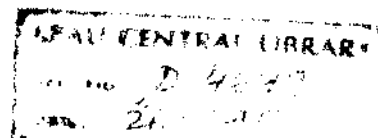


# STUDIES ON RING SPOT DISEASE OF SUGARCANE



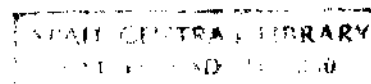
BY

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M. Sc (Ag)

THESIS SUBMITTED TO THE  
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IN PARTIAL FULFILMENT OF THE REQUIREMENTS  
FOR THE AWARD OF THE DEGREE OF  
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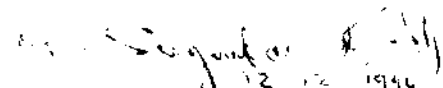
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**DECEMBER, 1994**

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Date : 12.12.1994

  
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This is to certify that the thesis entitled, **STUDIES ON RING SPOT DISEASE OF SUGARCANE** submitted in partial fulfilment of the requirements for the degree of **DOCTOR OF PHILOSOPHY IN AGRICULTURE** of the Andhra Pradesh Agricultural University, Hyderabad, is a record of the bonafide research work carried out by **Mr.M.ACHUTARAMA RAO** 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 the assistance and help received during the course of investigations have been duly acknowledged by the author of the thesis.

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## LIST OF CONTENTS

CHAPTER NO.	TITLE	PAGE NO.
	List of Tables	i
	List of Illustrations	vii
	List of Plates	ix
	List of abbreviations	xi
	Acknowledgements	xii
	Abstract	xiv
I	INTRODUCTION	1
II	REVIEW OF LITERATURE	5
2.1	Distribution	5
2.2	Symptomatology	8
2.3	Isolation of the pathogen and establishment of pathogenicity	11
2.4	Disease rating scale and assessment of the disease intensity	21
2.5	Survey for disease incidence	24
2.6	Methods for testing resistance to the disease	25
2.7	Factors affecting disease development	28
2.8	Variation in ring spot pathogen	34
2.9	Survival and spread of the pathogen	35
2.10	Host range	37
2.11	Varietal reaction	38
2.12	Losses due to the disease	41
2.13	Control of the disease with fungicides	45

Contd..

CHAPTER NO.	TITLE	PAGE NO.
<b>III</b>	<b>MATERIALS AND METHODS</b>	
3.1	General	49
3.1.1	Glassware	50
3.1.2	Chemicals	55
3.1.3	Sterilisation of glassware, media and soil	55
3.1.4	Surface sterilisation of leaf material	55
3.1.5	Media used for isolation and maintenance of fungi	56
3.1.6	Fungicides	59
3.1.7	Seed material of test varieties	59
3.1.8	Fertilizer application	59
3.1.9	Irrigation	59
3.1.10	Cultural operations	61
3.2	Experimental	
3.2.1	Symptomatology	61
3.2.2	Isolation and identification of pathogen and establishment of pathogenicity	62
3.2.3	Developing a disease rating scale for estimation of disease intensity	68
3.2.4	Survey for assessment of disease intensity	70
3.2.5	Inoculation and rapid screening techniques	71
3.2.6	Factors affecting disease development	74

Contd..

CHAPTER NO.	TITLE	PAGE NO.
3.2.7	Variation in the isolates of ring spot pathogen	82
3.2.8	Role of soil, host debris, cane setts, irrigation water, and leaf contact on the spread of disease	84
3.2.9	Host range studies	86
3.2.10	Reaction of sugarcane varieties/clones for resistance to ring spot disease	87
3.2.11	Chemical control	89
3.2.12	Losses due to the disease in different sugarcane varieties/clones	94
3.3	Replications	97
3.4	Recording observations	97
3.5	Statistical analysis of data	98
<b>IV</b>	<b>RESULTS</b>	
4.1	Symptomatology	99
4.2	Isolation and identification of pathogen and establishment of pathogenicity	110
4.3	Developing a disease rating scale for estimation of disease intensity	143
4.4	Survey for assessment of disease intensity	147
4.5	Inoculation and rapid screening techniques	155
4.6	Factors affecting disease development	161
4.6.1	Effect of different levels of nitrogen, phosphorus and potassium fertilizers on the extent of disease development	161

Contd..

CHAPTER NO.	TITLE	PAGE NO.
4.6.2	Relationship of nitrogen, phosphorus and potassium contents in the index tissues of resistant and susceptible varieties/clones on the extent of disease severity	169
4.6.3	Age of the crop and intensity of the disease	172
4.6.4	Effect of leaf orientation on the intensity of the disease	174
4.6.5	Moisture stress and water stagnation on disease intensity	177
4.6.6	Effect of silica content of leaves on disease intensity	179
4.7	Variation in ring spot pathogen	181
4.8	Role of soil, host debris, cane setts, irrigation water and leaf contact on the spread of disease	184
4.9	Host range studies	187
4.10	Reaction of sugarcane varieties/clones to ring spot disease	192
4.11	Chemical control	200
4.12	Losses due to ring spot disease in different sugarcane varieties/clones	214
V	DISCUSSION	240
VI	SUMMARY	297
	LITERATURE CITED	304
	APPENDICES	324
	VITA	333

## LIST OF TABLES

TABLE NO.	TITLE	PAGE NO.
1.	Distribution of ring spot disease in cane growing countries of the world.	7
2.	Mean meteorological data from 1981 to 1990 at Anakapalle	51
3.	Mean meteorological data for 1988-89 at Anakapalle	52
4.	Mean meteorological data for 1989-90 at Anakapalle	53
5.	Mean meteorological data for 1990-91 at Anakapalle	54
6.	Details of culture media used in the investigations	56
7.	Details of fungicides employed in the investigations	60
8.	Details of source of isolates of ring spot pathogen	83
9.	Number of spots on 1-8 fully opened leaves of different varieties	103
10.	Number of days taken for the first appearance of symptom, shape and size of ring spot in different sugarcane varieties/clones	107
11.	Fungi isolated from immature spots	113
12.	Fungi isolated from mature spots	115
13.	Cultural characters and spore measurements of different fungi isolated from ring spot lesions	119
14.	Growth and sporulation of ascospores and pycnidiospores of ring spot fungus on sterile host leaf bits of susceptible varieties	121

Contd..

TABLE NO.	TITLE	PAGE NO.
15.	Description and measurements of perithecia, asci, ascospores and pycnidia and pycnidiospores of ring spot fungus	123
16.	Pathogenicity tests with different fungi individually and in combination on different sugarcane varieties	135
17.	Growth and sporulation of <u>Leptosphaeria sacchari</u> and <u>Phyllosticta saccharicola</u> on various media	140
18.	Intensity of ring spot on different sugarcane varieties in different factory areas of coastal Andhra Pradesh, Telangana and Rayalaseema	149
19.	Per cent disease intensity with different inoculation methods in different sugarcane varieties	157
20.	Comparative efficacy of different screening techniques for ring spot in terms of per cent disease intensity	160
21.	Influence of nitrogen alone or in combination with phosphorus and potassium on the intensity of ring spot disease	164
22.	Disease intensity (mean) in relation to per cent NPK in index tissues of sugarcane cultivar Co 7219	166
23.	Disease intensity <u>vis a vis</u> NPK levels in index tissues of sugarcane cultivar Co 7219	167
24.	Per cent disease intensity in resistant and susceptible varieties/clones in relation to per cent leaf nitrogen, sheath phosphorus and potassium	170
25.	Simple correlations of leaf nitrogen, sheath phosphorus and potassium with per cent disease intensity	171

Contd..

TABLE NO.	TITLE	PAGE NO.
26.	Effect of age of plant on the intensity of ring spot disease	173
27.	Effect of orientation of leaf on cane stalk on the intensity of ring spot disease	175
28.	Simple correlation and linear regression relationship between angle of 1 to 8 functional leaves and per cent disease intensity	176
29.	Disease intensity under moisture stress, water stagnation and normal irrigation	178
30.	Effect of silica content of leaves on the intensity of ring spot disease	180
31.	Simple correlation and linear regression relationship between silica per cent in leaves and per cent disease intensity	181
32.	Cultural and morphological characters of six monoconidial isolates of <u>Phyllosticta saccharicola</u>	183
33.	Per cent disease intensity of six isolates of ring spot fungus on different varieties of sugarcane	185
34.	Extent of disease spread in terms of per cent disease intensity through various agencies	186
35.	Reaction of different plant species to artificial inoculation of <u>Phyllosticta saccharicola</u>	188
36.	Disease severity (PDI) in different sugarcane varieties/clones at different periods after inoculation with <u>Phyllosticta saccharicola</u> (1988-89)	193
37.	Reaction of sugarcane varieties/clones to ring spot disease by adult cane inoculation	195

Contd..

TABLE NO.	TITLE	PAGE NO.
38.	Reaction of sugarcane varieties/clones to ring spot disease by Rajoong inoculation	199
39.	Disease intensity (PDI) and reaction in different sugarcane varieties/clones under Rajoong method of inoculation and adult cane inoculation	201
40.	Effect of different concentrations of fungicides on the growth of <u>Phyllosticta saccharicola in vitro</u>	202
41.	Effect of three systemic fungicides at different concentrations on the growth of <u>Phyllosticta saccharicola</u>	203
42.	Effect of different fungicides on month wise incidence of ring spot disease of sugarcane from October to February	205
43.	Effect of different fungicides on the intensity of ring spot disease of sugarcane at harvest	207
44.	Effect of different fungicides on yield parameters and yield of sugarcane	209
45.	Effect of different fungicides on juice sucrose and different juice characteristics of sugarcane	211
46.	Influence of ring spot disease on the content of chlorophyll a and b in captaf sprayed and unsprayed canes of different varieties/clones of sugarcane	215
47.	Simple correlation between per cent disease intensity and chlorophyll a and b	216
48.	Per cent reduction of disease intensity in captaf sprayed canes over unsprayed canes in different sugarcane varieties/clones	218

Contd..

TABLE NO.	TITLE	PAGE NO.
49.	Per cent disease intensity and per cent reduction in length of millable cane, diameter and weight of cane in unsprayed over captaf sprayed canes	219
50.	Results of 't' test of length of millable cane, diameter and weight of cane (n=20)	221
51.	Relationship between per cent disease intensity and reduction in length of millable cane of unsprayed over captaf sprayed canes in twenty sugarcane varieties/clones	222
52.	Relationship between per cent disease intensity and reduction in diameter of unsprayed canes over captaf sprayed canes in twenty sugarcane varieties/clones	223
53.	Relationship between per cent disease intensity and reduction in weight of unsprayed canes over captaf sprayed canes in twenty sugarcane varieties/clones	224
54.	Simple correlation and linear regression relationship between per cent disease intensity and length of millable cane (LMC) diameter and weight of cane	226
55.	Per cent disease intensity in unsprayed cane and reduction/increase in total solids, juice sucrose, purity, glucose, electrical conductivity and pH in unsprayed over captaf sprayed cane juice	228
56.	Results of 't' test of the six juice characteristics of sprayed and unsprayed canes of twenty sugarcane varieties/clones	230
57.	Relationship between per cent disease intensity and reduction in total solids of cane juice of unsprayed canes over captaf sprayed canes in twenty sugarcane varieties/clones	231

Contd..

TABLE NO.	TITLE	PAGE NO
58.	Relationship between per cent disease intensity and reduction in juice sucrose of unsprayed canes over captaf sprayed canes in twenty sugarcane varieties/clones	231
59.	Relationship between per cent disease intensity and reduction in juice purity of unsprayed canes over captaf sprayed canes in twenty sugarcane varieties/clones	232
60.	Relationship between per cent disease intensity and increase in glucose of juice of unsprayed canes over captaf sprayed canes in twenty sugarcane varieties/clones	232
61.	Relationship between per cent disease intensity and increase in electrical conductivity (E.C.) of juice of unsprayed canes over captaf sprayed canes in twenty sugarcane varieties/clones	233
62.	Relationship between per cent disease intensity and decrease in pH in juice of unsprayed canes over captaf sprayed canes in twenty sugarcane varieties/clones	233
63.	Linear regression relationship between per cent disease intensity and the six qualitative characteristics of cane juice	234
64.	Progress of ring spot disease in maturity phase of the crop in captaf sprayed and unsprayed canes of different sugarcane varieties/clones	236
65.	Progress of reduction in cane weight and juice sucrose in unsprayed canes in maturity phase of different sugarcane varieties/clones	237

## LIST OF ILLUSTRATIONS

FIGURE NO.	TITLE	PAGE NO.
1.	Per cent disease intensity in different inoculation techniques	254
2.	Per cent disease intensity in different screening techniques	256
3.	Effect of different levels of nitrogen fertilizer alone or in combination with phosphorus and potassium on the intensity of ring spot	259
4.	Influence of nitrogen, phosphorus and potassium on the extent of ring spot intensity	260
5.	Relationship between per cent disease intensity and angle of the leaf in different sugarcane varieties/clones	265
6.	Disease intensity in different sugarcane varieties/clones under normal irrigation, moisture stress and water stagnation	267
7.	Relationship between per cent disease intensity and silica content in leaves of different sugarcane varieties/clones	269
8.	Reaction of different sugarcane varieties/clones to ring spot disease 1 to 8 months after inoculation	278
9.	Per cent disease intensity in different varieties/clones in Rajoong and adult cane methods of inoculation	281
10.	Effect of different fungicides on the intensity of ring spot disease in sugarcane cultivar Co 7219	282
11.	Effect of different fungicides on cane yield in sugarcane cultivar Co 7219	284
12.	Effect of different fungicides on per cent juice sucrose in sugarcane cultivar Co 7219	286

Contd..

FIGURE NO.	TITLE	PAGE NO.
13.	Relationship between per cent disease intensity in unsprayed cane and per cent reduction in (a) length of millable cane (b) diameter and (c) cane weight in unsprayed cane over sprayed cane	289
14.	Relationship between per cent disease intensity in unsprayed cane and certain qualitative characters of cane juice as reduction/increase in unsprayed over sprayed	292
15.	Progress of disease intensity in maturity phase of the crop	294
16.	Rate of reduction in cane weight and juice sucrose in maturity phase due to ring spot disease in different varieties/clones	295

## LIST OF PLATES

PLATE NO.	TITLE	PAGE NO.
1.	Healthy and ring spot affected sugar cane leaves	102
2.	Mature spots with reddish margin and black dot like perithecia in the straw coloured centre	102
3.	Variety Co 7219 affected by ring spot showing premature senescence of the leaves and a few green leaves at the top	105
4.	Variation in spotting in sugarcane varieties	112
5.	Single ascospore culture showing pinkish growth at centre and greyish black pycnidia of <u>Phyllosticta saccharicola</u> all around	118
6.	Single pycnidiospore culture of <u>Phyllosticta saccharicola</u> showing greyish black pycnidia	118
7.	Perithecia and ascospores developed in sterile host leaf bits X160	125
8.	Asci and Ascospores developed in sterile host leaf bits X640	125
9.	Ascospores(8) of <u>Leptosphaeria sacchari</u> arranged in asci X640	127
10.	Perithecia and ascospores of <u>L. sacchari</u> from natural lesions X160	129
11.	Perithecia, asci and ascospores from natural lesions X640	129
12.	Biguttulate pycnidiospores from natural lesions X640	131
13.	Cirrus of pycnidiospores from pycnidium of <u>P. saccharicola</u> in Culture X160	131
14.	Brown mass of pycnidiospores of <u>P. saccharicola</u> from Culture X640	133

Contd..

PLATE NO.	TITLE	PAGE NO.
15.	Biguttulate pycnidiospores of <u>P. saccharicola</u> from Culture X640	133
16.	Typical ring spot lesions developed on Co 7219 one and half months after inoculation with pycnidiospores of <u>P. saccharicola</u>	139
17.	Typical ring spot lesions developed on Co 7219 one and half months after inoculation with ascospores of <u>L. sacchari</u>	139
18.	Scale for estimating intensity of ring spot disease of sugarcane	146
19.	Inoculated plants of Co 7219 showing susceptible reaction in Rajoong method of screening	163
20.	Inoculated plants of 84A 155 showing resistant reaction in Rajoong method of screening	163
21.	Symptoms of ring spot on <u>Saccharum spontaneum</u> (Rellu) inoculated with <u>P. saccharicola</u>	191

## LIST OF ABBREVIATIONS

a.i.	:	Active ingredient
C	:	Celsius
CD	:	Critical Difference
cm <sup>2</sup>	:	Square centimetre
CMI	:	Commonwealth Mycological Institute
DAP	:	Days after planting
Fig.	:	Figure
g	:	gram
K	:	Potassium
m	:	metre
m <sup>2</sup>	:	Square metre
MAP	:	Months after planting
ml	:	milli litre
mm	:	milli metre
N	:	Nitrogen
No.	:	Number
nm	:	nano metre
P	:	Phosphorus
PDI	:	Per cent Disease Intensity
pH	:	Hydrogen ion concentration
ppm	:	parts per million
RARS	:	Regional Agricultural Research Station
RBD	:	Randomised Block Design
SEm	:	Standard Error mean
t ha <sup>-1</sup>	:	tonnes per hectare
µm	:	Micro metre

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Date : 12.12.1994

(M. ACHUTARAMA RAO)

## DECLARATION

I, M.ACHUTARAMA RAO hereby declare that the thesis entitled, STUDIES ON RING SPOT DISEASE OF SUGARCANE submitted to Andhra Pradesh 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 any part thereof has not been published earlier in any manner.

Date : .12.1994

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#### ABSTRACT

Ring spot of sugarcane caused by Leptosphaeria sacchari van Breda de Hann considered to be a minor disease became severe since 1985-86 with the introduction of commercial varieties like Co 7219, CoT 8201, Co 975 and CoR 8001. Detailed investigations were carried out on this disease from 1988 to 1991 at Regional Agricultural Research Station, Anakapalle, Visakhapatnam district, Andhra Pradesh, India.

Symptoms appeared only on leaf lamina but not on leaf sheath and stalk. The spots were oval to irregular with reddish brown margin and straw coloured centres within which black dot like perithecia were produced. The spots coalesce resulting in drying of leaf followed by premature senescence. The time of appearance of the disease and shape and size of the spot varied from variety to variety.

Perithecia were not produced in culture, but pycnidia and pycnidiospores of Phyllosticta saccharicola were produced abundantly on different media. Perithecia could however be produced on sterile leaf bits of varieties Co 7219, Co 7636 and CoT 8201 inoculated with ascospores when vitamins and sucrose were added and incubated at 24°C and 26°C and exposed to alternate light and darkness for 12 hours each for 18-21 days. Pycnidia of P. saccharicola developed later on these inoculated leaf bits. The spore measurement agreed with those described by earlier workers for L. sacchari and P. saccharicola. Pathogenicity tests with both the sexual and asexual forms produced typical ring spot symptoms. Out of several media tried, OMA + cane leaf extract and PDA + cane leaf extract were found better both for growth and sporulation of P. saccharicola.

For estimation of disease intensity a 0-9 scale was developed and used in the present study. The disease intensity was severe in coastal Andhra Pradesh and Telangana but negligible in Rayalaseema particularly in Renigunta area.

For inoculation, spraying conidial suspension on the leaves was preferred as it is easy and simple to operate. Screening by Rajoong method was preferred for its rapidity and reliability.

The disease intensity increased progressively and significantly with increased level of nitrogen from 0 to 112 and 112 to 224 kg ha<sup>-1</sup>. Application of potassium at 100 kg ha<sup>-1</sup> significantly reduced the disease intensity while phosphorus at the same level did not influence the disease severity.

Age of the crop had no effect on the disease intensity. Highly significant negative correlation was obtained between PDI and silica content and angle of the leaf. Irrespective of the variety disease intensity increased under moisture stress and water stagnation.

Pathogenic variation was not observed among the six isolates of ring spot fungus tested. Only Rellu and SES.594 both belonging to Saccharum spontaneum produced ring spot symptoms, while all other hosts tested did not take infection.

The disease was observed to spread mainly through contact of diseased leaf with healthy leaf and infected host debris on the surface of soil at the base of the clumps.

Eight varieties/clones viz., Co 85045, CoA 89081, 83A106, 84A155, 86A444, Co 85032, 87A142 and 87A279 were found resistant both under adult cane and Rajoong method of inoculation.

Captaf (0.3%), Blitox (0.4%), Bavistin (0.1%), Dithane M-45 (0.3%) and Topsin-M (0.1%) not only significantly reduced the disease in susceptible Co 7219 but also increased the juice quality significantly with slight increase in cane yield.

Chlorophyll a and b contents were significantly reduced due to the disease in susceptible varieties. Significant reduction in length of millable cane, diameter and weight of the cane was observed in unsprayed canes over captaf (0.3%) sprayed canes of the twenty varieties/ clones tested. Similar trend was observed with regard to juice sucrose and pH of juice. The invert sugar glucose and electrical conductivity increased in juice of unsprayed canes adversely affecting the quality of juice.

# INTRODUCTION

## CHAPTER I

### INTRODUCTION

Sugarcane (Saccharum spp. Hybrid) is an important commercial crop in many tropical areas of the world. It occupies a pride of place among the commercial crops not only in the state of Andhra Pradesh but also in the country. Sugarcane not only provides raw material to sugar industry but also at the village level for jaggery making. In recent years sugarcane is also used as energy source and for the production of ethanol, methanol and methane gas and a range of chemicals used in industries. Bagasse, a valuable by-product from sugar industry is being used in paper industry, news print and also for the production of furfural and rayon yarn.

In India, sugarcane occupies an area of 3.62 million hectares with an annual production of 230.83 million tonnes of cane according to final estimates for the production year 1992-93. In Andhra Pradesh, the crop is grown over an area of about 0.17 million hectares, producing about 12.35 million tonnes of cane (Goel et al., 1994) and the state occupies 5th rank for both area and production in India. Sugarcane is cultivated practically all over the state but large areas are concentrated in the districts of Visakhapatnam, Vizianagaram, Srikakulam, East Godavari, West Godavari, Krishna, Nizamabad, Medak and Chittoor. Although a major part of the crop area is under

irrigation, the crop is also raised under rainfed conditions in areas with relatively high rainfall.

Sugarcane is subject to attack by more than one hundred diseases of either fungal, bacterial, viral, mycoplasmal and other inanimate agents. The most significant of these diseases in India in terms of economic loss and wide spread distribution is red rot (Glomerella tucumanensis sp.eg.) followed by smut (Ustilago scitaminea Syd.) and grassy shoot (MLO). The foliage of sugarcane is attacked by a number of fungi that produce necrotic spots and lesions. Fifteen foliar fungal diseases were reported so far from Andhra Pradesh on sugarcane. Of these, eye spot (Bipolaris sacchari (Butl.) Shoemaker) and yellow spot (Mycovellosiella koepkei (Krüger) Deighton) are the two widely distributed and studied. Earlier, the ring spot (Leptosphaeria sacchari Van Breda de Hann) did not attract much attention of researchers in view of its 'minor importance' ther.

Ring spot caused by Leptosphaeria sacchari Van Breda de Hann was first reported by Krüger (1890) from Java. The disease has now been reported from 79 sugarcane growing countries of the world (Ricaud et al., 1989). In India, the occurrence of the disease has been reported from Andhra Pradesh (Chona, 1958), Uttar Pradesh, Bihar, Bengal, Assam, and Maharashtra (Singh, 1978). The

disease was observed on sugarcane varieties Co 419, Co 421, Co 449, Co 527 and Co 997 cultivated in Andhra Pradesh being conspicuous on Co 527 (Prakasam and Appalanarasaiah, 1965). The disease has been appearing on most of the cultivars of sugarcane in Andhra Pradesh for the last 3 decades in a mild form. The disease started appearing in a severe form since 1985-86 after the introduction of varieties Co 7219, CoT 8201, Co 975 and CoR 8001 on a commercial scale in different factory zones of coastal Andhra Pradesh and Telangana particularly under stress conditions. Numerous spots which appear on the leaves coalesce in due course resulting in reduction of net photosynthetic area. Barring 3 to 4 young leaves in the spindle, the remaining foliage is severely affected by the disease presenting a burnt appearance when the crop is viewed from a distance. The disease appears in severe proportions in the terminal stages of growth phase and prevails throughout the maturity phase reflecting on cane yield and juice quality. In view of the severe manifestation of the disease in certain cultivars, it is apprehended that it may prove yet another menace for successful cultivation of sugarcane in the state.

Not much research work has been attempted anywhere in the world, since ring spot was hitherto considered a minor disease. Realising the growing importance of this endemic disease in most of the cane

growing areas of the state, detailed investigations were taken up on the following aspects of the disease in the laboratory, glass house and field between 1988-1991 at Regional Agricultural Research Station, Andhra Pradesh Agricultural University, Anakapalle.

1. Symptomatology
2. Isolation and identification of pathogen and establishment of pathogenicity
3. Developing a disease rating scale for estimation of disease intensity
4. Survey for assessment of disease intensity
5. Inoculation and rapid screening techniques
6. Factors affecting disease development
7. Variation in the isolates of pathogen
8. Spread of the disease
9. Host range
10. Varietal reaction
11. Chemical control
12. Losses due to the disease

Results of investigations on the above aspects are presented and discussed in this thesis.

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# REVIEW OF LITERATURE

## CHAPTER II

### REVIEW OF LITERATURE

The available literature on various aspects of the present investigations on the ring spot disease and other foliar melonies caused by Leptosphaeria sp. on sugarcane and other crops is reviewed in this chapter.

#### 2.1 DISTRIBUTION

Ring spot is one of the older recorded diseases of sugarcane having been first reported in literature by Krüger (1890) from Java (Indonesia). He gave it the name "ring spot" which has since been retained. Later Van Breda de Hann (1892) reported that the fungus usually associated with the disease is Leptosphaeria sacchari which he considered as the causal organism. Since then several workers [Bancroft (1911) from West Indies and Java, Ashby (1912) from Jamaica, Vanderbijl (1921) from South Africa, Fawcett (1922) from Tukumán, North (1923) from Australia, Baissa (1936) from Madagascar, Matsumoto and Yamamoto (1936) from Taiwan, Caminha (1936) and Bitan Court (1938) from Brazil, Bourne (1934) from Florida, Bruner (1940) from Cuba, Muller (1941) from Venezuela, Deslandes (1944) from Amazonia, Resplandy et al. (1954) from Ivory Coast, Tarr (1957) from Sudan, Tabayoyong (1958) from Philippines, Hudson (1960) from Jamaica, Pan (1989) from Malasia, Echavez Badel (1990) from Puerto

Rico] reported the occurrence of the disease from various countries. The disease has now been reported from 79 sugarcane growing countries (Table 1) of the world (Ricaud et al., 1989).

Confirmatory reports from India on the occurrence of the disease are available from Andhra Pradesh (Chona, 1958); Bihar (Dutta, 1969); U.P., Bengal, Assam, Maharashtra and Gujarat (Singh, 1978); Kerala and Tamilnadu (Govindaswamy and Alagia Nagalingam, 1981).

In Andhra Pradesh, though the disease has been appearing on important cultivars like Co 419, Co 421, Co 527 and Co 997 for the last 3 decades in a mild form, the disease started appearing in a very severe form since 1985-86 on varieties Co 7219, CoT 8201, Co 975, Co 8021, Co 7704 and CoR 8001 in different areas (RARS, Anakapalle, 1992).

Leaf blight and leaf scorch, two other foliar diseases caused by Leptosphaeria taiwanensis and Leptosphaeria bicolor respectively were reported from different countries but reports of the latter melody only are there from India (Ricaud et al., 1989). According to him, leaf blight is restricted to Taiwan, Japan and Philippines while leaf scorch is reported from Argentina, Bangladesh, Cuba, India, Indochina, Japan, Nigeria, Panama, Papua-New Guinea, Philippines, South Africa, Taiwan, Thailand and Kenya.

Table 1: Distribution of ring spot disease in cane growing countries of the world

Name of the country	Name of the country	Name of the country
1. Andaman island	28. Guyana	55. Philippines
2. Angola	29. Hawaii	56. Puerto Rico
3. Antigua	30. Honduras	57. Reunion
4. Argentina	31. Hongkong	58. St. Kitts and Nevis
5. Australia	32. India	59. Samoa
6. Bangladesh	33. Indochina	60. Senegal
7. Barbados	34. Indonesia	61. Sierra Leone
8. Belize	35. Ivory coast	62. Solomon Is.
9. Benin	36. Jamaica	63. South Africa
10. Bolivia	37. Japan	64. Sri Lanka
11. Borneo	38. Kenya	65. Sudan
12. Brazil	39. Malagasy R.	66. Surinam
13. Burkina Faso	40. Malawi	67. Taiwan
14. Burma	41. Malaysia	68. Tanzania
15. Cambodia	42. Mali	69. Thailand
16. Cameroon	43. Martinique	70. Togo
17. Cent. Afr. R.	44. Mauritius	71. Trinidad
18. China	45. Mexico	72. Uganda
19. Colombia	46. Mozambique	73. Uruguay
20. Costa Rica	47. Nepal	74. U.S.A.
21. Cuba	48. Nicaragua	75. Vanuatu
22. Dominican R.	49. Niger	76. Venezuela
23. Egypt	50. Pakistan	77. Zaire
24. El Salvador	51. Panama	78. Zambia
25. Fiji	52. Papua-New Guinea	79. Zimbabwe
26. Ghana	53. Paraguay	
27. Guadeloupe	54. Peru	

## 2.2 SYMPTOMATOLOGY

The symptoms of ring spot disease were described in detail by Butler (1918), Bourne (1934), Abbott (1964), Dutta (1969) and Agnihotri (1990). The disease appears mainly on the leaf blades but infection some times also occurs on the leaf sheaths and stalks (Bourne, 1934 and Abbott, 1964). Although the disease most commonly affects only the older leaves, infection of the younger ones also may occur. Numerous spots appear on the leaf blade and the mature ones are conspicuous. At first they appear as dirty green areas of irregular shapes gradually becoming oval and purplish. They may vary in size in different sugarcane varieties. Butler (1918) and Bourne (1934) gave the lesion dimensions as 5x15 mm and 2.5-4 mm x 10-12 mm respectively and Matsumoto (1952) as 1-5 mm x 4-18 mm. Dutta (1969) reported that the average size of the spot is 7.0x4.0 mm while Agnihotri (1990) reported same measurement of spots as that of Bourne (1934). In the beginning, the shape of the spot is either oval or spherical but latter on it becomes irregular. As the spots enlarge, they become more irregular in outline and may coalesce to form large reddish brown patches. When a large portion of the leaf is affected, it withers and dies prematurely. The centre of the older spots usually become straw coloured, surrounded by well defined reddish margins and diffused discoloured zone. On the central straw coloured

portion of older spots, numerous pin head sized black fruiting bodies of Leptosphaeria sacchari in concentric rings and species of Phyllosticta are usually present.

The stalk lesions vary from somewhat oval or circular spots to irregular patches of considerable size (Bourne, 1934).

Regarding the course of development of the disease, Butler (1918) reported that the ring spot disease of sugarcane starts as small discoloured generally purple spots visible on both surfaces of leaf. The spots grow in size and the central portion dries up. The central straw coloured dried tissues are surrounded by a thin reddish brown band outside which there is some times a yellow areola merging into the green of leaf. The spots are scattered over the whole or part of the leaf blade. The spot is usually not regular but lobed or broken by angular projections and the union of neighbouring spots increases this irregularity. Ultimately a considerable part of leaf dries up in the neighbourhood of spots resulting in the premature withering of leaf. Dutta (1969) reported that the leaves of ring spot affected plants except a few top young ones, contain numerous small oval and purplish spots. The leaves dry up prematurely due to excessive spotting especially towards the apex of the lamina.

Two other species of Leptosphaeria also produce symptoms on sugarcane. Leaf blight caused by L.

taiwanensis produces first symptoms on the immature leaves in spindle as small narrow elliptical or elongate spindle shaped spots, yellowish in colour dotted with red, which are visible on both sides of the leaf. They develop into elongate reddish brown streaks which often coalesce into bands separated by narrow green portions of leaf. The diseased leaves die and become dried so that severely infected plants have a reddish brown appearance when viewed from a distance. The infection some times occurs on the sheath. The minute black fruiting bodies of the fungus develop in the margins of old lesions. Purplish red lesions may also be produced on the leaf sheaths especially in latter stages (Yen, 1964). Leaf scorch caused by L. bicolor produces very small red or reddish brown spots on the young leaves initially. The spots gradually elongate, assuming a more or less spindle shape with a definite yellowish halo. Later they coalesce and extend along the vascular bundles becoming spindle like streaks measuring 5-20 cm long and 0.3 to 1.0 cm wide, reddish brown at first and then straw coloured with dark red margins. In advanced stage, numerous minute black pycnidia develop in the dead tissues of the leaves. Occasionally straw coloured lesions occur on the upper part of leaf sheath but no pycnidial formation has been observed (LO and Leu, 1989).

2.3 ISOLATION OF THE PATHOGEN AND ESTABLISHMENT OF PATHOGENICITY

Van Breda de Hann (1892) considered the perithecial fungus he found associated with the lesions as the causal agent, which he described as Leptosphaeria sacchari. It belongs to the class Ascomycetes, Sub class: Loculoascomycetidae, order: Pleosporales and family pleosporaceae. He described that perithecia were formed before the spots shrivelled. They were brownish black in colour, spherical and thin walled and measured 140 μ in diameter containing 8 four celled spores with one thicker main cell and measured 20-24 x 3 μ. They became dark in colour when fully matured. He also described round, reddish brown conidiferous bodies which produced at their tips smoky brown, three to four celled, oval and slightly curved conidia resembling those of Dematium. He was not certain of the connection of this fungus with L. sacchari. Most authors have agreed with Van Breda de Hann's work on the description of symptoms of the disease but differences persisted with regard to descriptions of both the perithecial and conidial states of the fungus.

A few workers made mention of only the perfect state. Bancroft (1910) stated that the ascospores were 3 septate, 20-24 x 5 μ, brownish and that hyphae produced 3-4 septate curved spore on the leaf surface. Ashby (1912) described the asci, as containing 8, four celled hyaline

ascospores, 21-23 x 3.5-4.0  $\mu$ , constricted at the septa, each cell with an oil droplet and two median cells larger. According to Johnston (1917) the perithecia were spherical, thin walled, brown and 140  $\mu$  in diameter. The ascospores were 3 septate, brownish, 20-24 x 5  $\mu$  and the conidia were borne on the surface hyphae. Butler (1918) stated that fine hyaline hyphae of the parasite penetrate the leaf by entering directly into the cells. At a later stage the mycelium gives rise to conidial stage of the fungus. Perithecia are formed in large numbers chiefly within the rings but also to some extent outside them. They occur as tiny black dots arranged in rows between the fine veins of leaf and entirely buried in the leaf tissue except at the tip. They are visible only on the upper surface of the leaf where they open by a fine mouth (ostiole). He gave same measurements of perithecia and ascospores as stated by Johnston (1917) and described the perithecia as spherical with elongated club shaped asci. Each ascus contained 8 ascospores arranged in two or three rows. The spores were elongated, 3 septate, straight, rounded at the ends and were colourless at first and light brown later. Studies of Matsumoto (1952) revealed that the perithecia were at first subepidermal, later erumpent, spherical or sub globose, black with slightly papillate ostiole 130-150 x 140-170  $\mu$ . Asci cylindrical, slightly narrow at the base 54-85 x 10-15  $\mu$ , spores 8 per ascus

3

approximately 2 rowed, Oblong, 3 septate, constricted at the septa, hyaline or sub hyaline, 19-23 x 4.5 - 6  $\mu$ , paraphyses filamentous, about the same length as the asci, hyaline, 2-2.5  $\mu$  wide.

Several other workers gave varying accounts of the perfect and imperfect stages of the causal organism of ring spot disease. Hennings (1907) described the imperfect state as Phyllosticta saccharicola from the Belgian Congo. The pycnidia were hypophyllous 60-90  $\mu$  in diameter with oblong subfusoid, hyaline, 2-3 guttulate pycnospores, 10-13 x 3-3.5  $\mu$ . Fawcett (1922) in Tucuman reported that a Phyllosticta occurred on ring spot caused by the perithecial fungus (Leptosphaeria sacchari) and he believed it to be genetically related to the latter. Tucker (1925) from Puerto Rico described the imperfect state as Phyllosticta sacchari and suggested possible genetic connection between the pycnidia of this fungus and perithecia of L. sacchari which were often found in the same leaf spots developed on sugarcane. Bourne (1934) from Florida reported that isolations from ring spot made at various stages of spot development yielded number of fungi viz; Helminthosporium sacchari, Phyllosticta sp., Nigrospora sp., Leptosphaeria sacchari, Alternaria Sp. Initially he failed to produce ring spot symptoms by inoculating cane leaves with pure cultures of Leptosphaeria and Phyllosticta and thus opined that they

4

were not primarily causal agents but associated saprophytes. But when he inoculated leaves with the Helminthosporium in separate mixtures with the Leptosphaeria and Phyllosticta, typical ring spot lesions were produced by the Helminthosporium, Phyllosticta mixture, while infection by the Helminthosporium, Leptosphaeria mixture was no different from that produced by the eye spot organism alone. He concluded that H. ocellum (H. sacchari) was the primary cause of disease and L. sacchari was an associated saprophyte. However, Abbott (1964) opined that the eye spot fungus does not seem to be essential for initiating ring spot lesions in all cases since ring spot occurred in several sugarcane growing areas where H. sacchari has not been recorded.

Bourne made tissue plantings and single ascospore isolates from lesions of L. sacchari are obtained colonies on glucose potato agar which produced abundant Phyllosticta pycnidia. Bourne's description of the Phyllosticta state agreed with P. saccharicola described by Hennings (1907) and he was thus the first to offer proof of genetic connection between L. sacchari and Phyllosticta saccharicola. Proof that the two states were genetically connected was furnished by the fact that the cultures from single ascospores and pycnospores appeared to be identical and also because single ascospores yielded the perfect state at first on sterile sugarcane leaves and

later gave only the conidial state (Hudson, 1960; Abbott, 1964).

Matsumoto and Yamamoto (1936) reported from Japan that L. sacchari was found to produce abundant pycnidia of a species of Phyllosticta on sugarcane besides the perithecia on sterile leaves, thereby substantiating the genetic connection between the perfect and imperfect stages observed by Bourne (1934) in Florida. Luc (1953) observed numerous Phyllosticta saccharicola pycnidiospores measuring 9-14 x 3.5-4  $\mu$  on ring spot lesions. Resplandy et al. (1954) also reported that L. sacchari was commonly present along with a species of Phyllosticta on spots.

Hudson (1960) from Jamaica gave a description of conidial state of Leptosphaeria sacchari on stems and leaves of sugarcane and it was identified as Phyllosticta Sp. and thus his work provided further proof of the connection between the two fungi. He stated that the fungus persists saprophytically on old dead leaf tissues and the mycelium spreads from these and perithecia and pycnidia were produced often in great numbers all over the leaf surface. He described perithecia, Asci, Ascospores, Pycnidia and Pycnidiospores. The perithecia were small globose, pale brown with a papillate ostiole and measured 110-140  $\mu$ . Asci were numerous, cylindrical or club shaped, sessile, bitunicate with rounded apex, 8 spored and measured 60-72 x 9-13  $\mu$ . Ascospores were irregularly

biseriate in upper part of Ascus, almost hyaline, 3 septate with the second cell swollen. Each cell of ascospore had characteristically a single large guttule. The ascospores were 21-25  $\mu$  long and 4-6  $\mu$  wide at the swollen second cell and 3.5-5  $\mu$  else where. He also stated that single ascospores when transferred to oat meal agar produced pycnidia only but not perithecia.

Hudson made a mention of association of two more species of Leptosphaeria on sugarcane leaves in ring spot lesions each having 3 septate ascospores. L. eustomoides Sacc. could be distinguished from L. sacchari by its narrower, yellowish brown, non guttulate ascospores the end cells of which were more pointed and measured 16-22 x 4-5  $\mu$ . L. spetzazzinia sacc. and Syd. (Syn. L. sacchari Speg.) also occurred on the same host and had 3 septate ascospores, constricted at the centre, pale, olivaceous and measured 25 x 5-6  $\mu$ .

Ricaud et al. (1989) and Echavez Badel (1990) reported the association of L. sacchari and Phyllosticta saccharicola with ring spot disease of sugarcane.

Two more fungi were implicated in the incidence of ring spot disease. Vander Bijl (1921) stated that a Phoma was part of the life history of L. sacchari.

Yen and Chi (1953) considered L. sacchari as the causal agent of ring spot in Taiwan and referred to a

Cercospora stage of the fungus. They reported that perithecia grew well on sterilised green leaf blade of susceptible variety at optimum temperature of 26<sup>o</sup>.

Yen (1958) from Formosa reported the occurrence of Cercospora saccharicola causing ring spot symptoms and considered it as a possible fore runner of L. sacchari present as a saprophyte.

Research work on ring spot disease in India is meagre and only a few reports are available regarding aetiology of the disease.

Roy (1964) surveyed sugarcane area in Assam and isolated a pycnidial fungus, Phyllosticta sacchari from typical ring spot lesions.

Dutta (1969) reported Phyllosticta sp. and Leptosphaeria sp. on ring spot lesions. In many spots Leptosphaeria sp. always appeared later by a month or longer than Phyllosticta sp. He isolated ring spot pathogen on potato dextrose agar and oat meal agar by single spore method. Only the imperfect stage of the fungus appeared in culture and found it to be similar to Phyllosticta saccharicola.

