assessment once again negative ICS values were encountered. When the original values on the mean proportion of disease were multiplied by a constant (10^6), it gave positive ICS index for all dates of assessments (Table 41).

When the original values on the mean proportion of disease were multiplied by 10^6, it satisfied conditions to derive index of clumping and clumping size (Table 41). But, it lead to a simple displacement of the decimal point in the index of clumping size estimates on the four dates of assessment without any change in the values. The test with chi-square distribution (χ^2) on C became highly significant ($P \leq 0.01$) on all dates of assessment when original values multiplied by 10^6 were used, compared to highly significant C noted only on the third date of disease assessment (140 DAP) with ICS values, when original values multiplied by 10^3 were used. Very similar to what was observed with variance to mean ratio (VM) the values estimated for ICS with original mean proportion of disease as well as values multiplied by 10^3 and 10^6 showed changes from no aggregation to aggregation on all dates of disease assessment. This index was apparently not effective in discerning the spatial pattern.

4.12.2.4 Morisita's Index of Dispersion

As Morisita's index of dispersion (I_δ) is derived to measure the variability in the density among relatively large patches of individuals, this index was also calculated for each date of assessment of leaf blotch disease in all the sampling methods (Table 42). The departure value of Morisita's index (I_δ) from 1 (=random) is usually judged to be significant based on the estimates on I_δ^*. After estimating the departure value (I_δ^*) for

each date of leaf blotch disease assessment in turmeric in all the sampling methods, the significance of the I_δ^* values, was tested with chi-square (χ^2) for n-1 degree of freedom.

Table 42: Spatio-temporal changes in the Morisita's index (I_δ) and the departure value (I_δ^*) based on the proportion of leaf blotch disease estimated for different sampling methods

DAP		Vertical	Diagonal	Horizontal	Random
Original values on mean proportion of disease					
79	I_δ	4.00	4.00	5.00	13.01
	I_δ*	0.00	0.00	0.00	0.00
109	I_δ	4.05	4.05	5.09	13.75
	I_δ*	0.00	0.00	0.00	0.01
140	I_δ	-0.54	-0.55	-0.58	-0.44
	I_δ*	0.03	0.01	0.06	0.23
170	I_δ	-0.13	-0.13	-0.11	-0.09
	I_δ*	3.85	2.05	4.49	20.49
Original values on mean proportion of disease x 10^3					
79	I_δ	4.65	4.66	5.11	-34.75
	I_δ*	0.07	0.06	0.01	3.28
109	I_δ	1.03	1.06	0.99	1.04
	I_δ*	3.62	4.16	3.86	14.43
140	I_δ	1.01	1.00	1.02	1.02
	I_δ*	34.35[a]	14.08[a]	61.00[a]	231.24[a]
170	I_δ	1.00	1.00	1.00	1.00
	I_δ*	3.85	2.05	4.49	20.49
Original values on mean proportion of disease x 10^6					
79	I_δ	1.33	1.30	1.28	3.63
	I_δ*	67.71[a]	62.04[a]	12.38[a]	3276.93[a]
109	I_δ	1.19	1.22	1.17	1.24
	I_δ*	3624.67[a]	4158.25[a]	3860.60[a]	14426.27[a]
140	I_δ	1.01	1.00	1.02	1.03
	I_δ*	34351.47[a]	14078.00[a]	60997.79[a]	231235.85[a]
170	I_δ	1.00	1.00	1.00	1.00
	I_δ*	3849.43[a]	2051.44[a]	4487.21[a]	20486.62[a]

[a]Significant ($P \leq 0.01$)

The original low values on the mean proportion of leaf blotch disease gave negative values on the third and fourth dates of assessment (140 and 170 DAP). When the low values on the mean proportion of leaf blotch disease values were multiplied by 10^3 or 10^6, it lead to a simple displacement of the decimal point without any change in the values for both, Morisita's index (I_δ) and the departure value (I_δ^*) for each date of leaf blotch disease assessment in turmeric in all the sampling methods. The test with chi-square distribution (χ^2) on the departure value (I_δ^*) became highly significant ($P \leq 0.01$) on all dates of assessment when original values multiplied by 10^6 were used, compared to the highly significant departure value (I_δ^*) noted only on the third date of disease assessment (140 DAP) with values multiplied by 10^3.

Although the two indices of dispersion namely, index of clumping size (ICS) (Table 42) and Morisita's index (I_δ) (Table 41) varied, both clumping size and departure value I_δ^* were the same on all the dates irrespective of the original mean proportion of disease values or values multiplied by 10^3 or 10^6.

Further, a comparison was made between the Lloyd's index of patchiness (Table 40) and Morisita's index of dispersion (Table 42) estimated with values on mean proportion of disease multiplied by 10^6. Both indices (LIP and I_δ) showed a close match in the trend. Similar values and trend for both indices were encountered on all dates of leaf blotch disease in all the sampling methods.

DISCUSSION

CHAPTER V

DISCUSSION

Curcuma longa - Taphrina maculans pathosystem is one of the major causes for low yields in turmeric crop. The basic research done so far is scanty. There are contradictory reports on the survival and primary sources of inoculum by researchers (Upadhyayay and Pavgi, 1967a; and Kulkarni and Ahmed, 1968). Only preliminary observations on the secondary spread of leaf blotch and interaction of weather factors with the leaf blotch disease were reported (Upadhyayay and Pavgi, 1966; Ahmed and Kulkarni, 1968b). No information is available on the efficacy of new generation fungicides, despite their proven efficiency in controlling many other pathosystems. A comparatively greater attention was given to screening turmeric genotypes to identify resistance or susceptibility. (Nambiar *et.al.*1977; Philip and Nair,1981; Rao *et.al.*1992).

Analyses of temporal progress of certain pathosystems involving aerial pathogens were reported (Berger, 1981). However, the spatial and spatio-temporal investigations on the dynamic aerial pathogens were limited to a very few reports (Thal and Campbell, 1986). Neither temporal changes in epidemics nor the spatial pattern of disease development in *C. longa - T. maculana* pathosystem was ever studied.

The investigations in the present study with the defined objectives for a comprehensive understanding of the epidemiology in *C. longa - T. maculans* pathosystem and on the management of the disease were carried out at Agricultural

Research Station, Kovvur during 1988-89, 1999-2000 and 2000-2001. The results of these investigations are discussed in this chapter.

5.1 THE DISEASE

5.1.1 Symptoms

The appearance of symptoms, progression and symptomatology matched with the descriptions provided by earlier researchers (Butler,1918; Mundkar, 1949; Kulkarni and Ahmed, 1968). The unique observation in the present study is that the infection may extend into the petiolar canal (Plate 9). This petiolar canal infection by *T. maculans* was not reported earlier by any of the researchers.

The nomenclature pertaining to the name of the disease is not uniform as some earlier reports referred to it as leaf spot (Butler, 1911; McRae, 1917; Upadhyay and Pavgi, 1966; Ahmed and Kulkarni, 1968a), while the recent reports referred to it as leaf blotch (Nambiar, 1979; Rao and Rao, 1987; Rao, 1995). Hence, there is a need to fix the nomenclature for the disease and also make a distinction from the other foliar disease of turmeric namely, leaf spot caused by *Colletotrichum capsici* (Sydow) (Butler and Bisby). Since the observations on symptoms in this study as well as the descriptions of earlier workers satisfy the definition of blotch as, "irregular discolouration due to pustules produced by the pathogen following infection", it is appropriate to call the infection by *Taphrina maculans* on turmeric as leaf blotch.

5.1.2 Histopathological Observations

Thin transverse sections of the diseased tissue clearly brought out the presence of cuticle bound groups of naked asci (Plate 10) and mycelia embedded in the

intercellular spaces and walls of epidermis. These observations confirmed the characteristic feature of the causal organism and its identity as *Taphrina maculans*.

The octosporus ascospore colonies developed colonies of conidia by budding (Plates 12 and 13) on moist slides which when observed under a microscope, revealed the yeast like character of the fungus *in vitro*.

5.2 THE PATHOGEN

5.2.1 Etiology of the Pathogen

Among the methods of isolation of *T. maculans* from infected leaves, the method originally described by Martin (1925) in which infected leaf bit was stuck to the inside of the lid of a Petri plate containing nutrient medium was found superior to other methods. In the other methods attempted, the contaminant microorganisms hindered the isolation of the pathogen. Among the nutrient media, PDA was found ideal compared to nutrient agar, oat-meal agar and turmeric leaf extract agar. For isolation of the pathogen, a combination of pH 4.5 of the medium and temperature 20^{o} C was found to be ideal. The low pH (4.5) and incubation temperature (20^{0} C) might have favoured the competitive ability of *T. maculans* relative to microbial contaminants in the culture medium. The successful isolation of *T. maculans* (Ahmed and Kulkarni, 1968a), *T. deformans* (Mix, 1924) and *T. johansonii, T. communis, T. mirabilis, T. coryli* and *T. coerulescence* (Martin, 1925) were reported to depend on PDA, low pH and low temperature conditions. The acidic pH might have totally prevented bacterial contamination and enhanced the chances for the growth of the fungal pathogen.

The measurements on the dimensions of a population of spores of *T. maculans* were made using the formula of John (1970). The range in length and breadth of

naturally obtained spores (4.313 to 4.846 by 2.121 to 2.439 ìm) was within the range reported (4 to 6.5 by 2 to 2.8 ìm) by earlier workers (Butler, 1918; Kulkarni and Ahmed, 1968). The conidia in pure culture were bigger than the naturally obtained conidia (5.63 to 6.33 by 2.767 to 3.193 ìm). These conidia were also within the range of dimensions reported (6 to 7 by 3.5 to ìm) by Kulkarni and Ahmed (1968). The pink and yeast like colony characteristics of *T. maculans* observed in this study also matched with the descriptions of Pavgi and Upadhyay (1964) and Ahmed and Kulkarni (1968a). The naked asci with inter cellular mycelium and yeast like growth of the *T. maculans* in culture further confirmed the dimorphic nature of the fungus (Mix, 1924; Romano, 1966). Thus the identity of the pathogen as *Tapharina maculans* was confirmed.

The fungus was isolated from diseased tissue. Pathogenicity of this pure culture of *T. maculans* on the plants of a susceptible turmeric cultivar in contaminant-free pot culture was proved and established the etiology of the pathogen. Pavgi and Upadhyay (1964) and Ahmed and Kulkarni (1968a) also established the pathogenic ability of such pure cultures of *T. maculans* on turmeric plants.

5.2.2 Influence of pH and Temperature on Growth of *T. maculans* on Culture Medium

T. maculans was found to exhibit tolerance to a wide range of pH levels. When cultured on PDA, the limits to the tolerance of this fungus may probably extend beyond the range of pH tested i.e., 4.0 to 8.0. However, the optimum hydrogen ion concentration was found to be between 5.5 and 7.0, respectively on 10^{th} and 20^{th} day after inoculation (Table 2). The optimum incubation temperature was found to be 15 -

20^{0} C. The growth was limited at other temperature regimes. Temperature and pH did not have any interactive effect on the growth of the fungus (Table 3).

In a related species namely, *Exoascus deformans* (*Taphrina deformans*), Mix (1924) found that the optimum conditions for growth were reached with a temperature of 20^{0} C and pH 5.0. This study is the first to find out the optimum limits of the pH of growth medium and incubation temperature for *T. maculans*.

5.2.3 Variability Among Isolates of *T. maculans*

Upadhyay and Pavgi (1967b) considered a differential pathogenecity of the isolates relative to the naturally occurring infection by *T. maculans* on *C. amada.* Therefore, variability in the pathogen was speculated. In the present study, there was no perceptible difference in the isolates of *T. maculans* from the three turmeric-growing tracts of Andhra Pradesh either in cultural characters or in SDS-PAGE protein profiles (Plate 17). *Curcuma amada* was observed to be totally free of *T. maculans* infection even when surrounded by heavily infected turmeric cultivar.

5.3. SURVIVAL AND PRIMARY SOURCE OF INOCULUM OF THE PATHOGEN

The retrieval of *T. maculans* in culture from pretreated leaf debris of previous year's infected crop up to September and October suggested that the pathogen survived in the leaf debris and possibly acted as the primary source of inoculum for the next year's infection. Upadhyay and Pavgi (1967a) also demonstrated that *T. maculans* survived in infected turmeric leaves of previous crop up to January when stored at room temperature and that in the field, survival of the fungus was limited to the surface layers of soil.

In this study, the infected leaves were established to be a primary source of inoculum in pot culture also. This result is in contrast with the report of Ahmed and Kulkarni (1968b) who reported that leaf debris, seed rhizomes and soil from infected fields of previous year do not act as sources of primary inoculum.

This study conclusively proved that seed rhizomes from infected crop of the previous year are the best primary source of inoculum. The pathogen was retrieved with ease by plating washings of rhizomes on PDA compared to the other sources studied. Also the infection was noticed on plants that sprouted from untreated (not disinfected) rhizomes in pot culture earlier than on plants in pots incorporated with leaf debris and soil from infected fields. The pathogen might have possibly reached the rhizomes through the infected petiolar canal (Plate 9). In all probability, the spores deposited on rhizomes might have been desiccated gradually under ventilated storage. The desiccated spores of *Taphrina maculans* (Upadhyay and Pavgi, 1967a) and *T. deformans* (Mix, 1924) have been shown to attain heat resistance. Further, such inoculum in seed rhizomes might have encountered lesser competition than the inoculum in soil and explains the higher level of survival potential of the spores in the seed rhizomes.