According to Agnihotri (1990) ring spot is caused by L. sacchari. According to him the perithecia of the causal agent were spherical, thin walled, brown, measuring

140  $\mu$  in diameter. They contained numerous clavate, slender asci intermingled with paraphyses. Each ascus measuring 63-75 x 10  $\mu$  containing eight 3 septate brownish ascospores measuring 20-24 x 5  $\mu$ . The middle cells of the ascospores were darker than the end cells.

Two more species of Leptosphaeria cause diseases on sugarcane, L. taiwanensis causing leaf blight and L. bicolor causing leaf scorch. The etiological and related aspects are reviewed below.

Yen and Chi (1953) found that L. taiwanensis can be readily isolated and cultured on corn meal agar. The optimum temperatures for the growth of the culture was 25-30°C and perithecia developed in culture in 2-3 weeks.

Hsieh and Lew (1970) reported that out of several isolates of L. taiwanensis from different sugarcane varieties, only a few produced perithecia. Optimum temperature for ascospore formation was 24-26°C. They found that potato dextrose agar, oat meal agar and sugarcane leaf decoction agar were the best media for ascospore formation. They also reported that the lesions were induced by inoculating the ascospore from the isolate.

Leaf blight organism (L. taiwanensis) on potato glucose agar produced ascospores and pycnidia and pathogenicity was proved by inoculating ascospores and pycnidia (Wang, 1985).

Wang (1987) cultured different isolates of L. taiwanensis on potato dextrose agar (PDA). Only 20 per cent of them produced pycnidia and perithecia. However, abundant pycnidia and perithecia were produced when the cultures were kept at 10-30°C. PDA, PDA added with bean flour (2.2%), PDA with corn meal (1.9%) and PDA with oat meal (7.2%) were the best media for pycnidial and perithecial formation. Fourteen day old culture of L. taiwanensis on PDA produced Stagnospora and Phoma types of conidia in the same pycnidium. Both types of conidia also produced leaf blight symptoms when they were inoculated on sugarcane leaves.

Kaiser et al. (1979) in Kenya first reported that L. bicolor (teleomorph of Stagnospora sacchari) was associated with leaf scorch disease. Ascomata were observed occasionally in leaf scorch lesions on naturally infected leaves in association with pycnidia. Both states developed in pure cultures from single ascospores. The perfect stage did not develop on various nutrient media, but formed within 10-15 days on natural water agar containing autoclaved leaf pieces. Mycelial growth and pycnidial formation were more on oat meal agar and potato dextrose agar.

Chu and Tsai (1952) reported that both mycelia and pycnidia of Stagnospora sacchari grew abundantly on potato dextrose agar and cane leaf bean agar.

Wang and Lee (1980) stated that pycnidia of Stagnospora sacchari were abundant in potato sucrose agar added with sugarcane leaf (20%)

Literature pertaining to aetiological aspects of two diseases caused by Leptosphaeria spp. on two other crops viz., L. maculans causing black leg of rape and L. nodorum causing glume blotch of wheat are reviewed hereunder.

Wood and Barbetti (1977) reported that higher level of infection of Brassica campestris and Brassica rape seedlings was obtained with ascospores of L. maculans than pycnidiospores. Wittern and Krüger (1985) conducted green house experiments on testing resistance of 5 cultivars of rape against L. maculans using ascospores from old infected rape stalks, since ascospores did not form in culture. In general ascospores were more virulent than conidia.

Hampton et al. (1978) reported that pycnidia of Septoria nodorum (L. nodorum) were produced only when infected leaves were excised and placed on detached leaf culture under near U.V. light for 12 hours/day. They cultured conidia and ascospores on several agar media. Inoculations with both spore suspensions produced typical symptoms. Horellou et al. (1979) reported maximum sporulation of S. (Leptosphaeria) nodorum on czapecks Dox or

malt agar media when the dishes were kept under fluorescent light, U.V. for 12-16 hours/day. O'Reilly *et al.* (1987) in Ireland isolated perithecia typical of L. nodorum from glume blotch affected leaves on agar medium. Single ascospore cultures produced pycnidia typical of Septoria nodorum and the conidia of which caused necrotic lesions when sprayed on wheat seedlings.

#### 2.4 DISEASE RATING SCALE AND ASSESSMENT OF THE DISEASE INTENSITY

Hutchinson (1970) gave the following standardised rating system (0-9 system) for recording varietal resistance to sugarcane diseases which has been accepted in the year 1968 by the standing committee members of the International Society of Sugarcane Technologists at 13th Congress at Taiwan. This system formed the basis for estimating the intensity of foliar diseases by subsequent workers.

- 0 : Immune
- 1 : Very high resistant
- 2 : Highly resistant
- 3 : Resistant
- 4 : Intermediate - Resistant
- 5 : Intermediate
- 6 : Intermediate - Susceptible
- 7 : Susceptible

- 8 : Highly susceptible
- 9 : Very highly susceptible

However, different disease ratings followed by a few other workers are mentioned below.

In Taiwan, Leu (1968) screened sugarcane varieties for leaf scorch disease (L. bicolor) based on the following 1-9 scale.

- 1 : Highly resistant
- 2 : Resistant
- 3 : Moderately resistant
- 4 : Moderately susceptible
- 5 : Susceptible
- 6 : Susceptible
- 7 : Highly susceptible
- 8 : Highly susceptible
- 9 : Highly susceptible

Later Leu and Hsieh (1971) developed a 0-9 scale and rating varieties against leaf blight (L. taiwanensis) of sugarcane at Taiwan sugar experiment station is being done based on this scale.

- 0 : Very high resistant
- 1 : Highly resistant
- 2 : Highly resistant
- 3 : Resistant

- 4 : Resistant
- 5 : Moderately resistant
- 6 : Moderately susceptible
- 7 : Susceptible
- 8 : Susceptible
- 9 : Highly susceptible

Prakasam and Satyanarayana (1969) developed a scale for estimating the intensity of yellow spot disease of sugarcane and recognised 7 grades on the basis of per cent leaf area affected, i.e. 5, 10, 20, 40, 60, 80 and 100 per cent.

Mayee and Datar (1986) in their technical bulletin described a 0-9 scale of rating for sugarcane leaf spot diseases with special reference to rust.

Response Code No.	Response description
0	No visible infection.
1	Chlorotic flecks only.
2	Chlorotic flecks with red or brown centre. Centres may be necrotic.
3	Small to large irregularly shaped red to brown spots that may coalesce. Pustules absent.
4	Individual chlorotic or red spots with nonopened pustules.

- 5 Individual chlorotic or red spots with pustules opened and producing spores.
- 6 Blotches of leaf reddened or necrotic with pustules producing spores.
- 7 Coalesced red to brown blotches or spores covering much of the leaf surface from leaf edge to mid rib, may be on both sides of mid rib, and with pustules that are producing spores.
- 8 Pustules producing spores on chlorotic tissues, pustules with chlorotic halo.
- 9 Pustules producing spores on green tissues. Covers 51 per cent or more area.

## 2.5 SURVEY FOR DISEASE INCIDENCE

Chona (1958) reported severe incidence of ring spot disease of sugarcane on certain cane varieties in Talaparamba (Tamil nadu) and in certain coastal areas in Andhra Pradesh. Dutta (1969) from Ranchi noted that sugarcane variety M.16 (Red tana) was observed to be severely affected by ring spot disease every year, compared to other cultivars in Chota Nagapur. At Kanke the disease was observed to be severe during September and October of 1963 and 1965.

Working with leaf blight disease caused by L. taiwanensis Leu and Hsieh (1969) from Taiwan stated that the disease was more serious in Hualien but due to wide commercial cultivation of the resistant variety N.Co.310, leaf blight was rarely observed during the mid '50s and '60s. But with the introduction of new varieties like F.154 and F.157 the disease assumed epidemic proportion in Pintung, Yuchin and Taichung areas.

## 2.6 METHODS FOR TESTING RESISTANCE TO THE DISEASE

A review of work on ring spot disease showed that no attempts were ever made to develop a screening technique. Hence literature pertaining to foliar diseases on sugarcane and diseases on a few other crops caused by Leptosphaeria spp. is mentioned below.

Wismer and Koike (1967) developed a method for inciting brown spot disease (Cercospora longipes Butler) in Hawaii. Potted plants or plants grown in the field were used for the purpose. Top halves of the leaves of the plants that were watered well were clipped off and the whole crown was enclosed in an alkathene bag for subjecting to pre and post inoculation humidities. Inoculation was done by spraying a suspension of fungal conidia on the excised foliage. Prakasam and Satyanarayana (1969) adopted the above method for assessing the reaction of sugarcane varieties for yellow spot disease.

A rapid method of assessing resistance to leaf scorch disease (L. bicolor) under inoculated conditions was developed by Wang and Lee (1980) where results consistent with those from field trials were obtained. Conidial suspensions were either sprayed onto potted plants or dropped into the spindle. The plants kept in green house were exposed to simulated rainy conditions for 2 or 3 hours each day for a week and when inspected after 6 weeks, symptoms on the 3rd, 4th and 5th unfolded leaves were rated similar to those in the field trials.

Leu and Hsieh (1971) at Taiwan sugar experiment station studied artificial inoculation of sugarcane varieties in the field by means of an ascospore suspension of L. taiwanensis of  $2 \times 10^5$ /ml concentration by spraying on the leaves. Grading for the degree of resistance was performed twice at 2 and 4 weeks after inoculation. Wang and Lee (1982) made some improvements on the methods of testing sugarcane varietal resistance to leaf blight, the results of which were found to tally with field reaction. Four months old plants of different varieties planted in pots were artificially inoculated by spraying  $1 \times 10^5$ /ml conidial suspension in the green house, duly maintaining high humidity by spraying water over night. Initial symptoms appeared 4 to 5 days after inoculation and rating was done 2 and 4 weeks after inoculation.

Bernard and Liu (1980) compared three artificial inoculation methods for rust disease and concluded that wrapping young leaves with a piece of rust affected leaf appeared to be most effective. Liu (1982) described a detached leaf method for assessing varietal resistance to rust, where inoculated leaf pieces were maintained on a benzimidazole nutrient solution in a test tube. Of the 90 varieties tested, more than 95 per cent gave similar reaction to that observed under natural conditions.

Benedikz et al. (1981) developed a laboratory technique for screening wheat varieties for resistance to Septoria (L.) nodorum causing glume blotch of wheat. Cut pieces of 3 cm from first fully expanded leaf of 2 week old wheat seedlings were employed and results compared positively with those of field observations. Studies of Karjalainen (1985) revealed that field screening technique based on artificial inoculation in small pots with conidial suspension of S. nodorum showed that the most resistant entries were wild triticum spp. and late and tall varieties. Seedling plant tests, attached seedling leaves and detached leaves easily revealed the most resistant and most susceptible cultivars. The overall correlation between seedling and field tests was quite high. Verreet et al. (1987) stated that a conidial concentration of  $1 \times 10^6$  spores/ml was found optimum for screening varieties of wheat against S. (L.) nodorum.

Woodland and Hoffman (1988) while screening breeding lines of wheat against glume blotch disease (L. nodorum) adopted seed and spray inoculation methods and obtained similar results in both the methods.

**2.7 FACTORS AFFECTING DISEASE DEVELOPMENT**

Ring spot disease was favoured by cool, damp atmospheric conditions and locations in South Africa (Vanderbijl, 1921). Mosaic predisposed cane crop to ring spot in Tucuman (Fawcett, 1922). North (1923) recorded that in Australia ring spot appeared in a particular season such as winter and disappeared later. Tabayoyoung (1958) reported from Philippines that ring spot was maximum in June (10.27%) and minimum in December (4.99%).

Reports of the prevalence of the disease in conditions uncongenial to sugarcane are there. Baissa (1936) mentioned that in Madagascar ring spot affected sugarcane in unfavourable weather. Caminha (1936) from Brazil observed that ring spot disease attacked young shoots in wet soils. Ricaud (1969) observed wide spread occurrence of ring spot disease on many varieties of sugarcane in Mauritius due to extreme dry season which had prevailed, the driest on record for many years. He opined that water stress, perhaps, might have favoured infection by limiting the absorption of some essential nutrient. Echavez Badel (1990) reported that ring spot caused by L.

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sacchari is distributed throughout Puerto Rico, particularly in moist localities.

Butler (1918) reported that unfavourable conditions of soil and moisture appear to predispose the thick canes to ring spot disease while thin canes were never damaged. According to Agnihotri (1990) ring spot disease occurred mostly during the rainy season, particularly on the older leaves and continued till the harvest of canes.

Conditions that favoured incidence and spread of two other diseases caused by Leptosphaeria spp. on sugarcane are reviewed below.

Yen (1964) stated that leaf blight was most prevalent in the high rainfall areas of east coast of Taiwan throughout the year. Observations by Leu and Hsieh (1969) revealed that leaf blight disease occurred most intensively in Taiwan throughout the year regardless of age of the plant particularly during winter and early spring. However, in 1968, a combination of susceptible varieties, abundant inoculum and rainy weather caused considerable damage even in summer. Leu and Hsieh (1971) studied pattern of lesion development in leaf blight affected sugarcane leaves. Spraying ascospore suspension during rain gave better results than when done in dry weather. Lesions developed faster in mature than in younger leaves on the same plant.

Exconde (1963) reported that there was seasonal variation in leaf scorch severity, with the highest incidence in Negros in the January-July period which encompasses the rainy season. Lo and Leu (1989) from Taiwan stated that the development of initial streak lesion in the leaf scorch disease is influenced by the variety and environmental conditions. Susceptible varieties in dry weather developed streak formation rapidly and there was extensive discolouration of adjacent tissue. The whole crop developed a typical scorched appearance with the worst cases showing normal green leaf tissue only in the leaf spindle. Streak formation was most evident in spring and autumn when growth was only moderate. Winter temperatures were too low for the pathogen, while in summer, the rapid production of new leaves resulted in good symptom expression only on the older leaves well down in the canopy. Spring planted cane and ratoons showed less disease than cane planted in autumn (July-September). They also reported that in Philippines the disease was present throughout the year under different environmental conditions.

Literature pertaining to three more diseases caused by Leptosphaeria spp. on crops other than sugarcane viz., glume blotch of wheat, crown canker of rape and stem rot of paddy is reviewed below.

Extremely rainy weather and below average temperatures favoured severe attack of Septoria (Leptosphaeria) nodorum in wheat (Rowe, 1979; Svanold and Djurle, 1980; Jeger et al., 1981; Mittermeier and Hoffman, 1984). Scott et al. (1985) reported that the canopies of taller wheat varieties appeared to generate a less favourable microclimate for development of Leptosphaeria nodorum. Space occupied by canopies showed that taller varieties had lower canopy densities than shorter varieties. They also stated that reduced wetness of leaf surface contributed to the tendency for taller varieties to be less infected. Nevertheless microclimate effect was considered by the workers, to be only one of the several mechanisms that contributed to the tendency for taller varieties to be more resistant. Vanova and Benda (1988) reported from Ochrane that stress factors were important for infection of S. (L.) nodorum. Khoury and Kraus (1989) found in German Federal Republic that high disease rate of S. (L.) nodorum on wheat was favoured by a combination of weekly rainfall of more than 10 mm and relative humidity exceeding 90 per cent coupled with temperatures above 12°C.

Severe out breaks of stem rot of paddy (L. salvini) have been traced to weakening of the plant due to physiological imbalance, unusual drought or insect attack particularly stem borer incidence (Rangaswami,

1979). Li et al. (1984) observed that irrigation, nitrogen level, varietal resistance and amount of inoculum had significant effect on both incidence and severity of stem rot disease.

Barbetti (1975) while working with crown canker of rape (L. maculans) reported that temperature regime was a major factor determining the time of first appearance of the disease. Sundarmadi and Wallace (1984) conducted studies on rape seed and reported that ascospores of black leg pathogen (L. maculans) were main source of first infection in the crop while pycnidiospores which are easily spread by wind borne rain splash to other parts of plant or to nearby plants were responsible for the development of late cankers on the growing crop.

Information on the effect of major nutrients viz., nitrogen, phosphorus and potassium on the foliar diseases of sugarcane caused by Leptosphaeria Spp. is not available. Investigation on the effect of major nutrients on brown stripe of sugarcane caused by Cochliobolus stenospilus, indicated that (1) the disease was more severe on varieties growing in soils of low fertility, (2) the leaves and stalks of healthy varieties contained more silica than similar parts of diseased plants and (3) the disease was less severe when additional fertilizers particularly potash was applied to areas where the disease was prevalent (Ricaud et al., 1989). Rabindra and Kumaraswamy

(1978) from Mandya reported that application of potassium helped in minimising the incidence of eye spot disease of sugarcane (Bipolaris sacchari).

The effect of N, P and K fertilizers on the intensity of glume blotch of wheat and stem rot of rice caused by Leptosphaeria spp. are reviewed hereunder.

With increased nitrogen fertilizer the severity of glume blotch of wheat increased significantly (Olsson, 1979; Broschius et al., 1985). Nitrogen fertilizer alone or in combination with phosphorus fertilizer increased the infection by L. nodorum in wheat (Kharkova et al., 1984). Potassium fertilisation reduced the intensity of glume blotch in field tests in Bavaria (Siebold, 1980). According to Cunfer et al. (1980) glume blotch severity was positively correlated with phosphorus fertilizer rates and yield was negatively correlated. K and P and K interactions did not affect any variable measured except grain yield when increasing K rates lessened the detrimental effect of applied P.

High nitrogen levels increased the incidence and severity of stem rot of rice caused by Magnaporthe salvinii (Jain, 1977; Mayee et al., 1977; Nyvall, 1979; Rathaiah, 1990; Mehrotra and Aneja, 1990). Application of phosphorus individually or in combination with nitrogen increased the disease intensity (Jain, 1977; Rathaiah,

1990). Application of potassium fertilizer reduced stem rot severity with corresponding increase in grain and straw yields (Nyvall, 1979; Rathaiah, 1990; Jayaraj et al., 1991).

The role of silica in disease resistance to many leaf spot diseases of crops has been reported. However, its role in sugarcane against leaf spot diseases has not been studied in detail. Egan (1989) while working with brown stripe (Cochliobolus stenospilus) disease in sugarcane reported that leaves and stalks of resistant varieties contained more silica than in susceptible varieties. A few reports are there in literature regarding the role of silica in offering resistance to Leptosphaeria sp. According to Nonaka et al. (1958), increased silica content in rice plants decreased the stem rot severity. Leusch and Buchenauer (1984) reported that severity of glume blotch in wheat was negatively correlated with the silica content of leaves and glumes.

## 2.8 VARIATION IN RING SPOT PATHOGEN

No information is available on the variation of ring spot pathogen. Studies on the variability of Leptosphaeria spp. on sugarcane are very meagre. Kaiser et al. (1979) from Kenya reported that isolates of leaf scorch fungus (L. bicolor) varied in growth rate, sporulation and colony appearance. However, several

workers have conducted studies on the variability of Leptosphaeria spp. in different crops. Rufty et al. (1981a) and Allingham and Jackson (1981) reported variation in the virulence pattern of isolates of L. nodorum in wheat while Scott and Benedikz (1983) found no variation in the isolates of L. nodorum and L. avenaria. Humpherson-jones and Ainsworth (1984) detected no variation in the strains of L. maculans.

## 2.9 SURVIVAL AND SPREAD OF THE PATHOGEN

Abbott (1964) stated that transmission of ring spot was apparently by wind or rain borne spores which caused new infections. There was no evidence that the pathogen was transmitted by seed cane, although it might have been carried on portions of leaves or sheaths adhering to the cuttings. Transmission of the disease through infected old dead leaves with the aid of rain splashes was reported by Jones (1967).

Lo (1961) while working with Stagnospora sacchari (Leptosphaeria bicolor) observed that wind blown rain and dew were indispensable for the dissemination of conidia and conidia ooze in a gelatinous mass from the moist pycnidia and were dispersed by free water or rain from one field to another if high winds accompany the rain. Work of Matsumoto et al. (1955) indicated that sett transmission has not been positive even where there was

infected leaf tissue adhering to the setts and the pathogen did not seem to be able to reach and infect the emerging young shoot. Similarly, although conidia are washed on to the soil from leaves, it has not been possible to obtain infection from the soil either in the field or in laboratory tests.

The survival and spread of Leptosphaeria on other graminaceous plants are reviewed hereunder.

Dispersal of pycnidiospores of S. (L.) nodorum causing glume blotch of wheat was by rain splash, the droplets acting as dissemination units (Rapilly and Skajennikoff, 1974; Griffith and Hann, 1976). Holmes and Colhoun (1975) reported that wheat straw infected with S. nodorum acted as the source of inoculum.

Perithecia of L. nodorum developed on dead leaves of wheat stubbles soon after harvest. The ascospores liberated in the presence of free water on the dead leaf surface and blown off by wind initiate infection on young crops (Mehta, 1975; Sanderson and Hampton, 1978). Nyvall (1979) reported that L. nodorum survived as mycelium in live plants and pycnidia on infected residue.

Perithecia of Magnaporthe salvini on rice residue (Bockus et al., 1979) and sclerotia on straw and stubbles (Singh, 1978) were reported to be the source of infection for the stem rot of rice.

Ascospores of L. maculans, causing black leg of rape, discharged from crop residues were the main source of early cankers according to McGee (1977), Nyvall (1979) and Sundarmadi and Wallace (1984). Pycnidiospores which were easily splashed by rain were responsible for cankers in the growing rape crop (Nyvall, 1979; Sundarmadi and Wallace, 1984). Gladders and Musa (1980) observed that stubble debris of rape from the previous season was the main source of infection.

#### 2.10 HOST RANGE

Butler (1918) reported that the fungus L. sacchari was also found on a wild grass Saccharum spontaneum in Java. But Bourne (1934) stated that genotypes having high proportion of S. spontaneum blood are mostly resistant. Hudson (1960) from Jamaica observed that L. sacchari has not been noticed on any other host other than Saccharum sp. Misra et al. (1971) reported from Bihar that the ring spot fungus (L. sacchari) has also been found on Tiger grass (Saccharum munja Roxb.) which is considered genetically close to Saccharum spontaneum.

Kaiser et al. (1979) reported that L. bicolor causing leaf scorch on sugarcane was pathogenic to several varieties but not so to napier grass (Pennisetum purpureum), maize, rice and sorghum. Leaf scorch occurred

naturally on Miscanthus sinensis in Taiwan (Lo et al., 1953) while Sorghum bicolor and Andropogon sorghum were infected artificially. In Philippines the common wild cane Saccharum spontaneum L. has shown natural infection, while inoculations produced symptoms on Miscanthus sinensis Anders, Imperata cylindrica (L.) P. Beauv., Sorghum halepense (L.) pers. and Pennisitum purpureum Schum (Exconde, 1963).

## 2.11 VARIETAL REACTION

The most practical, efficient and economic control of ring spot as of other diseases of sugarcane is the replacement of susceptible varieties with resistant ones. Butler (1918) observed that thick canes were more susceptible than thin canes. Cook (1925) reported that in Puerto Rico, the control of ring spot disease was accomplished only through evolving resistant varieties. Bourne (1934) noted serious outbreaks in susceptible varieties in Barbados, Puerto Rico, Cuba and Florida and emphasized that resistance to ring spot should be taken into consideration by cane breeders. He made a study of susceptibility in the progenies of crosses carrying blood lines of Saccharum officinarum, S. barberi and S. spontaneum. He found that those combinations involving a high proportion of S. barberi blood were susceptible, while those with a high proportion of S. spontaneum were resistant.

Bruner (1940) from Cuba reported that sugarcane varieties POJ 2825 and POJ 2878 were resistant to ring spot. Matsumoto (1952) stated that F 108 at one time, an important commercial variety in Taiwan was highly susceptible. Sandhu et al. (1963) found that varieties Co 453, Co 617, Co 838 and S3 45/52 were highly susceptible to ring spot in Punjab and the incidence varied from 70-100 per cent.

Abbott (1964) reported that ring spot has never been considered of sufficient importance to justify control measures. However, if the disease was completely ignored in breeding programmes, it is possible that highly susceptible varieties might be advanced to commercial culture. Dutta (1969) reported from Ranchi that Mauritius variety M16 which is popularly known as red tana in Chota Nagapur was highly susceptible to Ring spot. Singh and Pavgi (1969) studied the relative susceptibility of several varieties against Leptosphaeria sacchari. Varieties B.O. 32, Co.245, Co 312, Co 314, Co 416, Co 445 and Co 1007 were tolerant to the disease while Co 313, Co 1138 and Bo 17 were susceptible. The degree of infection varied from 12 to 100 per cent. Singh (1978) stated that the late maturing thick canes are more susceptible to ring spot disease. Nyvall (1979) from Iowa recommended growing of resistant varieties of sugarcane for the control of ring spot. Echavaz Badel (1990) studied reaction of 6

released cultivars at Gurabo (Puerto Rico) under natural conditions. He found that two of the cultivars were resistant to ring spot throughout the first and second ratoon crops, but the others showed reduced resistance in the second ratoon crop possibly because of change in soil moisture.

The varietal reaction to leaf blight (L. taiwanensis) and leaf scorch (L. bicolor) diseases of sugarcane reported by several workers are reviewed below.

Leu and Hsieh (1969) reported from Taiwan that varieties F.146, F.153, F.154, F.157, F.160 were highly susceptible; N.Co.310 moderately resistant; F.148, F.152, F.156, F.158 resistant and H-37-1933 highly resistant to leaf blight. Lee et al. (1984) evaluated 21 new clones and 67 foreign varieties in Taiwan for resistance to leaf blight and found that 57.1 per cent of new clones and 62.7 per cent of foreign varieties were susceptible.

Chu et al. (1955) studied the reaction of cultivated and wild canes to leaf scorch disease. In Saccharum officinarum, S. spontaneum, S. sinense and S. robustum groups, most of the clones showed above average susceptibility. In the S. barberi group there was considerable variation in the susceptibility of canes. All wild relatives tested were very highly resistant. Exconde (1963) reported that H 37-1933 was highly susceptible to

leaf scorch in Philippines. Lo and Leu (1989) stated that virtual disappearance of leaf scorch in Taiwan was due to release of resistant variety N.Co.310. Lee and Fang (1991) evaluated 24 promising clones for their resistance to leaf scorch at Taiwan sugar experiment station. High resistance was found in 13 clones viz; 83-588, 87-3703, 84-964, 84-7688, 84-8842, LF 77-20, LF 77-359, LF 77-457, LF 77-1104, LF 77-1660, LF 77-1972, H 77-0682 and H 99-2751.

## 2.12 LOSSES DUE TO THE DISEASE

Reports of quantitative expression of losses due to the disease were not there from any quarter in the world excepting for a mention on the damage of the disease. Agnihotri (1990) stated that the losses caused by L. sacchari have not been worked out because it was presumed that the losses were almost negligible and the disease, by and large, was considered of minor importance but can be dangerous.

Butler (1918) opined that no considerable damage was caused by ring spot disease inspite of severe attacks in Burma on thick canes than on thin canes. North (1923) from Australia and Altson (1926) from Guiana reported severe outbreaks of ring spot. Bell (1929) reported that L. sacchari caused serious damage to seedlings in Hawaii. According to Bourne (1934), ring spot caused appreciable

damage in susceptible cultivars in Cuba, Barbados and Florida. Ocfemia (1938) stated that when ring spot disease became destructive on certain varieties, it caused entire crop in the field to appear rusty brown in colour due to premature death of leaves. Severe out breaks and heavy damages also have been reported from Cuba (Bruner, 1940), South Africa (D [odds], 1942) and Madagaskar (Moreau, 1949) and slight damage was reported from Venezula (Muller, 1941).

Chona (1958) reported that ring spot (L. sacchari) which was considered to be of only minor importance in the past in India has become serious and caused considerable damage to certain cane varieties at Talaparamba and in certain coastal areas of Andhra Pradesh. The disease was so serious in Andhra Pradesh during 1955-56 that it was claimed by the cane growers that it had interfered with Jaggery setting. Singh (1978) opined that L. sacchari may cause significant damage to late maturing thick cane varieties. He also stated that the plants are not killed, but due to reduction in effective leaf surface, there is indirect effect on the plant vigour resulting in reduced yield.

Reports on the extent of losses caused by two other foliar diseases on sugarcane caused by Leptosphaeria spp. indicate a different trend.

Extensive injury to the leaves of susceptible varieties due to leaf blight resulted in reduction in both tonnage and sucrose per cent (Yen, 1964). Severe outbreaks of leaf blight was observed in Pintung, Yuchin and Taichung areas of Taiwan in 1968-69 in more than 3000 ha in newly released varieties F.154 and F.157. There was a reduction of more than 50 per cent in cane yield when spring planted canes were severely infected at an early growing stage, while losses of 10-30 per cent were estimated when the disease was severe in growing season. The sucrose content was observed to be low in diseased plants as compared to healthy plants in the same field (Leu and Hsieh, 1969).

Lo and Ling (1950) from Taiwan made a comparative study of leaf scorch diseased and healthy stalks of variety Co 290 grown in the same field and found losses of 17 per cent and 13 per cent in cane and sugar yields respectively. Chu and Tsai (1952) reported that brix of cane juice decreased according to degree of severity of leaf scorch infection of plants but it varied from 3.7 to 16.3 per cent in different varieties. Lin (1952) stated that the loss in cane and sugar yields due to the disease vary with the variety and weather conditions. Exconde (1963) reported from Philippines that leaf scorch infection in H 37-1933 was so severe that some times only three of 10 open leaves remained green and stated that

under such conditions losses of sugar probably ranged from 10-30 per cent. Studies from Philippines by Sampang and Reyes (1980) on leaf scorch revealed a loss of approximately 25 per cent in both tonnage and sugar per hectare with the highly susceptible variety Phil 6111. These workers (Sampang and Reyes, 1985) reported that the critical stage of growth of cane affected by leaf scorch occurred 4-5 months after planting and the disease markedly reduced stalk height, diameter and number of internodes as well as number of green leaves thus resulting in a reduction in cane and sugar yields by 32.8 and 36.1 per cent respectively.

Losses encountered by diseases caused by Leptosphaeria spp. on wheat, rice and rape are detailed below.

Yield components were reduced with increased severity of infection of L. nodorum in wheat (Wafford and Whitbread, 1978), Luz (1984) from Brazil estimated 8 per cent loss in yield due to glume blotch in wheat. Roux, (1984) stated that yield losses were greater (21.2%) following inoculation, but it was less (15.4%) under natural infections. Karjalainen and Karjalainen (1990) studied the effects of S. (L.) nodorum on the grain yield and yield components of 3 spring wheat cultivars in Finland and reported that all yield components were affected and grain yields were reduced under low disease

stress by 3-5 per cent, while under severe disease stress, the reductions were 7-20 per cent.

Paracer and Luthra (1944) estimated 50-75 per cent losses in yield due to stem rot in rice (L. salvini) in case of severe infection in Punjab. Rangaswami (1979) stated that stem rot disease was known to cause losses upto 75 per cent in yield in Punjab while in other areas it causes hardly any loss.

Yield reduction in rape due to black leg (L. maculans) infection was reported by Barbetti (1975) Schuster (1980) and Petrie (1986). An epidemic of rape infected by L. maculans in U.S.A. was first reported by M. ngister et al. (1990) on various cultivars in about 100 ha resulting in a loss in yield of 80 per cent.

### 2.13 CONTROL OF THE DISEASE WITH FUNGICIDES

According to Agnihotri (1990) the need for developing chemical control measures has not been felt, as ring spot disease was thought to be of minor importance. The report of Govindaswamy and Alagia Nagalingam (1981) where, spraying of 1 per cent Bordeaux mixture was recommended, is the only available reference on the control of the disease with fungicides.

Mineral oil (0.1%) along with Dithane Z-78 was found effective in the control of leaf blight (L.

taiwanensis) disease of sugarcane in Taiwan (Lau and Hsieh, 1969).

As there is not much information available on the chemical control of Leptosphaeria on sugarcane, information available on control of Leptosphaeria spp. on other graminaceous crops is furnished briefly here under.

Spiertz (1973) from Netherlands reported that spraying with Maneb at 2 kg/ha twice before flowering and with Benomyl at 1 kg/ha twice after flowering greatly delayed the spread of L. nodorum causing glume blotch of wheat and increased grain yield. Wheat yields were 20 per cent higher in plots treated with Mancozeb or Mancozeb + Benomyl and 15 per cent higher with Benomyl alone and the infection of L. nodorum was reduced from 57 to 8-22 per cent (Jacobsen, 1977). Oppitz and Hoerer (1978) from S. Germany observed that Calixin (Tridemorph) and Thiophanate methyl effectively controlled glume blotch and increased grain yields. One application of Frumidor (Thiophanate methyl + Maneb) and Daconil (chlorothalonil) between ear emergence and flowering reduced the infection of L. nodorum in disease endangered areas in Switzerland (Jaggi, 1979). Siegle and Casutt (1979) from the same country stated that a single application of Dithane M-45 + Benlate between ear formation and beginning of flowering of wheat reduced infection by S. (L.) nodorum by 60 per cent and increased yield by 19 per cent. Difolatan (captafol) at

1.5 kg/ha sprayed between shooting of ears and flowering was recommended for the control of L. nodorum (Zwatz, 1979; Mielke and Ahlf, 1985). Saniau et al. (1981) from France reported that the compound Remidine plus (1.6% Fenarimol, 8% Carbendazim and 64% maneb), sprayed at 2.5 kg/ha was effective against S. (L.) nodorum on wheat. Barros et al. (1984) indicated that 3 sprays of mancozeb + triadimefon, starting from 45 days after emergence reduced the incidence of L. nodorum with increased yield. Vanova and Ben da (1988) obtained increased yields of 14.8 per cent and 19 per cent by spraying Tilt (Propiconazole) and Bayleton Triple (Triadimefon + Carbendazim + Captafol) respectively. They also reported that the fungicides based on mancozeb were also most effective against glume blotch. Foliar spray with Triadimefon (Bayleton 50% W.P.) and Mancozeb (Dithane M-45) reduced L. nodorum and increased grain yields (Christ and Frank, 1989). Eynard and Shepard (1990) studied five fungicides against L. nodorum both in vitro and in vivo. Chlorothalonil was much less effective in vitro than prochloraz, Hexaconazole, Flusilazole and Tebuconazole and similar results were obtained in vivo also. Field experiments in Denmark, France, Germany and U.K. revealed that at 125 g a.i./ha BAS 480F (a new triazole fungicide) controlled L. nodorum and increased grain yields in wheat (Saur et al., 1990).

Edifenphos, validamycin, carbendazim and Fuzi-one (Isoprothiolane) provided 75-94 per cent control of stem rot of rice caused by M. salvini (Li et al., 1984).

Ramsingh et al. (1988) tested six fungicides in vitro among which carbendazim and Thiophanate methyl effectively inhibited sclerotial germination and mycelial growth of Sclerotium oryzae and M. salvini. In field trials, the stem rot incidence could be reduced by spraying the crop once with Thiophanate methyl @ 875 g/ha at disease initiation. Spraying fungicides such as Triphenyl tin hydroxide on debris and stubble after harvest was recommended for the control of stem rot of rice (Mehrotra and Aneja. 1990).

Application of Bayleton CF (Triadimefon + Captafol) gave good control of L. avenaria causing leaf blotch and produced improvements in kernel weight and grain yield of oats (Jhonston et al., 1981).

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# MATERIALS AND METHODS

CHAPTER III  
MATERIALS AND METHODS

3.1 GENERAL

In view of the severe incidence of ring spot disease on sugarcane in several cane growing districts in Andhra Pradesh in recent years, its role in limiting cane production is sought to be assessed by conducting investigations on aspects such as the effect of the disease on yield and juice quality, varietal reaction, factors influencing the disease and chemical control. These studies were made between 1988-1991 in the laboratory, glass house and field at Regional Agricultural Research Station, Anakapalle, Andhra Pradesh (India). Observations presented in this thesis pertain to 1988-89 and 1989-90 crop seasons and the same are mentioned in the text. However, the experiment pertaining to estimation of losses was conducted during 1990-91 crop season. Geographically the experimental field is located at  $83^{\circ}-01'$  East longitude,  $18^{\circ}-45'$  North latitude. It is on the eastern side of peninsular India at an altitude of 28.62 meters above mean sea level and about 20 kilometers East from Bay of Bengal. The climate is tropical and humid. It receives rainfall both during South-west (June-September) and North-East (October-February) monsoons. The mean decennial rainfall in South-West and North-East monsoon periods is 638.6 mm and 289.7 mm, the total annual

rainfall being 1052.4 mm. The winter is mild during the months of December to February with minimum temperatures rarely touching 12°C. Summer is severe during the months of April-May with maximum temperature going upto 42°C. The relative humidity is high through out the year ranging from about 60 per cent to over 90 per cent, as the place is close to the sea. The monthly mean meteorological data at the site, for ten years (1981 to 1990) are given in Table 2. The relevant weather data recorded at Regional Agricultural Research Station, Anakapalle during the experimental period (1988-89, 1989-90 and 1990-91) are presented in Table 3, 4 and 5. The material used and the methods adopted in the present investigations are described in this chapter.

### 3.1.1 Glassware

The glassware used in the studies comprised of petriplates, Erlenmeyer conical flasks, test tubes, beakers etc. of Corning or Borosil make. It was thoroughly cleaned with a detergent and washed in running tap water. Cleaning solution prepared by dissolving potassium dichromate (100 g), water (1000 ml) and concentrated sulphuric acid (500 ml) was used whenever required. Cleaned glassware was finally rinsed in distilled water and air dried.

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Table 2: Mean meteorological data from 1961 to 1990 at Anakapalle

Months	Rain fall in mm	No. of rainy days	Temperature °C		Evapora- tion in mm	Average number of sunshine hours per day	Wind velocity in km per hour	Per cent relative humidity	
			Maximum	Minimum				7.00 hours	14.00 hours
January	9.00	1	29.9	17.1	3.2	6.6	4.2	88	75
February	22.20	2	28.9	18.6	4.9	6.3	4.4	90	47
March	33.20	1	34.2	21.2	3.1	5.2	4.8	80	42
April	22.40	2	36.0	24.9	6.8	4.4	6.9	87	51
May	110.00	5	36.5	26.3	6.8	6.7	6.3	89	57
June	114.00	7	35.5	26.4	5.7	5.1	6.0	91	50
July	173.00	10	33.2	29.5	4.7	4.9	5.6	87	65
August	153.00	10	32.7	25.7	4.7	4.8	5.1	84	66
September	111.00	10	33.0	24.9	4.7	6.4	3.2	90	68
October	219.40	8	32.1	23.2	4.3	4.3	3.4	87	61
November	75.00	3	30.7	20.3	3.6	5.0	4.7	88	51
December	4.00	1	29.9	17.8	3.9	6.3	5.4	84	47
Mean	106.70	51	32.72	22.69	4.57	6.19	4.99	86.87	56.25

Table 3: Mean meteorological data for 1988-89 at Anakapalle

Months	Rain fall in mm	No. of rainy days	Temperature °C Maximum	Evacota- tion in mm	Average number of sunshine hours per day	Wind velocity in km per hour	Per cent relative humidity	
							7.00 hours	14.00 hours
March	0.4	0	34.4	5.5	5.2	5.9	93	51
April	32.6	3	35.7	6.2	5.1	6.8	90	51
May	126.2	7	36.7	5.8	5.5	7.3	87	52
June	57.6	3	37.0	5.9	5.3	5.1	81	50
July	224.0	11	37.0	4.3	4.7	5.5	91	65
August	105.1	10	30.3	3.7	4.7	4.7	92	73
September	346.0	14	32.5	3.7	5.7	3.2	93	75
October	210.8	7	32.5	2.7	5.0	4.9	85	74
November	7.0	1	31.4	4.0	4.7	10.5	75	41
December	6.9	0	30.3	3.4	5.4	14.0	63	50
January	5.0	0	30.3	4.1	4.5	5.6	80	46
February	0.0	0	33.3	5.7	5.7	4.9	93	39
Year / Total	1097.0	55	33.12	4.39	5.46	4.94	87.87	55.12

Table 4: Mean meteorological data for 1989-90 at Anekapalle

Months	Rain fall in mm	No. of rainy days	Temperature °C		Evapora- tion in mm	Average number of sunshine hours per day	wind velocity in km per hour	Per cent relative humidity	
			Maximum	Minimum				7.00	14.00
March	18.8	1	34.4	20.7	5.8	9.7	5.4	88	41
April	2.9	0	36.3	25.1	7.1	8.9	5.6	88	49
May	36.1	3	37.2	27.4	5.4	7.2	7.1	87	53
June	54.2	7	35.1	25.5	5.6	4.8	5.8	80	54
July	151.5	12	33.3	25.7	4.9	6.0	5.3	81	51
August	123.4	9	32.9	25.4	5.0	4.4	4.8	86	50
September	142.4	17	32.9	24.5	3.9	5.7	3.9	87	51
October	26.0	3	33.9	23.3	5.0	8.9	3.6	84	54
November	2.7	1	32.1	18.9	4.4	8.6	3.9	75	42
December	5.8	1	29.4	16.7	4.0	7.7	4.1	87	43
January	6.4	1	32.2	15.9	4.2	9.1	3.1	88	37
February	135.9	8	31.8	19.1	4.4	8.4	3.3	81	45
Mean / Total	891.1	84	33.46	22.46	5.24	7.4	4.9	85.51	51.63

Table S: Mean meteorological data for 1990-91 at Anakapalle

Months	Rain fall in mm	No. of rainy days	Temperature °C		Evapora- tion in mm	Average number of sunshine hours per day	Wind velocity in km per hour	Per cent relative humidity	
			Maximum	Minimum				7.00 hours	14.00 hours
March	234.5	9	32.4	22.7	4.5	8.7	4.3	92	60
April	41.2	2	34.7	25.0	5.8	5.8	5.7	90	59
May	486.1	12	34.1	25.0	6.0	5.1	4.4	88	54
June	59.2	6	34.5	26.3	5.2	4.0	5.1	86	50
July	95.2	8	32.0	25.0	4.4	3.0	5.0	91	50
August	57.9	6	34.0	25.3	5.0	5.3	5.1	86	55
September	207.0	7	33.1	25.3	5.1	5.0	3.4	90	50
October	209.4	13	30.7	23.4	3.3	3.0	3.4	86	50
November	37.8	5	30.7	21.7	3.7	6.7	4.2	85	56
December	3.6	1	30.0	16.4	3.1	3.0	4.1	80	47
January	24.0	2	30.0	18.3	5.2	6.7	3.5	90	51
February	0.0	0	33.6	19.7	5.3	6.5	3.4	86	47
Mean/Total	1442.2	79	32.58	22.85	4.98	5.09	4.36	86.17	53.94

3.1.2 Chemicals

RESEARCH REPORT  
D 4649  
26 8-51

In all the studies, based on the experimental requirement, analytical or laboratory grade chemicals of British Drug House Company Limited, Sarabhai Chemicals Limited, Qualigens Fine Chemicals and Loba Chemicals were used unless otherwise stated.

3.1.3 Sterilisation of glassware, media and soil

Glassware kept in appropriate containers were sterilised in hot air oven at 160°C for 90 minutes. All the types of media and cane leaf bits of different varieties were sterilised at 15 PSI for 20 minutes in an autoclave. Soils were sterilised at 20 PSI for one and half hours on two alternate days.

3.1.4 Surface sterilisation of leaf material

The diseased leaf tissues of 8x2 cm were washed under running water for 10 minutes and transferred to blotting paper. They were cut into bits of 3x2 cm size with sterile razor blade and treated with 0.1 per cent aqueous solution of mercuric chloride for 45 seconds to one minute in laminar air flow chamber. Later they were washed in three changes of sterile distilled water.

### 3.1.5 Media used for isolation and maintenance of fungi

The culture media used in the studies along with their composition are furnished in Table 6. Streptopenicillin (Dicrysticin-S) at the rate of 500 ppm was added to the media unless otherwise stated, to avoid bacterial contaminations.

Table 6: Details of culture media used in the investigations

Media	Ingredients	Quantity
1. Potato Dextrose Agar (PDA)	Peeled potato slices	200 g
	Dextrose	20 g
	Agar	20 g
	Distilled water	1000 ml
2. Oat Meal Agar (OMA)	Oats	40 g
	Agar	20 g
	Distilled water	1000 ml
3. Czapek-Dox Agar	Na NO <sub>3</sub>	3.0 g
	KH <sub>2</sub> PO <sub>4</sub>	1.0 g
	Mg SO <sub>4</sub> , 7H <sub>2</sub> O	0.50 g
	Potassium chloride	0.50 g
	Fe SO <sub>4</sub> , 7 H <sub>2</sub> O	0.01 g
	Sucrose	30.0 g
	Agar	20.0 g
	Distilled water	1000 ml

Contd..

Media	Ingredients	Quantity
4. Richard's Agar	KNO <sub>3</sub>	10.0 g
	KH <sub>2</sub> PO <sub>4</sub>	5.0 g
	Mg SO <sub>4</sub> · 7 H <sub>2</sub> O	2.5 g
	Fe Cl <sub>3</sub>	0.02 g
	Sucrose	50.0 g
	Agar	20.0 g
	Distilled water	1000 ml
5. Bean Meal Agar	Bean Meal (Ground seeds of dwarf bean)	30.0 g
	Agar	20.0 g
	Distilled water	1000 ml
6. Cane Leaf Agar (CLA)	Cane leaf	50 g
	Dextrose	5 g
	Peptone	5 g
	Crystallised sugar	20 g
	Agar	20 g
Distilled water	1000 ml	
7. Carrot Agar	Carrot	20 g
	Glucose	30 g
	Agar	20 g
	Distilled water	1000 ml
8. Potato carrot Agar	Grated potato (potato slices)	20 g
	Grated carrot (carrot slices)	20 g

Contd..

Media	Ingredients	Quantity
	Agar	20 g
	Distilled water	1000 ml
9. Water Agar	Agar	20 g
	Distilled water	1000 ml
10. Corn Meal Agar	Corn meal Agar (Hi Media)	20 g
	Distilled water	1000 ml
11. Potato Sucrose Agar (PSA)	Peeled potato slices	200 g
	Sucrose	20 g
	Agar	20 g
	Distilled water	1000 ml
12. Potato Sucrose Agar + Oats	Ingredients as in PSA + Oats	10 g
13. Potato Sucrose Agar + Cane leaf extract	Ingredients as in PSA + cane leaf	20 g
14. Potato Dextrose Agar + Corn meal	Ingredients as in PDA + Corn meal	20 g
15. Potato Dextrose Agar + Cane leaf extract	Ingredients as in PDA + Cane leaf	20 g
16. Cane leaf Agar + oats	Ingredients as in CLA + Oats	10 g
17. Oat meal Agar + cane leaf extract	Ingredients as in OMA + Cane leaf	20 g

### 3.1.6 Fungicides

The fungicides used in the investigations are furnished in Table 7.

### 3.1.7 Seed material of test varieties

Required seed material of different varieties/clones was taken from 6 months old short crops raised from hot water treated setts at Regional Agricultural Research Station, Anakapalle as and when needed. The setts with sound buds were selected for use in the experimental work. Field and pot culture studies were conducted with the susceptible variety Co 7219 unless otherwise mentioned. Planting in field was done in March every year and the crop was harvested in February next year unless otherwise stated.

### 3.1.8 Fertilizer application

As per the recommendation for the region, only nitrogen, to supply  $112 \text{ kg ha}^{-1}$  in the form of urea, was applied to the crop in two equal halves at 45 and 90 days after planting unless otherwise mentioned. No other nutrients were given to the crop unless otherwise mentioned.

### 3.1.9 Irrigation

The crop in the field was irrigated as per the recommendations for the tract, at an interval of 7-8 days

Table 7: Details of fungicides employed in the investigation.

S.No.	Trade name	Common name	Chemical name	a.i.	Source / Company
1.	Bavistin	Carbendazim (MBC)	methyl-2-benzimidazole carbamate	50%	BASF India Ltd.
2.	Topsin-M	Thiophanate methyl	Dimethyl 4,4'-O-phenylenebis (3-thiophanate)	70% U.P.	Motilal Pesticides (India) Pvt.Ltd.
3.	Calixin	Tridemorph	1-tridecyl-2,6-dimethyl-morpholine	60% E.C.	BASF India Ltd.
4.	Bayleton	Triadimefon	1-(4-chlorophenoxy)-3,3-dimethyl-1H-1,2,4-triazol-1-yl)-2-butazole	25% U.P.	Bayer India Ltd.
5.	Kitazin	Kitazin	O,O-di-isopropyl-benzyl thiophosphate	40% E.C.	Pesticides India
6.	Hinosan	Edifenphos	O-methyl-S-S-diphenyl phosphoro-dithioate	50% E.C.	Bayer India Ltd.
7.	Blitox	Copper oxychloride	Copper oxychloride	50% U.P.	Rallis India Ltd.
8.	Captan	Captan	N-Trichloromethyl, (thio)-4-cyclohexene-1,2-dicarboximide	50% U.P.	Rallis India Ltd.
9.	Captafol	Foltag	Bis-N-(1,1,2,2-tetrachloroethyl, (thio)-4-cyclohexene-1,2-dicarboximide	40% U.P.	Rallis India Ltd.
10.	Dithane M-45 (Indofil M-45)	Mancozeb	Manganese ethylene bis dithio-carbamate-zinc	75% U.P.	Indofil Chemicals Limited
11.	Dithane Z-78	Zineb	Zinc ethylene bis dithio-carbamate	75% U.P.	Rallis India Ltd.
12.	Thiram	Thiram	tetra methyl thiuram disulphide	75% U.P.	Swarup Chemicals Private Limited
13.	Chloro thalonil	Safe guard	tetra chloro isophthalonit rile	75% U.P.	Chadra Chemicals Ltd.

during the formative phase and 21 days during the maturity phase with 13 irrigations. Only two irrigations were given during grand growth phase which coincided with monsoon time. In all, the crop received 15 irrigations.

### 3.1.10 Cultural operations

The weeds in the experimental plot were removed by manual labour one week before manuring. Earthing up for the crop was done by manual labour. Trash twist propping was done thrice to prevent the crop from lodging. Recommended plant protection measures were taken up particularly for the control of early shoot borer and scale insect. The crop was finally harvested by manual labour from pre fixed cane rows that were left undisturbed after recording stalk observations and cane yield.

## 3.2 EXPERIMENTAL

### 3.2.1 Symptomatology

#### 3.2.1.1 Symptoms

Development of spots in nature, starting from the initiation of the disease, was recorded on four varieties viz. Co 7219, CoT 8201, CoA 89081 and Co 85045 and described. Symptoms were recorded on all the leaves including the spindle leaf. However, at the time of harvest, the number of spots produced on top 1-8 fully opened leaves were counted in 50 plants of each variety and the average was worked out. The third leaf from top,

including spindle leaf was taken as the first fully opened leaf.

### **3.2.1.2 Size and shape of the spots in different sugarcane varieties**

To study the nature of spotting, (ie.) size and shape on different sugarcane varieties, 34 varieties/clones were planted each in 2 m length rows in the month of March, 1988. The pycnidiospore suspension was sprayed on leaves once during last week of August, 1988. The number of days taken for the first appearance of the disease after inoculation, shape and size of the spot in mm were recorded, 3 months after inoculation.

### **3.2.2 Isolation and identification of pathogen and establishment of pathogenicity**

#### **3.2.2.1 Isolation**

Leaf samples from naturally infected plants with immature spots and mature spots with black discolouration in the greyish centre were used for isolation.

#### **Isolation from immature spots**

Leaf bits of 3 x 2 cm size were surface sterilized and washed in sterile water. The leaf bits were then cut into small pieces with sterilised scissors and the bits were transferred to moist blotters and to different media (PDA, OMA, CLA, Richard's Agar and Czapek-

Dox agar) in sterile petriplates. These were incubated at  $28^{\circ} \pm 1^{\circ}\text{C}$  and the causal fungi were isolated and purified by monoconidial method (Pathak, 1984).

#### **Isolation from mature spots**

Microscopic observations of slides from mature lesions of ring spot revealed the continuous association of ascospores and pycnidiospores. For isolating these fungi, the leaf bits were surface sterilised for 45 seconds and washed with sterile water as quickly as possible. These sterile leaf bits were transferred to sterile petriplate in which few drops of sterile water was placed. The leaf bit was cut with a sterile razor into several small pieces in sterile water. These small pieces were then transferred to different media (PDA, OMA, CLA, Richards agar and Czapek-Dox agar) and incubated. A loop full of sterile water containing spores was streaked on the above media separately. The suspension was also flooded in sterile water agar plates. Single ascospores and pycnidiospores were picked up and transferred to different media mentioned above.

#### **3.2.2.2 Maintenance of isolated fungi**

The isolated fungi were maintained on PDA, OMA and OMA + cane leaf extract free from aerial contaminations. They were subcultured as and when needed.

### 3.2.2.3 Identification of isolated fungi

The isolated fungi were identified to the genus/species level based on the colony and cultural characters and spore measurements with the help of relevant monographs, illustrated books and CMI descriptions.

### 3.2.2.4 Production of ascospores in vitro

i) Healthy leaf bits of highly susceptible sugarcane varieties Co 7219 and Co 7636 were surface sterilised with 0.1% mercuric chloride in laminar air flow chamber for one minute and washed in 3 changes of sterile distilled water were placed one each at the centre of a sterile petriplate containing OMA, PDA and CLA. Ascospores collected from surface sterilised mature lesions taken in sterile water were flooded on sterile water agar separately. Ascospores lifted along with the agar bit were placed at the edge of the leaf bit in different media. One set of ten plates was incubated at ambient temperature in the laboratory and another set was exposed to alternate light and darkness for 12 hours each under 40 watts florescent tubes at  $28 \pm 1^{\circ}\text{C}$  for one and half months. The third set of plates were kept in growth chamber ten each at  $24^{\circ}\text{C}$ ,  $26^{\circ}\text{C}$  and  $28^{\circ}\text{C}$  for one and half months with alternating 12 hours light and 12 hours darkness at a distance of 45 cm.

ii) Ten grams of healthy leaf bits (without midrib) of susceptible sugarcane varieties viz. Co 7219, Co 7636, Co 8021 and CoT 8201 were taken in 150 ml earlenmeyer flasks and sterilised at 15 PSI for 20 minutes in an autoclave. To each flask five µg of Biotin, 10 µg of Thiamine hydrochloride and 2 ml of 0.1 per cent sucrose solution were added before autoclaving. Ascospores from naturally infected leaves and pycnidiospores obtained from the culture were transferred separately on to these leaf bits in each flask. Fifty such flasks were taken for each variety. One set of flasks was incubated at ambient temperature in the laboratory and the second set was subjected to alternating light and darkness of 12 hours each under 40 watts florescent tubes at  $28 \pm 1^{\circ}\text{C}$  for one and half months. The third set of flasks was kept in growth chamber five each at  $24^{\circ}\text{C}$ ,  $26^{\circ}\text{C}$  and  $28^{\circ}\text{C}$  for one and half months in alternating light (12 hours) and darkness (12 hours) at a distance of 45 cm. For each of the above treatments sterilised leaf bits without vitamins or sucrose served as control (5 flasks each).

#### **3.2.2.5 Pathogenicity tests**

The fungi isolated from the ring spot lesions were tested for their pathogenicity in greenhouse on potted plants of susceptible sugarcane varieties Co 7219, CoT 8201 and Co 7636.

The procedure adopted by Wismer and Koike (1967) for brown spot disease of sugarcane was followed with slight modifications. Potted plants of 4 months age were used for inoculation. The plants to be inoculated were watered well. The top halves of leaves were clipped off and the whole crown was enclosed in an alkathene bag for 24 hours to provide pre inoculation humidity. The leaves of the plants were spray inoculated, employing an atomiser, with a suspension of conidia of the test fungi either individually or in combination. Similarly plants were also spray inoculated with ascospores produced in the laboratory on the sterile host leaf bits and also from the naturally infected material. Another set of plants were inoculated by slightly wounding the leaves by pin prick method. Suitable controls were maintained by spraying sterile distilled water. The plants after inoculation were again enclosed in an alkathene bag for 72 hours to provide post inoculation humidity.

#### **Preparation of inoculum of test fungus for pathogenicity tests**

The inoculum was prepared from the cultures grown in the laboratory and also from the naturally infected cane leaves.

The fungus was cultured on 30 ml of potato dextrose broth in 150 ml Erlenmeyer flask at 26°C in alternating 12 hours light and 12 hours darkness for 10

days in growth chamber. The culture mats were removed, washed with sterile distilled water and blended with sterile distilled water @ 50 ml per mat at low speed in a blender. The mixture was filtered through a muslin cloth and the filtrate containing conidia and mycelial fragments was used as inoculum. Ascospores that developed on the sterile host leaf bits in the laboratory and also from the naturally infected material were also used for inoculation.

#### 3.2.2.6 Identification of a suitable medium for the pathogen

Different synthetic and non synthetic media as mentioned in 3.1.5 were tried, to identify a suitable one for optimum growth and sporulation of the pathogen. Ascospores and pycnidiospores from sterile water agar were transferred to different culture media separately and incubated at ambient temperature. The extent of sporulation in each media was measured with the help of haemocytometer. Since CMA + cane leaf extract supported good growth and sporulation of P. saccharicola in the present studies, this medium was used for maintenance of the fungus throughout the studies.

#### 3.2.2.7 Inoculum for field and other studies

The pycnidiospores of P. saccharicola obtained from the affected leaves of CO7219 on OMA + cane leaf

extract formed the inoculum. A concentration of  $1 \times 10^6$  spores/ml was used in all the studies unless otherwise stated.

#### 3.2.2.8 Cultural characters and measurement of ascospores and pycnidiospores

The cultural and morphological characters of both sexual and asexual stages of the fungus were recorded on different media. The measurements of perithecium, asci and ascospores that developed on healthy sterile cane leaf bits in the laboratory and from lesions in nature were recorded with the help of ocular and stage micrometers. For the asexual stage pycnidia and pycnidiospores obtained in culture and from lesions in nature were measured.

#### 3.2.3 Developing a disease rating scale for estimation of disease intensity

The per cent green leaf affected area at different intensities of disease was worked out with the help of leaf area meter and a 0-9 scale was developed specially in the present studies, for estimating the intensity of the disease. The details of 0-9 scale is given below:

Grade	Description
0	No disease symptoms.
1	Only small brown specks of 1 mm size at apical part of leaves, few to many, some times unrecognisable.
2	Slightly large, oval, greyish spots of 3 mm in diameter covering less than 1 per cent of leaf area.
3	Oval to irregular greyish spots of 4 mm diameter with reddish margin covering 1 to 5 per cent of leaf area. No necrotic spots.
4	Typical ring spot lesions develop. Spots with necrotic grey centre and reddish brown margins covering 5.1 to 10.0 per cent of leaf area.
5	Typical ring spot lesions spreading to middle portion of the leaf covering 10.1 to 20.0 per cent of the leaf area.
6	Lesions as in scale 5, have a tendency to coalesce, spreading towards basal portion of the leaf affecting 20.1 to 30.0 per cent of leaf area.
7	Large quickly expanding lesions, irregular because of fusion, brown margins and most of the affected area blighted covering 30.1 to 50.0 per cent of leaf area.
8	Symptoms as in scale 7, affecting 50.1 to 60.0 per cent of the leaf area. Premature senescence of the leaf.
9	More than 60.0 per cent of the leaf area affected and premature senescence of the leaf.

For assessment of disease intensity, 20 millable canes tagged at random formed the sample. The disease intensity in all the studies was recorded on the top 1 to 8 fully opened leaves (RAPS, Anakapalle, 1989) with the aid of the scale. All leaves considered for assessment

were assumed to be equal in size. The per cent disease intensity (PDI) was calculated using the following formula suggested by Mayee and Datar (1986).

$$PDI = \frac{0(X_0) + 1(X_1) + 2(X_2) + \dots}{X_0 + X_1 + X_2 + \dots} \times 100$$

..... x maximum grade used

where,

X represent the number of diseased entities within a sampling unit in the respective class or grade such as 0, 1, 2 ..... 9

**3.2.4 Survey for assessment of disease intensity**

To study the incidence and intensity of ring spot disease on different cultivated varieties in different cane growing areas of the three regions of the state, survey was conducted during 1988-89 and 1989-90 in sugar factory areas of Podhar, Renigunta, Bobbili, Vuyyuru, Nellore, Anakapalle and Chelluru. The fields were selected at random in each factory area. Field visits were undertaken during the maturity phase of the crop (8-9 months age).

In each field, observations were made on 20 millable canes at 4 places at random on the diagonal line of the field (Singh, 1984). The relevant observations made on crop variety, extent of holding, type of soil, type of crop (plant or ratoon) and grade on 0-9 scale were recorded in a proforma. Information on the date of

planting and plant protection measures adopted was also collected from farmers and factory agricultural staff. A total of 98 fields in 34 villages of 7 factory zones were surveyed.

### 3.2.5 Inoculation and rapid screening techniques

#### 3.2.5.1 Inoculation techniques

Different methods of artificial inoculation were tried to find out an appropriate method. The study was conducted in green house with four susceptible varieties Co 7219, CoT 8201, CO 975 and Co 7636. Two budded setts of the test varieties were planted in pots in April. The leaves were inoculated during last week of August. The plants were watered before and after inoculation. Pre and post inoculation humidities were maintained. The inoculation methods employed were as follows:

1. Spraying leaves with conidial suspension
2. Clipping the top halves of leaves and spraying conidial suspension
3. Clipping the top halves of leaves and slightly wounding them by pin prick method and spraying conidial suspension
4. As in method 3 + spraying water with continuous atomiser on inoculated plants for 2 hours every day upto 7 days from 4th day to simulate rain, conditions.

5. Mixing celite in inoculum and rubbing gently over the leaves.
6. Inserting small pieces of pycnidia bearing leaf tissues into the spindle in the month of August and spraying water (Leu, 1968).
7. Squirtng spore suspension into the spindle in August (Wang and Lee, 1980) and spraying water.
8. Wrapping 3-6 leaves with a piece of ring spot affected leaf during August (Bernard and Liu, 1980).

The data on the intensity of the disease in each treatment were recorded 6 months after inoculation.

#### 3.2.5.2 Rapid screening techniques

Effective methods for determining the reaction of varieties to ring spot disease are essential. Different screening methods mentioned below were tested in pot culture, field and laboratory. Eight varieties/clones viz., C07219, C0T8201, Co7636, Co85044, 84A155, Co85045, CcA89081 and 86A444 were included in the study.

1. Two budded setts were planted in pots in April. The first and fourth inoculation techniques mentioned in 3.2.5.1 were employed.
2. Rajoong inoculation.

Three budded setts were planted vertically in the pot during June. The bottom most bud was scooped out before planting and the lower internode was pressed into the soil. The lateral buds sprout up and such setts with shoots are called "Rajoongs". There will be no shoot roots and shoots survive with the sett roots put forth from the node immersed in the soil. The leaves of the shoots were spray inoculated with the spore suspension in the last week of August. Pots were watered profusely before and after inoculation and were sprayed with sterile water for 2 hours every day for one week starting from 4th day after inoculation. The intensity of the disease was recorded in each variety 6 months after inoculation.

### 3. Detached leaf method

Healthy leaf pieces of 1.5 cm were cut from test varieties and washed thoroughly with tap water and then with sterile distilled water. They were transferred to sterile moist blotters. The leaf pieces were inoculated by dipping in spore suspension and also by spraying with atomiser and maintained on a nutrient solution in 25x200 mm test tubes (Liu, 1982).

### 4. Screening under natural conditions

The test varieties were planted in the field in the month of March in two rows of 3 m length each. The susceptible variety Co7219 was planted in one row after

each test variety and also all around the plot. The disease intensity in each variety was recorded in February, 6 months after inoculation.

### 3.2.6 Factors affecting disease development

The studies were conducted both in field and green house.

#### 3.2.6.1 Effect of application of different levels of Nitrogen, Phosphorus and Potassium fertilizers on the extent of disease development.

To assess the effect of different levels of nitrogen (N), phosphorus (P) and potash (K) fertilizers applied to the crop on the intensity of disease development, a randomised replicated field trial (Factorial R.B.D.) was conducted using variety Co 7219 with the following four levels of nitrogen in the form of urea, two levels of phosphorus in the form of single super phosphate and two levels of potash in the form of muriate of potash.

#### **Nitrogen**

- N<sub>0</sub> : no nitrogen
- N<sub>1</sub> : 112 kg ha<sup>-1</sup>
- N<sub>2</sub> : 224 kg ha<sup>-1</sup>
- N<sub>3</sub> : 336 kg ha<sup>-1</sup>

### Phosphorus

$P_0$  : No phosphorus

$P_1$  : 100 kg ha<sup>-1</sup>

### Potash

$K_0$  : No potash

$K_1$  : 100 kg ha<sup>-1</sup>

Potash and phosphorus fertilizers were applied basally at the time of planting in furrows. Nitrogen was applied in two equal splits at 45 and 90 days after planting (DAP). The treatments were randomised and replicated thrice. Size of each treatmental plot was kept at 38.4 m<sup>2</sup> with a net plot area of 28.8 m<sup>2</sup> for recording observations. Spraying with spore suspension was done in the month of August in the central four rows. The PDI was recorded in each treatment on 0-9 scale starting from November till the crop was harvested in February. Since mid lamina portion and sheaths of 3-6 leaves (with spindle leaf as first and counting downward) are index tissues, the mid lamina nitrogen, sheath phosphorus and potash were estimated in all the treatments at 240 DAP (Clements, 1953) following standard procedures.

#### 3.2.6.1 i) Estimation of leaf nitrogen

For estimating per cent nitrogen, 3-6 leaves from top were used. Mid lamina portion excluding the mid rib was used for estimation.

Oven dried and finely powdered leaf material (0.1 g) was used for nitrogen estimation. The standard micro kjeldahl method was adopted for nitrogen estimation and the nitrogen content was expressed as per cent on oven dry basis (AOAC, 1975).

### Digestion

A weighed quantity (0.1 g) of oven dried, powdered leaf material was taken in heat resistant tubes. To this 3 ml of concentrated  $H_2SO_4$  was added and kept for half an hour. The test tube was then heated on flame till thick fumes came out. Then the flame was removed and a few drops of  $H_2O_2$  were added. The same process was repeated till the solution turned colourless indicating the completion of digestion.

### Distillation

The digested material was carefully transferred to a distillation unit and 10 ml of 45% NaOH solution was added. This solution was then steam boiled and the distillate containing the liberated ammonia was collected over 4 per cent boric acid containing mixed indicator (0.5 g of Bromocresol green and 0.1 g of methyl red indicators in 100 ml of 95% alcohol). The ammonia absorbed in boric acid was titrated against N/50  $H_2SO_4$  and the titre value (T.V.) was noted.

The per cent of nitrogen in each sample was calculated using the formula

$$\text{T.V.} \times 0.28 \times \frac{100}{\text{Weight of leaf sample (0.1 g)}}$$

### 3.2.6.1 ii) Sheath phosphorus

Leaf sheaths were collected from 3-6 leaves and oven dried at 70°C. Phosphorus was estimated by Vanado-Molybdo-phosphoric yellow colour method in nitric acid medium as described by Koeing and Johnson (1942) in wet digests and expressed as per cent on oven dry basis.

One gram of oven dried sheath material was digested in 10 ml of triacid mixture prepared in 9:2:1 proportion of nitric, sulphuric and perchloric acids respectively. The extract was made upto 100 ml volume. Ten ml of the extract was taken into a 50 ml volumetric flask to which 10 ml of Bartons reagent (Ammonium vandate + Ammonium molybdate) was added and then the volume was made upto 50 ml. The contents of the flask were shaken and kept for half an hour. The intensity of the yellow colour was read with the help of calorimeter using blue filter (420 nm). A blank was run simultaneously. A standard curve was drawn with different concentrations of standard phosphate solution using the same reagents mentioned

above. The per cent phosphorus in 3-6 sheaths was determined with the help of standard curve using the formula

$$\text{Per cent P} = \frac{R}{10^6} \times \frac{\text{Total volume made up (50ml)}}{\text{aliquot taken (10 ml)}} \times \frac{100}{\text{Weight of sample (lg)}}$$

where R = ppm of P present in the sample corresponding to the standard curve

**3.2.6.1 iii) Sheath potassium**

Potassium in wet digest of 3-6 sheaths was estimated using Flame Photometer method (Jackson, 1973) and expressed as per cent on oven dry basis.

One gram of oven dried sheath material was digested in 10 ml of triacid mixture.

The triacid extract was made upto 50 ml and fed to the flame photometer using potassium filter. The potassium in the sample was determined with the help of the standard curve drawn with different concentrations of potassium. The per cent potassium is determined as follows.

$$\text{Per cent K} = \frac{R}{10^6} \times \frac{\text{Volume made (50ml)}}{\text{Weight of plant sample taken (lg)}} \times 100$$

R = ppm of K present in the sample corresponding to the standard curve

### 3.2.6.2 Relationship of Nitrogen, Phosphorus and Potassium contents in the index tissues of resistant and susceptible varieties on the extent of disease severity

To study the relationship of NPK contents in the index tissues of plants of resistant and susceptible varieties, a trial was conducted. Five resistant viz., CoA 89081, 84A155, Co 85045, 86A444, 83A106 and five susceptible viz., Co 7219, CoT 8201, Co 7636, Co 975, Co 85044 varieties/clones were planted in the field each in 4 rows of 5 m length in the month of March. Only nitrogen @  $112 \text{ kg ha}^{-1}$  in the form of urea was applied to the crop in two equal doses at 45 and 90 days after planting. Inoculation was done in the central 2 rows leaving one row on either side as border. Leaf and sheath samples were collected from 3-6 leaves at 240 DAP from the inoculated canes of all the varieties/clones and analysed for nitrogen, phosphorus and potassium. Disease intensity in each variety was recorded at harvest during February.

### 3.2.6.3 Age of the crop and intensity of the disease

To study the effect of age of the crop on the intensity of the disease, a field trial was conducted during 1988-89 and 1989-90. The test variety Co 7219 was planted in the field at monthly intervals starting from February to June every season. The variety was planted in

6 rows of 5 m length every month. Inoculation with conidial suspension was done at 3 months age of the crop. The disease intensity was recorded starting from initiation of symptoms (September) till harvest of the crop (February).

#### **3.2.6.4 Effect of leaf orientation on the intensity of disease**

To study the effect of leaf orientation on the intensity of the disease 10 varieties/clones (84A155, 87A142, 82V12, 86A444, 87A279, 87A140, CoA 8901, Co 85032, 83A106 and 87A3) having erect leaves and 10 varieties/clones (Co 7219, Co 7636, CoT 8201, Co 7706, Co 975, Co 85044, Co 8021, 84A86, 84A125 and CoA 8401) having drooping leaves were inoculated with conidial suspension at the age of 5 1/2 months. The disease intensity was recorded 6 months after inoculation. The angle between the leaf sheath and laminar portion at the back of ligule of 1 to 8 functional leaves on each stalk was also measured in 20 canes in all the test varieties at harvest and average was worked out.

#### **3.2.6.5 Moisture stress and water stagnation**

The effect of moisture stress and water stagnation on the intensity of the disease was studied in green house, in comparison with normal irrigation with ten varieties/clones viz., Co 7219, CoT 8201, Co 7636, Co

85044, Co 975, 84A155, 86A444, Co 85045, CoA 89081 and 83A106. Moisture stress and water stagnation were created in pots as detailed below.

#### **Moisture stress**

Initially, irrigation was provided at frequent intervals until germination and establishment of plants. later the plants were subjected to moisture stress. The plants were irrigated sparingly when they showed loss of turgidity and tendency for drying.

#### **Water stagnation**

After establishment of plants, the drain holes in the pots were plugged and the plants were subjected to water stagnation for 90 days (August to October).

The plants in the above varieties were sprayed with conidial suspension in the first week of September at the age of 5 months and the intensity of the disease was recorded 6 months after inoculation. Suitable controls with normal irrigation were maintained.

#### **3.2.6.6 Effect of silica content of leaves on disease intensity**

The silica content in the leaves of five resistant (CoA 89081, 84A155, Co 85045, 86A444, 83A106) and five susceptible (Co 7219, CoT 8201, Co 7636, Co 975,

Co 85044) varieties/clones was estimated by gravimetric method (AOAC, 1975). Ten g of leaf material of each test variety were ignited in flat bottom platinum dish in muffle at 500-550°C until the residue was white. To this residue 10 ml of hydrochloric acid was added and boiled for 2 minutes and evaporated to dryness and heated on steam bath for 3 hours. Again 5 ml of hydrochloric acid was added and boiled for 2 minutes. Then 50 ml of water was added and heated on steam bath for 5 minutes. The contents were filtered through Whatmann No.42 filter paper and washed thoroughly. The filter paper along with the residue was ignited at 500-550°C and the residue was weighed. The silica content present in the leaf was expressed in per cent on dry weight basis.

### **3.2.7 Variation in the isolates of ring spot pathogen**

For understanding the variability in ring spot pathogen, isolates obtained from different varieties and locations were studied for their cultural, morphological and pathological characters.

#### **Details of the isolates**

The details of the isolates studied are furnished in Table 8.

Table 8: Details of source of isolates of ring spot pathogen

Sugarcane variety	Size of the spot	Locality	Number assigned to the isolate
Co 7706	Big (14.9x3.7 mm)	Nagulapalle	1
Co 7704	Big (12.9x3.6 mm)	Chagallu	2
Co 7219	Medium (8.9x3.2 mm)	Anakapalle	3
CoT 8201	Medium (8.4x3.1 mm)	Bochan	4
CoA 89082	Small (6.2x1.6 mm)	Anakapalle	5
Co 419	Small (4.8x1.3 mm)	Amadalavalasa	6

3.2.7.1 Isolation

Cultures were obtained from the affected leaf samples on OMA by single spore isolation by following the procedure indicated in 3.2.2. The plates were incubated at 28±1°C. Morphological characters and spore measurements were recorded in 10 days old cultures of all the isolates. Measurements of asci and ascospores were recorded from those obtained from host leaf bits.

3.2.7.2 Pathogenic variation

The pathogenic variation, if any, among the isolates, was assessed by simultaneously inoculating them

separately by spraying conidial suspension ( $10^6$  spores/ml) on different varieties during last week of August (170 DAP). The varieties/clones inoculated were CoA 89081, 84A155, Co 85045, Co 7219, CoT 8201, Co 85038, Co 8220, Co 7706, CoA 7602, Co 975, Co 85044, Co 7636, CoA 8401, Co 419 and Co 62175. Canes in two rows of two m length were inoculated per isolate per variety. The assessment of the disease was made on 0-9 scale, 6 months after inoculation.

### **3.2.8 Role of soil, host debris, cane setts, irrigation water and leaf contact on the spread of disease**

The role of soil, host debris, cane setts with infected leaf tissues, irrigation water and contact with diseased leaf tissues, in disease spread, was examined. The study was conducted in pots with two susceptible varieties, viz., Co 7219 and Co 7636 during 1988-89 and 1989-90 with the following treatments.

#### **a) Sick soil**

Setts from healthy canes of test varieties were planted in soil collected from adjacent to the severely diseased clumps.

#### **b) Host debris**

i) Diseased leaves with advanced symptoms were collected during April and chaffed with a chaff

cutter and incorporated in sterilized soil in pots during May. Setts from healthy canes were then planted during June in the pots.

- ii) Chaffed debris from previously collected 6 months old infected leaves was spread over the sterilized soil at the base of clump in pots in September when the crop was 3 months of age.

**c) Cane setts with infected leaf tissue**

Setts from infected cane along with the infected leaf tissue adhering to it, were taken and planted in pots.

**d) Irrigation water**

Healthy setts were planted in pots with sterilized soil in June. Normal watering was done upto August. From the last week of August, pots were watered once in a week with water mixed with 200 ml of spore suspension of P. saccharicola prepared by soaking diseased leaf tissues and enriched with a 10 day old culture of the fungus. This was done for one month.

**e) Contact with diseased leaf tissue**

Heavily infected leaves were kept in contact with healthy leaves at the age of 4 months during October and were sprayed with sterile distilled water every alternate day for one month.

### 3.2.9 Host range studies

Twenty plant species belonging both to monocots and dicots were tested for their reaction by inoculating with pycnidiospores produced in ten day old culture.

Plant species included in the study were :

- 1a Saccharum spontaneum L. (Rellu)
- 1b S. spontaneum L. (SES.594-wild cane)
- 2 Pennisetum glaucum (L.) R.Br.
- 3 Sorghum vulgare (Pers.)
- 4 Sorghum halepense (L.) Pers.
- 5 Panicum maximum Jacq.
- 6 Zea mays L.
- 7 Eleusine coracana (L.) Gaertn
- 8 Oriza sativa L.
- 9 Erianthus arundinaceus Retz.
- 10 Phragmites karka (Retz.) Trin. ex Steud.
- 11 Sesamum indicum L.
- 12 Euphorbia hirta L.
- 13 Cyperus rotundus L.
- 14 Cynodon dactylon (L.) Pers.
- 15 Digitaria sanguinalis (L.) Scop.
- 16 Eclipta alba (L.) Hassk.
- 17 Trianthema portulacastrum L.
- 18 Croton bipolarandes L.
- 19 Justicea simplex D. Don.
- 20 Cymbopogon nardus Rendle

All the above plant species planted in July were raised in pots in the glass house and inoculated when 40-45 days old. The plants in one set of pots were inoculated by rubbing inoculum on the leaves after wounding with celite and another set was inoculated by spraying conidial suspension on the leaves. Suitable controls were maintained by spraying with sterile water. Observations were made from 3rd day onwards upto 30 days after inoculation.

### **3.2.10 Reaction of sugarcane varieties/clones for resistance to ring spot disease**

#### **3.2.10.1 Determination of appropriate time for assessment of the disease**

Twenty sugarcane varieties/clones were planted in the field in March, 1988 to determine proper time for assessing the reaction of varieties after inoculation. Inoculation with conidial suspension was done during August. Twenty standing canes were tagged at random for each variety/clone for recording the disease intensity at 1, 2, 3, 4, 5, 6, 7 and 8 months after inoculation on 0-9 scale as described in 3.2.3.

#### **3.2.10.2 Screening of popular and promising varieties/clones by adult cane inoculation**

A total of 60 popular and promising varieties/clones, including commercial standards, were

screened for their resistance to ring spot disease during 1988-89 and 1989-90 in observational plots. The varieties/clones were planted during March and manured as per the recommendations. Inoculation was done with conidial suspension during August on a cloudy day without any rain, taking advantage of humid weather. The crop was irrigated thoroughly, before and after inoculation. Twenty standing canes were tagged at random and the disease intensity was recorded on 0-9 scale in each variety, twice, three and six months after inoculation. Varieties/clones were classified into different reaction groups based on PDI using the rating scale developed in the present studies (4.3).

#### **3.2.10.3 Varietal reaction by Rajoong inoculation**

Forty varieties/clones were screened during 1988-89 and 1989-90. Three budded setts of the test varieties were planted vertically in the field in June as described in 3.2.5.2. The leaves were inoculated by spraying conidial suspension in the last week of August. The intensity of the disease was recorded twice in each variety at 3 and 6 months after inoculation in twenty canes tagged randomly and compared with that recorded on adult cane inoculation.

### 3.2.11 Chemical control

#### 3.2.11.1 In vitro tests

##### **Preliminary screening of systemic and non systemic fungicides**

The efficacy of thirteen fungicides was tested against ring spot pathogen (Phyllosticta saccharicola) in vitro employing poisoned food technique (Nene and Thapliyal, 1979).

Oat agar medium was prepared in 150 ml Erlenmeyer flasks and sterilized. Thirteen fungicides (vide 3.1.6) with different concentrations viz., 1000 ppm, 500 ppm, 200 ppm and 100 ppm were prepared and mixed thoroughly with the media by stirring in laminar air flow chamber. The medium was then poured in sterile petriplates. Small discs of 7 mm size of 10 days old test fungal culture grown on oat agar medium were cut with a sterile cork borer and transferred aseptically to the centre of each petriplate and incubated at  $28 \pm 1^{\circ}\text{C}$ . Suitable checks were maintained without fungicide. The colony diameter measured every 24 hours was compared with that of check 10 days after inoculation (time taken to cover the entire plate in control) to determine fungitoxicity. Three systemic fungicides viz., Bavistin, Topsan-M and Catixin which were found effective at 100 ppm were also tried at

50 ppm, 20 ppm, 10 ppm and 5 ppm following the same procedure.

### 3.2.11.2 In vivo tests

**Efficacy of systemic and non systemic fungicides in the control of ring spot disease, cane yield and juice quality**

To study the effect of different fungicides which were found effective in vitro, on the intensity of ring spot disease, cane yield and juice quality, a randomised replicated field trial (RBD) was conducted consecutively for two crop seasons (1988-89 and 1989-90) with the following fungicides on weight by weight basis of the formulation.

Bavistin 0.1%	Blitox 0.4%
Calixin 0.1%	Captaf 0.3%
Topsin M 0.1%	Dithane M-45 0.3%

Susceptible sugarcane variety Co 7219 was planted in the field in the month of March every year. Size of each treatmental plot was kept at 57.6 m<sup>2</sup> with a net plot size of 38.4 m<sup>2</sup> for recording observations. The comparative efficacy of fungicides was tested under conditions of artificial ephiphytotics created by evening spray of aqueous suspension of conidia of ring spot pathogen on two successive days. The experimental plot was irrigated frequently to keep the microclimate sufficiently humid.

The first spraying of fungicides was done in the third week of September, when the disease was first observed in the experimental plot, followed by two more applications at an interval of one month. All sprayings were made at the rate of 1500 litres of spray fluid per ha by foot sprayer.

The disease intensity was recorded before each spray and subsequently at monthly intervals till harvest. Cane yield parameters and juice characteristics were also recorded at the time of harvest, following standard procedures as given below :

#### **Length of millable cane (LMC)**

Canes severed at the third leaf sheath attachment were designated as millable. Length in cm of such stalks in sprayed and unsprayed plots was recorded. Twenty canes in each treatment, collected at random, formed the sample.

#### **Diameter of the cane**

The diameter of the cane in cm at harvest was taken from top, middle and bottom portions of twenty canes in each treatment and means were worked out.

#### **Cane yield**

The cane yield from the net plot area of each treatment was recorded separately. The recorded cane yield (kg/plot) was converted into  $t\ ha^{-1}$ .

## Juice characteristics

The juice from sprayed and unsprayed canes was analysed for various chemical characteristics, namely, total solids, sucrose, purity, glucose, pH and electrical conductivity (E.C.). Ten canes in each treatment were topped by severing at the third leaf joint to get millable canes. The canes were crushed and samples of juice were drawn for determining various constituents after filtering through a fine muslin cloth by adopting standard methods as detailed below :

### a) Apparent total solids or brix

Apparent solids were determined by suspending the brix hydrometer in the raw juice. The brix spindle used was the one calibrated in degrees brix at  $27.5^{\circ}\text{C}$  and the reading was corrected to this temperature by referring to tables (Browne and Zerban, 1941).