In this investigation, efforts to isolate *T. maculans* from soil collected from infected fields were not successful. However, the soil from infected fields of previous year was found to be a source of primary inoculum in pot culture albeit in only 25 to 50% of pots tested. Upadhyay and Pavgi (1967a) demonstrated that blotch spores washed down from infection spots during the crop season were liable for desiccation with the start of summer and acquired tolerance to high temperature. *T. maculans* is thus

capable of perpetuating in soil. A related species of *Taphrina, T. deformans* was not found to have a similar capability to perpetuate in soil (Mix, 1924).

T. maculans was found to survive on *Canna* plants (Plates 18 and 19) which are present along the bunds in fields round the year. This is the first finding that *Canna* sp is another host of *T. maculans* and possibly contributes to the primary inoculum. Plants like *Curcuma amada, C. angustifolia, Zingiber casumunar, Z. zerumbat* and *Hedychium* (Butler, 1918) and *Z. meioga* (Mix, 1949) were reported to suffer from *T. maculans* infection. Upadhyay and Pavgi (1967b) expressed doubt on the ability of *T. maculans* to infect *C. amada.* In this study also, *C. amada* was found to be totally free from *T. maculans* infection even under a high inoculum density.

The present study in addition to the earlier reports established the many avenues available for the survival of *T. maculans*. The other species of *Taphrina*, which infect only the crops in temperate regions have not been reported to perpetuate in as many ways. The findings on the primary source of inoculum thus explain the unfailing incidence of *Taphrina* leaf blotch disease when a susceptible turmeric cultivar is introduced into a new area far away from the traditional tracts.

5.4 GENOTYPE REACTION

In the present study, 28 turmeric cultivars mostly belonging to intermediary maturity duration (8 months) and 8 belonging to short duration (7 months) were found susceptible to *T. maculans* infection. In all 25 cultivars were found susceptible to *C. capsici,* while 6 other cultivars were found resistant to both *T. maculans* and *C. capsici.* The cultivars susceptible to *T. maculans* in general, did not vary much in their disease severity (Table 7). These observations are similar to those made by Sarma and

Dakshinamurthy (1962). Rao *et al.*(1992) also reported that cultivars belonging to intermediate duration were susceptible and all short duration types were resistant to leaf blotch disease. Reddy *et al.*(1963) and Rao *et al.*(1992) suggested that a genotype is resistant to only one of the two diseases. But Philip and Nair (1981) reported simultaneous infection by both the pathogens on nineteen genotypes. In the present study, no genotype was found infected by both the foliar pathogens of turmeric, *T. maculans* and *C. capsici.* A genotype susceptible to either of the pathogens was invariably resistant to the other.

5.4.1 Variability Between Groups of Cultivars Susceptible to Leaf Blotch or Leaf Spot

In this study, the turmeric genotypes were classified into two major groups based on their reaction to the foliar pathogens. There were distinct and visually perceivable differences between the two groups of cultivars susceptible either to *T. maculans* or *C. capsci.* These differences were in the site of infection and disease progress, as well as in the leaf colour, and in the colour and shape of finger rhizomes. The curcumin content in rhizomes of the leaf blotch infected cultivars was also found to be higher than that in the rhizomes of cultivars susceptible to leaf spot. These observations recorded by Rao (2000) on curcumin content in the rhizomes of turmeric genotypes indicated that the genotypes susceptible to leaf blotch disease contained higher curcumin in the rhizomes than in the rhizomes of Armoor and Duggirala, which are susceptible to leaf spot disease. Narasimhudu (1999) however, did not find significant differences in the curcumin content in turmeric cultivars susceptible to *Colletotrichum* leaf spot, and *T. maculans* leaf blotch.

It is difficult for a Plant Pathologist or a Horticulturist to identify the leaf blotch susceptible, leaf spot susceptible and resistant genotype based on a few morphological characters. Hence it was necessary to apply a method by which identification and classification of genotypes would be possible based on morphological characters and obtain a confirmation on grouping based on qualitative assessment of disease reaction to the two foliar diseases.

The multivariate analysis using the simple Euclidean distance statistic (Kendall, 1986) provides a useful measure for separating the genotypes into clusters. The clusters thus formed can then be verified if they are matching with the groups made based on qualitative assessment for disease reaction to the leaf blotch and leaf spot. In the present study, the 67 cultivars studied were distributed into four clusters based on Euclidean distance and Ward's minimum variance dendrogram (Fig.2 and Table 8). All the genotypes except one in the cluster I with the lowest intra-cluster distance (Fig. 3) were susceptible to leaf blotch disease. The leaf spot susceptible cultivars and cultivars resistant to both the foliar diseases were distributed in the three remaining clusters. It was thus possible to segregate the cultivars susceptible to leaf blotch disease to a great extent since they were more uniform than the cultivars susceptible to leaf spot and cultivars that were resistant to both the foliar diseases. Leaf length, weight, length and length/girth ratio of finger rhizomes recorded in leaf blotch susceptible cultivars significantly differed with those recorded in cultivars susceptible to leaf spot, and cultivars resistant to both foliar diseases. Similar such significant differences in plant height and number of mother rhizomes were also recorded in this study.

Initially groups were formed in the turmeric cultivars based on qualitative assessment of disease reaction to leaf blotch and leaf spot. Later, clusters were formed in turmeric cultivars based on morphological characters. A comparison of clusters and groups so formed was made for the first time in this study. However, classification of turmeric cultivars based on Mahalanobis' D^2 values resulted in clustering of cultivars with similar duration (Rao, 2000). The cluster with cultivars of intermediate duration in that study matched with the cluster with cultivars susceptible to leaf blotch identified in this study.

The study of isoenzyme pattern by gel electrophoresis of leaf extracts of turmeric cultivars susceptible to *Taphrina* leaf blotch and *Colletotrichum* leaf spot also revealed the possibility of separating the turmeric genotypes susceptible to leaf blotch from the turmeric genotypes susceptible to leaf spot (Plates 22 and 23). Such differentiation can be made in the laboratory. This technique could be used as an effective and quick laboratory tool for differentiation of two groups of genotypes.

5. 5 TIME OF PLANTING AND DISEASE SEVERITY

No information is available as on date, on the influence of planting time on leaf blotch severity in turmeric. In this study, the final leaf blotch severity in turmeric, in general, decreased with delay in planting time (Table 12), from the first fortnight of May to the second fortnight of August. This may be due to the greater availability of ontogenically ready site of infection in early plantings than in the successive later plantings. Further, the vegetative growth of the plants was also substantially less in the plantings made after first fortnight of July compared to the growth of plants in the earlier plantings. The yield of fresh rhizomes, in general, also decreased with the delay

in planting time. The reduction in yield with delay in planting time from first fortnight of July may be due to insufficient vegetative growth phase. By developing proper agronomic package for delayed planting, it is possible to reduce the damage from *T. maculans* and minimize the yield reduction. Narsimhudu (1999), however, reported an increase in severity of leaf spot caused by *C. capsici* and a decrease in yield of rhizome with delay in planting time up to the end of July while the planting made in the first week of August showed a decreased severity.