### b) Sucrose

Sucrose was determined by direct polarisation of clarified, undiluted juice (Meade, 1964). To 100 ml of juice, 3 to 4 grams of dry, basic lead acetate was added, the liquid stirred and filtered into a clean, dry beaker. The clean filtrate was polarised in a 200 mm tube with light filtered through a layer of potassium bichromate solution. The sucrose per cent was read from tables

(Browne and Zerban, 1941) with uncorrected brix and polariscoppe readings.

**c) Juice purity**

Coefficient of purity was calculated based on the following formula

$$\text{Purity} = \frac{\text{Per cent sucrose}}{\text{Brix (corrected)}} \times 100$$

**d) Glucose**

Glucose, one of the reducing sugars in juice, was estimated by the usual volumetric method using methylene blue as internal indicator. Ten ml of Fehling's solution (5 ml each of A and B) was reduced with the juice taken in a burette till the blue colour just changed. Three to four drops of methylene blue were then added and the titration completed by vigorously boiling the liquid for 1 to 2 minutes. The volume of juice consumed, was noted and the reducing sugar present calculated on the basis that 100 ml of Fehling's solution was reduced by 0.4941 g of invert sugar. This was expressed as per cent in the juice by weight (Browne and Zerban, 1941). The titration was repeated with diluted juice whenever the titre value was less than 5 ml and the result was checked.

#### e) Hydrogen ion concentration (pH)

pH of undiluted juices was directly read with Elico pH meter using glass electrode (Jackson, 1973).

#### f) Electrical conductivity

The electrical conductivity of cane juice gives the mineral matter and changed organic compound content of cane juices. This was determined using conductivity bridge adopting the procedure indicated by Jackson (1973).

About 30 ml of strained juice was taken in a 100 ml beaker and the electrode of the conductivity bridge was dipped in the cane juice taking due care to see that the juice entered the bulb of the conductivity bridge. Then the reading was recorded and expressed as m.mhos/cm<sup>2</sup>.

### 3.2.12 Losses due to the disease in different sugarcane varieties/clones

#### 3.2.12.1 Loss in cane weight and juice quality

The effect of the disease on the cane weight and juice quality was studied in twenty sugarcane varieties/clones under sprayed and unsprayed conditions. The varieties/clones studied were Co 419, Co 975, Co 7219, Co 7636, Co 7706, Co 8205, Co 8220, Co 62175, Co 85038, Co 85044, Co 85045, CoA 7602, CoA 8401, CoA 8402, CoA 89081, CoT 8201, 83A106, 83A214, 84A155 and 86A444. All the

varieties/clones were planted in field in March, 1990 and grown under normal irrigated conditions. Inoculation by spraying conidial suspension was done in the month of August. Captaf 0.3% which was found most effective in the present studies was sprayed thrice at monthly interval from September onwards. The disease intensity was recorded in February before harvest. As regards to weight of canes, twenty canes were weighed for each treatment and the average weight of each cane is reported. Length and diameter of the cane and juice quality parameters were recorded in all the varieties/clones in both sprayed and unsprayed treatments as per the procedures indicated in 3.2.11.

#### 3.2.12.2 Rate of reduction in cane weight and juice sucrose in different sugarcane varieties/clones in maturity phase

To study the rate of reduction due to the disease in cane weight and juice quality in different varieties in maturity phase (November to February), the cane weight and juice sucrose were recorded every month starting from third month after inoculation till 6 months (i.e. at harvest) in February in five resistant (CoA 89081, 84A155, 86A444, Co 85045 and 83A 106) and five susceptible (Co 7219, CoT 8201, Co 7636, Co 975 and Co 85044) varieties/clones both in sprayed and unsprayed treatments mentioned at 3.2.12.1. Twenty canes in each treatment collected at

random formed the sample. The disease intensity was also recorded.

### 3.2.12.3 Loss of chlorophyll content

Twenty canes in the above varieties were tagged randomly in both sprayed and unsprayed treatments. The chlorophyll (chlorophyll a and chlorophyll b) content was estimated 4 months after inoculation adopting the following procedure suggested by Witham et al. (1971). Disease intensity was also recorded on 0-9 scale.

#### Estimation of chlorophyll

One gram of fresh leaves cut into small pieces was taken into a clean mortar and homogenized to a fine pulp with 40 ml of 80 per cent acetone and a pinch of  $\text{CaCO}_3$ . The supernatant was decanted and filtered through a buchner funnel using whatman number 42 filter paper. The extraction was repeated by adding fresh 30 ml of 80 per cent acetone. Then the contents from the mortar were transferred to the Buchner funnel and the pulp was grinded and washed again with acetone until it becomes colourless. The mortar and the sides of the funnel were rinsed with 10 ml of acetone to ensure that all the chlorophyll was collected. Then the final volume of the filtrate was made up to 100 ml by adding sufficient quantity of 80 per cent acetone. The optical density of chlorophyll extract was read in a spectrophotometer set at 645 and 663 nm

using 80 per cent acetone solvent as blank. The concentration of chlorophyll a and b in mg per g of tissue is calculated by using the following formulae:

mg chlorophyll a/g tissue =

$$12.7 (D663) - 2.69 (D645) \times \frac{V}{1000 \times W}$$

mg chlorophyll b/g tissue =

$$22.9 (D645) - 4.68 (D663) \times \frac{V}{1000 \times W}$$

where

- D : Optical density at respective nm  
 V : Final volume of the 80 per cent acetone-chlorophyll content  
 W : Fresh weight in g of the tissue

### 3.3 REPLICATIONS

Unless otherwise mentioned, the treatments were replicated thrice in all the experiments.

### 3.4 RECORDING OF OBSERVATIONS

Unless otherwise mentioned, all observations were recorded in the central rows leaving borders on either side of the plot. The disease intensity was recorded on 0-9 scale 3 months and 6 months after inoculation. Data were recorded in 20 millable canes tagged at random. Data

on yield and juice quality parameters were recorded at the time of harvest during February. Cane yields in the net plots were recorded in kg and expressed as  $t\ ha^{-1}$ .

### 3.5 STATISTICAL ANALYSIS OF DATA

The data from the experiments were statistically analysed following the procedures suggested by Snedecor and Cochran (1967) and Panse and Sukhatme (1978). In the tables, both actual percentage values and their corresponding transformed values have been given. Wherever the interaction of the treatments was involved the data were statistically analysed. To examine the relationship between certain characters, simple correlation and Chi-square ( $X^2$ ) were worked out. Paired 't' test and regressions were worked out for certain quantitative and qualitative characters.

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# RESULTS

## CHAPTER IV

### RESULTS

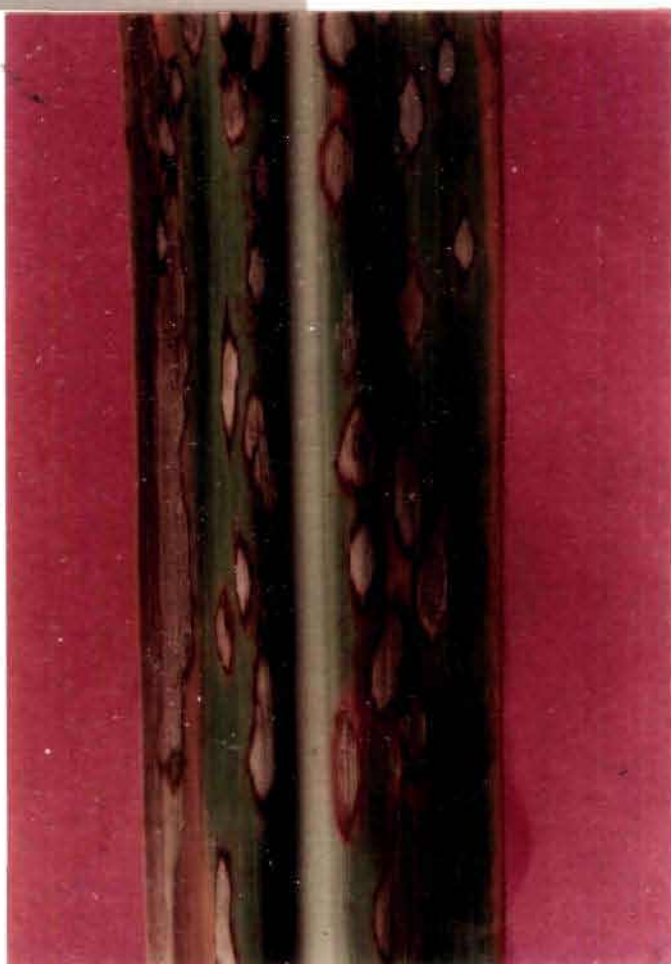
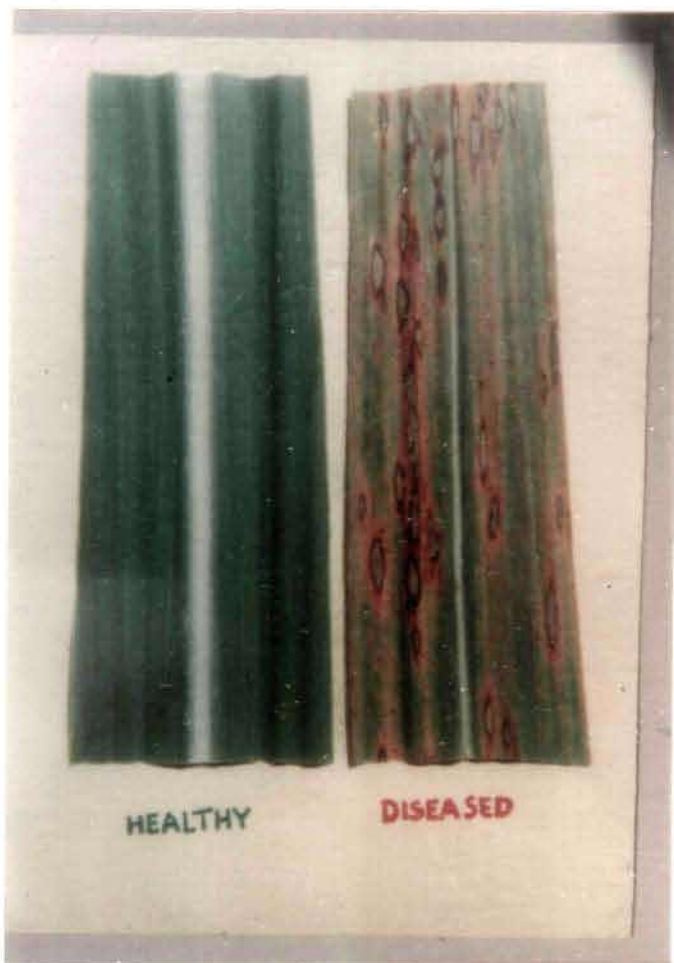
#### 4.1 SYMPTOMATOLOGY

##### 4.1.1 Symptoms

The symptoms of ring spot disease as observed in nature on four varieties viz., Co 7219, CoT 8201, CoA 89081 and Co 85045 planted in March, 1988 are described below.

The growth of the spots starting from the initiation of the disease was recorded in the above mentioned four varieties. Symptoms developed only on the laminar region but not on leaf sheath and cane stalk. The first symptom of the disease appeared on eleventh or twelfth order leaf from top during the last week of August (i.e.,)  $5\frac{1}{2}$  months after planting (MAP) in varieties Co 7219 and CoT 8201 while in CoA 89081 and Co 85045, it appeared one and half months later. The spots first appeared as small discoloured specks on the tip of the leaf blade which gradually turned purplish oval or spherical within 15 days and were visible on both the surfaces of the leaf. At this stage they measured 0.5-1.0 x 0.5 mm. These purplish spots grew in size and developed dry necrotic area at the centre of the spot. The margin consisted of a narrow reddish purple or brownish band of varying width and sometimes surrounded by an yellowish

hale diffusing into green colour of the healthy portion (Plate 1). The straw colour dry centre was sharply marked off by the surrounding coloured ring. In Co 7219 and CoT 8201, the ring was usually irregular but lobed or broken with angular projections and the union of neighbouring spots increased this irregularity. Ultimately considerable part of the leaf tip dried up in about 23-25 days. Later the disease spread on the middle portion of the leaf by the end of September ( 6½ MAP ) by which time, the disease appeared on the 9th order leaf from the top. By the end of October ( 7½ MAP) when there were usually heavy rains, the disease had spread rapidly on 7th and 8th order leaves covering entire leaf area and the top half portion of 6th order leaf (Table 3 at Para 3.1). This situation further lead to premature senescence of 7th and 8th order leaves on varieties Co 7219 and CoT 8201. By the first fortnight of October (7 MAP) on the older spots of Co 7219 and CoT 8201 perithecia developed in large numbers chiefly within the rings as tiny black dots arranged in rows and entirely buried in leaf tissue. The perithecia were visible only on the upper surface of the leaf (Plate 2). In CoA 89081 and Co 85045 perithecia appeared two and half months later (i.e.,) by the end of December ( 9½ MAP). Fully mature spots in the above four varieties measured about 5.2 - 8.9 x 1.1 - 3.2 mm. In Co 7219 and CoT 8201, the disease spread was more during November and December. The disease appeared on the fourth



order leaf by the end of December (9½ MAP). Except 3, 4 leaves on the top, the remaining foliage dried up by the first week of February (i.e.,) 1½ MAP (Plate 3). But in CoA 89081 and Co 85045 only few spots were produced by this time that too on 8th and 9th order leaves. The entire leaf portion of 1 to 7 order leaves and basal portion of 8th and 9th order leaves were free from disease. No premature senescence was observed due to the disease on these two varieties. The average number of spots produced on 1-8 fully opened leaves of the four varieties by the first week of February are furnished in Table 9.

Table 9: Number of spots on 1-8 fully opened leaves of different varieties

Variety	Average number of spots produced on 1-8 fully opened leaves							
	1	2	3	4	5	6	7	8
Co 7219	0	14	49	146	287	436	512	491
CoT 8201	0	8	28	107	191	343	394	417
CoA 89081	0	0	0	0	0	0	16	21
Co 85045	0	0	0	0	0	0	19	24

The data presented in Table 9 indicate that more number of spots were produced in the varieties Co 7219 and CoT 8201 from 3rd to 8th fully opened leaves. No spots were observed on 1st fully opened leaf on these two varie-



ties. On bottom (5th to 8th) leaves of these two varieties maximum number of spots were produced leading to premature senescence of the leaves. In CoA 89081 and Co 85045 1 to 6 fully opened leaves on the top were free from the disease while only a few (16 to 24) spots were produced on 7th and 8th fully opened leaves. No drying of leaves was observed in these two varieties.

#### 4.1.2 Size and shape of spots in different sugarcane varieties/clones

The number of days taken for the first appearance of the symptom after inoculation and nature of spotting were noted on thirty four varieties/clones under artificially inoculated conditions. The shape of the spot and length and breadth of the spot in mm were recorded in each variety 3 months after inoculation. The data are presented in Table 10.

The data presented in Table 10 indicate that in nine varieties/clones viz., CoA 8401, Co 6907, Co 7704, 83A93, CoT 8201, Co 85044, Co 7636, Co 7219 and Co 8021 the disease appeared early within 15 days after inoculation. The first appearance of the disease was delayed in four varieties/clones viz., CoA 89081, Co 85045, 84A155 and 86A444 beyond 40 days after inoculation. In the remaining varieties/clones the disease appeared between 18 and 34 days after inoculation.

Table 10: Number of days taken for the first appearance of symptom, shape and size of ring spot in different sugarcane varieties/clones

Sl. No.	Variety/ clone	No. of days taken for the first appearance of the symptom	Shape of the spot	Average size of the spot in mm	
				Length	Breadth
1.	Co 419	20	Small, oval and irregular	4.8	1.3
2.	Co 975	18	Oval and irregular	9.7	2.9
3.	Co 997	34	Oval and irregular	12.5	3.5
4.	Co 6907	15	Linear and narrow	9.1	1.4
5.	Co 7219	11	Oval and irregular	8.9	2.4
6.	Co 7636	11	Spindle and irregular	8.7	2.6
7.	Co 7704	9	Oval and irregular	12.9	2.6
8.	Co 7706	18	Spindle and irregular	14.9	2.7
9.	Co 7803	31	Small, oval	3.8	1.1
10.	Co 8012	32	Oval and irregular	7.8	2.8
11.	Co 8013	18	Linear, narrow and irregular	8.8	1.7
12.	Co 8021	15	Oval and irregular	7.8	2.9
13.	Co 8212	22	Linear and narrow	11.5	1.3
14.	Co 8220	32	Spindle with pointed ends	11.6	3.0
15.	Co 62175	28	Linear and narrow	8.9	1.8
16.	Co 85044	11	Spindle and irregular	7.1	2.1
17.	Co 85045	42	Narrow and linear	8.9	2.1
18.	CoA 7602	18	Spindle and irregular	9.6	2.8
19.	CoA 8401	11	Linear and narrow	9.2	1.5

Contd..

Sl. No.	Variety/ clone	No. of days taken for the first appearance of the symptoms	Shape of the spot	Average size of the spot in mm	
				Length	Breadth
20.	CoA 88081	20	Spindle and irregular	10.5	4
21.	CoA 89081	43	Oval and irregular	5.2	5
22.	CoA 89082	24	Oval and irregular	6.2	1.6
23.	CoA 89083	24	Oval, narrow and irregular	5.3	1.4
24.	CoT 8201	14	Oval and irregular	8.4	3.1
25.	83A 60	22	Small, oval	3.7	1.2
26.	83A 93	15	Linear, narrow and irregular	8.6	1.7
27.	83A 159	24	Minute, dot like with less straw centre	2.9	0.7
28.	83A 214	32	Spindle and irregular	13.6	1.1
29.	83A 758	34	Spindle and irregular	10.5	1.9
30.	84A 132	22	Spindle and irregular	10.5	1.6
31.	84A 155	45	Oval and irregular	5.9	1.6
32.	84A 191	28	Linear and narrow	16.1	1.7
33.	86A 444	41	Oval and irregular	5.6	1.5
34.	88A 87	28	Oval and irregular	9.3	1.9

Different shapes of the spots were observed in different varieties/clones as seen in the Table. Peculiar dotted spots with inconspicuous straw centre was observed in the clone 83A159. Small oval spots were observed in three varieties/clones 83A60, Co 7803 and Co 419. In six varieties/clones viz., 84A191, CoA 8401, Co 6907, Co 8212, Co 62175, and Co 85045 the spots were linear and narrow. Similar types of spots were noticed in Co 8013 and 83A93 but they were occasionally irregular. In majority of varieties/clones viz., Co 7704, Co 997, CoT 8201, CoA 89081, CoA 89082, CoA 89083, Co 8012, 88A87, Co 975, Co 7219, 84A155, 86A444 and Co 8021 the spots were oval and irregular. Spindle shaped and irregular spots were observed in 9 varieties/clones viz., Co 7706, 83A214, CoA 7602, CoA 88081, 84A132, Co 85044, Co 8220, Co 7636 and 83A758.

The length of the spot was more than 10 mm in ten varieties/clones viz., Co 7706, 83A214, 84A191, Co 7704, Co 997, Co 8212, CoA 88081, 84A132, Co 8220 and 83A758 while it was small measuring less than 5 mm in length in four varieties/clones viz., Co 7803, Co 419, 83A60 and 83A159. The breadth of the spot was more than 3 mm in 6 varieties viz., Co 7706, Co 7704, CoT 8201, CoA 88081, Co 8220 and Co 7219 while it was less than 1 mm in clone 83A159. In four varieties viz., Co 6907, Co 8013, Co 85045 and Co 8212 eventhough the length of the spot was more

than 8 mm, the breadth was less than 1.5 mm giving the spot a narrow elongated appearance. Remaining varieties/clones showed medium sized spots measuring 5.1 to 9.7 mm in length and 1.4 to 2.9 mm in breadth. The first appearance of the symptom and shape and size of the spots varied from variety to variety (Plate 4).

#### 4.2 ISOLATION AND IDENTIFICATION OF PATHOGEN AND ESTABLISHMENT OF PATHOGENICITY

##### 4.2.1 Isolation

Isolations were made from immature and mature spots of the leaf samples of naturally infected plants.

##### Isolation from immature spots

Leaf bits from young lesions were surface sterilised as described at para 3.2.2.1 and transferred to PDA, OMA, CLA, Richards agar, CZapek-Dox agar and incubated for 10 days at  $28 \pm 1^{\circ}\text{C}$ . The results of isolations are furnished in Table 11.

The results indicate that majority of isolations (29.6%) yielded Nigrospora sp. followed by Curvularia sp. (24.7%), on all the media tested. Phyllosticta sp. constituted 23.9 per cent while Bipolaris sp. was obtained in 21.8 per cent of isolations.

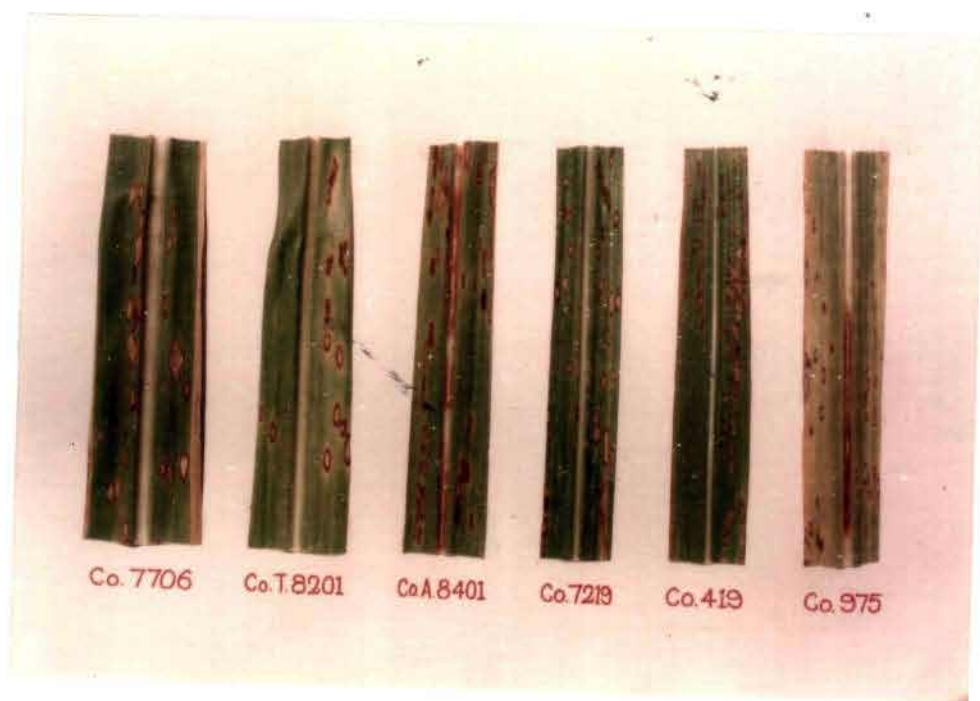


Table 11: Fungi isolated from immature spots

Medium	Number of leaf bits used for isolation	Number yielding		
		<u>Bipolaris</u> sp.	<u>Nigrospora</u> sp.	<u>Curvularia</u> sp.
PDA	230	51	65	56
OMA	215	49	61	54
CLA	205	46	64	51
Pichards Agar	165	36	53	46
Czapek-Dox Agar	150	28	45	41
Total	965	210 (21.8)	286 (29.6)	238 (24.7)

Figures in parenthesis indicate percentage

### Isolation from mature spots

Microscopic observations of slides from mature spots with black discolouration in greyish centre revealed the continuous association of ascospores and pycnidiospores. Isolations were made from mature spots on different media by transferring very small leaf bits and streaking spore suspension as described at para 3.2.2.1. The results of these isolations are presented in Table 12.

The results indicate that the majority of isolations yielded both Phyllosticta sp. and Leptosphaeria sp. No other organism was isolated from mature spots.

The colour and growth of the mycelium varied initially in both the species. The mycelium of Phyllosticta sp. (anamorph) was white and fast growing while that of Leptosphaeria sp. (teleomorph) was slow growing. Although the colour of the mycelium of the latter was white to start with it was deep seated in the medium, later turned pinkish with sparse aerial mycelium. Finally both the cultures turned greyish black producing abundant pycnidia and pycnidiospores one month later. Perithecia were not produced in any of the media employed in the study.

Streaking spore suspension on different media yielded Phyllosticta sp. Leptosphaeria sp. in the same plate.

Table 12: Fungi isolated from mature spots

Medium	Number of leaf bits used for isolation	<u>Phyllosticta</u> sp.	<u>Leptosphaeria</u> sp.	<u>Phyllosticta</u> sp. + <u>Leptosphaeria</u> sp.
PDA	120	31	37	52
OMA	120	44	30	46
CLA	120	31	27	62
Richards Agar	120	36	34	45
Czapek-Dox Agar	120	27	21	72

Single ascospores and pycnidiospores were isolated from sterile water agar plates and transferred to OMA, PDA, CLA, Richards agar and Czapek-Dox agar. The mycelial growth from ascospores was very slow and deep seated in the medium. Initially the mycelium was white but within 2 days it turned pinkish or umber coloured with sparse areial mycelium and later turned greyish black. No perithecia or ascospore production was observed on any of the media studied but pycnidia and pycnidiospores were produced one month after isolation from single ascospore culture (Plate 5). The growth from single pycnidiospores was fast on all the media except Czapek-Dox agar. The mycelium was white, profuse and later turned greyish black. Small, dark, roundish pycnidia appeared on the surface of the mycelium and the medium (plate 6). Only the imperfect stage of the fungus appeared in culture.

#### 4.2.2 Identification of isolated fungi

Four fungal cultures were isolated from ring spot lesions. The cultural characters and spore measurements were recorded. The fungi were identified at the genus/species level with the help of illustrated books and CMI descriptions. The details of cultural characters and spore measurements are furnished in Table 13.

#### 4.2.3 Production of ascospores in vitro

Ascospores from sterile water agar were lifted and placed on the edges of sterile leaf bits of suscepti-



Table 13: Cultural characters and spore measurements of different fungi isolates from rice spot lesions

Fungal culture	Culture characters	Spore measurements in $\mu\text{m}$		Fungus identification
		Length	Breadth	
1	Colonies effuse, grey, often hairy; Mycelium mostly immersed; stroma often present; conidiophores in tufts, unbranched, septate, straight or flexuous, olivaceous brown near the base and paler towards the apex, characteristically bent with knee joints at regular intervals; conidia variable in size, 3-10 celled, slightly curved, bulge in the middle and tapering towards ends, dark brown; vilum dark; conidial germination bipolar.	48-64	~12	<u>Bipolaris sacchari</u>
2	Colonies at first white, turning black later; mycelium immersed or partly superficial; stroma none; conidiophores branched, flexuous, brown, smooth; conidia solitary, with violent discharge mechanism, acrogenous, simple, spherical, black, shining, smooth, one celled.	18-22 in diameter		<u>Nigrospora sacchari</u>
3	Colonies effuse, dark olive grey, velvety; mycelium immersed; stromata rare; conidiophores straight or flexuous, often geniculate, brown, smooth; conidia solitary, curved, broadly fusiform, 3 septate, dark brown, end cells paler, second cell enlarged.	18-28	9-17	<u>Curcularia lunata</u>
4	Mycelium profuse, white, later turning greyish black, immersed in the substrate; pycnidia thin walled, immersed, globose, hard textured, dark brown, embedded in subepidermal stroma, ostiolate, 62-90 $\mu\text{m}$ in diameter; conidiogenous cells short, cylindrical forming blasto conidia in basipetal succession; conidia abundant, individually hyaline, pale brown in mass, unicellular, aseptate, ellipsoid to subfusoid, straight or curved, two guttules at either end, surrounded by a slime layer and with an apical aperture, exude from osti- ticle in a cirrus; germination bipolar.	10-12.5	4-5	<u>Phylllosticta saccharicola</u>

ble varieties Co 7219 and Co 7636. The leaf bits were placed at the centre of the petridishes containing OMA, PDA and CLA as described at para 3.2.2.4. The growth of the fungus was very slow, covering the leaf bit and surrounding medium in a week. Initially the mycelium was white but turned pinkish in 3 days. No perithecia and ascospore production was observed in any plate kept at ambient temperature even after one and half months. Similarly there was no perithecial and ascospore production when the plates were exposed to different temperatures (24, 26 or 28°C) under alternate 12 hours light and 12 hours darkness for one and half months but pycnidia of Phyllosticta sp. were produced one and half months after inoculation in all the plates.

In another experiment single ascospores and pycnidiospores from sterilised mature spots were transferred to sterile leaf bits of four susceptible varieties, namely Co 8021, CoT 8201, Co 7219 and Co 7636 in Erlenmeyer flasks with and without vitamins and sucrose as per the details given at para 3.2.2.4. The results are presented in Table 14.

The results indicate that perithecia and ascospore production was observed mostly on the sterile host leaf bits of varieties Co 7219, Co 7636 and CoT 8201, to which vitamins and sucrose were added and incubated at 24°C and 26°C in growth chamber under alternate 12 hours

Table 14: Growth and sporulation of ascospores and pycnidiospores of ring spot fungus on sterile host leaf bits of susceptible varieties

Treatment	Sterile host leaf bits with vitamins and sucrose				Sterile host leaf bits without vitamins and sucrose			
	Co 8021	CoT 8201	Co 7219	Co 7636	Co 8021	CoT 8201	Co 7219	Co 7636
	Asco- spores	Pycni- spores	Asco- spores	Pycni- spores	Asco- spores	Pycni- spores	Asco- spores	Pycni- spores
1. Flasks kept at ambient temperature	-	+	-	+	-	+	-	+
2. Flasks at 28°C and subjected to 12 hours of alternate light and darkness	-	-	-	-	-	-	-	-
3. Flasks at 24°C in growth chamber and subjected to 12 hours alternate light and darkness	-	-	+	+	-	-	-	-
4. Flasks at 26°C in growth chamber and subjected to 12 hours alternate light and darkness	-	+	+	+	-	-	-	-
5. Flasks at 28°C in growth chamber and subjected to 12 hours alternate light and darkness	-	+	-	+	-	+	-	+

+) Sporulation absent in corresponding treatment

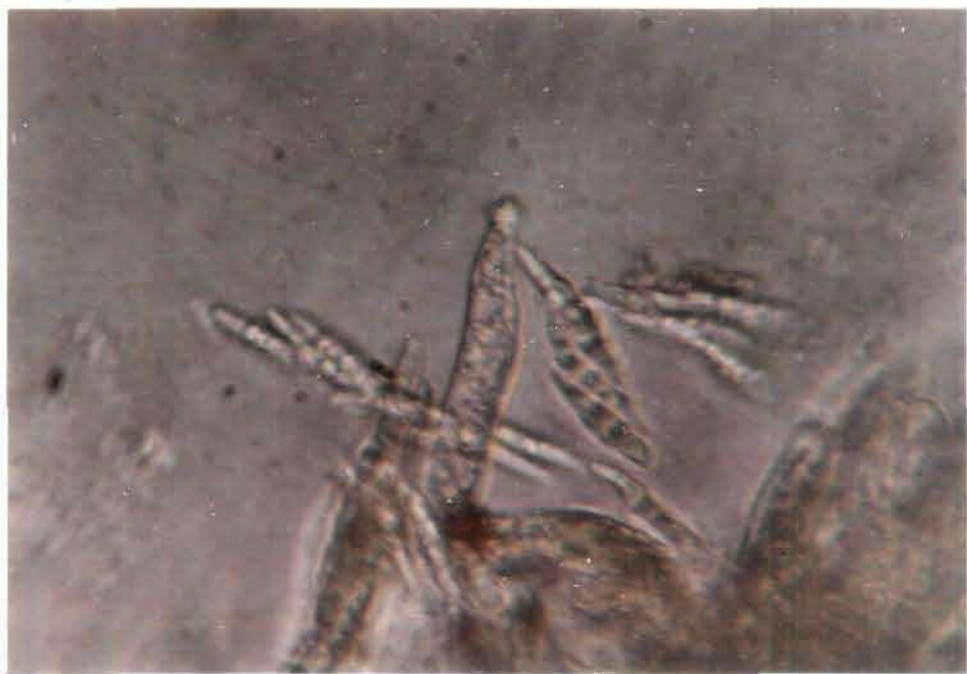
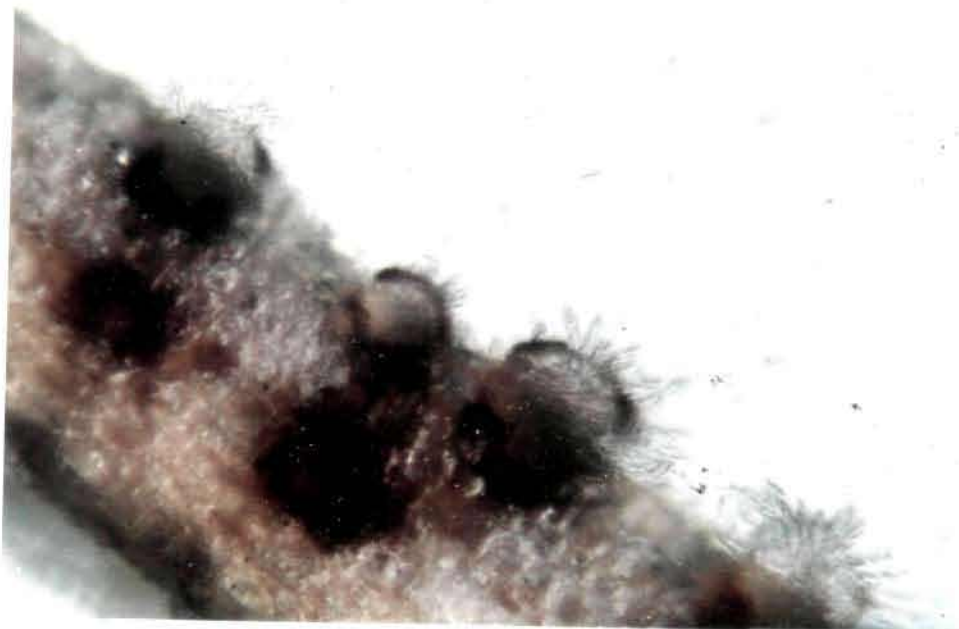
ight and 12 hours darkness. But, in the leaf bits of Co 7636 the ascospore production was also observed at 26°C without vitamins and sucrose. Ascospore production was noticed only on the leaf bits of two varieties viz., Co 7219 and Co 7636 at 24°C, 21 days after inoculation (treatment No.3). At 26°C (treatment no.4) ascospore production was observed on leaf bits of 3 varieties viz., Co 7219, Co 7636 and CoT 8201, 18 days after inoculation. Profuse pycnidiospores were produced from these leaf bits, three weeks later. No ascospores were produced in the leaf bits of Co 8021 in any treatment. Except at 24°C and 26°C ascospore production was not noticed at any other temperature. Pycnidiospore production was observed in almost all the treatments and varieties except in Co 8021 and CoT 8201 at 24°C. Thus the ascospores yielded the perfect state at first on sterile cane leaf bits and later gave only the conidial state suggesting proof of connection between these two fungi.

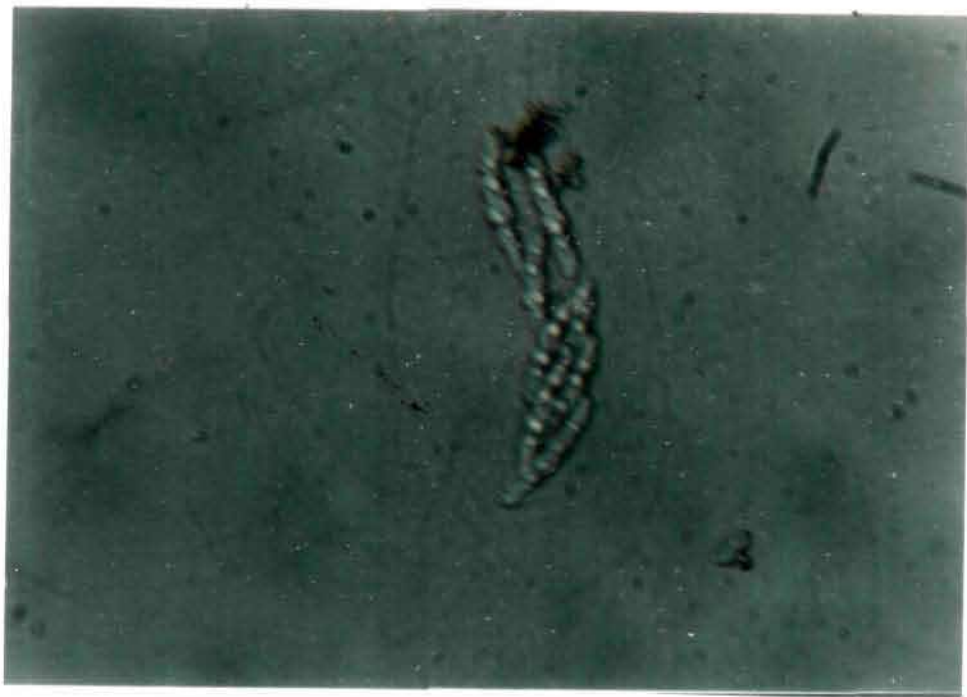
#### 4.2.4 Description and measurements of perithecia, asci and ascospores and pycnidia and pycnidiospores

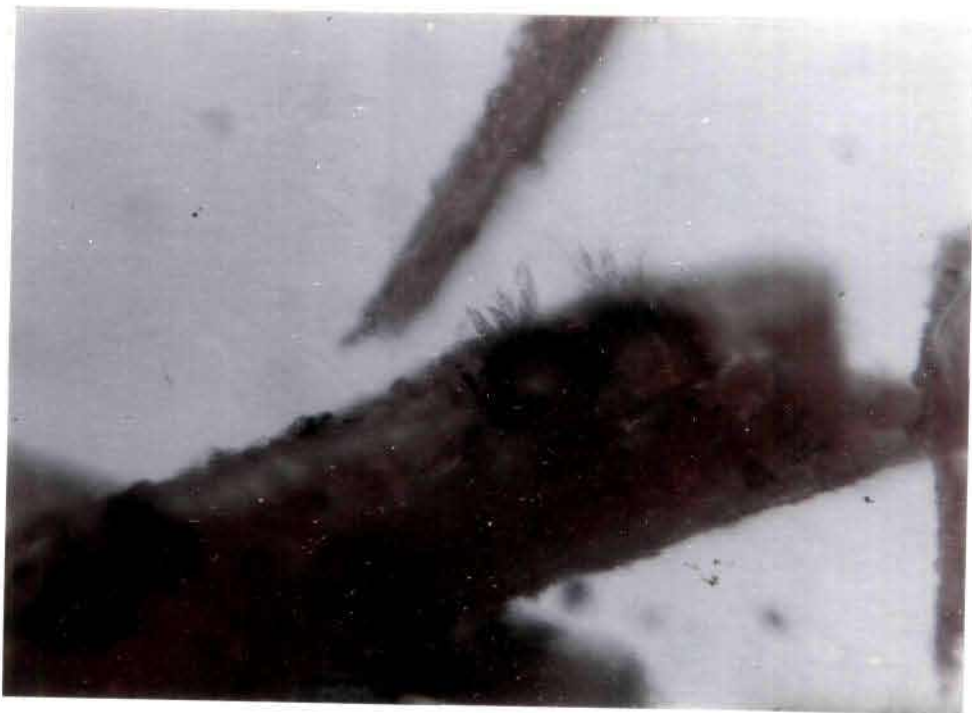
The descriptions and measurements of perithecia, asci, ascospores and pycnidia and pycnidiospores that developed on sterile healthy leaf bits of Co 7219 in the laboratory and on host in nature were recorded (Plates 7 to 15). The details are furnished in Table 15.