5.6 PLANT POPULATION DENSITY AND DISEASE SEVERITY

Spacing adopted between plants and between rows is well known to influence the disease development (Nagarajan and Muralidharan, 1995). Venkat Rao and Muralidharan (1982b) also found less rice blast severity at wider planting, which resulted in higher grain yield. In the present study also, turmeric plant population density had a significant effect on leaf blotch severity (Tables 14a, 14b, 14c). A decrease in plant population density through widening the distance between rows and plants decreased the leaf blotch severity. The surface wind currents can easily penetrate at wider interplant spaces and blow away the inoculum from the plot that is otherwise confined mostly below the canopy level. In contrast a close covering by crop foliage at closer plantings prevents spore movement, dissipation and loss. Also at low plant density, the microclimate within the crop canopy may not be as congenial for pathogen multiplication and infection as at higher plant density.

The plant density also influenced the vegetative growth characters in turmeric cultivars susceptible to leaf blotch (Table 16a, 16b). The increased plant height and leaf length at high plant population densities may be due to an elongated growth under

competition for sunlight. At low plant population density, the greater number of tillers observed might have resulted from crop reaction to adjust to the available space.

The yield of fresh rhizomes in turmeric cultivar susceptible to leaf blotch was also influenced by the plant population density. The yield of rhizomes decreased with a decrease in plant population density despite reduced disease severity (Tables 14, 17). It is, therefore, apparent that an optimum plant population density of 49383 plants ha^{-1}, at a spacing of 45 x 45 cm must be maintained to avoid damage from *T. maculans* and yet harvest high yields in turmeric.

5.7 DISEASE MANAGEMENT WITH FUNGICIDES

The earlier attempts on management of leaf blotch disease in turmeric were mostly confined to use of protectant fungicides like zineb, copper oxychloride, captan, cuman (Nirwan *et.al.*1974) and Dithane Z-78, Dithane M-45, Bavistin and Cuman L (Srivastava and Gupta, 1977).

In the present study, for the first time, systemic fungicides like bitertenol, propiconazole and tridemorph besides protectant fungicides like chlorothalonil, kitazin and copper oxychloride were evaluated for their efficacy on leaf blotch disease. Propiconazole, bitertenol and chlorothalonil provided the best disease control (Table 18). Tridemorph, kitazin and copper oxychloride also reduced the leaf blotch severity. The efficacy of chlorothalonil in control of peach leaf curl caused by *T. deformans* was well demonstrated (Tate *et al.*1991; Ko *et.al.*1998). Bitertenol and tridemorph (Mehdi *et al.*1994) were also found very effective in the control of peach leaf curl caused by *T. deformans*. Tate *et al.*(1991), however, found the DMI (demethylation inhibitor) fungicides including propiconazole to be effective in controlling *T. deformans* only at

very high doses. For the fungicidal control of peach leaf curl, copper fungicides were found highly effective (Pierce, 1900; Schwabe and Williams, 1999; Tate *et al.*1991 , Montero *et al.*1992). However, the commonly available copper oxychloride in the present investigation when applied at 0.25% showed a slight phytotoxicity on turmeric. Verma (1986) also reported similar phytotoxic effects of copper oxychloride (Fytolan) on turmeric.

5.8 MICRONUTRIENTS AND LEAF BLOTCH SEVERITY

The micronutrients, zinc sulphate (0.2%) and ferrous sulphate not only reduced the leaf blotch severity but also increased the rhizome yield in turmeric (Tables 20, 21). It is possible that the two micronutrients might have altered the physiology of the susceptible turmeric plants because of which infection by *Taphrina maculans* was reduced and the yield was increased.

5.8.1 Yield Loss and Comparative Economics of Application of Fungicides and Micronutrients

Yield loss in turmeric due to infection by *T. maculans* was suspected by Butler (1918) and hence recommended control measures. However, no estimates were ever made on the yield loss caused by *T. maculans* in turmeric crop. In the present investigation, the differential efficacy of fungicides recorded on blotch severity (Table 18) and yield (Table 19) in turmeric was used to derive a yield loss prediction model. The model reveals that for every 1% increase in PDI, there would be a loss of 308 kg ha^{-1} in yield. Such a loss is substantial to farmers as turmeric is a commercial crop. Compared to the yield in the best fungicide treatment, propiconazole (0.1%), the yield

loss was as high as 35% in the control plots. Hence, timely and effective management practices should be adopted to prevent yield loss by *T. maculans* in turmeric.

The present study clearly demonstrated that high economic returns could be realized with the use of fungicides and micronutrient to control leaf blotch on turmeric crop (Table 22). The best economic return with C:B ratio of 8.15 was obtained with the application of fungicide, propiconazole (0.1%). Similarly, micronutrient, zinc sulphate also gave a high C:B ratio of 7.44.

5.9 BARRIER CROPS AND LEAF BLOTCH SEVERITY

Rainsing a barrier crop surrounding main crop is well known to prevent epidemic spread of many pathogens (Nagarajan and Muralidharan, 1995). However, the attempts made in the present investigation to contain secondary spread of inoculum by raising barrier crops all round the leaf blotch susceptible turmeric cultivar were not successful in reducing damage from *T. maculans*. Raising barrier crops resulted in an actual increase in disease severity and this led to a decrease in the rhizome yield in turmeric (Tables 23, 24). As evident from the observations of spore trap, the inoculum of *T. maculans* remained below the crop canopy level until infection reached the apical leaves. Therefore, barrier crop did not apparently allow inoculum to scatter but helped to retain within the crop canopy resulting in a greater disease severity on turmeric. This is confirmed further by the fact that the yield in turmeric plots surrounded by barrier crops was less than the yield recorded in plots without any barrier crop cover. The significant reduction in yield may not entirely be due to disease alone, as the competition posed by the barrier crop might have also contributed to such a reduction in yield.

5.10 LEAF BLOTCH ON TURMERIC CROP AND ITS RELATIONSHIP WITH WEATHER

If a susceptible host and pathogen occur together, disease develops only as permitted by the physical environment (Fry, 1982). During the progress in time of the epidemic, cycles of events occur repeatedly in which inoculum is produced, dispersed, intercepted and some surviving propagules infect. As a consequence of this multiplication, the disease intensifies and can reduce crop yields (Butt and Royale, 1990). The severity depends upon the multiplicity of environmental factors, both biological and physical. Weather represents atmospheric conditions such as temperature, rainfall, cloudiness, relative humidity and wind at a short period of time like a day (Nagarajan and Muralidharan, 1995).