Table 15: Description and measurements of perithecia, asci, ascospores, conidia, pycnidia and pycnidiospores of *Phoma* leaf fungus

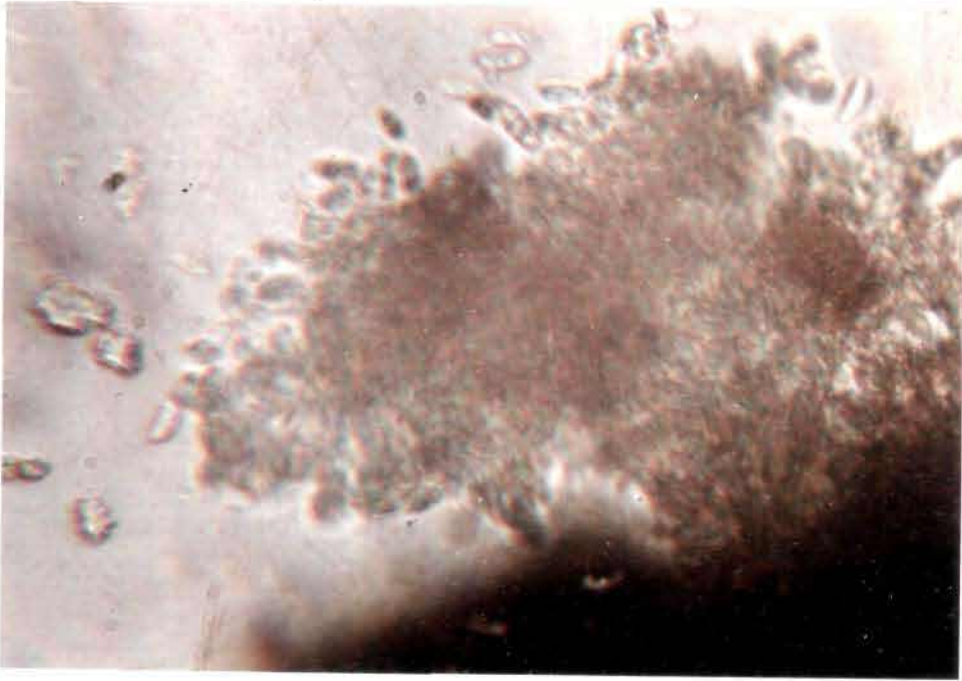
Sexual and asexual stages	Description	Measurements (µm)			
		Sterile host leaf bits in the lab.		Natural lesions	
		Length	Breadth	Length	Breadth
Perithecia	Immersed, small, spherical, brownish black with protruding papillate ostiole.	111-135 in diameter		115-136 in diameter	
Asci	Numerous, cylindrical or club shaped, sessile, intermingled with paraphyses, ascus wall thick, bitunicate with rounded apex, slightly narrowed at the base, 8 spored.	62-70	9-13	62-70	10-13
Ascospores	Irregularly biseriolate in lower part of ascus, hyaline, ellipsoid, ends broadly, obtuse, 2 septate, constricted slightly at the septa, second cell slightly swollen and each cell with a single large guttule.	36-27	4-6	31-26	4-5
Pycnidia	Immersed, globose, dark brown membranous, ostiolate.	70-94 in diameter		66-94 in diameter	
Pycnidiospores	Abundant, conidia individually hyaline, pale brown in mass, single celled with 2 guttules at either end, aseptate, ellipsoid to sub fusoid, straight or curved germination bipolar.	10-17	3-4	10-14	3-4











The diameter of perithecia and length and breadth of asci and ascospores tallied with the measurements of Leptosphaeria sacchari (Jones, 1967) of CMI descriptions. The measurements of pycnidia and pycnidiospores tallied with those of Phyllosticta saccharicola described by Hennings (1907) and CMI. Thus the results of the present study clearly indicate that teliomorph of ring spot fungus is L. sacchari van Breda de Hann and the anamorph is P. saccharicola P. Henn. which is in confirmity with the report compiled by the International Society of Sugarcane Technologists (ISSCT) standing committee on sugarcane diseases (Ricaud et al., 1989).

#### 4.2.5 Pathogenicity tests

The fungi isolated from the ring spot lesions were tested individually and in combination, for their pathogenicity on potted plants of susceptible varieties Co 7219, CoT 8201 and Co 7636 as described at para 3.2.2.5. The details of pathogenicity tests as obtained by spray inoculation are furnished in Table 16. Similar observations were made in respect of pin prick method of inoculation.

The results indicate that no typical ring spot symptoms were produced on any variety with Bipolaris sacchari, Curvularia lunata and Nigrospora sacchari either alone or in combination; but typical ring spot lesions

Table 16: Pathogenicity tests with different fungi individually and in combination on different sugarcane varieties

Fungi inoculated	Symptoms produced on		
	Co 719	71-8291	a 7636
<u>Bipolaris sacchari</u>	No symptoms produced	Small specks produced initially, later turn reddish brown	Small red specks produced
<u>Nigrospora sacchari</u>	Small pin head sized chlorotic specks produced later turned purplish	No symptoms produced	Small pin head sized reddish spots appeared on the entire lamina
<u>Curvularia lunata</u>	No symptoms produced	No symptoms produced	Small purplish spots appeared 20 days after inoculation
<u>Phyllosticta saccharicola</u>	Small specks formed four days after inoculation and enlarged into spots after 8 days. Typical ring spot lesions developed 13 days after inoculation	Small specks formed six days after inoculation and enlarged into small spots in 11 days. Typical ring spot lesions developed in 16 days after inoculation	Small specks formed four days after inoculation and enlarged into spots after 8 days. Typical ring spot lesions developed 13 days after inoculation
<u>B. sacchari + C. lunata</u>	No symptoms produced	Small reddish specks produced 10 days after inoculation	Small reddish specks produced 3 days after inoculation
<u>B. sacchari + N. sacchari</u>	No symptoms produced	No symptoms produced	Small reddish spots produced 8 days after inoculation
<u>C. lunata + N. sacchari</u>	Small pin head sized purplish specks produced	No symptoms produced	Small pin head sized purplish specks produced
<u>B. sacchari + P. saccharicola</u>	Typical ring spot lesions developed 20 days after inoculation	Typical ring spot lesions developed 23 days after inoculation	Typical ring spot lesions developed in 18 days
<u>C. lunata + P. saccharicola</u>	Typical ring spot lesions developed in 18 days	Typical ring spot lesions developed in 21 days	Typical ring spot lesions developed in 18 days

Contd..

Fungi inoculated	Symptoms produced on		
	Co 2819	al 6201	Co 2650
<u>N.sacchari</u> + <u>P.saccharicola</u>	Characteristic ring spot lesions developed in 20 days	Characteristic ring spot lesions developed in 22 days	Characteristic ring spot lesions developed in 19 days
<u>Leptosphaeria sacchari</u> (Ascospores from sterile host leaf bits in laboratory)	Small specks formed 8 days after inoculation and enlarged into small spots in 12 days. Typical ring spot lesions developed after 20 days	Small specks formed in 6 days and enlarged into small spots in 12 days. Typical ring spot lesions developed after 22 days	Small specks formed in 6 days and enlarged to small spots in 10 days. Typical ring spot lesions developed in 20 days
<u>L. sacchari</u> (Ascospores from naturally infected lesions)	Small specks formed in 5 days and enlarged into small spots in 9 days. Typical ring spot lesions developed in 17 days	Small specks formed in 7 days and enlarged into small spots in 10 days. Typical ring spot lesions developed in 19 days	Small specks formed in 5 days and developed into small spots in 9 days. Typical ring spot lesions formed in 17 days

developed in all the test varieties with P. saccharicola in 13-16 days after inoculation (Plate 16). B. sacchari, C. lunata and N. sacchari in combination with P. saccharicola also developed typical ring spot lesions but the symptom expression was slightly delayed (16-23 days). Ring spot lesions also appeared on all the three test varieties when ascospores from sterile host leaf bits (in vitro) and also those from naturally infected material were inoculated (Plate 17). Characteristic ring spot lesions developed in 17-19 days with ascospores from natural lesions while it was slightly delayed with ascospores from sterile host leaf bits (20-22 days). The results indicate that ascospores of L. sacchari and conidia of P. saccharicola only could incite ring spot on the leaves of the three test varieties (Plates 16 and 17) of sugarcane, proving Koch's postulates.

#### 4.2.6 Identification of a suitable medium for the pathogen

Single ascospores and pycnidiospores from sterile water agar were transferred to different culture media to identify a medium suitable for better multiplication of both the fungi based on growth and sporulation. The observations are furnished in Table 17.

The results indicate that mycelial growth of L. sacchari was observed on almost all the media but, it was



Table 17: Growth and sporulation of Leptosphaeria sacchari and Phyllosticta saccharicola on various media

Media	Cultural characters		Number of pycnidiospores/ml
	<u>L. sacchari</u>	<u>P. saccharicola</u>	
FDA	Initially slow growing white colonies which soon turned pink to amber with sparse aerial mycelium, deep seated in medium and finally turning greyish black	Fast growing white profuse mycelial growth, later turned grey and finally greyish black	$2.69 \times 10^7$
DMA	Same as above	Same as above	$2.82 \times 10^7$
Richards Agar	Same as above	Same as above	$1.88 \times 10^7$
Czapiek-Dox Agar	Same as above	Slow growing white mycelium and later turned greyish black	$4.5 \times 10^5$
Bear meal Agar	Fast growing white colonies which soon turned pink, deep seated in medium and finally turning greyish black	Same as above	$3.8 \times 10^5$
Cane leaf Agar	Slow growing white mycelial colonies which later turned to greyish black	Fast growing white mycelium which later turned to greyish black	$2.47 \times 10^6$
Carrot Agar	Same as above	Slow growing white mycelium and later turned greyish black	$4.3 \times 10^5$
Potato carrot Agar	Same as above	Fast growing white mycelium which later turned to greyish black	$2.07 \times 10^6$
Corn meal Agar	Fast growing white colonies which later turned pinkish and finally greyish black	Slow growing white colonies and later turned greyish black	$4.2 \times 10^5$
Potato sucrose Agar (PSA)	Initially slow growing white colonies which quickly turned pinkish, deep seated and later turned greyish black	Fast growing white mycelium which later turned greyish black	$2.23 \times 10^6$

Contd..

Media	Cultural characters		Number of pycnidiospores/ml
	<u>L. sacchari</u>	<u>P. saccharicola</u>	
PSA + Oats	Same as above	Same as above	$2.41 \times 10^6$
PSA + cane leaf extract	Same as above	Same as above	$2.57 \times 10^6$
POA + corn meal	Fast growing white mycelium, rapidly turning pinkish, deep seated in medium and later turned greyish black	Same as above	$2.18 \times 10^6$
POA + cane leaf extract	Slow growing white mycelium which later turned greyish black	Fast growing white mycelium which later turned greyish black	$3.17 \times 10^6$
Cane leaf Agar + oats	Same as above	Fast growing white mycelium, finally turning greyish black	$2.71 \times 10^6$
GMA + cane leaf extract	Slow growing white mycelial colonies rapidly turning pinkish deep seated in medium and later turned greyish black	Fast growing white mycelium, profuse, later turning grey and then greyish black	$3.28 \times 10^6$

\*Both the cultures produced Pycnidia and pycnidiospores one month after isolation

fast only on bean meal agar, corn meal agar and PDA + corn meal at ambient temperature. On the remaining media, the growth was very slow. Perithecia and ascospores were not produced on any of the media even after one month but pycnidia and pycnidiospores were produced one month after inoculation from single ascospore cultures.

The growth of Phyllosticta saccharicola was observed to be fast on PDA, OMA, Richards agar, CLA, Potato carrot agar, PSA, PSA + oats, PSA + cane leaf extract, PSA + corn meal, PDA + cane leaf extract, CLA + oats, OMA + cane leaf extract at ambient temperature but, it was slow on Czapek-Dox agar, bean meal agar, corn meal agar and carrot agar. Maximum sporulation was obtained on OMA + cane leaf extract ( $3.28 \times 10^6$  spores/ml) and PDA + cane leaf extract ( $3.17 \times 10^6$  spores/ml). In the remaining media the extent of sporulation was in the range of  $3.8 \times 10^5$  to  $2.82 \times 10^6$  spores/ml.

The results indicate that corn meal and bean meal agar supported good growth of L. sacchari but not sporulation, while PDA + cane leaf extract and OMA + cane leaf extract were better for both growth and sporulation of P. saccharicola as compared to other media. Hence, for further studies cultures of P. saccharicola were maintained on OMA + cane leaf extract.

#### 4.3 DEVELOPING A DISEASE RATING SCALE FOR ESTIMATION OF DISEASE INTENSITY

The per cent green leaf area affected at different intensities of disease was worked out with the help of leaf area meter and the following 0-9 scale was prepared specially for estimating the intensity of the disease (Plate 18).

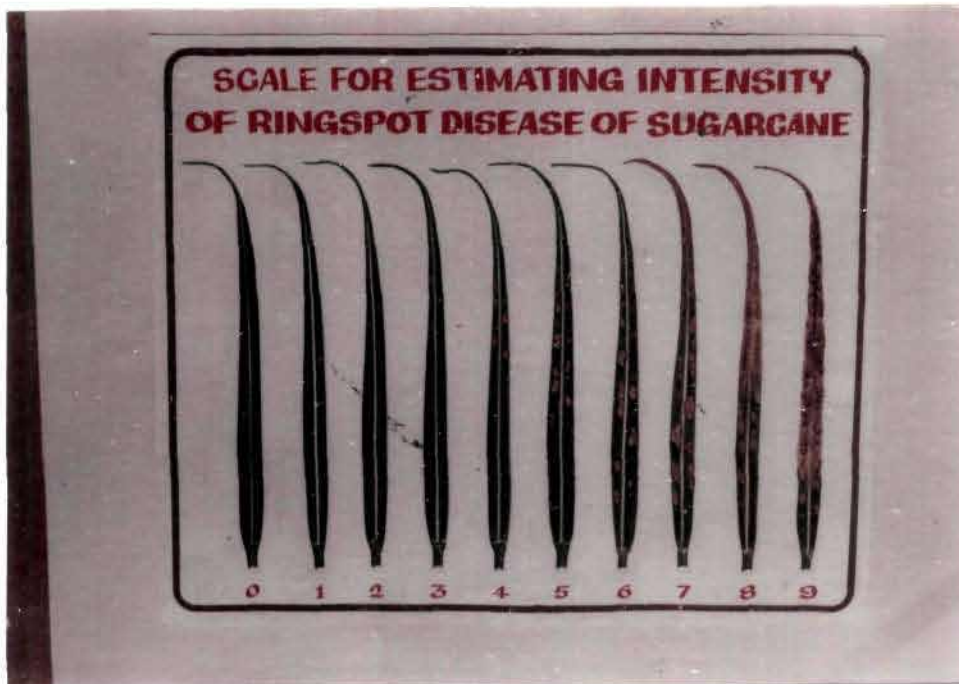
##### 0-9 scale for estimation of disease intensity

Grade	Description
0	No disease symptoms.
1	Only small brown specks of 1 mm size at apical part of leaves, few to many, sometimes unrecognisable.
2	Slightly large, oval, greyish spots of 3 mm diameter covering less than 1 per cent of leaf area.
3	Oval to irregular greyish spots of 4 mm diameter with reddish margin covering 1 to 5 per cent of leaf area. No necrotic spots.
4	Typical ring spot lesions develop. Spots with necrotic grey centre and reddish brown margins covering 5.1 to 10.0 per cent of leaf area.

Contd..

Grade	Description
5	Typical ring spot lesions spreading to middle portion of the leaf covering 10.1 to 20.0 per cent of the leaf area.
6	Lesions as in scale 5, have a tendency to coalesce, spreading towards basal portion of the leaf affecting 20.1 to 30.1 per cent of leaf area.
7	Large quickly expanding lesions, irregular because of fusion, brown margins and most of the affected area blighted covering 30.1 to 50.0 per cent of the leaf area.
8	Symptoms as in scale 7, affecting 50.1 to 60.0 per cent of the leaf area. Premature senescence of the leaf.
9	More than 60.0 per cent of the leaf area affected and premature senescence of the leaf.

The disease incidence in all the studies was recorded on 0-9 scale in 1 to 8 fully opened functional leaves and the PDI was worked out using the formula



mentioned at para 3.2.3. Varieties/clones were classified into different reaction groups based on PDI using the rating scale as detailed below.

#### Rating scale

Rating	PDI	Reaction
0	0.0	Immune
1	0.1 to 1.0	Highly resistant
2	1.1 to 5.0	
3	5.1 to 10.0	Resistant
4	10.1 to 15.0	Moderately resistant
5	15.1 to 20.0	Moderately susceptible
6	20.1 to 30.0	
7	30.1 to 40.0	Susceptible
8	40.1 to 50.0	
9	More than 50.0	Highly susceptible

#### 4.4 SURVEY FOR ASSESSMENT OF DISEASE INTENSITY

Survey was conducted in sugarcane fields during 1988-89 and 1989-90 in coastal Andhra Pradesh (Bobbili, Vuyyuru, Nellore, Chelluru and Anakapalle factory areas), Telangana (Bodhan) and Rayalaseema (Renigunta) regions, as

per the details given at para 3.2.4, to study the intensity of ring spot disease on different cultivated sugarcane varieties under natural conditions. The information pertaining to extent of holding, type of soil, variety grown, type of crop (plant or ratoon) and the intensity of the disease recorded are furnished in Table 18.

In coastal Andhra Pradesh survey was conducted in 25 villages of five factory zones.

In Bobbili factory area, the crop is grown under rainfed conditions in sandy and clay loams. The intensity of the disease was recorded in 3 varieties both in plant and ratoon. The per cent intensity of the disease in different varieties ranged from 33.2 to 62.6 in plant crops while it was 24.7 to 73.7 per cent in ratoons. The disease intensity was observed to be high in ratoon plots of Co 7219 (73.7%), Co 975 (64.2%), Co 527 (61.2%) and CoT 8201 (51.8%). The plot of Co 7219 in Alajangi village which was grown under rainfed conditions and received high doses of nitrogen fertilizer was severely affected by the disease (73.7%).

In Vuyyuru factory area where sugarcane is cultivated in clay loams and sandy loams, survey was conducted in 9 villages and the intensity of the disease was recorded in 6 varieties in 16 holdings. The disease intensity was more in clay loam as compared to sandy loam.

Table 18: Intensity of ring spot on different sugarcane varieties in different factor areas of coastal Andhra Pradesh, Telangana and Rayalaseema

Region	Factory area	Village	Extent of holding	Type of soil	Variety	Plant, ratoon	Yield
Coastal Andhra Pradesh	Bobbili	Birihi	1.00 ac	Clay loam	Co 527	R	61.2
			0.70 ac	Clay loam	Co 6907	R	33.2
			1.00 ac	Clay loam	Co 7219	R	62.7
			0.75 ac	Sandy loam	Co 8013	R	31.4
		Alajanqi	0.75 ac	Clay loam	Co 527	R	43.7
			0.60 ac	Sandy loam	Co 975	R	40.8
			0.90 ac	Clay loam	Co 7219	R	73.0
			0.40 ac	Sandy loam	Co 8013	R	24.7
		Kasaipet	0.50 ac	Sandy loam	Co 527	R	51.2
			0.75 ac	Clay loam	Co 975	R	64.2
			0.75 ac	Clay loam	Co 6907	R	35.4
			0.50 ac	Clay loam	Co 7219	R	55.6
	Seethanagarani	0.60 ac	Clay loam	Co 8013	R	34.8	
		0.70 ac	Clay loam	Co 7219	R	67.3	
		0.25 ac	Sandy loam	Co 7508	R	28.5	
		0.50 ac	Clay loam	CoA 7602	R	47.8	
	Vuyyuru	Hanumanthapuram	1.00 ac	Clay loam	Co 6907	R	26.4
			0.60 ac	Sandy loam	Co 8013	R	33.8
			1.25 ac	Sandy loam	CoC 671	R	21.2
		Meduru	0.75 ac	Sandy loam	Co 6907	R	27.2
			1.25 ac	Clay loam	Co 7805	R	19.6
			0.75 ac	Clay loam	CoC 671	R	14.7
		Kalavanni	1.25 ac	Clay loam	Co 7805	R	48.1
		Punatipadu	0.75 ac	Clay loam	Co 7219	R	11.7
Tennuru		0.80 ac	Sandy loam	Co 6907	R	23.8	
		1.25 ac	Sandy loam	Co 8013	R	26.9	
		0.50 ac	Clay loam	CoA 8401	R	11.7	
Ajinapadu		1.00 ac	Clay loam	Co 6907	R	31.7	
	1.00 ac	Clay loam	Co 7219	R	48.7		
Yakanuru	1.00 ac	Sandy loam	Co 8013	R	28.7		
Chagantipadu	1.25 ac	Sandy loam	Co 7219	R	58.6		
Kallarvaripalem	0.80 ac	Clay loam	Co 7219	R	48.7		

Contd.

Region	Factory area	Village	Extent of holding	Type of soil	Variety	Planting season	PHI	
Nellore	Panace mandyu		1.00 ac	Clay loam	Co 7219	R	60.4	
			0.75 ac	Clay loam	Co 8021	R	47.7	
			0.75 ac	Clay loam	Co 571	R	50.2	
	Paturu		0.75 ac	Sandy loam	Co 8021	R	72.0	
			1.50 ac	Clay loam	Co 571	R	29.6	
			1.00 ac	Clay loam	CoT 8201	R	46.3	
	Rebala		3.75 ac	Clay loam	Co 8021	R	40.6	
			1.80 ac	Clay loam	CoT 8201	R	43.4	
	Chelluru	Draksharamam		1.00 ac	Clay loam	Co 7219	R	58.2
				1.25 ac	Clay loam	Co 7805	R	46.2
				0.75 ac	Clay loam	CoA 89081	P	5.2
		Dulla		1.60 ac	Clay loam	Co 7219	R	20.2
0.50 ac				Clay loam	Co 8013	P	29.6	
0.75 ac				Clay loam	82V 12	P	11.4	
Ryali			0.80 ac	Clay loam	Co 7219	R	52.2	
			0.75 ac	Clay loam	Co 7805	R	41.6	
			1.00 ac	Sandy loam	82V 12	R	12.1	
Ankampalem			1.00 ac	Sandy loam	Co 6907	R	21.2	
			3.60 ac	Clay loam	Co 7219	R	67.8	
			1.50 ac	Clay loam	Co 7805	R	47.3	
			1.00 ac	Clay loam	CoA 89081	R	5.8	
Chelluru			1.00 ac	Clay loam	Co 6907	R	22.7	
			0.75 ac	Clay loam	Co 7219	R	52.4	
			1.50 ac	Clay loam	Co 7805	R	47.1	
Anakapalle		Nagulapalle		0.75 ac	Clay loam	Co 7219	R	64.6
				0.50 ac	Clay loam	Co 7706	R	19.2
	0.75 ac			Clay loam	CoA 7602	R	16.2	
	Munagabaka		0.70 ac	Clay loam	Co 7706	R	50.4	
			0.75 ac	Sandy loam	CoA 7602	R	30.6	
	Thunmapala		1.00 ac	Clay loam	Co 7219	P	51.3	
0.80 ac			Sandy loam	CoA 7602	P	51.2		
0.75 ac			Clay loam	CoT 8201	R	40.6		
Marturu			0.75 ac	Sandy loam	CoA 7602	R	30.4	

Contd..

Region	Factory area	Village	Extent of holding	Type of soil	Variety	Plant/ration	Yield		
Telangana	Bodhan	Atchanna-palle	0.60 ac	Clay	Co 6907	R	21.4		
			1.00 ac	Clay	Co 7219	P	48.4		
			0.75 ac	Clay	Co 8014	P	36.4		
		Bardipur	1.00 ac	Clay	Co 419	P	18.7		
			0.50 ac	Clay	Co 740	R	66.8		
			0.75 ac	Clay	Co 7219	R	64.1		
		Langdapur	1.00 ac	Clay	Co 6907	R	29.6		
			1.50 ac	Clay	Co 7219	P	63.2		
			3.80 ac	Clay	Co 8014	P	29.1		
			3.75 ac	Clay	CoT 8201	P	48.5		
		Rajapalli	1.00 ac	Clay	Co 6907	P	31.7		
			1.00 ac	Clay	Co 7219	P	61.0		
			0.75 ac	Clay	CoC 671	P	21.9		
			1.25 ac	Clay	CoT 8201	P	51.3		
		Bodhan farm	0.80 ac	Sandy loam	Co 6907	P	11.3		
			1.00 ac	Clay loam	Co 7219	P	52.5		
			0.50 ac	Sandy loam	CoC 671	P	14.7		
			0.15 ac	Clay	BIF 50R	P	41.4		
		Rayalaseema	Renigunta	Vadamalpet	1.00 ac	Sandy	Co 7219	P	13.1
					0.50 ac	Sandy	Co 8014	P	6.2
Vengalera Kandriga	1.00 ac			Red sandy	Co 997	R	0.0		
	0.75 ac			Red sandy	Co 6304	P	0.0		
	0.75 ac			Red sandy	CoT 8201	R	9.5		
Chandragiri	1.00 ac			Red sandy	CoT 8201	R	8.7		
Perumalle- palle farm	0.75 ac			Sandy	Co 7219	P	9.3		
	0.05 ac			Sandy	Co 7621	P	0.0		
	0.05 ac			Sandy	Co 7808	P	0.0		
	0.05 ac			Sandy	Co 8009	P	0.0		
	0.05 ac			Sandy	Co 8017	P	0.0		
	0.75 ac			Sandy	Co 8021	P	0.0		
	0.05 ac			Sandy	Co 8132	P	0.0		
1.00 ac	Sandy			CoT 8201	P	8.1			

P = Plant crop

R = Ratoon crop

The per cent intensity of the disease ranged from 21.2 to 57.5 in plant crop while it was 23.6 to 68.2 in ratoons irrespective of the variety. Maximum disease intensity (68.2%) was observed in ratoon plot of Co 7219 in Aginapadu village where the plot was inundated with water. In Punadipadu village the plant crop of same variety which received double the dose of recommended nitrogen, recorded 61.3 per cent intensity. Next to Co 7219, two varieties viz., Co 7805 (48.2%) and CoA 8401 (41.7%) recorded high intensity of the disease in the area. The most popular early maturing variety of the area viz., Co 6907 recorded disease intensity of 23.6 to 31.2 per cent. The lowest incidence of the disease was recorded in plant crop of Co C671 (21.2%) in Hanumanthapuram village.

In Nellore factory area the disease intensity was recorded in 8 holdings, in 3 villages where the crop is mostly grown in clay loams. Maximum intensity was recorded in Co 7219 (60.4%) in Damaramadugu village where there was water stagnation in the field. Two popular varieties viz.; CoC 671 and CoT 8201 which occupied larger area in the factory zone recorded 29.8 to 30.2 per cent and 43.4 to 46.3 per cent respectively. The intensity of the disease in Co 8021 ranged from 32.9 per cent in sandy loam soil to 42.7 per cent in clay loam soil.

In Chelluru factory area survey was conducted in 16 holdings in 5 villages. The crop is cultivated in clay

loams and sandy loams. Maximum disease intensity was recorded in Co 7219 in Dulla (70.2%) and Ankampalem (67.8%) villages where the fields were stagnated with water and manured with three times the recommended dose of nitrogen fertilizer. Another important mid late maturing variety Co 7805 recorded an intensity of 41.6 per cent to 47.3 per cent. Least incidence of the disease was recorded in two varieties viz., CoA 89081 (5.2 to 5.8%) and 82V12 (11.4 to 12.1%) irrespective of high nitrogen fertilization to the crop. The disease intensity was observed to be more (70.2%) in clay loams as compared to sandy loams (12.1%).

The intensity of the disease was recorded in Anakapalle factory area in 9 holdings. The crop is grown in clay loams and sandy loams. Variety Co 7219 recorded maximum disease intensity (64.6%) in Nagulapalle village where the crop was manured with area twice the recommended dose. Variety CoT 8201 recorded 40.6 PDI. The variety CoA 7602 which occupied maximum area in the factory zone recorded 30.4 to 36.2 PDI. The late maturing good jaggery variety Co 7706 recorded 29.2 to 30.4 PDI.

In Telangana region, out of 18 holdings surveyed in Bodhan area, where the crop is grown mostly in clay soils, maximum disease intensity was recorded in Berdipur village in the varieties Co 7219 (64.1%) and Co 740 (69.8%) where the crop received double the dose of

recommended nitrogen. The PDI ranged from 16.7 to 63.2 in plant crop, while it was 21.4 to 69.8 in ratoon crop irrespective of the variety. The variety Co 7219 recorded high disease intensity (48.4 to 64.1%) followed by CoT 8201 (48.5 to 54.3%). The most popular early maturity variety Co 6907 recorded moderate incidence of the disease ranging from 14.5 to 29.6 per cent. In the remaining varieties viz., Co 8014 (26.4 to 29.1%), CoC 671 (16.7 to 23.9%), Co 419 (18.7%), 81R508 (47.4%), the disease intensity varied from 16.7 to 47.4 per cent.

In Rayalaseema region, the disease intensity was recorded in 14 holdings in 4 villages where the soil type is sandy/red sandy. In general, the disease intensity was very low even in susceptible varieties like Co 7219 and CoT 8201. Out of 11 varieties observed, only 3 viz., Co 7219 (9.3 to 10.1%), CoT 8201 (8.1 to 9.5%) and Co 8014 (6.2%) manifested the disease incidence. The other 8 varieties were free from the disease.

In almost all the holdings surveyed in the three regions, the crop was planted between January-March and ratooned between December-March. In most of the holdings in coastal Andhra Pradesh, particularly in Chelluru and Vuyyuru factory areas the crop in majority of the varieties received high doses of nitrogen fertilizers. In only a few holdings potash and phosphatic fertilizers

were applied. In Vuyyuru zone alone, the crop in most of the holdings was raised from hot water treated seed. Except sprays for the control of early shoot borer and scale insect in some holdings, no other plant protection measures were taken up in grown up crop in any factory zone.

The survey in the three zones of Andhra Pradesh, thus clearly indicate that the ring spot disease intensity was found to be severe in coastal Andhra Pradesh and Telangana and negligible in Rayalaseema particularly in Renigunta factory area. The disease intensity was also observed to be more under stress conditions (both under rainfed and water stagnated conditions) particularly in clay loam soils where high doses of nitrogen fertilizers were applied. Certain important commercial varieties viz., Co 7219, CoT 8201, Co 975, Co 740, Co 7805 and Co 8021 recorded maximum disease intensity in most of the holdings while two varieties CoA 89081 and 82V12 recorded lowest incidence of the disease. In most of the areas ratoon crops recorded more disease intensity than plant crops of sugarcane.

**4.5 INOCULATION AND RAPID SCREENING TECHNIQUES**

**4.5.1 Inoculation techniques**

Experiments were conducted as detailed at para 3.2.5.1 to evaluate different methods of inoculation for

evolving a suitable inoculation technique. The following eight inoculation methods were employed in the study.

1. Spraying leaves with conidial suspension ( $I_1$ ).
2. Clipping top halves of leaves and spraying conidial suspension ( $I_2$ ).
3. Clipping the top halves of leaves and slightly wounding them and spraying conidial suspension ( $I_3$ ).
4. As in method 3+ spraying water from 4th day after inoculation upto 7th day ( $I_4$ ).
5. Mixing celite in inoculum and rubbing gently over the leaves ( $I_5$ ).
6. Inserting small piece of pycnidia bearing leaf tissues into the spindle and spraying water ( $I_6$ ).
7. Squirting the spore suspension into the spindle ( $I_7$ ).
8. Wrapping 3-6 leaves with a piece of ring spot affected leaf ( $I_8$ ).

Transformed ( $\sqrt{X+1}$ ) values of PDI were analysed as suggested by Mostellier and Youtz (1961). The data are furnished in Table 19.

Table 19: Per cent disease intensity with different inoculation methods in different sugarcane varieties

Inoculation method	PDI in varieties				Mean for methods of inoculation
	Co-7219	Co-7636	Co-8201	Co-975	
I <sub>1</sub>	60.95 (7.87)	71.40 (8.51)	49.90 (7.20)	48.00 (7.00)	57.81 (7.64)
I <sub>2</sub>	60.60 (7.85)	70.95 (8.49)	49.60 (7.12)	48.90 (7.06)	57.51 (7.60)
I <sub>3</sub>	61.20 (7.89)	71.80 (8.53)	49.30 (7.09)	49.55 (7.11)	57.96 (7.65)
I <sub>4</sub>	62.40 (7.96)	72.70 (8.59)	49.70 (7.12)	48.30 (7.02)	58.28 (7.67)
I <sub>5</sub>	58.90 (7.74)	68.80 (8.36)	49.30 (7.38)	46.40 (6.89)	55.10 (7.44)
I <sub>6</sub>	5.30 (2.22)	11.10 (3.48)	5.40 (2.33)	5.70 (2.59)	7.90 (2.76)
I <sub>7</sub>	8.10 (3.02)	10.20 (3.35)	4.90 (2.42)	4.40 (2.33)	6.90 (2.78)
I <sub>8</sub>	4.70 (2.39)	5.90 (2.63)	5.10 (2.34)	6.0 (3.00)	5.47 (2.01)
Mean for varieties	40.78 (5.99)	47.86 (6.47)	32.40 (5.30)	31.41 (5.12)	

Figures in parenthesis are  $\sqrt{x+1}$  transformed values

### Conclusions

Particulars	Whether significant or not	S.E.M. <sub>±</sub>	CD at 5%
Varieties	Yes	0.037	0.106
Inoculation methods	Yes	0.052	0.149
Varieties x inoculation methods	Yes	0.104	0.299

The data in Table 19 clearly indicate that the inoculation methods  $I_1$ ,  $I_2$ ,  $I_3$  and  $I_4$  were on par and were significantly superior to other four methods. The PDI in all the four methods ( $I_1$  to  $I_4$ ) of inoculation was high and ranged from 60.6 to 62.4 in Co 7219; 70.95 to 72.7 in Co 7636; 49.3 to 50.9 in CoT 8201; 48.0 to 49.55 in Co 975. In the inoculation method 5 where celite was mixed with inoculum and rubbed on the leaf, the intensity of the disease was more in all the varieties (46.3 to 68.8%) and was significantly superior to other 3 methods ( $I_6$ ,  $I_7$  and  $I_8$ ). The severity of the disease was low in all the test varieties in methods  $I_6$  (5.4 to 11.1%),  $I_7$  (4.4 to 10.2%) and  $I_8$  (0.0 to 5.9%).

Out of the four inoculation methods which were found significantly superior and on par with each other, the method of spraying conidial suspension ( $1 \times 10^6$  spores/ml) on the leaves- $I_1$  was preferred as it is easy and simple to operate.

#### 4.5.2 Rapid screening techniques

Different screening methods were tried as described at para 3.2.5.2 to identify a reliable and rapid screening method for determining the reaction of varieties to ring spot disease. The methods include (a) spraying conidial suspension on leaves ( $S_1$ ), (b) clipping, pin pricking and spraying of conidial suspension on leaves

followed by water spray ( $S_2$ ) (c) Rajooong inoculation ( $S_3$ ) (d) Detached leaf inoculation ( $S_4$ ), (e) Screening under natural conditions ( $S_5$ ). The original PDI values were transformed adopting  $\sqrt{x+1}$  (where  $x$  = original PDI value) and subjected to statistical analysis. The data are presented in Table 20.

Among the five screening methods tried, three methods  $S_1$ ,  $S_2$  and  $S_3$  were on par with each other and were significantly superior to other two methods. In the remaining two methods,  $S_5$  was significantly superior to  $S_4$ . Minimum disease intensity (6.24%) was observed in  $S_4$ , where detached leaves were inoculated.

The disease intensity was observed to be uniformly high in the three screening methods  $S_1$ ,  $S_2$  and  $S_3$  as compared to  $S_4$  and  $S_5$  in all the test varieties/clones. Out of 8 varieties/clones tested, four varieties Co 7219, CoT 8201, Co 85044 and Co 7636 exhibited more than 50 PDI while it was less than 10.0 per cent in four varieties/clones namely CoA 89081, 84A155, Co 85045 and 86A444. In the screening method  $S_5$  where the varieties were screened under natural conditions, four varieties (Co 7219, CoT 8201, Co 85044 and Co 7636) recorded more than 40 PDI while two other clones 84A155 and 86A444 did not manifest the disease at all. In the screening method  $S_4$  where the detached leaves of the varieties were inoculated, minimum disease intensity was observed in Co 7219

Table 20: Comparative efficacy of different screening techniques for ring spot in terms of per cent disease intensity

Screening technique	POI in varieties/clones								Mean for screening method
	Co 7219	Co 7636	CoT 8201	Co 85044	94A 155	CoA 89081	Co 85045	86A 444	
S <sub>1</sub> Spraying conidial suspension	61.3 (7.90)	70.6 (8.48)	51.1 (7.22)	69.2 (8.36)	5.9 (2.63)	8.1 (3.02)	8.4 (3.07)	6.1 (2.66)	35.11 (5.42)
S <sub>2</sub> Clipping leaves, pin prickling, spraying conidial suspension followed by water spray	62.6 (7.96)	71.6 (8.52)	52.3 (7.30)	70.4 (8.45)	6.4 (2.72)	9.4 (3.23)	9.5 (3.24)	7.2 (2.87)	36.18 (5.53)
S <sub>3</sub> Bag seed inoculation	61.6 (7.93)	70.4 (8.45)	51.7 (7.26)	69.7 (8.41)	6.0 (2.56)	7.1 (2.57)	8.1 (2.85)	7.1 (2.86)	35.06 (5.42)
S <sub>4</sub> Bagged leaf inoculation	45.2 (4.04)	48.2 (4.38)	44.3 (3.51)	49.7 (4.44)	3.2 (1.36)	4.7 (1.69)	6.0 (2.26)	7.8 (2.90)	30.14 (4.12)
S <sub>5</sub> Screening under natural conditions	44.6 (6.75)	58.7 (7.73)	40.1 (6.41)	56.2 (7.56)	3.0 (1.30)	4.1 (1.75)	2.2 (1.79)	6.2 (3.00)	25.49 (4.23)
Mean for varietal/clones	48.1 (6.82)	57.94 (7.51)	41.9 (6.34)	56.9 (7.45)	3.7 (2.01)	6.04 (2.55)	6.64 (2.45)	4.38 (2.37)	

Figures in parenthesis are (X+1) transformed values

Conclusions

Screening technique	Whether significant or not	D.F. at 5%	CO at 5%
Varieties/clones	Yes	0.0512	0.145
Inoculation method	Yes	0.0001	0.0001
Varietal/clonal interaction	Yes	0.0001	0.0001

(15.3%), Co 7636 (18.2%), CoT 8201 (1.3%), Co 85044 (18.7%) and CoA 89081 (1.7%). The remaining 3 varieties/ clones viz. Co 85045, 84A155 and 86A444 did not show even incipient infection.

The results show that the three screening methods  $S_1$ ,  $S_2$  and  $S_3$  are superior to  $S_4$  and  $S_5$ . Out of these three methods which are at par,  $S_3$  or Rajoong method of screening (Plates 19 and 20) is preferred as it is rapid, reliable and saves two months time. The setts germinate faster and the growth of the crop is slightly quick in this method when compared to the other two methods.

#### 4.6 FACTORS AFFECTING DISEASE DEVELOPMENT

##### 4.6.1 Effect of different levels of Nitrogen, Phosphorus and Potassium fertilizers on the extent of disease development

The individual and integrated effects of nitrogen (0 ( $N_0$ ), 112 ( $N_1$ ), 224 ( $N_2$ ), 336 ( $N_3$ )  $\text{kg ha}^{-1}$ ), phosphorus (0 ( $P_0$ ), 100 ( $P_1$ )  $\text{kg ha}^{-1}$ ) and potassium (0 ( $K_0$ ), 100 ( $K_1$ )  $\text{kg ha}^{-1}$ ) fertilizers on the intensity of disease development in Co 7219 was studied in the field in two crop seasons as per the details given at para 3.2.6.1. The data on the PDI assessed in different treatments six months after inoculation were statistically analysed and presented in Table 21.



Table 21: Influence of nitrogen alone or in combination with phosphorus and potassium on the intensity of ring spot disease

Levels of P and K	1988-89						1989-90					
	P <sub>0</sub>		P <sub>1</sub>		Mean for N level		P <sub>0</sub>		P <sub>1</sub>		Mean for N level	
	K <sub>0</sub>	K <sub>1</sub>	K <sub>0</sub>	K <sub>1</sub>	K <sub>0</sub>	K <sub>1</sub>	K <sub>0</sub>	K <sub>1</sub>	K <sub>0</sub>	K <sub>1</sub>	K <sub>0</sub>	K <sub>1</sub>
N <sub>0</sub>	33.9	30.0	34.0	29.7	31.8	31.8	30.1	27.2	31.0	27.1	29.1	29.1
N <sub>1</sub>	52.3	44.3	51.5	40.1	46.1	46.1	47.0	41.0	40.0	41.0	44.1	44.1
N <sub>2</sub>	67.1	60.8	67.0	61.5	64.1	64.1	65.1	58.1	62.7	59.1	60.6	60.6
N <sub>3</sub>	69.2	61.9	67.3	62.9	65.5	65.5	65.0	57.8	64.3	58.7	61.0	61.0
Means for P and K levels	P <sub>0</sub> = 52.5		P <sub>1</sub> = 42.5		K <sub>0</sub> = 55.4		K <sub>1</sub> = 44.0		P <sub>0</sub> = 48.9		K <sub>0</sub> = 51.1	
	P <sub>1</sub> = 52.4		P <sub>0</sub> = 44.0		K <sub>0</sub> = 47.0		K <sub>1</sub> = 50.4					

Conclusions

- S.E.m.<sub>±</sub> for nitrogen C.D. at 5% 0.5570
- S.E.m.<sub>±</sub> for phosphorus 1.61
- S.E.m.<sub>±</sub> for potash 0.3939
- C.D. at 5% 0.3939
- S.E.m.<sub>±</sub> for N x P 1.14
- S.E.m.<sub>±</sub> for N x P 0.7877
- S.E.m.<sub>±</sub> for N x P 0.7877
- C.D. at 5% 2.27
- S.E.m.<sub>±</sub> for P x K 0.5570
- S.E.m.<sub>±</sub> for N x P x K 1.1140

The disease intensity was less in the treatments where nitrogen was not applied. Disease intensity increased progressively and significantly with increased level of nitrogenous fertilizer from 0 to 112 and from 112 to 224 kg ha<sup>-1</sup>. The differences in disease intensity between N<sub>2</sub> and N<sub>3</sub> levels were however not significant, though slight increase in the incidence was noted.

Application of phosphorus at 100 kg ha<sup>-1</sup> did not influence the severity of disease incidence while application of potash at 100 kg ha<sup>-1</sup> reduced the disease incidence significantly in both the crop seasons. Interaction between N and K was significant, but the same effect was not perceptible between NP, PK and NPK.

The data relating to per cent nitrogen in 3-6 leaves, phosphorus and potash in 3-6 sheaths at 240 days age of the crop in relation to disease intensity in different treatments are furnished in Tables 22 and 23. Multiple regressions were worked out with the above data and the equations are presented in Table 22.

The leaf nitrogen content tended to increase with increase in the level of applied nitrogen and the disease intensity increased correspondingly with increase in the leaf nitrogen content. Slight improvement in the phosphorus content of 3-6 sheaths was noticed as a result of phosphate application but the effect of the element on

Table 22: Disease intensity (Mean) in relation to per cent NPK in index disease of sugarcane cultivar Co. 3219

Treatment	1988-89				1989-90			
	POI	% Nitro- gen	% Sulfur	% Potas- sium	POI	% Nitro- gen	% Sulfur	% Potas- sium
N <sub>0</sub> P <sub>0</sub> K <sub>0</sub>	33.90	1.20	0.073	1.90	30.5	1.25	0.070	1.92
N <sub>0</sub> P <sub>1</sub> K <sub>1</sub>	29.67	1.21	0.087	2.17	27.4	1.19	0.083	2.38
N <sub>0</sub> P <sub>1</sub> K <sub>0</sub>	34.63	1.24	0.087	1.89	31.0	1.20	0.087	1.83
N <sub>0</sub> P <sub>0</sub> K <sub>1</sub>	29.97	1.27	0.070	2.45	27.2	1.26	0.063	2.45
Mean	31.80	1.24	0.079	2.14	29.02	1.23	0.076	2.15
N <sub>1</sub> P <sub>0</sub> K <sub>0</sub>	52.33	1.81	0.073	1.90	47.8	1.79	0.080	1.87
N <sub>1</sub> P <sub>1</sub> K <sub>1</sub>	45.10	1.83	0.093	2.41	41.8	1.82	0.083	2.27
N <sub>1</sub> P <sub>1</sub> K <sub>0</sub>	51.53	1.80	0.083	1.81	46.6	1.78	0.087	1.79
N <sub>1</sub> P <sub>0</sub> K <sub>1</sub>	44.27	1.82	0.068	2.39	41.2	1.81	0.067	2.34
Mean	48.30	1.82	0.079	2.13	44.35	1.80	0.079	2.07
N <sub>2</sub> P <sub>0</sub> K <sub>0</sub>	67.10	1.91	0.060	1.91	63.1	1.90	0.057	1.81
N <sub>2</sub> P <sub>1</sub> K <sub>1</sub>	61.53	1.92	0.086	2.35	59.2	1.89	0.083	2.24
N <sub>2</sub> P <sub>1</sub> K <sub>0</sub>	67.07	1.91	0.080	1.83	62.7	1.87	0.087	1.84
N <sub>2</sub> P <sub>0</sub> K <sub>1</sub>	60.76	1.90	0.053	2.30	58.1	1.88	0.067	2.29
Mean	64.11	1.915	0.071	2.12	60.78	1.89	0.074	2.04
N <sub>3</sub> P <sub>0</sub> K <sub>0</sub>	69.87	1.97	0.063	1.80	65.6	1.94	0.053	1.81
N <sub>3</sub> P <sub>1</sub> K <sub>1</sub>	62.90	1.96	0.083	2.39	58.7	1.93	0.080	2.34
N <sub>3</sub> P <sub>1</sub> K <sub>0</sub>	67.27	1.96	0.090	1.83	64.7	1.92	0.087	1.96
N <sub>3</sub> P <sub>0</sub> K <sub>1</sub>	61.87	1.97	0.066	2.44	57.8	1.94	0.067	2.33
Mean	65.47	1.965	0.075	2.14	61.6	1.93	0.072	2.11

$$Y = 8.4 + 43.6 X_1 - 85 X_2 - 11.8 X_3 \quad Y = 5.6 + 42.4 X_1 - 75 X_2 - 11.3 X_3$$

$$R^2 = 89.2\%$$

$$R^2 = 85.1\%$$

Table 23: Disease intensity, vis à vis NPK levels in index tissues of sugarcane cultivar Co 7249

Level of element	1988-89		1989-90	
	Disease intensity	% of element in index tissues	Disease intensity	% of element in index tissues
N <sub>0</sub>	32.0	1.24	29.1	1.23
N <sub>1</sub>	48.3	1.82	44.4	1.80
N <sub>2</sub>	64.1	1.92	60.4	1.89
N <sub>3</sub>	65.5	1.97	61.5	1.93
P <sub>0</sub>	50.8	0.065	48.9	0.065
P <sub>1</sub>	52.4	0.087	49.0	0.084
K <sub>0</sub>	55.4	1.87	51.4	1.85
K <sub>1</sub>	49.5	2.39	46.4	2.32

the disease intensity was not appreciable. The sheath potassium content in the index tissues increased due to application of potassium and resulted in decreased PDI. Thus application of N, P and K fertilizers to the paddy plants resulted in higher contents of these nutrient elements in the index tissues. The increased leaf nitrogen content contributed for higher incidence of the disease while the sheath potassium content reduced the intensity of the disease in both the crop seasons. Phosphorus content in sheaths appeared to have had no appreciable effect on the disease intensity.

Multiple regressions were worked out between the disease intensity and the leaf nitrogen ( $X_1$ ), sheath phosphorus ( $X_2$ ) and potassium ( $X_3$ ) contents for both the crop seasons. The combined role of these three factors accounted for variation in disease intensity to the extent of 89.2 and 85.9 per cent during 1988-89 and 1989-90 respectively.

The regression coefficients for leaf nitrogen were positive and significant while it was negative and significant for sheath potassium in both the crop seasons indicating positive association of leaf nitrogen and negative association of sheath potassium with disease intensity.

#### 4.6.2 Relationship of Nitrogen, Phosphorus and Potassium contents in the index tissues of resistant and susceptible varieties/clones on the extent of disease severity

The relationship of NPK contents in the index tissues of 240 days old plants of five resistant (86A444, 84A155, CoA 89081, Co 85045, 83A106) and five susceptible (Co 7219, CoT 8201, Co 975, Co 7636, Co 85044) varieties/clones on disease severity was studied under recommended fertilizer application ( $112 \text{ kg N ha}^{-1}$ ) and as per the details given at para 3.2.6.2. The data on per cent leaf nitrogen, sheath phosphorus and sheath potassium in all the test varieties/clones along with the disease intensity recorded at the time of harvest in February are furnished in Table 24.

The results indicate that the disease intensity in resistant varieties/clones was in the range of 6.1 to 9.8 per cent while it was 43.2 to 74.6 per cent in susceptible varieties. The per cent nitrogen in the leaves of resistant varieties/clones was less (1.778 to 1.796) as compared to susceptible varieties (1.814 to 1.838%). There was no variation in sheath phosphorus between resistant and susceptible varieties/clones. The per cent sheath potassium was noted to be more (1.96 to 2.08%) in resistant varieties/clones when compared to susceptible varieties (1.79 to 1.88%). Thus, increased nitrogen

Table 24: Per cent disease intensity in resistant and susceptible varieties/clones in relation to per cent leaf nitrogen, sheath phosphorus and potassium

S.No.	Variety/ clone	Soil type	PDI	% N	% P	% K
<b>Resistant</b>						
1.	86A 444	Clay loam	6.4	1.786	0.065	2.08
2.	84A 155	"	6.1	1.784	0.060	2.06
3.	CoA 89081	"	9.8	1.792	0.070	1.98
4.	Co 85045	"	8.9	1.796	0.065	1.96
5.	83A 106	"	7.4	1.778	0.070	2.01
	Mean		8.12	1.787	0.066	2.01
<b>Susceptible</b>						
6.	Co 7219	"	59.2	1.822	0.065	1.86
7.	CoT 8201	"	52.4	1.818	0.070	1.88
8.	Co 975	"	43.2	1.814	0.070	1.86
9.	Co 7636	"	74.6	1.832	0.070	1.81
10.	Co 85044	"	70.2	1.838	0.075	1.79
	Mean		59.92	1.825	0.070	1.84

content in leaves resulted in increased disease severity while increased sheath potassium reduced the disease severity.

The relation of per cent leaf nitrogen, sheath phosphorus and potassium with PDI at harvest as revealed through simple correlations are presented in Table 25.

Table 25: Simple correlations of leaf nitrogen, sheath phosphorus and potassium with per cent disease intensity

Varieties	Leaf nitrogen per cent	Sheath phosphorus per cent	Sheath Potassium per cent
Resistant (n = 5)	0.2393	0.6035	-0.8787**
Susceptible (n = 5)	0.9300**	-0.1333	-0.8212*

\*\* Significant at 1% level

\* Significant at 5% level

Significant negative correlation was obtained between PDI and sheath potassium per cent in resistant varieties/clones. In susceptible varieties, highly significant positive correlation (at 1% level) between per cent leaf nitrogen and PDI and significant negative correlation (at 5% level) between per cent sheath potassium and per cent disease intensity were observed.

#### 4.6.3 Age of the crop and intensity of the disease

To study the effect of age of the crop on the intensity of the disease, a field trial was conducted with staggered plantings of the variety Co 7219 during 1988-89 and 1989-90.

The test variety was planted in the field at monthly intervals from February to June every crop season and inoculated when three months old as described at para 3.2.6.3. The disease intensity was recorded at all dates of planting, starting from initiation of symptoms till harvest of the crop every crop season.

To examine the relationship between disease intensity and age of the plant, Chi Square ( $\chi^2$ ) was also worked out. The details of disease intensity related to each month of planting are furnished in Table 26.

The data presented in Table 26 indicate that irrespective of date of planting in both the crop seasons, the disease appeared in September only (4.1 to 5.3%) and gradually increased upto February (47.7 to 53.1%). At all dates of planting disease intensity increased rapidly from October through December and the increase was nominal in January and February. This pattern has also been established statistically by  $\chi^2$  test. The  $\chi^2$  observed was significantly lower than  $\chi^2$  from tables indicating that

Table 26: Effect of age of plant on the intensity of ring spot disease

Month of planting	1988-89 PDI						1989-90 PDI					
	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.
February	5.1	22.6	35.6	47.7	50.7	51.7	5.5	18.7	31.6	45.8	46.4	47.7
March	5.3	23.0	34.1	47.8	51.7	53.1	6.1	21.4	35.2	40.4	43.1	44.8
April	4.9	23.6	35.8	45.9	48.2	49.7	5.2	24.1	38.4	46.7	48.9	50.1
May	4.1	21.4	32.8	42.6	45.4	47.7	4.8	22.6	33.7	41.6	45.7	46.7
June	4.2	22.0	33.8	44.6	48.9	50.7	6.9	24.1	36.2	43.4	47.2	48.5

 $\chi^2$  observed : 0.4149

 $\chi^2$  observed : 1.4070

 $\chi^2$  from tables ( $P = 0.05$ ) = 51.416

the disease intensity was not dependent on the age of the plant.

4.6.4 Effect of leaf orientation on the intensity of the disease

The effect of orientation of leaf on cane stalk on the intensity of the disease was studied in 20 sugarcane varieties/clones. The data recorded at harvest, in February on the leaf orientation, intensity of the disease and the mean angle of 1 to 8 functional leaves as per the procedure described at para 3.2.6.4 are presented in Table 27.

The mean angle of 1 to 8 functional leaves varied from 79.0° to 81.6° in erect leaved varieties. In varieties with drooping leaves it varied from 59.8° to 66.8°. The results presented in the Table 27 indicate that there was significantly low disease intensity in varieties having erect leaves with more angle as compared to varieties having drooping leaves with less angle. The intensity of the disease in the varieties having erect leaves was in the range of 6.7 to 9.9 per cent while it was 32.7 to 72.7 per cent in varieties with drooping leaves.

Table 27: Effect of orientation of leaf on cane stalk on the intensity of ring spot disease

S.No.	Variety/ clone	Leaf orientation	Mean angle in $^{\circ}$ of 1 to 8 func- tional leaves	PDI
1.	Co 85045	Erect	81.0	8.8
2.	86A 444	Erect	81.2	6.7
3.	87A 279	Erect	79.8	8.3
4.	87A 140	Erect	79.6	8.9
5.	CoA 89081	Erect	79.0	8.6
6.	87A 3	Erect	80.2	9.4
7.	Co 85032	Erect	81.2	7.6
8.	83A 106	Erect	79.2	9.1
9.	87A 142	Erect	81.6	7.3
10.	82V 12	Erect	80.0	9.9
11.	Co 7219	Drooping	65.6	57.6
12.	Co 7636	Drooping	61.6	71.2
13.	CoT 8201	Drooping	66.8	48.9
14.	Co 7706	Drooping	65.6	36.7
15.	Co 975	Drooping	66.2	41.1
16.	Co 85044	Drooping	63.2	64.8
17.	Co 8021	Drooping	64.2	40.9
18.	84A 86	Drooping	59.8	72.7
19.	CoA 88081	Drooping	66.4	32.7
20.	CoA 8401	Drooping	65.8	51.2
S.Em $\pm$ for varieties			0.8316	
C.D. at 5%			2.374	

The relationship between the angle of the leaves and PDI was studied through simple correlation and linear regression and the data are presented in Table 28.

Table 28: Simple correlation and linear regression relationship between angle of 1 to 8 functional leaves and per cent disease intensity

Particulars ----- Characters	Simple correlation coefficient	Regression equation**	'F' value
Erect leaves n=10	-0.6431*	-	-
Drooping leaves n=10	-0.8047*	-	-
Combined	-0.9580**	$\hat{Y}=234.28-2.82X$	189.79

\* Significant at 5% level

\*\* Significant at 1% level

'F' value from tables (P=0.05) = 4.4

The correlation of angles of 1 to 8 functional leaves of erect and droopy leaved varieties with PDI individually was negative and significant at 5 per cent level. In the combined analysis also, the correlation was negative and highly significant at 1 per cent level.

The regression coefficient was observed to be significant indicating that the variation in disease intensity is also dependent on the angle of the leaves. With every one degree increase in the angle of the leaves the disease intensity decreased by 2.82 per cent.

Thus the results show that the erect leaf orientation in varieties is an important factor and plays a dominant role for low incidence of ring spot disease.

#### **4.6.5 Moisture stress and water stagnation on disease intensity**

The effect of moisture stress and water stagnation on the intensity of the disease was studied in comparison with normal irrigation in green house with ten sugarcane varieties/clones. Moisture stress and water stagnation was created in pots as detailed at para 3.2.6.5. The data recorded on the intensity of the disease in each variety/clone under moisture stress, water stagnation and normal irrigation during February are furnished in Table 29.

The data furnished in Table 29 indicate that there was significant increase in disease intensity in all the varieties/clones both under moisture stress and water stagnation as compared to normal irrigation except in clone 84A155. In this clone, significant increase in disease intensity was obtained only under moisture stress over normal irrigation. The difference in the intensity of the disease between moisture stress and water stagnation is significant only in four (Co 7219, CoT 8201, Co 7636 and Co 85044) of ten varieties/clones, but in the remaining varieties/clones, the intensity was on par with

Table 29: Disease intensity under moisture stress, water stagnation and normal irrigation

Variety/ clone	PDI			Mean for varieties
	Normal irrigation	Moisture stress	Water stagnation	
Co 975	43.2	58.4	59.7	53.8
Co 7219	57.9	70.6	68.4	65.6
Co 7636	65.2	81.7	79.8	75.6
Co 85044	64.9	77.7	74.8	72.5
Co 85045	8.4	10.2	11.7	10.1
CoT 8201	47.9	62.4	59.9	56.7
CoA 89081	8.1	12.1	12.9	11.0
83A 106	9.1	12.7	12.3	11.4
84A 155	5.9	8.1	7.2	7.1
86A 444	6.1	7.3	8.1	7.3
Mean for treatment	31.7	40.2	39.5	

### Conclusions

Particulars	Whether signi- ficant or not	S.Em. <sub>t</sub>	CD at 5%
Varieties/ clones	Yes	0.8829	2.553
Irrigation types	Yes	0.4836	1.398
Varieties x irrigation types	Yes	1.5292	4.421

each other. In six varieties/clones viz., Co 7219, CoT 8201, Co 7636, Co 85044, 84A155 and 83A106, the increase in disease intensity over normal irrigation was observed to be more under moisture stress as compared to water stagnation. But in the remaining four varieties/clones viz., Co 975, CoA 89081, Co 85045 and 86A444 the increase was more under water stagnation.

Irrespective of the variety/clone the disease intensity increased under moisture stress and water stagnation compared to under normal irrigation.

#### **4.6.6 Effect of silica content of leaves on disease intensity**

The effect of silica content of the leaves on the intensity of the disease was studied in 5 resistant and 5 susceptible varieties/clones as per the procedure mentioned at para 3.2.6.6. Observations made at the time of harvest in February are furnished in Table 30.

Silica per cent in leaves of susceptible varieties is lower (1.42 to 1.71%) as compared to resistant varieties/clones (2.01 to 2.14%). Increase in the silica content resulted in low disease intensity (4.6 to 7.8%) in all the resistant varieties/clones while decrease in silica content resulted in high disease intensity (40.2 to 66.2%) in susceptible varieties. The silica content was observed to be lowest in the variety Co 85044 (1.42%)

followed by Co 7636 (1.49%) where they recorded more than 60 PDI. Maximum silica content was observed in the clone 84A155 (2.14%) followed by 86A444 (2.08%) and both the clones recorded less than 6 PDI.

Table 30: Effect of silica content of leaves on the intensity of ring spot disease

Varieties/ clones	Per cent silica content in leaves	PDI
<b>Susceptible</b>		
1. Co 7219	1.56	53.8
2. Co 7636	1.49	66.2
3. CoT 8201	1.71	46.2
4. Co 85044	1.42	62.2
5. Co 975	1.77	40.1
<b>Resistant</b>		
1. 84A 155	2.14	4.6
2. CoA 89081	2.04	7.4
3. 86A 444	2.08	5.7
4. Co 85045	2.03	7.5
5. 83A 106	2.01	7.8

The relation of mean per cent silica content of leaves with PDI at harvest in resistant and susceptible varieties/clones as revealed through simple correlation and linear regression are presented in Table 31.

Table 31: Simple correlation and linear regression relationship between silica per cent in leaves and per cent disease intensity

Particulars	PDI
Simple correlation coefficient**	-0.9860
Regression equation**	$\hat{Y} = -203.45 - 94.96X$
'F' value	279.64

\*\* Significant at 1% level

'F' value from tables (P=0.05) = 5.32

The correlation worked out disclosed that silica content in leaves bore a significant negative correlation with PDI. The regression coefficient was observed to be significant indicating that the variation in disease intensity is dependent on the range of silica content in the leaves. With every 0.1 per cent increase in the silica content of leaves the disease intensity decreased by 9.5 per cent.

The results thus indicate, that silica content in leaves appears to be one of the factors that is offering resistance to the varieties/clones.

#### 4.7 VARIATION IN RING SPOT PATHOGEN

The cultural, morphological and pathological characters of six isolates of ring spot fungus were studied to understand variability, if any of the pathogen.

Isolates were collected from Nagulapalle, Chagallu, Anakapalle, Amadalavalasa areas of Coastal Andhra Pradesh and Bodhan area of Telangana as described at para 3.2.4.

#### 4.7.1 Cultural and morphological characters

Six isolates collected from Co 7706, Co 7704, Co 7219, CoT 8201, CoA 89082 and Co 419 grew well on oat agar medium. The radial growth in 100 mm petriplates was completed by 10 days after inoculation. The cultural and morphological characters are furnished in Table 32.

No appreciable difference were seen either in cultural characters or in the size of the sexual and asexual spores of six isolates studied. The pycnidia measured 86.0-96.7  $\mu\text{m}$  in diameter while the pycnidiospores measured 10.8-12.7 x 3.1-3.6  $\mu\text{m}$ . The asci were sessile, 8 spored and measured 66.3-71.6 x 11.5-12.5  $\mu\text{m}$ . The ascospores were 3 septate, guttulate, with second cell slightly swollen and measured 20.0-23.8 x 4.1-5.4  $\mu\text{m}$ . These measurements fall within the group range reported by Abbott (1964) for L. sacchari.

#### 4.7.2 Pathogenic variation in the isolates of ring spot pathogen

To study the pathogenic variation of the ring spot fungus, the isolates were inoculated simultaneously by spraying conidial suspension on different varieties as

Table 32: Cultural and morphological characters of six monoonidial isolates of *Phyllosticta saccharicola*

Isolate No.	Culony diameter in mm	Morphological and cultural characters	Spore-lation / ml	Measurement in $\mu\text{m}$						
				Pycnidium mean diameter	Pycnidiospore Mean length	Pycnidiospore Mean breadth	Asci Mean length	Asci Mean breadth	Ascospore Mean length	Ascospore Mean breadth
1	100	Profuse white mycelial growth which later turned to pink or amber colour. Small dark roundish pycnidia appeared on the surface of the mycelium and also embedded in the medium.	$2.72 \times 10^6$	64.5	15.6	3.3	66.3	11.9	20.0	4.1
2	94	Same as above	$2.53 \times 10^6$	66.0	16.0	3.4	67.7	11.5	20.5	4.2
3	100	Same as above	$2.31 \times 10^6$	60.4	15.0	3.6	60.0	10.8	22.0	4.0
4	100	Same as above	$2.67 \times 10^6$	96.7	12.7	3.2	71.6	12.1	22.5	4.5
5	100	Same as above	$2.56 \times 10^6$	85.7	12.0	3.1	70.0	11.5	22.5	4.1
6	97	Same as above	$2.48 \times 10^6$	92.1	12.7	3.1	71.4	12.1	23.5	4.7

described at para 3.2.7.2. The PDI of each isolate on different varieties is given in Table 33.

Scrutiny of the data show that there is no difference in the disease intensity between the isolates. The virulence pattern of all six isolates appeared to be similar in all the test varieties. The results indicate the absence of any pathogenic variation among the six isolates tested.

#### 4.8 ROLE OF SOIL, HOST DEBRIS, CANE SETTS, IRRIGATION WATER AND LEAF CONTACT ON THE SPREAD OF DISEASE

The role of some agencies like soil, host debris, cane setts with infected leaf tissue, irrigation water and contact of affected leaf with healthy leaf in the spread of the disease was studied for two seasons and the procedure adopted is given at para 3.2.8. The PDI recorded in each treatment at harvest was transformed adopting  $\sqrt{X+1}$  and subjected to statistical analysis. The data are presented in Table 34.

It is very clear from the results obtained that among the six agencies tested, contact of diseased leaf with healthy leaf followed by water spray, was found to be the major source of spread (52.3 to 64.9%) in both the seasons and was significantly superior to the other five treatments. Host debris (6 months old) spread at the base

Table 33: Per cent disease intensity of six isolates of ring spot fungus on different varieties of sorghum

Variety	Isolate number						Mean for variety
	1	2	3	4	5	6	
Co 419	34.72	57.18	59.22	56.91	56.72	59.76	51.67
Co 975	48.72	50.10	47.12	53.61	47.56	46.18	48.68
Co 7219	54.28	58.89	60.78	56.67	57.69	59.16	57.91
Co 7636	71.28	67.25	70.28	69.17	68.12	72.35	69.81
Co 7706	37.92	42.22	41.27	39.61	40.62	36.78	39.71
Co 8220	26.41	51.21	30.12	39.72	37.61	51.56	39.43
Co 62175	37.63	40.59	39.78	36.56	41.28	39.37	39.17
Co 85038	27.56	30.61	28.74	33.54	29.76	32.48	30.45
Co 85044	64.78	63.46	62.72	65.81	65.12	62.14	64.67
Co 85045	7.94	8.45	9.33	7.56	8.94	7.72	8.32
CoI 8201	48.62	45.72	50.18	51.42	48.18	49.67	48.91
CoA 7602	43.21	37.62	40.26	41.34	44.26	39.92	41.40
CoA 8401	57.12	55.62	55.64	51.62	54.78	53.21	53.99
CoA 89081	9.11	8.61	9.61	8.22	7.89	9.39	8.81
84A 155	5.22	4.61	6.61	6.12	5.83	5.53	5.65
Mean for the isolate	38.30	38.79	39.32	38.56	39.18	39.04	

Table 34: Extent of disease spread in terms of percent disease intensity through various agencies

Treatment	1988-89			1989-90		
	POI in varieties			POI in varieties		
	Co 7219	Co 7636	Mean for treatment	Co 7219	Co 7636	Mean for treatment
1. Healthy setts planted in healthy soil	0.0 (1.00)	0.0 (1.00)	0.0 (1.00)	0.0 (1.00)	0.0 (1.00)	0.0 (1.00)
2. Healthy setts planted in soil taken from a highly affected clump	3.3 (1.00)	0.0 (1.00)	0.0 (1.00)	0.0 (1.00)	0.0 (1.00)	0.0 (1.00)
3. Healthy setts planted in the soil after incorporation of debris	0.0 (1.00)	0.0 (1.00)	0.0 (1.00)	0.0 (1.00)	0.0 (1.00)	0.0 (1.00)
4. Healthy setts planted in the soil and chaffed debris spread at the base of the clump	20.07 (4.65)	21.67 (4.54)	20.87 (4.69)	21.07 (4.71)	29.17 (5.49)	25.12 (5.10)
5. Cane setts with infected leaf tissue	0.0 (1.00)	0.0 (1.00)	0.0 (1.00)	0.0 (1.00)	0.0 (1.00)	0.0 (1.00)
6. Irrigation water	0.0 (1.00)	0.0 (1.00)	0.0 (1.00)	0.0 (1.00)	0.0 (1.00)	0.0 (1.00)
7. Contact of diseased leaf with healthy leaf followed by water spray	52.27 (7.57)	62.91 (7.99)	59.59 (7.78)	52.30 (7.30)	64.90 (8.12)	58.60 (7.71)
Mean for variety	10.99 (2.46)	13.22 (2.65)		13.50 (2.43)	13.44 (2.66)	

Figures in parenthesis are  $\sqrt{X+1}$  transformed values

### Conclusions

Particulars	1988-89	1989-90
S.E.m.± for treatments	0.057	0.0472
C.D. at 5%	0.20	0.13
S.E.m.± for varieties	0.0172	0.025
C.D. at 5%	0.36	0.50
S.E.m.± for varieties x treatments	0.0472	0.057
C.D. at 5%	0.13	0.19

of the clump also helped in the spread of the disease to a considerable extent (20.7 to 29.7%) in both the varieties tested. The variety Co 7636 recorded disease severity which was significantly more over that of Co 7219 in both the seasons. The other four agencies tested viz., soil adjacent to a highly affected clump, diseased host debris incorporated in the soil, cane setts with infected leaf tissue and irrigation water failed to spread the disease.

#### 4.9 HOST RANGE STUDIES

##### 4.9.1 Reaction of different plant species to P. saccharicola

Forty to forty five day old plants of twenty species raised in the glass house were inoculated with P. saccharicola as per the procedure described at para 3.9. Observations were made from third day onwards upto 30 days after inoculation. The results are presented in Table 35.

Out of twenty plant species inoculated, only Saccharum spontaneum (Relu and SES.594) produced symptoms thus being a host under artificial inoculation (Plate 21). The pathogen from these lesions was reisolated from this host (both type of plants) confirming that this plant species can harbour the ring spot fungus.

Table 35: Reaction of different plant species to artificial inoculation of Phyllosticta saccharicola

Plant species	Presence (+) or absence (-) of symptoms with each inoculation method		Description of symptoms
	method		
	Rubbing inoculum after wounding	Spraying inoculum	
1a. <u>Saccharum spontaneum</u> L. (Rellu)	+	+	Minute purple spots appeared 10 days after inoculation, later the spots enlarged and turned oval. The centre of the spot was straw coloured with black dots. Spots were irregularly elongated, scattered over the whole or part of the leaf blade, coalesced leading to drying and withering of leaf prematurely. The size of the spots was smaller compared to those on <u>Saccharum officinarum</u> .
1b. <u>S. spontaneum</u> (SES.594)	+	+	Symptoms appeared 15 days after inoculation and similar to those produced on <u>S. spontaneum</u> L. (Rellu)
2. <u>Pennisetum glaucum</u> (L.) R.Br.	-	-	-
3. <u>Sorghum vulgare</u> Pers.	-	-	-
4. <u>Sorghum halepense</u> (L.) Pers.	-	-	-
5. <u>Panicum maximum</u> Jacq.	-	-	..
6. <u>Zea mays</u> L.	-	-	..
7. <u>Eleusine coracana</u> (L.) Gaertn	-	-	..
8. <u>Oriza sativa</u> L.	-	-	..
9. <u>Erianthus arundanaceus</u> Retz.	-	-	..
10. <u>Phragmites karka</u> (Retz.) Trin. ex Steud.	-	-	-
11. <u>Sesamum indicum</u> L.	-	-	-
12. <u>Euphorbia hirta</u> L.	-	-	-
13. <u>Cyperus rotundus</u> L.	-	-	-

Contd..

Plant species	Presence (+) or absence (-) of symptoms with each inoculation method		Reproduction of symptoms
	Rubbing inoculum after wounding	Spraying inoculum	
14. <u>Cynodon dactylon</u> (L.) Pers.	-	-	-
15. <u>Digitaria sanguinalis</u> (L.) Scop.	-	-	-
16. <u>Eclipta alba</u> (L.) Hassk.	-	-	-
17. <u>Trianthema portulacastrum</u> L.	-	-	-
18. <u>Croton bipolarandae</u> L.	-	-	-
19. <u>Justicia simplex</u> D.Don	-	-	-
20. <u>Cymbopogon nardus</u> Rendle.	-	-	-



#### 4.10 REACTION OF SUGARCANE VARIETIES/CLONES TO RING SPOT DISEASE

##### 4.10.1 Determination of appropriate time for assessment of disease

To fix up a proper time for assessing the reaction of sugarcane varieties after inoculation, disease counts were made at 1, 2, 3, 4, 5, 6, 7 and 8 months after inoculation in standing canes of 20 varieties/clones planted in March, 1988. Assessment of the disease was based on 0-9 scale and PDI was worked out as indicated at para 3.2.3. The data are furnished in Table 36.

A critical scrutiny of the data show that 9 out of 20 varieties/clones (Co 419, Co 975, Co 7219, Co 7636, Co 7706, CoA 7602, CoT 8201, CoA 8401 and Co 85044) manifested disease incidence in September i.e., one month after inoculation. Out of the remaining 11, nine varieties/clones (Co 8205, Co 8220, Co 62175, Co 85038, Co 85045, CoA 8402, CoA 89081, 83A106 and 83A214) exhibited disease incidence 2 months after inoculation i.e., in October. The other 2 clones, 84A155 and 86A444 manifested disease incidence in November i.e., 3 months after inoculation. In most of the varieties/clones the disease intensity increased substantially during 2nd, 3rd and 4th months after inoculation due to intermittent rains received during October and November (Table 3 at para

Table 36: Disease severity (POI) in different sugarcane varieties/clones at different periods after inoculation with *Phyllosticta saccharicola* (1986-89)

S.No.	Time Variety/ clone	POI Months after inoculation							
		1 September	2 October	3 November	4 December	5 January	6 February	7 March	8 April
1.	Co 419	4.6	14.3	24.1	32.4	36.6	38.6	35.1	30.1
2.	Co 979	4.3	12.8	14.9	38.1	46.4	45.1	46.4	36.1
3.	Co 7219	5.9	21.3	40.7	52.1	54.2	58.7	53.1	47.3
4.	Co 7636	9.4	35.2	53.1	64.4	68.7	71.4	64.2	53.4
5.	Co 7706	5.2	15.6	22.8	33.7	37.9	40.1	36.2	32.3
6.	Co 8205	0.0	1.2	4.8	16.6	18.8	20.2	18.1	16.8
7.	Co 8220	0.0	4.1	12.4	20.9	24.7	26.7	23.1	20.3
8.	Co 62175	0.0	6.8	14.9	30.4	33.3	37.2	34.1	30.9
9.	Co 85038	0.0	5.5	14.1	22.7	26.1	29.2	25.7	22.6
10.	Co 85044	7.8	28.1	48.7	60.4	64.7	68.2	61.4	52.1
11.	Co 85045	0.0	2.2	4.9	7.3	8.9	9.7	8.3	6.6
12.	CoA 7602	3.8	11.2	23.6	34.1	38.8	40.9	36.6	31.1
13.	CoA 8401	5.6	26.6	35.6	45.6	48.9	52.3	47.4	41.6
14.	CoA 8402	0.0	2.2	9.7	17.3	18.9	20.3	17.6	14.7
15.	CoA 89081	0.0	3.6	5.2	7.1	6.4	9.2	8.1	6.1
16.	CoT 8201	5.1	16.2	32.4	44.6	48.2	52.5	47.6	40.7
17.	83A 106	0.0	1.4	2.6	7.4	8.4	9.3	8.5	7.1
18.	83A 214	0.0	3.2	9.1	16.2	18.1	19.8	15.1	14.4
19.	84A 155	0.0	0.0	2.2	3.9	5.4	6.4	5.5	3.3
20.	86A 444	0.0	0.0	2.4	4.3	5.2	6.1	5.4	4.1

3.1). The disease intensity further increased during January and February which period corresponds to 5th and 6th months after inoculation in all the test varieties. There was dew fall during this period. However, the increase was not so marked. The disease showed a fall during March and April months i.e., 7th and 8th months after inoculation due to rise in temperatures. Thus, it is clear from the table that the disease intensity increased upto 6th month (February) after inoculation and reached peak in all the test varieties during this month and gradually slid down during 7th and 8th month (March and April) after inoculation. Thus, assessment made six months after inoculation i.e., in February was found to be the best time for categorising varieties into different resistance groups.

#### **4.10.2 Screening of popular and promising varieties/ clones by adult cane inoculation**

Sixty popular and promising varieties/clones including commercial standards were screened for their resistance to ring spot disease during 1988-89 and 1989-90 as detailed at para 3.2.10.2. The data regarding PDI assessed 3 and 6 months after inoculation and rating/category based on the PDI values recorded six months after inoculation at the eleventh month age of the crop are presented in Table 37.

Table 37: Reaction of sugarcane varieties/clones to ring spot disease by adult cane inoculation

1988-89										1989-90			
Sl. No.	Variety/ clone	PDI 3 months after inocula- tion	PDI 6 months after inocula- tion	Rating at 6th month	Reaction at 6th month	Sl. No.	Variety/ Clone	PDI 3 months after inocula- tion	PDI 6 months after inocula- tion	Rating at 6th month	Reaction at 6th month		
1.	Co 419	26.7	37.9	7	S	1.	Co 7219	34.8	50.2	9	H.S.		
2.	Co 975	26.7	43.2	8	S	2.	Co 8011	8.3	17.8	5	M.S.		
3.	Co 997	7.8	17.6	5	M.S.	3.	Co 8012	7.4	18.9	5	M.S.		
4.	Co 9907	24.1	40.2	8	S	4.	Co 8121	26.7	45.2	8	S		
5.	Co 7219	41.9	58.7	9	H.S.	5.	Co 8139	16.3	30.7	7	S		
6.	Co 7636	54.7	69.4	9	H.S.	6.	Co 8211	15.9	37.8	7	S		
7.	Co 7706	21.1	39.2	7	S	7.	Co 85032	2.6	8.2	3	R		
8.	Co 8013	13.3	33.4	7	S	8.	Co 85046	10.6	17.4	5	M.S.		
9.	Co 8021	25.6	43.7	8	S	9.	CoA 89081	3.8	8.9	7	R		
10.	Co 8116	23.0	41.2	8	S	10.	CoA 89082	15.8	24.4	6	M.S.		
11.	Co 8205	6.6	20.7	6	M.S.	11.	CoA 89083	13.4	25.8	6	H.S.		
12.	Co 8212	16.3	26.4	6	M.S.	12.	CoI 8201	29.2	43.8	8	S		
13.	Co 8220	11.9	27.8	6	M.S.	13.	81A 132	9.1	17.8	5	M.S.		
14.	Co 82172	13.7	35.8	7	S	14.	82A 30	28.9	54.1	9	H.S.		
15.	Co 85055	20.0	35.2	7	S	15.	82A 44	10.4	29.5	6	M.S.		
16.	Co 85038	13.9	26.9	6	M.S.	16.	83A 69	6.3	14.9	4	M.R.		
17.	Co 85044	50.2	66.3	9	H.S.	17.	83A 93	15.6	42.7	8	S		
18.	Co 85045	4.4	9.4	3	R	18.	83A 124	18.7	32.4	7	S		
19.	CoC 671	23.3	33.8	7	S	19.	84A 42	22.2	39.1	7	S		
20.	CoA 7602	22.2	39.9	7	S	20.	84A 86	49.1	73.2	9	H.S.		

Contd..

		1988-89				1989-90					
Sl. No.	Variety/ clone	PDI 3 months after inocula- tion	PDI 6 months after inocula- tion	Rating at 6th month	Reaction at 6th month	Sl. No.	Variety/ Clone	PDI 3 months after inocula- tion	PDI 6 months after inocula- tion	Rating at 6th month	Reaction at 6th month
21.	CoA 8401	34.8	50.7	9	H.S.	21.	84A 110	28.7	51.2	9	H.S.
22.	CoA 8402	7.6	17.3	5	H.S.	22.	84A 140	14.9	26.7	6	H.S.
23.	CoA 88081	21.8	35.1	7	S	23.	84A 155	1.8	5.6	3	R
24.	CoA 89081	4.7	9.1	3	R	24.	84A 191	7.1	17.6	5	H.S.
25.	CoA 8201	30.0	50.4	9	H.S.	25.	85A 123	3.7	11.2	4	H.S.
26.	83A 75	30.1	50.4	9	H.S.	26.	85A 256	14.8	24.5	6	H.S.
27.	83A 106	3.0	9.8	3	R	27.	CoA 89085	27.6	41.1	8	S
28.	83A 214	7.2	17.9	5	H.S.	28.	87A 142	2.6	6.8	3	R
29.	84A 155	2.6	6.1	3	R	29.	87A 279	2.3	7.4	3	R
30.	86A 444	2.4	6.2	3	R	30.	80K 41	13.3	34.6	7	S
	Mean	19.72	33.33				Mean	15.33	28.65		

S : Resistant, M.R. : Moderately resistant; H.S. : Moderately susceptible; S : Susceptible;  
H.S. : Highly susceptible

The results indicate that there was substantial increase in PDI in all the test varieties/clones from 3 to 6 months after inoculation in both the crop seasons and thus confirm the observation made in the previous study that the proper time for assessment of the reaction of the variety is six months after inoculation. During 1988-89, out of 30 varieties/clones tested, five varieties/clones viz., Co 85045, CoA 89081, 83A106, 84A155 and 86A444 manifested less than 10 PDI and were rated as resistant. Six varieties/clones (Co 7219, CoT 8201, CoA 8401, Co 7636, Co 85044 and 83A75) exhibited more than 50 PDI and were rated as highly susceptible. Among the highly susceptible varieties Co 7636 showed maximum PDI of 69.4. The remaining 19 varieties/clones were rated as moderately susceptible to susceptible.

During 1989-90, five varieties/clones (Co 85032, CoA 89081, 84A155, 87A142 and 87A279) showed resistant reaction out of 30 varieties/clones tested. Only four varieties (Co 7219, 82A30, 84A86 and 84A110) exhibited highly susceptible reaction. Among these, 84A86 manifested maximum disease severity (73.2 PDI). Two clones viz., 83A69 and 85A123 manifested more than 10 but less than 20 PDI and were rated as moderately resistant. The remaining 19 varieties/clones reacted as moderately susceptible to susceptible. Four varieties/clones (CoA 89081, 84A155, Co 7219 and CoT 8201) which were included in both the crop seasons gave consistent reaction.

#### 4.10.3 Varietal reaction by Rajoong method of inoculation

Forty varieties/clones which were tested under mature cane inoculation were also tested by Rajoong method of inoculation during 1988-89 and 1989-90. The per cent disease intensity recorded 3 and 6 months after inoculation and disease rating and reaction 6 months after inoculation eight months after planting are presented in Table 38.

The results indicate that the disease intensity increased in all the test varieties from 3 to 6 months after inoculation. During 1988-89, 3 varieties/clones CoA 89081, 86A444 and 84A155 showed resistant reaction while five varieties Co 7219, CoA 8401, Co 8021, Co 7636 and CoT 8201 showed highly susceptible reaction. Remaining twelve varieties/clones showed moderately susceptible to susceptible reaction. During 1989-90, 4 varieties/clones CoA 89081, 84A155, 87A142 and 87A279 reacted as resistant while two varieties Co 7219 and Co 8121 showed highly susceptible reaction. The variety CoT 8201 which reacted as highly susceptible during 1988-89, showed susceptible reaction during 1989-90. Only one clone 85A123 gave moderately resistant reaction. The remaining 13 varieties/clones showed moderately susceptible to susceptible reaction.