Interpretation of multiple linear regression equation is limited by several considerations. Its application to the joint observation of independent or determining variable (weather variables like temperature, rain fall, rainy day and relative humidity) on dependent variable or response variable (disease, PDI) will have importance of contribution of each independent variable to PDI. In contrast to regression, correlation measures the degree of association between variables of equal status; there need be no causing effect. In analyzing the relationship, after performing correlation analysis, stepwise regression method was followed to provide for the inclusion and deletion of independent variables at each step.

The best-fit regression equations assembled by computer programmes for each year of this study did not agree with one another. The prediction of disease severity (PDI) made with the best-fit equation of one year for the remaining two years of study

did not fully agree with the actually observed disease severity of leaf blotch disease (Table 31). This is mainly due to the fact that the independent variables showed a high degree of inter-correlation (stickiness).

The interpretation of multiple linear regressions is limited by several considerations. First, evidence of regression is not evidence of causation (Butt and Rolaye, 1990). Preliminary observations on temperature, wetness and humidity were reported to have an influence on infection of *T. maculans* on turmeric (Upadhyay and Pavgi, 1966; Ahmed and Kulkarni, 1968b). Hence, relationship identified in the present study between disease severity with maximum temperature, minimum temperature in 1998-99 (Table 26), maximum temperature, rainfall and relative humidity (0800h) in 1999-2000 (Table 28), and minimum temperature, rainfall and relative humidity (0800h) during 2000-01 (Table 30) cannot be ignored as meaningless.

In laboratory or growth chamber studies, the control exercised by experimental design and experiment allows the response to each treatment to be measured independently, so that the variables are not inter-correlated. This situation is changed in the present study, when features of *T. maculans* epidemic are measured in holistic field experiments in which multitudes of uncontrolled factors operate and interact simultaneously. The extensive inter-correlation in natural *Curcuma longa-T. maculans* pathosystem made the identification of key variables very difficult. Yet, each of the four independent variables, namely, maximum temperature, minimum temperature, rainfall and relative humidity (0800h), showed a high degree of stickiness as well as exerted influence on leaf blotch disease index, in at least two of the three years studied.

The range (between the maximum and minimum) in each of these variables was very much limited. This might have led to invalidating predictions as estimated in this study. Further work for several more seasons may be needed to take into consideration all the other features of the weather variables to derive a perfect leaf blotch prediction model.

5.11 TEMPORAL ANALYSIS OF *T. maculans* EPIDEMICS ON *C. longa*

The description and accurate analysis of the dynamic process of plant disease increase is essential to understand the rapidness at which the epidemic progresses (Van der Plank, 1963; Kranz, 1990). It also provides clues as to when it would be effective to make an intervention for the management of disease. The characteristics of an epidemic can be discerned from disease progress curve, which is likened as the signature of the epidemic (Campbell and Madden, 1990). The main tools employed in the analysis of plant disease epidemics have been the transformation equations describing disease progress with time which help in the linearization of disease progress curves (Van der Plank, 1963). It is essential to linearize disease progress curves to determine epidemic speed, to project future disease and to estimate initial disease (Berger, 1981).

The disease progress in *C. longa-T. maculans* pathosystem in time was studied for two years in six cultivars. The data on disease severity, as per cent disease index (PDI), recorded several times from the first appearance of disease at about 10 day interval were used for analyzing the *T. maculans* epidemic on *C. longa*.

An epidemic can be defined as a change in disease (incidence or severity) in a host population over time and space (Kranz, 1974b). The fundamental way of depicting a plant disease epidemic is to plot disease level at several times or distances. The plot

of disease versus time, the disease progress curve, summarizes the interaction of pathogen, host and environment in disease development (Van der Plank, 1963; Kranz, 1974a, 1978). In this study, the disease progress curves for the 2 epidemics on 6 cultivars were plotted over time using PDI (Fig.10 & 11). There was a difference in the time of first appearance of disease between 1998-99 and 1999-2000. The disease onset in 1998-99 was 109 DAP, while it was 64 DAP in 1999-2000. The delay in the onset of disease during 1998-99 may be attributed to the incidence of extremely high temperature regimes in May 1998 (> 47° C for 15 consecutive days) without any summer rains. During the length of the epidemic only progressive phase was observed, in both the years i.e., the disease continued to increase throughout the course of the epidemics on turmeric. In general, the epidemic reached its mid point in about 60 days from the onset of disease in all the cultivars during 1998-99 and 50 days after onset of disease in 1999-2000.

Inspection of disease progress curves in the two epidemics did not show a levelling off of PDI at any time. But the disease progress curves showed subtle and extremely small fluctuations. The mean PDI at each time of observation was taken to calculate the small changes (*dy/dt*) and plotted versus time (Fig. 12 and 13). The absolute rate (*dy/dt*) revealed that the leaf blotch continued to increase at a minimum absolute rate of about 0.002 or slightly less than 0.002 (Fig. 12 and 13). Although there were 4-6 peaks in *dy/dt* versus time graph, the maximum inflection point of 0.01 was reached between 179 and 199 DAP in 1998-99 and between 114 and 135 DAP in 1999-2000, which coincided with the middle part of the respective epidemics.

This study showed that *T. maculans* caused a continuous increase in disease throughout the epidemic. Van der Plank (1963) suggested for the application of a correction factor (1- proportion of disease) to consider the changing quantum of available host. The equation proposed by Van der Plank to calculate apparent rate of disease development helps to compare and estimate speed of epidemics. The maximum apparent rate of infection (*r*) was reached 189 DAP in 1998-99 (0.017) and 124 DAP in 1999-2000 (0.016) (Table 33). The rate of leaf blotch disease increase in turmeric crop was very low during both the years. The description of the leaf blotch epidemics on turmeric gathered from the disease progress curves and estimates on absolute rate and apparent rate of increase in disease during the two epidemics fits the classification of Kranz (1974a) as one having a highly linear growth with no substrate constraints and with more or less constant (*r*) values.

The disease progress curve is now almost universally considered the standard representation of disease development over time in a plant population. After data are plotted, it is common to fit the observations to one of the relatively simple models discussed in this chapter. A disease progress model is simply a way of summarizing an epidemic. Model parameters then can be used to compare and classify epidemics. The quantification of disease progress curves, as well as other biological phenomena, is done with the use of mathematical or statistical models (Madden, 1980; Rouse, 1985). The value of a model is its "potential for bringing out relationships which are not obvious from the data alone" (Pruitt *et al.*1979). There are many types of models that can be used to describe a disease progress curve.