Table 38: Reaction of sugarcane varieties/clones to ring spot disease by Rajoung inoculation

1988-89										1989-90			
Sl. No.	Variety, clone	PDI 3 months after inoculation	PDI 6 months after inoculation	Rating at 6th month	Reaction at 6th month	Sl. No.	Variety, Clone	PDI 3 months after inoculation	PDI 6 months after inoculation	Rating at 6th month	Reaction at 6th month		
1.	Co 419	28.7	41.1	8	S	1.	Co 7219	42.3	55.7	9	H.S.		
2.	Co 975	33.3	47.6	8	S	2.	Co 8011	9.9	20.7	9	M.S.		
3.	Co 997	8.6	19.1	5	M.S.	3.	Co 8121	32.7	50.8	9	H.S.		
4.	Co 6907	27.2	43.7	8	S	4.	Co 8211	19.8	43.0	6	S		
5.	Co 7219	49.6	64.5	9	H.S.	5.	Co 85046	10.9	21.1	6	M.S.		
6.	Co 7636	58.9	75.8	9	H.S.	6.	CoA 89081	4.4	9.7	3	R		
7.	Co 7706	24.7	43.6	8	S	7.	CoA 89082	19.5	26.7	6	H.C.		
8.	Co 8013	19.8	36.2	7	S	8.	CoA 89083	15.7	29.0	6	M.S.		
9.	Co 8021	31.7	50.2	9	M.S.	9.	CoA 89084	16.9	28.6	6	M.S.		
10.	Co 8205	8.4	23.9	6	M.S.	10.	CoA 89085	31.7	46.3	8	S		
11.	Co 8212	14.2	29.8	6	M.S.	11.	CoT 8201	33.1	47.9	8	S		
12.	Co 85038	14.1	29.2	6	M.S.	12.	81A 132	11.1	19.0	3	H.S.		
13.	CoA 8401	39.6	54.6	9	H.S.	13.	83A 69	8.9	18.4	2	M.S.		
14.	CoA 8402	10.4	18.9	5	M.S.	14.	83A 124	22.7	37.4	7	S		
15.	CoA 88081	26.1	38.2	7	S	15.	84A 140	17.3	29.8	6	M.S.		
16.	CoA 89081	3.5	9.5	3	R	16.	84A 155	4.7	6.4	3	R		
17.	CoT 8201	36.5	54.7	9	H.S.	17.	84A 191	10.6	19.1	5	M.S.		
18.	83A 214	9.2	20.8	6	M.S.	18.	85A 123	6.3	14.7	4	M.R.		
19.	84A 155	2.9	6.9	3	R	19.	87A 142	3.4	7.7	3	R		
20.	86A 444	3.2	7.1	3	R	20.	87A 279	3.6	8.8	3	R		

R : Resistant; M.R. : Moderately resistant; M.S. : Moderately susceptible; S : Susceptible; H.S. : Highly susceptible

The disease intensity recorded in 40 varieties/clones during 1988-89 and 1989-90 in Rajoong method of inoculation was compared with adult cane inoculation. The PDI values and reaction of each variety in both the screening methods are furnished in Table 39.

The data presented in the Table 39 indicate that the PDI in Rajoong method of inoculation was slightly more than adult cane inoculation in all the varieties in both the crop seasons. More than ninety per cent of the varieties/clones showed same reaction in both the methods of screening. To examine the relationship between these two methods Chi square ( $\chi^2$ ) was worked out.

The observed  $\chi^2$  was less than the  $\chi^2$  from the tables indicating that both the methods are independent and give similar type of reaction. The results thus confirm that Rajoong method of screening is preferable to adult cane screening as it is rapid and the time taken for obtaining the reaction could be reduced by 3 months.

#### 4.11 CHEMICAL CONTROL

##### 4.11.1 In vitro test

##### 4.11.1.1 Preliminary screening

With a view to findout effective fungicides against P. saccharicola in vitro thirteen fungicides were tried employing poisoned food technique as described at para 3.2.11.1. The observations recorded are furnished in Table 40.

Table 39: Disease intensity (PDI) and reaction in different sugarcane varieties/clones under Rajoongs method of inoculation and adult cane inoculation

1988-89										1989-90									
Sl. No.	Variety/ Clone	Rajoongs			Adult cane			Sl. No.	Variety/ Clone	Rajoongs			Adult cane						
		PDI 8 months age of crop	Re- action	Re- action	PDI 11 months age of crop	Re- action	Re- action			PDI 8 months age of crop	Re- action	Re- action	PDI 11 months age of crop	Re- action	Re- action				
1.	Co 419	41.1	S	S	37.9	S	1.	Co 7219	55.7	H.S.	H.S.	50.2	H.S.	H.S.					
2.	Co 975	47.6	S	S	43.2	S	2.	Co 8011	20.7	M.S.	M.S.	17.8	M.S.	M.S.					
3.	Co 997	19.1	H.S.	H.S.	17.6	M.S.	3.	Co 8121	50.6	H.S.	H.S.	45.2	S	S					
4.	Co 8907	43.7	S	S	40.2	S	4.	Co 8211	41.6	S	S	37.8	S	S					
5.	Co 7219	64.5	H.S.	H.S.	58.7	H.S.	5.	Co 85046	21.1	M.S.	M.S.	17.1	M.S.	M.S.					
6.	Co 7636	75.8	H.S.	H.S.	69.4	H.S.	6.	CoA 89081	9.7	R	R	8.9	R	R					
7.	Co 7706	43.6	S	S	39.2	S	7.	CoA 89082	28.7	M.S.	M.S.	24.4	M.S.	M.S.					
8.	Co 8013	36.2	S	S	33.4	S	8.	CoA 89083	29.6	M.S.	M.S.	25.8	M.S.	M.S.					
9.	Co 8021	50.2	H.S.	H.S.	43.7	S	9.	CoA 89084	28.6	M.S.	M.S.	24.5	M.S.	M.S.					
10.	Co 8205	23.9	M.S.	M.S.	20.7	M.S.	10.	CoA 89085	46.3	S	S	41.1	S	S					
11.	Co 8212	29.8	M.S.	M.S.	26.4	M.S.	11.	CoI 8201	47.7	S	S	43.8	S	S					
12.	Co 85038	29.2	M.S.	M.S.	26.9	M.S.	12.	81A 132	19.6	M.S.	M.S.	17.8	M.S.	M.S.					
13.	CoA 8401	54.6	H.S.	H.S.	50.7	H.S.	13.	83A 69	18.4	M.S.	M.S.	14.7	M.R.	M.R.					
14.	CoA 8402	18.9	M.S.	M.S.	17.7	M.S.	14.	83A 124	37.4	S	S	32.4	S	S					
15.	CoA 88081	38.2	S	S	35.1	S	15.	84A 146	29.6	M.S.	M.S.	26.7	M.S.	M.S.					
16.	CoA 89081	7.6	R	R	9.1	S	16.	84A 155	6.3	R	R	2.6	R	R					
17.	CoI 8201	54.7	H.S.	H.S.	50.4	H.S.	17.	84A 191	19.1	M.S.	M.S.	17.6	M.S.	M.S.					
18.	83A 214	20.8	M.S.	M.S.	17.7	M.S.	18.	85A 123	14.7	M.R.	M.R.	11.2	M.R.	M.R.					
19.	84A 155	6.9	R	R	6.1	R	19.	87A 142	7.7	P	P	4.8	P	P					
20.	86A 444	7.1	R	R	6.3	R	20.	87A 279	8.8	P	P	7.1	P	P					

R : Resistant; M.R. : Moderately resistant; M.S. : Moderately susceptible; S : Susceptible; H.S. : Highly susceptible

$\chi^2$  observed = 0.1607  $\chi^2$  observed = 0.6601  
 $\chi^2$  from tables : 30.134  $\chi^2$  from tables : 30.144  
 P = 0.057 P = 0.011

Table 40: Effect of different concentrations of fungicides on the growth of Phyllosticta saccharicola in vitro

Fungicide	*Colony diameter of <u>P. saccharicola</u> in mm 10 days after inoculation at different concentrations			
	100 ppm	200 ppm	500 ppm	1000 ppm
Bavistin	0	0	0	0
Topsin-M	0	0	0	0
Calixin	0	0	0	0
Bayleton	71	0	0	0
Kitazin	100	97	89	82
Hinosan	97	92	86	81
Blitox	61	0	0	0
Captaf	52	0	0	0
Captafol	57	0	0	0
Dithane M-45	68	0	0	0
Dithane Z-78	72	28	0	0
Thiram	98	94	91	84
Chlorothalonil	76	34	0	0
Control	100	100	100	100

\* Mean of three replications

Bavistin, Topsin-M and Calixin were effective at 100 ppm. Five fungicides viz., Captaf, Dithane M-45, Blitox, Captafol and Bayleton were found effective at 200 ppm. Dithane Z-78 and Chlorothalonil completely inhibited the growth at 500 ppm but were ineffective at 100 and 200 ppm. The remaining three fungicides viz., Kitazin, Hincsan and Thiram were ineffective even at 1000 ppm.

#### 4.11.1.2 Effect of three systemic fungicides at different concentrations

The three systemic fungicides viz., Bavistin, Topsin-M and Calixin which were found effective at 100 ppm in the previous study were tested at lower concentrations to find out the minimal effective concentration by poisoned food technique. The data are presented in Table 41.

Table 41: Effect of three systemic fungicides at different concentrations on the growth of Phyllosticta saccharicola

Concentration in ppm	*Colony diameter of <u>P. saccharicola</u> in mm 10 days after inoculation		
	Bavistin	Topsin-M	Calixin
5	46	71	92
10	0	37	68
20	0	0	44
50	0	0	0
Control	100	100	100

\* Mean of three replications

The results indicate that none of the three fungicides were effective at 5 ppm; the lowest concentration tried. Bavistin was effective at 10 ppm while Topsin-M and Calixin were effective at 20 and 50 ppm respectively.

#### In vivo tests

##### 4.11.2 Efficacy of systemic and nonsystemic fungicides in the control of ring spot disease, cane yield and juice quality

Six fungicides which were found to inhibit P. saccharicola in vitro were selected for use as foliar sprays to study their effect on the disease intensity, cane yield and juice quality during 1988-89 and 1989-90 and the details are described at para 3.2.11.2.

##### 4.11.2.1 Effect of fungicides on the intensity of ring spot

The data on the intensity of the disease recorded every crop season at monthly intervals from October till harvest in February are furnished in Table 42.

The data presented in Table 42 clearly indicate that the disease intensity in both the crop seasons increased from October till harvest in February in all the treatments. The increase in the intensity was progressive between October and December while it was only marginal

Table 42: Effect of different fungicides on month wise incidence of ring spot disease of sugarcane from October to February

Treatment	Concentration	PDI											
		1988-89						1989-90					
		October	November	December	January	February	October	November	December	January	February		
Captaf	0.3%	10.6	20.1	24.4	27.3	28.0	8.4	16.9	21.1	23.7	24.6		
Blitox	0.4%	10.9	21.4	26.8	29.6	30.5	8.9	18.2	23.1	25.6	26.7		
Dithane M-45	0.3%	12.8	25.5	29.8	33.6	35.0	9.7	20.2	25.7	28.3	30.0		
Bavistin	0.1%	12.3	23.3	28.7	31.2	32.2	9.1	19.3	24.2	27.4	28.6		
Topsin-M	0.1%	13.7	26.4	31.6	34.9	36.9	12.1	23.6	29.0	31.5	32.5		
Calixin	0.1%	19.7	35.4	45.8	49.6	52.1	15.9	29.9	37.4	41.9	45.1		
Control		22.4	37.9	47.1	51.1	54.5	17.4	30.3	38.9	43.8	46.2		

24

between December to February in all the treatments. The disease in sprayed plots in both the crop seasons was low in all the months of observations as compared to untreated control except in Calixin. The intensity with Calixin was on par with control. With a single spray in September, the disease intensity was reduced to a considerable extent in October in both the crop seasons as compared to untreated control and resulted in low incidence in subsequent months. The data on the intensity of the disease at harvest in February in all the treatments were statistically analysed and furnished in Table 43.

The results in Table 43 reveal that all the fungicides except Calixin reduced the intensity of the disease significantly in comparison with control in both the crop seasons. Among the five fungicides which significantly reduced the disease, Captaf appeared to be most effective followed by Blitox, Bavistin, Dithane M-45 and Topsin-M. Captaf was significantly superior to Dithane M-45, Topsin-M and Calixin and at par with Blitox and Bavistin in both the crop seasons. Calixin was noted to be ineffective in the control of the disease in vivo during both the crop seasons.

Maximum reduction of the disease (46.8 to 48.6%) was obtained with Captaf followed by Blitox (42.2 to 44.0%), Bavistin (38.1 to 40.9%), Dithane M-45 (35.1 to

Table 43: Effect of different fungicides on the intensity of ring spot disease of sugarcane at harvest

Treatment	Concentration	PDI		Per cent reduction in disease intensity over control	
		1988-89	1989-90	1988-89	1989-90
Captaf	0.3%	28.0	24.6	48.6	46.8
Blitox	0.4%	30.5	26.7	44.0	42.2
Dithane M-45	0.3%	35.0	30.0	35.8	35.1
Bavistin	0.1%	32.2	28.6	40.9	38.1
Topsin-M	0.1%	36.9	32.5	32.3	29.7
Calixin	0.1%	52.1	45.1	4.4	2.4
Control		54.5	46.2		
SEm. <sub>t</sub> for treatment		1.6672	1.4284		
C.D. at 5%		5.137	4.401		

35.8%) and Topsin-M (29.7 to 32.3%) during both the crop seasons. Least reduction (2.4 to 4.4%) was obtained with Calixin. In general, the disease intensity was observed to be low during 1989-90 in all the treatments.

#### 4.11.2.2 Effect of fungicides on cane yield

The data on cane yield and its attributes viz., length of millable cane and diameter of the cane recorded at the time of harvest are furnished in Table 44.

It is evident from the results in the Table 44 that the length of cane in both the crop seasons increased in all the sprayed plots over control. Maximum increase was obtained with Captaf (8.0 and 7.2%) followed by Blitox (6.69 and 6.04%), Bavistin (5.6 and 5.3%), Dithane M-45 (3.73 and 3.70%) and Topsin-M (2.9 and 2.7%) in both the crop seasons. Marginal increase in length (0.24 and 0.67%) was obtained with Calixin. However, the increase of length of canes was not statistically significant.

The diameter of the cane in the sprayed plots increased in both the crop seasons as compared to control but it was statistically significant only during 1989-90. Significant increase in diameter was obtained with only 3 fungicides viz., Captaf, Blitox and Bavistin. Maximum increase in diameter was observed with Captaf (9.64 and 9.68%) followed by Blitox (6.02 and 5.65%) Bavistin (6.02 and 4.84%), Dithane M-45 (4.42 and 2.82%) and Topsin-M

Table 44: Effect of different fungicides on yield parameters and yield of sugarcane

Treatment	Concentration	Length of cane in cm		Diameter of the cane in cm		Yield t ha <sup>-1</sup>	
		1988-89	1989-90	1988-89	1989-90	1988-89	1989-90
Captaf	0.3%	313.0 (8.01)	321.5 (7.20)	2.73 (9.64)	2.72 (9.68)	105.99 (8.82)	108.51 (5.76)
Blitox	0.4%	309.2 (6.69)	318.0 (6.04)	2.64 (6.02)	2.62 (5.65)	105.47 (8.29)	108.07 (5.33)
Dithane M-45	0.3%	300.6 (3.73)	311.0 (3.70)	2.60 (4.42)	2.55 (2.82)	102.69 (5.43)	105.30 (2.63)
Bavistin	0.1%	305.9 (5.56)	315.8 (5.30)	2.64 (6.02)	2.60 (4.84)	104.77 (7.57)	107.55 (4.82)
Topsin-M	0.1%	298.2 (2.90)	308.0 (2.70)	2.60 (4.42)	2.55 (2.82)	102.00 (4.72)	104.69 (2.04)
Calixin	0.1%	290.5 (0.24)	301.9 (0.67)	2.51 (0.80)	2.52 (1.61)	97.48 (0.08)	103.56 (0.94)
Control		289.8	299.9	2.49	2.48	97.40	102.60
SEM.±		8.2593	6.8112	0.0569	0.0332	8.4689	11.2616
C.D. at 5%		NS	NS	NS	0.102	NS	NS

Figures in parentheses indicate per cent increase over control

(4.42 and 2.82%) in both the crop seasons. In Calixin treated plot marginal increase (0.8 and 1.61%) was obtained.

The cane yield ( $t\ ha^{-1}$ ) in all the sprayed plots though noted to be more as compared to untreated control, none of the fungicides gave significant increase in both the crop seasons. Out of six test fungicides, Captaf gave maximum increase (8.82 and 5.7%) followed by Blitox (8.29 and 5.33%), Bavistin (7.57 and 4.82%), Dithane M-45 (5.43 and 2.63%) and Topsin-M (4.72 and 2.04%) in both the crop seasons. The increase in cane yield in Calixin treated plot was negligible compared to the untreated plot (0.08 and 0.94%).

Increase in cane yield of more than  $5\ t\ ha^{-1}$  was obtained with Captaf, Blitox, Bavistin and Dithane M-45 during 1988-89 and Captaf and Blitox during 1989-90. The yield differences between untreated control and sprayed plots was more during 1988-89 compared to 1989-90, as disease incidence was low during the latter year in all the treatments including control.

#### **4.11.2.3 Effect of fungicides on juice quality**

The data on juice sucrose and other juice characteristics are presented in Table 45.

Table 45: Effect of different fungicides on juice sucrose and different juice characteristics of sugarcane

Treatment	Concentration	Total solids		Per cent juice sucrose		Per cent purity of juice		Per cent glucose		Electrical conductivity m.mhos/cm		pH*	
		1988-89	1989-90	1988-89	1989-90	1988-89	1989-90	1988-89	1989-90	1988-89	1989-90	1988-89	1989-90
Captaf	0.3%	20.4 (13.97)	20.4 (12.71)	19.08 (18.66)	18.96 (16.46)	93.54 (4.10)	92.93 (3.52)	0.287 (38.94)	0.285 (31.65)	2.332 (26.06)	2.279 (29.14)	5.27 (0.24)	5.35 (0.31)
Blitox	0.4%	20.3 (13.41)	20.3 (12.15)	18.99 (18.10)	18.88 (15.97)	93.71 (4.26)	92.63 (3.41)	0.294 (37.45)	0.292 (29.98)	2.703 (14.30)	2.712 (15.67)	5.23 (0.20)	5.34 (0.30)
Dithane M-45	0.3%	19.8 (10.61)	20.1 (11.95)	18.74 (16.54)	18.55 (13.94)	94.49 (5.15)	92.30 (2.82)	0.300 (36.17)	0.255 (29.26)	2.730 (13.44)	2.756 (14.30)	5.30 (0.27)	5.41 (0.37)
Bavistin	0.1%	20.1 (12.29)	20.2 (11.60)	18.92 (17.66)	18.72 (14.99)	94.01 (4.62)	92.54 (3.09)	0.253 (36.60)	0.299 (28.30)	2.694 (14.58)	2.738 (14.86)	5.27 (0.24)	5.32 (0.26)
Trosin-M	0.1%	19.8 (10.61)	20.0 (10.50)	18.69 (16.23)	18.46 (13.39)	94.24 (4.87)	92.09 (2.86)	0.364 (42.55)	0.329 (21.10)	2.403 (13.40)	2.491 (22.54)	5.23 (0.20)	5.27 (0.23)
Calixin	0.1%	18.8 (5.03)	19.5 (7.73)	17.42 (8.33)	17.86 (9.71)	92.56 (3.12)	91.90 (2.37)	0.349 (25.74)	0.323 (22.54)	2.880 (8.69)	2.879 (10.48)	5.40 (0.17)	5.22 (0.18)
Control		17.9	18.1	18.08	18.26	89.86	89.77	0.471	0.417	3.724	3.217	5.03	5.04
SEM <sub>t</sub>		0.2772	0.2811	0.1697	0.2856	0.4750	0.3463	0.0150	0.0055	0.3310	0.0429	0.0300	0.0526
C.D. at 5%		0.8840	0.8661	0.5844	0.8800	1.4635	1.0669	0.0479	0.0169	0.5970	0.1318	0.0521	0.1520

Figures in parentheses indicate per cent increase/decrease over control

\* Difference over control

Significant increase in total solids was obtained with all the fungicides over control. The fungicides Captaf, Blitox, Bavistin, Dithane M-45 and Topsin-M were at par with one another in both the crop seasons. The per cent increase ranged from 3.03 to 13.97 during 1988-89 while it was 7.73 to 12.71 during 1989-90. Maximum increase was obtained with Captaf (13.97% and 12.71%) while it was minimum with Calixin (5.03 and 7.73%).

The per cent juice sucrose significantly increased with all the test fungicides as compared to control. Except Calixin, all other fungicides were on par with each other. The increase in per cent juice sucrose ranged from 8.33 to 18.66 per cent during 1988-89, while it was 9.71 to 16.46 per cent during 1989-90. Maximum increase was obtained with Captaf (18.66 and 16.46%) followed by Blitox (18.10 and 15.97%), Bavistin (17.66 and 14.99%), Dithane M-45 (16.54 and 13.94%) and Topsin-M (16.23 and 13.39%). Minimum increase was obtained with Calixin (8.33 and 9.71%).

Significant increase in purity of juices was obtained with all the test fungicides over control. The purity increased by 3.12 to 5.15% during 1988-89 and 2.37 to 3.52% during 1989-90. Maximum purity of 5.15% was obtained with Dithane M-45 during 1988-89 while it was 3.52% with Captaf during 1989-90. Minimum increase was

obtained with Calixin in both the crop seasons as compared to other fungicides.

The glucose content was significantly reduced with all the test fungicides over control. The per cent decrease ranged from 22.55 to 38.94 during 1988-89 while it was 21.10 to 31.65 per cent during 1989-90. Maximum decrease was obtained with Captaf in both the crop seasons and significantly superior to all the other fungicides.

Significant increase in electrical conductivity was obtained with all the fungicides. The per cent decrease ranged from 8.69 to 26.06 per cent during 1988-89 and 10.48 to 29.14 per cent during 1989-90. Maximum decrease was obtained with Captaf (26.06 and 29.14%) followed by Topsin-M (23.8 and 22.54%) in both the crop seasons.

The hydrogen ion concentration (pH) has significantly increased with all the test fungicides over control. The increase in pH was 0.17 to 0.27 during 1988-89 and 0.18 to 0.37 during 1989-90. Maximum increase in pH was obtained with Dithane M-45 (0.27 and 0.37) followed by Captaf (0.24 to 0.31) in both the crop seasons.

The results indicate that all the test fungicides except Calixin significantly reduced the disease intensity over control. The length of cane, diameter of the cane and cane yield increased with all the test fungicides but they

were not statistically significant. Significant increase in total solids, juice sucrose, purity of juice and pH and significant decrease in per cent glucose and electrical conductivity were obtained with all the test fungicides over control. Five out of six fungicides viz., Captaf, Blitox, Bavistin, Dithane M-45 and Topsin-M were found effective in reducing ring spot disease in that order.

#### 4.12 LOSSES DUE TO RING SPOT DISEASE IN DIFFERENT SUGARCANE VARIETIES/CLONES

##### 4.12.1 Loss of chlorophyll due to the disease

To study the effect of ring spot disease on chlorophyll content of sugarcane leaves, chlorophyll a and b were estimated in five resistant (84A155, CoA 89081, 86A444, Co 85045 and 83A106) and five susceptible (Co 7219, Co 7636, CoT 8201, Co 85044 and Co 975) varieties/clones 4 months after inoculation in both sprayed (captaf 0.3%) and unsprayed treatments as per the procedure described at para 3.2.12.3. The data recorded on PDI and chlorophyll a and b contents in sprayed and unsprayed canes of both resistant and susceptible varieties/clones are furnished in Table 46.

The results clearly indicate that increased disease intensity in unsprayed canes of susceptible varieties resulted in less content of chlorophyll a and b as compared to sprayed canes. In resistant varieties/

Table 46: Influence of ring spot disease on the content of chlorophyll a and b in captaf sprayed and unsprayed canes of different varieties/clones of sugarcane

variety	PDI		Chlorophyll a mg/g		Chlorophyll b mg/g		Per cent reduction
	Sprayed	Unsprayed	Sprayed	Unsprayed	Sprayed	Unsprayed	
<b>Susceptible</b>							
Co 7219	25.8	53.8	0.459	0.255	0.299	0.169	43.48
Co 7636	48.3	66.2	0.924	0.703	0.279	0.142	49.11
Co 18201	22.4	46.2	0.506	0.290	0.296	0.188	36.48
Co 89044	29.6	62.2	0.451	0.215	0.266	0.157	48.49
Co 975	20.2	37.6	0.496	0.298	0.298	0.214	29.19
<b>Resistant</b>							
84A 155	3.6	4.6	0.676	0.650	0.309	0.274	9.33
Co A89081	4.9	7.8	0.674	0.644	0.321	0.294	8.41
86 A444	3.4	4.7	0.606	0.585	0.326	0.314	3.68
Co 85045	4.0	7.6	0.596	0.559	0.317	0.306	2.84
83 A106	4.2	7.8	0.594	0.604	0.302	0.281	4.75

clones there was not much variation in the chlorophyll a and b between sprayed and unsprayed canes. In susceptible varieties, the per cent reduction in chlorophyll a, in unsprayed canes ranged from 39.91 to 52.53 while it ranged from 28.19 to 49.11 for chlorophyll b. In resistant varieties/clones the per cent reduction in chlorophyll a (1.37 to 5.25) and chlorophyll b (2.84 to 8.41) in unsprayed canes was low compared to that in susceptible varieties.

The relationship between PDI and per cent reduction in chlorophyll a and b was worked out by simple correlation and the data are presented in Table 47.

Table 47: Simple correlation between per cent disease intensity and chlorophyll a and b

Varieties/ clones	Per cent reduction in chlorophyll a over sprayed	Per cent reduction in chlorophyll b over sprayed
Resistant n=5	+0.6891	+0.4214
Susceptible n=5	+0.9645**	+0.9855**

\*\* Significant at 1% level

Although there was positive correlation in resistant varieties/clones between PDI and reduction in chlorophyll a and b in unsprayed treatment, it was not significant. But in susceptible varieties highly significant positive correlation was obtained in unsprayed treatment.

91

These results clearly indicate that the disease causes considerable loss in both chlorophyll a and b contents of sugarcane leaves especially in susceptible varieties.

#### 4.12.2.1 Loss in length of millable cane, diameter of cane and cane weight

The effect of disease in respect of length of millable cane, diameter of the cane and cane weight was studied in twenty varieties/clones in both Captaf (0.3%) sprayed and unsprayed treatments. Length of millable cane, diameter and weight of cane were recorded in twenty canes at random in each variety/clone in both sprayed and unsprayed treatments as per the procedure described at para 3.2.12.1.

The PDI in all the test varieties/clones in both sprayed and unsprayed treatments, recorded at the time of harvest is presented in Table 48. The particulars of per cent reduction in cane length (cm), diameter of the cane (cm) and cane weight (kg) of sprayed and unsprayed canes in respect of 20 varieties/clones recorded six months after inoculation at harvest are furnished in Table 49 and detailed data in Appendix A to C.

The data in Table 48 clearly indicate that the disease intensity was reduced considerably (40.50 to 56.89%) in sprayed canes of all the test varieties/clones.

Table 48: Per cent reduction of disease intensity in captaf sprayed canes over unsprayed canes in different sugarcane varieties/clones

Variety/ clone	PDI		Per cent reduction in sprayed canes
	Sprayed	Unsprayed	
Co 419	20.4	38.6	47.15
Co 975	22.2	45.3	50.99
Co 7219	28.5	58.4	49.31
Co 7636	29.4	68.2	56.89
Co 7706	19.6	40.8	51.96
Co 8205	10.7	18.9	43.39
Co 8220	16.6	27.9	40.50
Co 62175	19.4	37.5	48.40
Co 85038	17.2	29.1	40.89
Co 85044	28.8	66.3	56.56
Co 85045	4.2	9.0	53.33
CoA 7602	19.6	41.1	52.31
CoA 8401	28.1	53.2	47.18
CoA 8402	9.7	20.4	52.45
CoA 89081	4.5	9.4	52.12
CoT 8201	23.9	47.2	49.36
83A 106	4.7	9.9	52.53
83A 214	11.3	20.6	45.15
84A 155	2.8	6.4	56.25
86A 444	3.1	6.8	54.41
Mean	16.24	32.76	50.06

Table 49: Per cent disease intensity and per cent reduction in length of millable cane, diameter and weight of cane in unsprayed over captaf sprayed canes

Variety/ clone	PDI	Per cent reduction in		
		Length of millable cane	Diameter of the cane	Cane weight
Co 419	38.6	3.72	6.34	5.04
Co 975	45.3	4.24	7.95	6.94
Co 7219	58.4	7.81	9.45	8.42
Co 7636	68.2	11.71	11.40	11.71
Co 7706	40.8	3.82	4.92	5.07
Co 8205	18.9	1.73	3.48	2.94
Co 8220	27.9	2.44	4.83	2.76
Co 62175	37.6	2.69	5.61	3.63
Co 85038	29.1	2.71	4.56	3.57
Co 85044	66.3	9.13	12.12	11.85
Co 85045	9.0	0.40	1.06	0.65
Co A7602	41.1	3.77	5.83	5.34
CoA 8401	53.2	4.71	6.49	7.21
CoA 8402	20.4	2.00	3.33	3.75
CoA 89081	9.4	0.92	2.17	0.78
CoT 8201	49.2	4.81	8.90	6.36
83A 106	9.9	1.10	2.49	0.72
83A 214	20.6	2.01	2.19	2.54
84A 155	6.4	0.00	0.00	0.00
86A 444	6.8	0.00	0.00	0.00

More than 50 per cent reduction in the intensity was observed in eleven out of 20 varieties/clones tested.

There was reduction in the length of cane (0.40 to 11.71%), diameter of the cane (1.06 to 12.12%) and cane weight (0.65 to 11.85%) in unsprayed canes of 18 varieties/clones (Table 49). More than 10 per cent reduction in cane weight was observed in Co 85044 and Co 7636. In two clones, 84A155 and 86A444, no reduction was observed in all the three yield attributes due to the disease.

To examine whether the differences in the three quantitative characters between sprayed and unsprayed canes are significant or not, paired 't' test was conducted and the data are presented in Table 50.

The results of the 't' test for the three quantitative characters - Length of millable cane, stalk diameter and cane weight - clearly show that the differences are significant.

To observe the relationship between the extent of disease and per cent reduction in cane length, diameter of the cane and cane weight in unsprayed canes over sprayed canes, Chi-square ( $\chi^2$ ) was worked and the data are presented in Table 51 to 53.

The observed  $\chi^2$  values (Tables 51 to 53) were significantly higher than the  $\chi^2$  from tables indicating

Table 50: Results of 't' test of length of millable cane, diameter and weight of cane (n=20)

Character	Sprayed cane	Unsprayed cane	Difference	Observed value of 't'	't' from tables (P=0.05)	Whether significant or not
Length of millable cane (cm)	293.1	282.8	10.3	5.32	2.09	Yes
Diameter of cane (cm)	2.79	2.64	0.15	6.66	2.09	Yes
Cane weight (kg)	1.214	1.157	0.057	4.37	2.09	Yes

Table 51: Relationship between per cent disease intensity and reduction in length of millable cane of unsprayed over captaf sprayed canes in twenty sugarcane varieties/clones

PDI	Number of varieties/clones				Total
	Per cent reduction in length of millable cane in unsprayed over sprayed canes		Per cent reduction in length of millable cane		
	2.0 or below	4.1 to 6.0	6.1 to 8.0	8.1 to 10.0	10.1 to 12.0
10 below	5	-	-	-	5
10.1 to 20.0	1	-	-	-	1
20.1 to 30.0	1	1	-	-	4
30.1 to 40.0	-	2	-	-	2
40.1 to 50.0	-	2	2	-	4
50.1 to 60.0	-	-	1	-	2
60.1 to 70.0	-	-	-	1	2
Total	7	7	3	1	20
$\chi^2$ observed = 54.82					$\chi^2$ from tables (P=0.05) = 16.49

Table 52: Relationship between per cent disease intensity and reduction in diameter of unsprayed canes over captaf sprayed canes in twenty sugar-cane varieties/clones

PDI	Number of varieties/clones						Total
	Per cent reduction in diameter of unsprayed canes over sprayed canes						
	2.0 or below	4.1 to 6.00	6.1 to 8.00	8.1 to 10.0	10.1 to 12.0	12.1 to 14.0	
10 below	3	2	-	-	-	-	5
10.1 to 20.0	-	1	-	-	-	-	1
20.1 to 30.0	-	2	2	-	-	-	4
30.1 to 40.0	-	-	1	1	-	-	2
40.1 to 50.0	-	-	2	1	1	-	4
50.1 to 60.0	-	-	-	1	1	-	2
60.1 to 70.0	-	-	-	-	-	1	1
Total	3	5	5	3	2	1	20

$\chi^2$  observed = 48.93       $\chi^2$  from tables (P = 0.05) = 18.49

Table 53: Relationship between per cent disease intensity and reduction in weight of unsprayed canes over captaf sprayed canes in twenty sugarcane varieties/clones

PDI	Number of varieties/clones				Total
	Per cent reduction in cane weight in unsprayed canes over sprayed canes				
	2.0 or below	4.1 to 6.0	6.1 to 8.0	8.1 to 10.1 to 12.0	
10 below	5	-	-	-	5
10.1 to 20.0	-	1	-	-	1
20.1 to 30.0	-	4	-	-	4
30.1 to 40.0	-	1	1	-	2
40.1 to 50.0	-	-	2	2	4
50.1 to 60.0	-	-	1	1	2
60.1 to 70.0	-	-	-	2	2
Total	5	6	3	1	20

$\chi^2$  observed = 70.03       $\chi^2$  from tables (P = 0.05) = 16.49

that length of millable cane, diameter of cane and cane weight are dependent on the extent of severity of ring spot disease in a variety/clone.

The simple correlations and linear regressions between PDI and per cent reduction in the three quantitative characters of cane were worked out and the data are presented in Table 54.

All the three correlation coefficients were found to be positive and highly significant. Similarly the regression coefficients were also observed to be significant, indicating the deleterious effects of the disease on the three economic attributes.

With every one unit increase in PDI value, the reduction in length of millable cane, diameter and weight of cane increased by 0.14 cm, 0.17 cm and 0.17 kg respectively.

#### 4.12.2.2 Loss in quality of cane juice

The effect of the disease on various qualitative characters of juice viz., total solids, sucrose per cent in juice, juice purity, glucose, pH and electrical conductivity (E.C.) was determined in different varieties under sprayed and unsprayed conditions as described at para 3.2.11.2. Particulars of the reduction/increase in the six juice characteristics in unsprayed diseased canes

Table 54: Simple correlation and linear regression relationship between per cent disease intensity and length of millable cane (LMC), diameter and weight of cane

Particulars	Per cent reduction in LMC over sprayed canes	Per cent reduction in diameter of cane over sprayed canes	Per cent reduction in cane weight over sprayed canes
Simple correlation coefficient**	+ 0.24	+ 0.97	+ 0.97
Regression equation	$\hat{Y} = -1.22 + 0.14x$	$\hat{Y} = -0.40 + 0.17x$	$\hat{Y} = -1.05 + 0.17x$
'F' value	151.06	270.1	332.7

'F' from tables (P = 0.05) = 4.4  
 \*\* Significant at 1% level

as compared to sprayed canes in respect of 20 varieties/ clones recorded, six months after inoculation are furnished in Table 55 and the detailed data in Appendix D to f.

The data in Table 55 clearly indicate that the total solids and per cent juice sucrose were reduced in unsprayed canes of all the twenty varieties/clones under test. The per cent reduction in total solids was from 0.47 to 18.05 while it was 0.51 to 22.88 for per cent juice sucrose. More than 15 per cent reduction in juice sucrose was observed in 5 varieties viz., CoT 8201, Co 7219, CoA 8401, Co 85044 and Co 7636. The juice purity was also affected in the unsprayed canes of eighteen out of twentys varieties/clones tested. In two clones viz., 84A155 and 86A444, the juice purity was not affected by the disease. A fall in pH of juice was observed in almost all the varieties/clones tested except in three viz., 84A155 and 86A444 and Co 85045.

Rise in glucose and electrical conductivity of juice was observed in unsprayed canes of all the test varieties/clones. The rise in glucose content ranged from 0.01 to 0.25 units while it was 0.01 to 1.00 unit for electrical conductivity.

To see whether the differences in the various juice characteristics between sprayed and unsprayed canes

Table 55: Per cent disease infection in unsprayed mango and treatment increase in total solids, juice content, acidity, vitamin C, electrical conductivity, and Brix unsprayed over control sprayed mango juice.

Variety/ clone	PHI	Per cent reduction in total solids	Per cent reduction in juice sugars	Per cent reduction in juice acidity	Increase in glucose (units)	Increase in Brix	Gain in pH
Co 419	38.6	10.18	6.21	0.29	0.11	0.34	0.10
Co 975	45.3	11.84	13.97	2.64	0.13	1.54	0.10
Co 7219	58.4	15.30	16.64	4.49	0.20	0.80	0.13
Co 7536	60.2	16.05	22.86	7.21	0.25	1.00	1.27
Co 7706	40.8	9.79	11.57	4.06	0.11	0.41	1.17
Co 8206	18.9	3.08	6.11	5.31	0.05	0.18	1.01
Co 8220	27.9	3.98	9.50	5.74	0.07	0.24	1.11
Co 62175	37.6	8.02	9.46	1.59	1.10	0.30	1.17
Co 85038	29.1	5.00	6.95	4.17	1.08	0.26	1.16
Co 85044	66.3	16.84	31.65	7.78	0.24	0.58	0.21
Co 85045	9.0	1.17	1.36	0.37	1.02	0.01	1.04
CoA 7602	41.1	10.16	13.76	3.64	0.11	0.43	1.11
CoA 8401	53.2	11.97	16.75	2.47	0.16	0.70	1.14
CoA 8402	20.4	3.77	6.35	2.60	0.06	0.20	1.01
CoA 89081	9.4	2.37	2.61	1.75	0.04	0.10	1.03
CoT 9201	49.2	11.56	15.11	4.06	0.14	0.70	1.14
83A 106	9.9	2.47	3.24	0.94	0.03	0.10	1.03
83A 216	20.6	3.63	6.05	2.52	0.07	0.21	0.16
84A 155	6.4	0.47	0.61	0.16	0.01	0.01	0.01
86A 446	6.8	1.02	0.45	0.09	0.01	0.01	0.01

are significant or not 't' test was conducted and the data are presented in Table 56.

The differences between the various characters of juice from sprayed and unsprayed canes are found to be significant.

To study the relationship between the disease intensity and (1) Per cent reduction in total solids (2) Per cent reduction in juice sucrose, (3) Per cent reduction in purity of juice (4) Per cent increase in glucose (5) Per cent increase in E.C. and (6) decrease in pH, Chi square ( $\chi^2$ ) was calculated and the data are presented in Tables 57 to 62.

The extent of depression in total solids, juice sucrose, purity and pH and increase in glucose and E.C. in different varieties was found to be associated with the extent of severity of the disease as can be seen from the significant Chi square values in Tables 57 to 62.

The regression of either fall or increase of the various qualitative characters of juice in unsprayed canes on PDI exhibited by the varieties/clones is presented in Table 63.