Both logistic and Gompertz models linearized the leaf blotch disease progress curves in all the six turmeric cultivars in both the years with high R^2 values (Table 34). The rate parameter of the logistic model was about 0.01 in the two epidemics (1998-99 and 1999-2000) of *Taphrina maculans* on *Curcuma longa*. The doubling time estimated using the rate parameter in the logistic model (Fig. 16) did not differ among the six cultivars. The leaf blotch doubling time varied from 29 to 32 days in 1998-99 and from 32 to 35 days during 1999-2000 i.e., the disease doubles approximately every month. In an epidemic lasting 130-140 days as observed in this study, the pathogen can easily complete 4-5 cycles leaving aside other overlapping disease cycles. This is in perfect agreement with the observed time for the completion of incubation and latent periods (Tables 5, 6), which is 25 to 30 days in *Curcuma longa–Taphrina maculans* pathosystem.

To discern finer changes, if any, in the disease progress that might have still remained undetected, further estimates in the area under disease progress curve (AUDPC) values were made. The AUDPC values calculated did not differ among the six cultivars (Fig. 17). The AUDPC values did not show any other additional characteristic feature of the epidemic other than what was discerned in the models.

By analyzing temporal changes in *Oryza sativa-Pyricularia grisea* pathosystem, Venkata Rao and Muralidharan (1982a) obtained clues to reduce the number of fungicide applications to control the rice blast epidemic on both leaf and panicle neck phases.

The in-depth analysis of leaf blotch epidemic has shown a progressive increase of disease at a slow rate and identified the inflection point to occur at the midpoint of

the epidemic. Therefore, a single and effective intervention with a fungicide application before the occurrence of inflection point i.e., at about 40 days after the onset of the disease might be warranted to prevent the disease from attaining serious levels.

5.12 SPATIAL ANALYSIS OF TEMPORAL CHANGES OF LEAF BLOTCH EPIDEMIC IN TURMERIC

Plant pathologists have directed a large portion of their efforts to study the disease progress over time, but have, generally neglected the spatial aspects of the disease. This might be due mainly to the lack of adequate methods (Gilligan, 1982; Jeger, 1990). Knowledge of disease progression over time and disease pattern in space are important as they affect progression of disease epidemic and the consequences in fields (Campbell, 1986; Campbell and Noe, 1985). Spatial pattern analysis is useful in providing insight on the dynamics of the host, pathogen and environment interactions within a pathosystem. It also provides a basis for determining the size of sample and sampling method. In a few reports by Plant Pathologists, spatial pattern is generally described as ranging from regular to random and then on to aggregated (Campbell and Pennypacker, 1980; Gilligan, 1982). Ecologists to study spatial pattern have used some analytical tools like the indices of dispersion. Indices have been developed to measure the degree of aggregation in a population. The few reports on spatial aspects of diseases are mostly confined to soil-borne plant diseases and virus diseases (Schuh *et.al.*1988; Madden *et.al.*1987b). Hence an analysis of the spatial pattern of leaf blotch disease in turmeric in relation to the temporal changes was attempted to understand epidemic progress over time and space.The data on proportion of diseased leaf area was

accurately and precisely mapped systematically for this purpose instead of using simpler scale of per cent disease index to estimate disease severity on plants.

For any accurate analysis of the spatial pattern of plant diseases, a most suitable sampling method has to be determined (Mihail and Alcorn, 1987). The most common unit adopted is the quadrat, its size varying with crop density, extent of cropped area, and nature of the disease studies. Rows and even individual plants are also considered as units depending on the type of analysis made. The most common method in locating quadrats is by systematic path selection (Lin *et al.*1979; Mihail and Alcorn, 1987).

In a disease survey of a field crop, a common practice is to collect samples at a constant interval along-path of predetermined shape (Lin *et al.*1979). Basu *et al.*(1977) suggested that when disease incidence was below 20% or above 80%, 10 equally spaced sites located along a W-shaped path covering entire field or a quadrat demarcated with in the field at 10 equally spaced sites along the 90 m diagonal gave essentially equivalent results. Lin *et al.*(1979) concluded in a simulation experiment, that the precision for all the methods was of the same magnitude under random conditions and differed considerably for clustered distribution.

On the basis of such information on sampling methods, vertical, diagonal, horizontal and random sampling methods were employed in the present investigation, with regrouping of quadrats into blocks in the direction of sampling methods. In this study, evidence gathered indicated that random sampling method was the most suitable, because it provided for sampling in the entire field. Data from all the quadrats treated as blocks was used in the analysis, which represented the entire population in the field. Further, in the first and last assessments the maximum values for mean proportion of

disease were recorded only in random sampling method (Fig. 18). The mean disease proportion in random sampling method significantly varied among the blocks (quadrats) in the assessments on 79 and 109 DAP. Such significant differences detected the uneven severity of leaf blotch disease in the field up to 109 days after planting rhizomes in the field. In the last two dates of assessments on 140th and 170th DAP, the blocks showed no significant differences in the mean proportion of disease. Therefore, beyond 109 days, an even or uniform spread of the leaf blotch disease was evident in the epidemic on turmeric crop.

Lin *et al.*(1979) considered coefficient of variation as an important statistical measure in determining the efficiency of sampling methods. In random sampling method, the coefficient of variation in the mean proportion of disease estimated on all dates of assessment was low compared to the other methods. In random sampling method data from all the 13 quadrats were used. In vertical or diagonal sampling data from 8 quadrats, and in horizontal sampling data from 10 quadrats were used. Yet, random sampling method showed lowest values for CV% (Fig. 19).

Using these four sampling methods, further attempts were made to study the temporal changes in the spatial pattern of leaf blotch disease on turmeric crop in the field. The indices of dispersion generally used in the field of Ecology can be adopted in Plant Pathology to ascertain the departure from randomness (Jeger, 1990; Campbell and Madden, 1990; Nagarajan and Muralidharan, 1995). The indices calculated in this investigation were variance to mean ratio (VM), Lloyd's index of patchiness (LIP), index of clumping or cluster size (ICS) and Morisita's index of dispersion (I_δ).

The variance to mean ratio (VM) in the *C. longa* - *T. maculans* showed no temporal changes and suggested a regular spatial pattern of disease occurrence. However, the disease proportion values were too low. When these original low values were multiplied by a constant (10^3 or 10^6), it resulted in a mere displacement of decimal point in the VM estimates (Table 39) and changes result from regular pattern to increasing level of aggregation. For a similar reason, the index of clumping size (ICS) and the test with χ^2 distribution on clump size (C) were also found to be apparently not suitable for discerning any spatial pattern in leaf blotch disease in the turmeric crop (Table 41).

The two other indices that were also estimated included Lloyd's (LIP) and Morisita (I_δ) indices. They did not show any major deviation or alteration following multiplication of the original values on mean proportion of disease by 10^3 or 10^6. Lloyd's index of patchiness and Morisita's index of dispersion (I_δ) helped to discern temporal changes in the spatial pattern of leaf blotch disease, in the turmeric field. Lloyd's index of patchiness and Morisita's index showed a similar trend in the values estimated on all the dates of disease assessment in all the four sampling methods studied (Tables 40 and 42). Such a similarity between these two indices has already been reported in a study on the spatial pattern of virus diseases (Madden *et al.*1987b). The values for both the Lloyd's index of patchiness and Morisita's index were much greater than unity on the first assessment (79 DAP), which indicated a high level of aggregation in the field. In the next date of assessment (109 DAP), the values for the two indices decreased but still remained at little more than 1, indicating a much-reduced spatial aggregation of leaf blotch disease in the turmeric field. In the third and fourth

assessments on 140 and 170 DAP, the values for both indices reached 1, indicating randomness in the spatial pattern of the disease. Therefore, the two indices in the present study conclusively showed a similar and unchanging spatio-temporal trend from an initial aggregation to final random pattern of leaf blotch disease in the field.

In a study of the spatial pattern of alfalfa leaf spot, Thal and Campbell (1986) found the disease severity to be random in the initial stages which became aggregated in the later stages of epidemic based on variance to mean ratio (VM) and Morisita's index (I_δ). They concluded that the aggregation in the later stages was primarily because of auto-infection by the pathogen *Leptosphaerulina briosiana* due to the mucilagenous spores.

Van der Plank (1946) postulated that clusters of infected plants indicated that a pathogen is predominantly spreading from plant to plant, provided that individual samples be within homogenous areas. On the other hand, random pattern of diseased plants although indicated no or insignificant spread from plant to plant, it does not necessarily indicate the source of inoculum (Madden *et al.* 1982).

In the present study, however, the spatial aggregation was in the initial stages. The investigations on the source of primary inoculum showed clearly the infected seed rhizomes and the weed host *(Canna* spp) as the initiator of leaf blotch epidemic in fields. In this study soil and leaf debris were also shown to harbour the pathogen. But the intense summer heat and change in the choice of field every year to grow turmeric may prevent such sources to act as primary sources. Therefore, the initial aggregation might have arisen as a result of the development of multiple foci of infection due to primary source of inoculum in seed rhizomes or from *Canna* sp. Although spatial

pattern was aggregated on 79 days after planting seed rhizomes, the heavy inoculum led to fast spread through autoinfection within the crop canopy. Several overlapping cycles of infections are quickly completed during the epidemic. This apparently resulted in a regular and uniform pattern of disease as confirmed by the fact that both Lloyd's index of patchiness and Morisita's index (I_δ) remained equal or less than 1.0. The study on the temporal changes in the spatial pattern of leaf blotch disease in turmeric field provided another effective clue that can be exploited in the disease management. The leaf blotch disease was aggregated in the initial stages of epidemic at 79 DAP, which spread and became uniform beyond 109 days after planting suggesting that any management intervention should be made around 100 - 110 days after planting in fields.

Proper selection of seed rhizomes free of pathogen and removal *Canna* plants will definitely help to control the disease onset and initial progress. This can reduce the intensity of an epidemic and delay its occurrence. Certain weather variables like maximum temperature, minimum temperature, rainfall and relative humidity (0800h) showed a definite influence on the leaf blotch severity. Therefore, besides intervention in the cultural practices, an intervention, if needed, through a fungicide particularly between 100 and 110 days after planting rhizomes would definitely help control the leaf blotch disease that can cause considerable yield loss. Such a possibility of an intervention that arise from this epidemiological investigation would be highly profitable to turmeric growers as these interventions which cost very little to adopt, will prevent yield and monetary losses.

5.13 CONCLUSIONS

In *C. longa-T. maculans* pathosystem, the causal organism was established to possess many avenues like infected rhizomes, leaf debris and soil for survival. Although leaf debris and soil from previous year's infected fields may enable a survival of the pathogen, their role as primary source for the infection in the succeeding year's crop is doubtful, as the turmeric crop is invariably shifted to a new field not grown to it in the earlier season.

In such a situation, the contaminated or infected seed rhizomes, therefore, appear to be the major source of primary inoculum. *Canna* plants along the bund and in the neighbourhood of turmeric field also provide additionally the primary inoculum as established by the cross-inoculation to turmeric plants. Disinfection of seed rhizomes with fungicides and removal of *Canna* plants in the vicinity of turmeric field can positively limit the load of primary inoculum and thereby delay and restrict the progress of the epidemic in *C. longa-T. maculans* pathosystem.

T. maculans on turmeric completes one life cycle i.e., from spore to spore, in 25-30 days and hence should be completing 4-5 cycles from the time of onset of disease. The ascospores and blastospores produced would remain within the crop canopy and cause repeated infections on the progressively maturing leaves.

By planting cultivars resistant to *Taphrina* leaf blotch, the leaf blotch disease can be avoided. But most of these blotch resistant cultivars were found susceptible to *Colletotrichum* leaf spot, which also causes a substantial loss. The few cultivars found resistant to both the foliar diseases need to be subjected to rigorous inoculation tests to confirm their resistance to the two foliar pathogens. There is also a need to develop

suitable agronomic practices for these resistant cultivars before they are recommended for cultivation. This investigation has shown that it is possible to identify the leaf blotch susceptible cultivars and leaf spot susceptible cultivars by their rhizome characteristics, even if their reaction to the two foliar diseases is unknown. Using such methods, a resistant cultivar may be chosen for cultivation in endemic areas specific to one or the other foliar diseases.

Planting turmeric late in the second fortnight of July and maintaining a plant population of 49383 plants ha^{-1} at 45 x 45 cm spacing would be ideal to reduce leaf blotch severity and harvest economically high yields.

Four weather variables such as maximum temperature, minimum temperature, rainfall and relative humidity (0800h) were found to influence the disease severity on turmeric. Although best-fit stepwise regression equations were identified, when they were used to predict disease severity in other years, the predicted and observed severities did not agree totally. At best they could predict partially only for one year. The problem was the stickiness encountered in the weather variables. Further, only for two years out of the three years studied, each of the identified predictor-variable occurred in the best-fit equations. This study indicated that further work on these variables under both field and controlled conditions is warranted for developing accurate prediction equations.

Raising any barrier crop all around the main crop instead of restricting actually increased the disease severity on turmeric crop. This was identified to be mainly due to the aid provided by barrier crop in containing the inoculum within the turmeric crop canopy.

The yield loss in untreated turmeric field due to *T. maculans* infection can be as high as 35% and such a loss can be avoided by a timely intervention with fungicide application. Foliar spray application of zinc sulphate and ferrous sulphate has been shown to reduce leaf blotch severity and improve crop growth.

Temporal and spatial analysis of the *C. longa–T. maculans* pathosystem revealed that the maximum inflection point (temporal) as well as spatial aggregation reach maximum levels around 110-120 DAP in the turmeric field. Therefore, timing an intervention by a fungicide application at 100 DAP may help to check the disease and prevent yield loss.

This study on *C. longa–T. maculans* pathosystem would serve as model for studying the temporal and spatial progress of other pathosystems, since there is very little or no information on these aspects on tropical crop plant diseases, particularly in India.

SUMMARY

CHAPTER VI

SUMMARY

The investigations on the epidemiology and management of leaf blotch disease of turmeric were carried out with the agenda of the set objectives at the Agricultural Research Station, Kovvur during 1998-99, 1999-2000 and 2000-01. The important findings of the investigations are summarized hereunder.

The isolation of the pathogen *Taphrina maculans* was difficult, as it required rigid cultural conditions. Nevertheless, it was isolated at a temperature of 20^0 C on PDA with pH adjusted to 4.5, which helped to prevent bacterial contaminants. The identity of the fungus isolated from leaf blotch tissue was confirmed by comparing conidial measurements with previous reports and by proving its pathogenicity. Potato dextrose agar was found ideal for growth of *T. maculans*. The optimum pH of the culture medium was around 5.5 and the optimum incubation temperature was between 15 and 20^0 C. SDS-PAGE protein profiles revealed no differences among the isolates of *Taphrina maculans* obtained from the three turmeric growing regions in Andhra Pradesh.

The pathogen was shown to possess many avenues to perpetuate from one season to the other. This study demonstrated that leaf debris, seed rhizomes and soil from infected fields of the previous year served as primary sources of inoculum. A search for alternative hosts led to detection of *Canna* sp. infected by *T. maculans*. The isolate from *Canna* was proved to cross-infect susceptible turmeric cultivars. Therefore,

Canna can contribute to the primary inoculum for the development of *Curcuma longa-Taphrina maculans* pathosystem.

The incubation period (*P*) in this pathosystem varied with the cultivars; but the latent period (*p*) and infectious period (*i*) did not vary. The incubation period (*P*) was 20 to 25 days, the latent period (*p*) was 5 to 6 days and the infectious period (*i*) was 24 to 25 days in different susceptible cultivars.

Most cultivars in intermediate maturity duration group and a few in the short duration group were found susceptible to *Taphrina* leaf blotch. The disease severity as measured by per cent disease index varied between 66 and 76. The turmeric cultivars susceptible to *Taphrina maculans* and *Colletotrichum capsici* exhibited visually perceivable differences and they were documented. The turmeric cultivars susceptible to *Taphrina maculans* leaf blotch were found to be more uniform based on Euclidean D^2 distance technique performed with 13 morphological characters. These cultivars were taller, had longer leaves and recorded a smaller length/girth ratio of finger rhizomes than the cultivars susceptible to *Colletotrichum* leaf spot. The curcumin content was higher in the rhizomes of leaf blotch susceptible cultivars compared to that in the rhizomes of cultivars susceptible to leaf spot. The cultivars susceptible to leaf blotch were separated from those susceptible to leaf spot based on isoenzyme patterns of polyphenol oxidase and esterase derived through polyacrylamide gel electrophoresis.

Cultural practices like the time of planting and plant population density influenced the leaf blotch severity and yield in turmeric cultivars. Any delay in the planting time decreased leaf blotch severity; it also resulted in a decrease in yield. A

decrease in plant population from 98765 plants ha^{-1} (normal) to 24691 plants ha^{-1} resulted in a decrease in leaf blotch severity and yield of turmeric.

The systemic demethylation inhibitor fungicides, propiconazole (0.1%) and biterternol (0.1%) besides the protectant chlorothalonil (0.2%) were highly effective in reducing leaf blotch disease severity and increasing the yield in turmeric. The best economic return was realized with propiconazole (C/B ratio: 8.15). Micronutrients zinc sulphate (0.2%) and ferrous sulphate (0.2%) also reduced the disease severity and increased the yield. The barrier crops *Amorphophallus, Colocasia* and leaf blotch resistant turmeric cultivar (KTS 8) were ineffective in reducing the disease severity. Raising them around leaf blotch susceptible turmeric crop resulted in increase in the disease severity and a decrease in yield.

Using the differential severity of leaf blotch and yield recorded with different fungicides in the fungicide control experiment, a regression model for estimation of yield loss was constructed. From this model it was estimated that for 1% increase in severity, there would be a yield loss of 308 kg ha^{-1}. In comparison with the best fungicide treatment, the yield loss in control plots was 35%.

Six weather variables namely, maximum temperature, minimum temperature, rainfall, rainy days, and relative humidity at 0800 and 1400 hours were found to exert a definite influence on the leaf blotch disease severity (PDI) in turmeric crop. Step-down multiple regression equations were constructed and the best-fit models were identified for each year study. Only four weather variables, namely maximum temperature, minimum temperature, rainfall and relative humidity (0800h) occurred in the best-fit models. When the best-fit equations were used to predict PDI and test with actually

recorded observations on PDI, predictions and observations did not totally agree. The correlation matrix showed a lot of stickiness among the weather variables. Each of the four identified weather variables occurred in the best-fit model for only two years. Further, the range (maximum and minimum) in each of the weather variables was limited. Yet, this study clearly showed the influence of some weather variables on leaf blotch disease. Further studies with these identified weather variables in the field as well as under controlled conditions in a greenhouse might help in accurately predicting the leaf blotch disease severity on turmeric.

The studies on temporal changes in disease progress showed that there was a steady and rather slow increase from the first appearance of leaf blotch at about 2-3 months after planting rhizomes in the field. The growth curve followed a typical sigmoid pattern. Logistic or Gompertz transformation of the proportion of the disease linearized the curve to some extent. Calculation on the absolute rate of disease growth showed fluctuations. Five to six peaks in the absolute rate were discernible, but the rate of increase was very low. Each peak roughly coincided with about 20-30 days time. With regression models on the transformed disease proportion over time, the rate parameter for disease growth was arrived. The doubling time for disease estimated using this derived rate for each cultivar showed no significant differences among the cultivars and between years. The doubling time for leaf blotch disease in turmeric crop ranged from 30 to 33 days. The area under disease progress estimated for the two epidemics showed distinct level of disease occurrence in the two years. The epidemic in 1998-99 was more intensive than the one in 1999-2000.

Spatial analysis of the temporal changes in the leaf blotch disease in turmeric crop showed interesting and useful information. The leaf blotch disease in turmeric occurred 79 days after planting rhizomes. Random sampling method was found to be the best for collection of samples for recording proportion of blotched leaf area for use in the spatial analysis of temporal changes. The indices of dispersion, Lloyd's index of patchiness (LIP) and Morisita's index (I_a) clearly explained the changes in the spatial pattern of leaf blotch disease. Leaf blotch disease showed an aggregation at the initial assessments (79 and 109 DAP) and became random at later stages (140 and 170 DAP) of the epidemic.

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*Original not seen

Literature cited is presented as per the guidelines of Acharya NG Ranga Agricultural University.