A close examination of the data indicate that the variation in total solids, per cent juice sucrose, purity of juice, glucose content, pH and E.C. is dependent on the range in disease intensity as can be seen from the

Table 56: Results of 't' test of the six Juice characteristics of sprayed and unsprayed canes of twenty sugarcane varieties/clones

Character Particulars	Total solids	Sucrose per cent	Juice purity	Per cent glucose	pH	E.C.
Sprayed Cane	20.0	18.13	90.82	0.43	5.28	2.49
Unsprayed cane	18.4	16.30	88.17	0.53	5.18	2.88
Difference	1.6	1.83	2.65	0.10	0.10	0.39
Observed 't' value	6.08	6.69	6.39	6.30	6.34	5.39
t value from tables (P=0.05)	2.09	2.09	2.09	2.09	2.09	2.09
Whether significant OR not	Yes	Yes	Yes	Yes	Yes	Yes

Table 57: Relationship between per cent disease intensity and reduction in total solids of cane juice of unsprayed canes over captaf sprayed canes in twenty sugarcane varieties/clones

POI	Number of varieties/clones					Total
	Per cent reduction in total solids of unsprayed over sprayed cane juice					
	4.0 or below	4.1 to 6.00	8.1 to 10.00	12.1 to 16.0	16.1 to 20.0	
10 below	5	-	-	-	-	5
10.1 to 20.0	1	-	-	-	-	1
20.1 to 30.0	2	1	-	-	-	4
30.1 to 40.0	-	-	2	-	-	2
40.1 to 50.0	-	-	4	-	-	4
50.1 to 60.0	-	-	1	1	-	2
60.1 to 70.0	-	-	-	-	2	2
<b>Total</b>	<b>9</b>	<b>1</b>	<b>7</b>	<b>1</b>	<b>2</b>	<b>20</b>

$\chi^2_{2}$  observed = 51.46       $\chi^2$  from tables (P=0.05) = 13.85

Table 58: Relationship between per cent disease intensity, and reduction in juice sucrose of unsprayed canes over captaf sprayed canes in twenty sugarcane varieties/clones

POI	Number of varieties/clones					Total
	Per cent reduction in juice sucrose in unsprayed cane juice over sprayed cane juice					
	4.0 or below	4.1 to 6.00	8.1 to 12.0	12.1 to 16.0	16.1 to 20.0	
10 below	5	-	-	-	-	5
10.1 to 20.0	-	-	1	-	-	1
20.1 to 30.0	-	2	2	-	-	4
30.1 to 40.0	-	-	2	-	-	2
40.1 to 50.0	-	-	1	3	-	4
50.1 to 60.0	-	-	-	-	2	2
60.1 to 70.0	-	-	-	-	-	2
<b>Total</b>	<b>5</b>	<b>2</b>	<b>6</b>	<b>3</b>	<b>2</b>	<b>20</b>

$\chi^2_{2}$  observed = 79.17       $\chi^2$  from tables (P=0.05) = 18.44

Table 59: Relationship between per cent disease intensity and reduction in juice purity of unsprayed canes over capital sprayed canes in twenty sugarcane varieties/clones

PCI	Number of varieties/clones							Total
	Per cent reduction in purity of juice in unsprayed canes over sprayed canes							
	0.0 to 1.0	1.1 to 2.0	2.1 to 3.0	3.1 to 4.0	4.1 to 5.0	5.1 to 6.0	6.1 to 7.0	
10 below	5	-	-	-	-	-	-	5
10.1 to 20.0	-	-	-	-	-	1	-	1
20.1 to 30.0	-	-	2	-	1	1	-	4
30.1 to 40.0	1	1	-	-	-	-	-	2
40.1 to 50.0	-	-	1	1	2	-	-	4
50.1 to 60.0	-	-	1	-	1	-	-	2
60.1 to 70.0	-	-	-	-	-	1	1	2
<b>Total</b>	<b>6</b>	<b>1</b>	<b>4</b>	<b>2</b>	<b>4</b>	<b>3</b>	<b>1</b>	<b>20</b>

$\chi^2$  observed = 52.5

$\chi^2$  from tables ( $P=0.05$ ) = 13.49

Table 60: Relationship between per cent disease intensity and increase in glucose of juice of unsprayed canes over capital sprayed canes in twenty sugarcane varieties/clones

PCI	Number of varieties/clones					Total
	Per cent increase in glucose in juice of unsprayed canes over sprayed					
	20.1 or below	20.1 to 40.0	40.1 to 60.0	60.1 to 80.0	80.1 to 100.0	
10 below	5	-	-	-	-	5
10.1 to 20.0	1	-	-	-	-	1
20.1 to 30.0	1	-	-	-	-	1
30.1 to 40.0	-	2	-	-	-	2
40.1 to 50.0	1	2	1	-	-	4
50.1 to 60.0	-	-	1	1	-	2
60.1 to 70.0	-	-	-	1	1	2
<b>Total</b>	<b>11</b>	<b>4</b>	<b>2</b>	<b>2</b>	<b>1</b>	<b>20</b>

$\chi^2$  observed = 41.14

$\chi^2$  from tables ( $P=0.05$ ) = 13.89

Table 61: Relationship between per cent disease intensity and increase in electrical conductivity (E.C.) of juice of unsprayed canes over captaf sprayed canes in twenty sugarcane varieties/clones

PDI	Number of varieties/clones					Total
	Per cent increase in electrical conductivity in juice of unsprayed canes over sprayed					
	10.0 or below	10.1 to 20.0	20.1 to 30.0	30.1 to 40.0	40.1 to 50.0	
10 below	5	-	-	-	-	5
10.1 to 20.0	1	-	-	-	-	1
20.1 to 30.0	2	1	-	-	-	3
30.1 to 40.0	-	2	-	-	-	2
40.1 to 50.0	-	1	2	1	-	4
50.1 to 60.0	-	-	1	1	-	2
60.1 to 70.0	-	-	-	1	1	2
<b>Total</b>	<b>8</b>	<b>4</b>	<b>3</b>	<b>3</b>	<b>1</b>	<b>20</b>

$\chi^2$  observed = 39.17       $\chi^2$  from tables (P=0.05) = 13.83

Table 62: Relationship between per cent disease intensity and decrease in pH in juice of unsprayed canes over captaf sprayed canes in twenty sugarcane varieties/clones

PDI	Number of varieties/clones				Total
	Decrease in pH (units) values of juice of unsprayed canes over sprayed				
	0.00 to 0.05	0.06 to 0.10	0.11 to 0.15	0.16 to 0.20	
10 below	1	-	-	-	1
10.1 to 20.0	1	-	-	-	1
20.1 to 30.0	1	3	-	-	4
30.1 to 40.0	-	1	1	-	2
40.1 to 50.0	-	2	2	-	4
50.1 to 60.0	-	-	1	-	1
60.1 to 70.0	-	-	-	2	2
<b>Total</b>	<b>4</b>	<b>6</b>	<b>4</b>	<b>2</b>	<b>16</b>

$\chi^2$  observed = 44.66       $\chi^2$  from tables (P=0.05) = 5.39

Table 63: Linear regression relationship between per cent disease intensity and the six qualitative characteristics of cane juice

Particulars Character	Simple correlation coefficient	Regression equation	'F' value
Reduction in total solids	0.98**	$\hat{Y} = -1.33 + 0.27x$	484.76
Reduction in sucrose	0.98**	$\hat{Y} = -0.66 + 0.33x$	806.76
Reduction in juice purity	0.66**	$\hat{Y} = 0.71 + 0.07x$	13.66
Increase in glucose	0.98**	$\hat{Y} = -0.015 + 0.004x$	489.71
Fall in pH	0.95**	$\hat{Y} = -0.005 + 0.003x$	164.62
Increase in E.C.	0.97**	$\hat{Y} = -0.13 + 0.015x$	353.44

'F' from tables (P = 0.05) = 4.4

\*\* Significant at 1% level

significant correlation coefficients and regression coefficients.

With every one unit increase in PDI value the reduction in total solids, sucrose content, purity and pH of juices increased by 0.27, 0.33, 0.07 and 0.003 units respectively. Similarly the increase in glucose and E.C. was found to have increased by 0.004 and 0.015 units respectively with every one unit increase in PDI value.

#### 4.12.3 Rate of reduction in cane weight and juice sucrose in different varieties/clones in maturity phase of the crop

The rate of reduction in cane weight and juice sucrose in different varieties/clones in the maturity phase of the crop was studied as described at para 3.2.12.2. The disease intensity recorded in each variety/clone at monthly intervals from November to February both in sprayed and unsprayed treatments are furnished in Table 64. The data on progress of reduction in cane weight and juice sucrose in unsprayed canes over sprayed canes in all the test varieties/clones are given in Table 65.

The data in Table 64 indicate that there was progressive increase in the intensity of the disease in the maturity phase of the crop (November to February) in all the test varieties/clones. The increase was not so

Table 64: Progress of ring spot disease in maturity phase of the crop in captaf sprayed and unsprayed canes of different sugarcane varieties/clones

S.No. Variety/ clone	Per cent disease intensity							
	November, 1990 Sprayed	Unsp- rayed	December, 1990 Sprayed	Unsp- rayed	January, 1991 Sprayed	Unsp- rayed	February, 1991 Sprayed	Unsp- rayed
<b>Resistant</b>								
1. 84A 155	0.8	2.2	1.7	3.9	2.3	5.4	2.5	6.4
2. 86A 444	0.9	2.4	1.9	4.2	2.6	5.6	3.1	6.8
3. CoA 89061	2.2	5.2	3.5	7.1	4.1	8.2	4.5	9.0
4. Co 85045	2.1	4.9	3.4	7.2	4.3	8.9	4.7	9.7
5. 83A 106	1.4	3.2	3.4	7.4	4.2	9.0	4.7	9.9
<b>Susceptible</b>								
1. Co 7219	21.1	40.4	25.5	51.2	28.1	55.3	28.6	58.4
2. CoT 8201	13.2	29.4	18.4	40.6	22.1	45.2	23.9	49.2
3. Co 975	11.6	25.9	17.9	37.2	20.9	41.4	22.2	45.3
4. Co 85044	19.6	46.7	25.2	58.2	27.7	63.6	28.5	67.3
5. Co 7636	23.7	51.2	27.7	62.1	30.0	67.9	31.4	70.2

Table 65: Progress of reduction in cane weight and juice sucrose in unsprayed canes in maturity phase of different sugarcane varieties/clones

Sl. No.	Variety/ clone	November 1990			December 1990			January 1991			February 1991		
		% reduction in cane weight in unsprayed	% reduction in juice sucrose in unsprayed	% reduction in cane weight in unsprayed	% reduction in juice sucrose in unsprayed	% reduction in cane weight in unsprayed	% reduction in juice sucrose in unsprayed	% reduction in cane weight in unsprayed	% reduction in juice sucrose in unsprayed	% reduction in cane weight in unsprayed	% reduction in juice sucrose in unsprayed	% reduction in cane weight in unsprayed	% reduction in juice sucrose in unsprayed
<b>Resistant</b>													
1.	54P 155	0.0	0.13	0.0	0.14	0.0	0.74	0.0	0.0	0.0	0.0	0.0	
2.	86A 444	0.0	0.25	0.0	0.70	0.0	1.08	0.0	0.0	0.0	0.0	0.0	
3.	Co 869081	0.0	1.24	0.41	2.19	0.68	2.62	0.78	0.0	0.0	0.0	0.0	
4.	Co 85045	0.0	1.57	0.10	2.28	0.25	2.64	0.72	0.0	0.0	0.0	0.0	
5.	83A 106	0.10	1.60	0.36	2.82	0.49	2.94	0.96	0.0	0.0	0.0	0.0	
<b>Susceptible</b>													
1.	Co 7200	5.26	14.47	7.42	16.06	8.01	16.46	8.42	16.00	0.0	0.0	0.0	
2.	CoT 8201	0.74	12.54	5.45	13.61	5.95	13.81	6.56	14.04	0.0	0.0	0.0	
3.	Co 875	2.86	12.40	6.19	13.48	6.62	13.71	6.94	13.87	0.0	0.0	0.0	
4.	Co 85044	4.82	19.37	10.33	20.74	11.28	21.29	11.65	21.63	0.0	0.0	0.0	
5.	Co 7634	6.04	20.44	10.71	22.23	11.41	24.68	11.71	25.00	0.0	0.0	0.0	

marked in sprayed and unsprayed canes of resistant varieties/clones. The per cent intensity of the disease in resistant varieties/clones ranged from 0.8 to 4.7 in sprayed canes while it was 2.2 to 9.9 in unsprayed canes from November to February. In susceptible varieties, the PDI was observed to be more in sprayed (11.6 to 23.7) and unsprayed (25.9 to 51.2) canes even in November which gradually increased from 22.2 to 31.4 per cent in sprayed and 45.3 to 70.2 per cent in unsprayed canes till harvest in February. There was marked increase in the intensity of disease between November and December while the increase was comparatively less in January and February.

With progressive increase in the disease intensity, there was progressive reduction in cane weight and juice quality in all the test varieties/clones especially in susceptible ones (Table 65). The per cent reduction in cane weight and juice sucrose in unsprayed canes of resistant varieties/clones from November to February was marginal and ranged from 0.0 to 0.96 per cent and 0.13 to 3.14 per cent respectively. But in susceptible varieties the reduction in cane weight and juice sucrose was more and ranged from 2.74 to 11.85 per cent and 12.40 to 22.88 per cent respectively from November till February. The cane weight was not affected in two resistant clones viz., 84A155 and 86A444 during November and December while a slight reduction was noted during January and February in

all the varieties/clones except 84A155. But the per cent reduction in juice sucrose gradually increased from November onwards only to a limited extent (0.13 to 0.96%). In susceptible varieties, the reduction in cane weight and juice sucrose in unsprayed canes was conspicuous even in November which rapidly increased upto December. The increase in reduction was marginal between January and February. Thus, the disease gradually increased from November to February causing progressive reduction in cane weight and juice sucrose.

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# DISCUSSION

## CHAPTER V

### DISCUSSION

The results of the present study on ring spot disease of sugarcane incited by Leptosphaeria sacchari Van Breda de Hann conducted at Regional Agricultural Research Station, Anakapalle (Andhra Pradesh) are discussed in this chapter in the light of the literature available and conclusions drawn.

#### 5.1 SYMPTOMATOLOGY

In the present study, symptoms were observed only on the leaf blade of all the varieties studied. However, Bourne (1934) and Abbott (1964) observed the disease in nature on leaf blade and occasionally on leaf sheath and cane stalk. The absence of symptoms on leaf sheath and cane stalk in the present study might be due to varietal behaviour.

In the present investigations, the course of development of symptoms over a period of time was studied in four sugarcane varieties viz. Co 7219, CoT 8201, CoA 89081 and Co 85045. The disease appeared first on 11th or 12th order leaf from top during last week of August at the age of 5 months in Co 7219 and CoT 8201. Spots which appeared initially as small specks gradually enlarged, turned oval or spherical with purplish colouration in 15 days. Dry necrotic centres with margins that were narrow

reddish purple or brownish, continuous or broken with angular projections all round appeared. Subsequently a diffusing yellow halo at the perimeter also appeared. In due course the spots coalesced, resulting in premature senescence of the leaves, particularly in the varieties Co 7219 and CoT 8201. In the case of the other two varieties the spread of the disease was not so rapid after the initial specking. At harvest, during February, when the crop was 11 months old, the entire foliage except three to four top leaves, dried up in Co 7219 and CoT 8201. This pattern of disease development observed in the present study is in agreement with descriptions of Edgerton (1955), Abbott (1964), Dutta (1969) and Agnihotri (1990).

The disease spread was faster during October and November months, at the fag end of the growth phase which coincided with heavy rains. Such severity of ring spot disease during rainy weather was reported by Agnihotri (1990).

Maximum number of spots were produced on bottom leaves of Co 7219 and CoT 8201, especially towards the tip of the lamina, leading to premature senescence of the leaves. In CoA 89081 and Co 85045 only a few spots were produced even on the older leaves (Table 9). This may be due to differential tolerance of these two varieties to the ring spot pathogen. Leu and Hsieh (1969), while working with leaf blight of sugarcane, reported very few

lesions on highly resistant varieties and as the susceptibility increased, the lesions increased in their number, finally covering the leaves completely. Premature senescence of older leaves due to excessive spotting, especially towards the apical portion of the lamina observed in the study was also reported by Butler (1918), Abbott (1964), Dutta (1969) and Agnihotri (1990).

The size and shape of the spots and incubation period varied in all the thirty four varieties/clones tested (Table 10).

The size of the spots (0.9-3.7 mm x 2.9-14.9 mm) in these studies varied considerably from variety to variety. Variation in the size of the spots in ring spot disease of sugarcane (1-5 mm x 4-18 mm) was reported by several workers (Butler, 1918; Bourne, 1934; Matsumoto, 1952 and Dutta, 1969). These reports and the present observations suggest wide variation in the size of the spots.

Different shapes of the spots viz., oval, spindle, linear, irregular and dot like were observed in different test varieties/clones in these investigations. According to Hudson (1960) and Agnihotri (1990) the shape of the spot was either oval or spherical at the beginning which later became irregular. Referring to stalk lesions, Bourne (1934) described somewhat oval or circular spots to

irregular patches of considerable size, but in the present study no such stalk lesions were observed in any variety/clone.

The incubation period under unoculated conditions (9 to 45 days) varied from variety to variety. The delay in the appearance of the first symptom of the disease in CoA 89081, Co 85045, 86A444 and 84A155 may be due to the relative tolerance of these varieties/clones which is also confirmed in the present varietal reaction studies. Longer incubation periods in resistant varieties for smut disease of sugarcane (Prasada Rao *et al.* 1979) and glume blotch of wheat (Cunfer *et al.*, 1988) were reported earlier.

## 5.2 ISOLATION AND IDENTIFICATION OF PATHOGEN AND ESTABLISHMENT OF PATHOGENICITY

### 5.2.1 Isolation

In the present investigations, isolations from immature spots yielded Nigrospora sacchari, Curvularia lunata, P. saccharicola and Bipolaris sacchari. A majority of the isolations from mature spots yielded both P. saccharicola and L. sacchari on different media (Table 12). No fruiting bodies were observed in the immature lesions, during the very early stages of spot development and isolations at this stage yielded a number of fungi including P. saccharicola but not L. sacchari. At a more

advanced stage, minute black dots against a greyish back ground in the lesions were conspicuously visible to the naked eye. Microscopic examination of these revealed the presence of both pycnidia and perithecia. Isolations from these lesions yielded both P. saccharicola and L. sacchari. Bourne (1934) and Hudson (1960) isolated a number of fungi from ring spot lesions viz. H. sacchari, Phyllosticta sp., N. sacchari, L. sacchari, Coniothyrium sp., Spondylocladium sp. and Alternaria sp. Association of Phyllosticta sp. and L. sacchari with mature ring spot lesions was also reported by several workers (Hennings, 1907; Fawcett, 1922; Tucker, 1925; Matsumoto and Yamamoto, 1936; Luc, 1953; Roy, 1964; Ricaude 1989; Echavez Badel, 1990).

### 5.2.2 Pathogenicity tests

Inoculations with B. sacchari, C. lunata and N. sacchari either individually or in combination did not result in ring spot lesions on any variety tested. But P. saccharicola, either individually or in combination with the above three fungi, produced typical ring spot lesions in all the three varieties viz. Co 7219, CoT 8201 and Co 7636 in 13-23 days after inoculation (Table 16). Ascospores of L. sacchari produced on sterile host leaf bits in the laboratory and also from naturally infected material produced typical ring spot symptoms in 17-22 days after inoculation in all the three varieties (Table 16). Koch's

postulates were established with P. saccharicola and L. sacchari. Bourne (1934) obtained typical ring spot symptoms with Phyllosticta sp. only in combination with Helminthosporium sp. But, Abbott (1964) opined that Helminthosporium did not seem to be essential for inciting ring spot lesions in all the cases, since ring spot occurs in several cane growing areas where H. sacchari has not been always recorded. Production of typical ring spot symptoms on sugarcane with the conidia of P. saccharicola (Hennings, 1907; Luc, 1953; Hudson, 1960; Dutta, 1969) and ascospores of L. sacchari (Martin, 1938; Yen and Chi, 1953; Matsumoto, 1952; Hudson, 1960) has been reported earlier. Thus, the results obtained in the present studies confirm the findings of these earlier workers.

### 5.2.3 Identification of a suitable medium for the pathogen

During the course of the present investigations, several culture media were tested to study the growth and sporulation of P. saccharicola and L. sacchari.

In case of P. saccharicola the mycelial growth from single pycnidiospore was fast on most of the media. However, PDA + cane leaf extract, OMA + cane leaf extract supported best growth and sporulation at ambient temperature (Table 17). Initially the mycelium which was white and profuse later turned greyish black with dark roundish

pycnidial bodies both on the surface and also embedded in the medium. Dutta (1969) obtained similar results working with this fungus. The pycnidia were globose and ostio- late, measuring 70-94  $\mu\text{m}$  in diameter and contained abundant hyaline, single celled pycnidiospores measuring 10-13  $\mu\text{m}$  x 3-4  $\mu\text{m}$  with 2 guttules at either end. Measure- ments of pycnidia and pycnidiospores of P. saccharicola agree with those reported earlier by Jones (1967) in CMI descriptions (No.145) and also by Hennings (1907) and Dutta (1969).

Efforts to culture single ascospore of L. sacchari on several synthetic and non-synthetic media yielded only mycelial growth but no single medium supported perithecial formation. Mycelial growth in general was very slow on all the media tested, reaching a maximum diameter of 80 mm only after 15-20 days. However, the growth was slightly faster in corn meal agar, bean meal agar and PDA + corn meal. Initially the mycelium was white but within two days it turned pinkish or umber coloured, deep seated in the medium with sparse aerial growth and finally turned greyish black (Table 17). Abundant pycnidia of P. saccharicola were produced on one month old cultures. These results are in agreement with those reported by earlier workers (Bourne, 1934; Hudson, 1960 and Dutta, 1969).

5.2.4 Production of ascospores in vitro

Attempts to obtain perithecia and ascospores in synthetic and non-synthetic media failed, however, when agar blocks containing single ascospores were transferred to sterile host leaf bits of sugarcane varieties Co 7219, Co 7636 and CoT 8261 enriched with biotin, thiamine hydrochloride and sucrose and exposed for 12 hours period of alternate light and darkness at 24<sup>o</sup>-26<sup>o</sup>C, abundant perithecia with asci and ascospores were produced in 18-21 days (Table 14). Profuse pycnidia of P. saccharicola were also produced from these leaf bits three weeks later. In the leaf bits of Co 7636 the perithecial production was observed at 26<sup>o</sup>C even without addition of vitamins and sucrose. It appears from these observations that the agar media are lacking in certain essential nutritional requirements which the host is able to provide and stimulate the formation of perithecia. Perithecial production on host leaf bits at 26<sup>o</sup>C for L. sacchari (Yen and Chi, 1953) and 24<sup>o</sup>-26<sup>o</sup>C for L. taiwanensis (Hsieh and Lew, 1990) has been reported earlier. The results of the present investigation are in conformity with the findings and the observations of Bourne (1934), Matsumoto and Yamamoto (1936) and Hudson (1960) who had earlier offered proof of genetic connection between P. saccharicola and L. sacchari.

The perithecia were spherical, immersed in the substratum, papillate, ostiolate and measured 111-138  $\mu\text{m}$  in diameter. The asci were numerous, cylindrical or club shaped, sessile, intermingled with paraphyses and measured 62-70  $\mu\text{m}$  x 9-13  $\mu\text{m}$ . The 8 four celled ascospores were hyaline, ellipsoid with a single large guttule for each cell and measured 18-24  $\mu\text{m}$  x 4-6  $\mu\text{m}$  (Table 15). These descriptions and measurements of perithecia, asci and ascospores tallied with those given for L. sacchari (Jones, 1967) in CMI descriptions (No.145) and those reported by Van Breda de Hann (1892); Bancroft (1910); Hudson (1960); Abbott (1964) and Agnihotri (1990).

Thus, the results of the present study clearly indicate that the telionorph of rang spot fungus is L. sacchari Van Breda de Hann and anamorph is P. saccharicola, P: Hennings. These findings are similar to those adopted by the International Society of Sugarcane Technologists standing committee on sugarcane diseases (Ricaud ~~et al~~ 1989).

### 5.3 DEVELOPING A DISEASE RATING SCALE FOR ESTIMATION OF DISEASE INTENSITY

For estimation of disease intensity, a 0-9 scale was developed based on the per cent green leaf area affected and PDI was worked out using the formula suggested by Mayee and Datar (1986). This 0-9 scale was

developed on the basis of the recommendations of ISSCT Standing Committee on Sugarcane diseases because of its obvious advantages in computerisation, analysis and retrieval systems (Hutchinson, 1970). This system has already been accepted by pathologists in many countries and has been found to be easy to use. It has already formed the basis for several international compilations of disease ratings. Based on the PDI, varieties/clones recording upto 10 per cent disease intensity were graded as immune to resistant and above 30 per cent as susceptible to highly susceptible.

The 0-9 scale has also been recommended for leaf blight of sugarcane (L. taiwanensis) in Taiwan (Leu and Hsieh, 1971) and rust of sugarcane in India (Mayec and Datar, 1986).

#### 5.4 SURVEY FOR ASSESSMENT OF DISEASE INTENSITY

Field survey was conducted in different factory areas in coastal Andhra Pradesh, Telangana and Rayalaseema regions (Table 18). Aspects like soil type, variety of cane, nature of crop (plant or ratoon) and disease intensity were taken into consideration. Intensity of ring spot was, in general, more in coastal Andhra Pradesh compared to Telangana and Rayalaseema regions. Even in coastal Andhra Pradesh, the disease was observed in the most severe form in Bobbili factory area, particularly in

the ratoon crop of Co 7219 (73.7%). Some other varieties like Co 975, Co 527 and CoT 8201 also recorded more than 50 per cent PDI in different villages. Such severe incidence of ring spot in this area may be attributed to the following factors.

1. In Bobbili area the sugarcane crop is usually planted during January-February and later depend entirely on rains resulting in moisture stress condition in pre and post-monsoon periods.
2. In some villages, sugarcane was also planted in June after receipt of rains leading to juvenile susceptibility.
3. Application of high doses of nitrogenous fertilizers.

Outbreak of ring spot in Mauritius under water stress conditions on different varieties was reported by Ricaud (1969). Disease incidence was observed to be more in ratoons than in plant crops in this area. Increased intensity of ring spot disease in ratoons was also reported by Echavez Badel (1990) from Puerto Rico under stress conditions.

In Vuyyuru, Nellore, Chelluru and Anakapalle factory areas the disease incidence was observed to be high in certain varieties viz., Co 7219, CoT 8201, Co 7805

and Co 8021 indicating their susceptibility. The AUD ranged from 21.2 to 68.2 in Vuyyuru, 29.8 to 60.4 in Nellore, 5.2 to 70.2 in Chelluru and 29.2 to 64.6 in Anakapalle factory areas. High incidence of the disease was also noticed when the crop was grown in low lying areas with poor drainage facilities. The water stagnation in the field might have created congenial microclimate (high relative humidity) for rapid multiplication and spread of the pathogen. The occurrence and severity of ring spot disease under moist conditions were reported by Caminha (1936) and Echavez Badel (1990).

The intensity of the disease was observed to be high in plots where excessive nitrogenous fertilizer was applied. In these factory areas, most of the cultivators apply two to three times the recommended dose of N fertilizer which might have resulted in excessive succulent vegetative growth and increased disease intensity. In Chelluru factory area varieties CoA 89081 and 82V12 recorded less incidence irrespective of high nitrogen fertilization indicating their resistance. The disease intensity was observed to be more in clay loams compared to sandy loams in this factory area. The comparative high soil moisture content in clay loams might have played an important role on the microclimate and thereby on the intensity of the disease.

271

In Telangana region, though the disease intensity ranged from 16.7 to 69.8 per cent, high incidence of the disease was observed only in Berdipur village where the cultivators applied high doses of nitrogenous fertilizer in the moisture retentive clayey soils.

In Rayalaseema, particularly in Renigunta factory area, the disease intensity was observed to be low even in susceptible varieties (0 to 10.1%) inspite of good rainfall extending upto December. This might perhaps be due to the lack of adequate initial inoculum coupled with unfavourable microclimate as a result of low soil moisture retentivity in sandy and red sandy soils which predominate in this area.

## 5.5 INOCULATION AND RAPID SCREENING TECHNIQUES

### 5.5.1 Inoculation techniques

Though ring spot is amenable to control by fungicides, the most practical, efficient and economic means to combat the disease is by the development of resistant varieties. Abbott (1964) observes that "If the disease is ignored completely in breeding programmes, highly susceptible cultures might be advanced to commercial cultivation and in this context varietal resistance seems to offer an effective measure of control". Growing resistant varieties for the control of ring spot disease has also been recommended by other workers (Cook,

1925; Bourne, 1934 and Nyvall, 1979). The need for a reliable inoculation and screening technique to employ in segregating resistant and susceptible clones thus assumes greater importance.

In the present studies, all inoculations for screening were conducted by using conidial suspension of P. saccharicola, since this fungus was found to sporulate profusely on OMA + cane leaf extract, L. sacchari although produced typical ring spot lesions, was not used in the studies as it failed to sporulate in synthetic and non synthetic media.

Out of eight inoculation methods tried (Table 19) four methods - a. spraying conidial suspension on the leaves, b. clipping top halves of leaves and spraying conidial suspension, c. clipping top halves of leaves, pinpricking and spraying inoculum, d. clipping, pinpricking, spraying inoculum followed by water spray were found to be significantly superior to other methods in reproducing the disease, though among themselves the four methods, were at par (Fig.1). Out of these four methods, spraying conidial suspension on whole leaves was preferred as it is operationally easy and simple. A similar technique was employed in screening sugarcane varieties against L. bicolor (Leu and Hsieh, 1971; Wang and Lee, 1982) and wheat varieties against L. nodorum (Rufy et

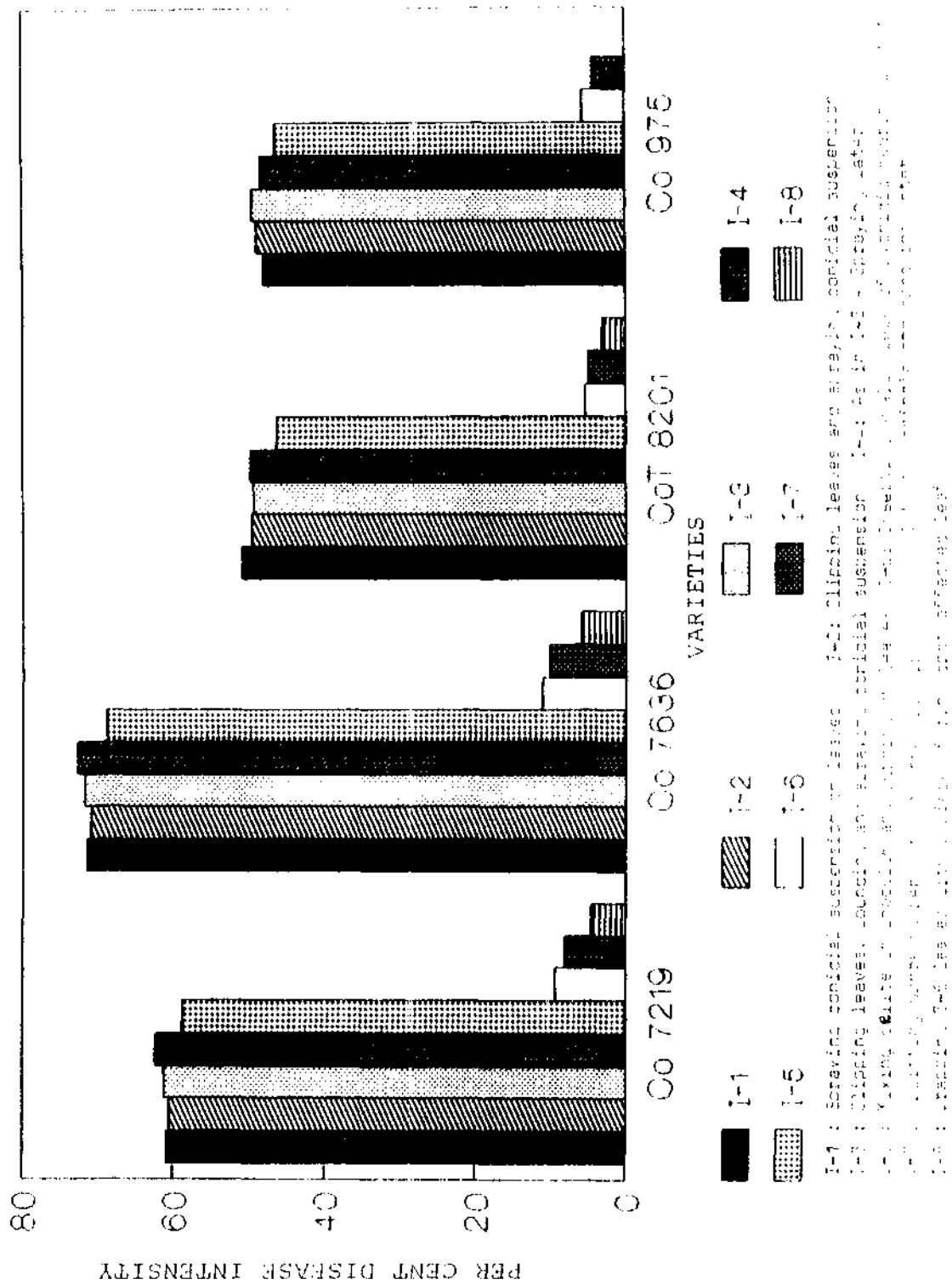


Fig.1: PER CENT DISEASE INTENSITY IN DIFFERENT INOCULATION TECHNIQUES

al., 1981<sup>b</sup>, Karjalainen, 1985; Verreet et al., 1987; Wendland and Hoffmann, 1988).

### 5.5.2 Rapid screening techniques

Reliable and less time consuming methods for determining the reaction of varieties to ring spot are essential. Out of five screening techniques tried in the present investigation (4.5.2), 3 techniques - (a) spraying inoculum on the leaves of potted plants, (b) clipping leaves, pinpricking, spraying inoculum followed by water spray, (c) Rajoong inoculation were effective and were on par with each other and significantly superior to other two methods (detached leaf method and screening under natural conditions). The disease intensity was observed to be uniformly high in all these three methods (Table 20 and Fig. 2). Among these three methods, Rajoong method of screening was preferred, as it is rapid, reliable and saves 2 months time for obtaining the reaction of a variety. The setts germinate sooner and growth of the crop is slightly fast in this method compared to other two methods. Further, more number of setts can be accommodated in an unit area. Rapid screening of sugarcane varieties to red rot by Rajoong method has been recommended by Prasada Rao (1984).

In the screening method where detached leaves of the varieties were inoculated, minimum disease intensity



was observed even in susceptible varieties. Similar was the experience of Nass and Johnston (1985) while working with glume blotch disease (L. nodorum) of wheat. When screening was done under natural conditions, two clones viz., 84A155 and 86A444 did not manifest the disease. As this method mostly depends on climatic conditions, it is difficult to get consistent results. Leu and Hsieh (1969) experienced the same while working with leaf blight (L. taiwanensis) of sugarcane in Taiwan.

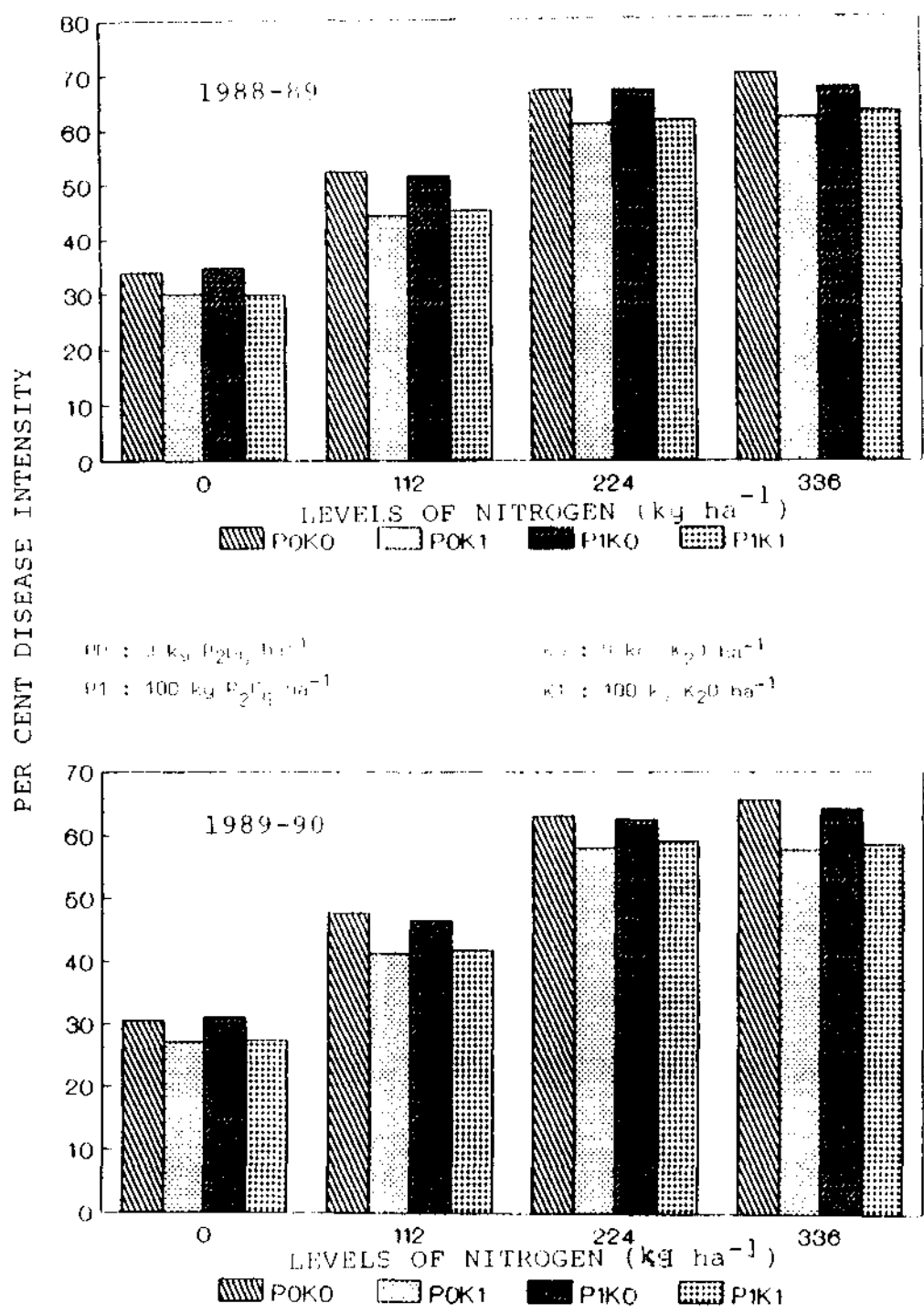
## 5.6 FACTORS AFFECTING DISEASE DEVELOPMENT

### 5.6.1 Effect of different levels of Nitrogen, Phosphorus and Potassium on the intensity of the disease

In the present study, nitrogen had significant effect on the disease severity. The increase in N level from 0 to 112 and 112 to 224 kg ha<sup>-1</sup> resulted in a progressive increase in disease in the variety Co 7219 (Fig. 3 and 4). Positive association was observed between PDI and leaf nitrogen (Tables 21 to 25). The relationship of nitrogen status of the host with the extent of disease in several crops has been well documented. Positive correlation between leaf nitrogen and rust severity in sugarcane was reported by Anderson and Dean (1986). Increased nitrogen fertilization is reported to increase severity of stem rot of rice caused by Magnaporthe (Lepto-

sphaeria) salvini (Jain, 1977; Mayee et al., 1977; Rangaswamy, 1979; Nyvall, 1979; Li et al., 1984; Ou, 1985; Rathaiah, 1990; Mahotra and Anoja, 1990) and glume blotch of wheat caused by L. nodorum (Olsson, 1979; Broscious et al., 1985; Kharkova et al., 1984). Higher doses of nitrogen results in succulent vegetative growth and the plants are more vulnerable to infection. The results in the current study indicated that application of either NP or NPK did not reduce the disease severity and the interactions were not significant. Increase in soluble nitrogen in rice leaves has been reported to enhance susceptibility to blast pathogen by many workers (Suryanarayanan, 1958; Zsoldos, 1962). Higher N levels would have increased the susceptibility of plant for better pathogenic development.

Application of phosphorus at  $100 \text{ kg ha}^{-1}$  in the present study, did not influence the disease (Fig. 4). Several workers reported increase in disease intensity due to Leptosphaeria spp. in several crops with the application of P. In stem rot of rice, increase in disease intensity with phosphate application was reported by Jain (1977), Rangaswami (1979), Ou (1985) and Rathaiah (1990). Phosphorus alone or in combination with N fertilizer is reported to have increased the infection of L. nodorum (Cunfer et al., 1980; Kharkova et al., 1984) in wheat.



**Fig.3: EFFECT OF DIFFERENT LEVELS OF NITROGEN FERTILIZER ALONE OR IN COMBINATION WITH PHOSPHORUS AND POTASSIUM ON THE INTENSITY OF RING SPOT**



Fig.4: INFLUENCE OF NITROGEN, PHOSPHORUS AND POTASSIUM ON THE EXTENT OF RING SPOT INTENSITY

11

The results of the present investigations have clearly indicated that K nutrition imparts tolerance to sugarcane against ring spot (Table 21 to 23). Application of potassium at  $100 \text{ kg ha}^{-1}$  either alone or along with different doses of N significantly reduced the disease intensity in the variety Co 7219 (Fig.3 and 4). Negative association was observed between FDI and sheath potassium. Numerous instances are there where application of potassium reduced the incidence of diseases. These include eye spot (Rabindra and Kumaraswamy, 1978) and brown stripe in sugarcane (Egan, 1989), stem rot (Keim and Webster, 1974; Jain, 1977; Subramanian and Balasubramanian, 1977; Nyvall, 1979; Rathaiah, 1990; Jayaraj et al., 1991) and sheath blight (Kannaiyan and Prasad, 1978) in rice and glume blotch of wheat (Siebold, 1980; Cunfer et al., 1980). The present findings are in agreement with the observations of other workers. Potash nutrition is known to cause physiological changes in plants leading to disease resistance (Ramasamy and Prasad, 1975). Last (1956) showed that potassium affects the host metabolism and that high levels of potassium retarded the growth of Fusarium in rice, the host crop. The results of present investigations indicate that potassium application plays a major role in reducing the intensity of ring spot disease.

The role of NPK in relation to disease intensity in Co 7219 was further confirmed in a set of nine varie-

ties (CoT 8201, Co 975, Co 7636, Co 85044, 86A444, 84A155, CoA 89081, Co 85045 and 83A106) having differential reaction to the disease (Table 24 and 25). However, further studies are required to ascertain the effect of higher doses of nitrogen than the recommended level in resistant varieties and lower doses of nitrogen in susceptible varieties with different levels of potassium on disease intensity.

The significant findings of the present study in relation to host nutrition for the management of the disease are as follows.

- a) When ring spot susceptible varieties like Co 7219, Co 975 and CoT 8201 are cultivated, it should be seen that only the recommended dose of nitrogen for the area is applied and excessive application avoided.
- b) Application of potassium ( $K_2O$ ) at  $100 \text{ kg ha}^{-1}$  should be advocated in responsive soils of drought prone areas to mitigate possible losses due to ring spot.

#### **5.6.2 Age of the crop and intensity of the disease**

Irrespective of the age of the crop, disease incidence was noticed in September (4.1 to 5.3%) which gradually increased (47.7 to 53.1%) upto February (Table 26). The results indicated that age of the crop had no effect on the incidence or severity of the disease. This

suggests that high rainfall coupled with high relative humidity and relatively low temperatures over previous months might be conducive for the initiation of the disease during the first fortnight of September, irrespective of the age of the crop. Vander bijl (1921) reported that ring spot was favoured by cool, damp, atmospheric conditions. Initiation and severity of ring spot disease during September and October months were reported by Dutta (1969). Leu and Hsieh (1969) stated that leaf blight (Leptosphaeria taiwanensis) of sugarcane occurred most intensively in Taiwan throughout the year, regardless of the age of the plant, particularly during winter and early spring. High disease rate of Septoria (L) nodorum on wheat was favoured by a combination of extreme rainy weather with high relative humidity and below average temperatures (Rowe, 1979; Svanold and Djurle, 1980; Jeger et al., 1981; Mittermeier and Hoffman, 1984, Khoury and Kraz, 1989).

### 5.6.3 Effect of leaf orientation on the intensity of the disease

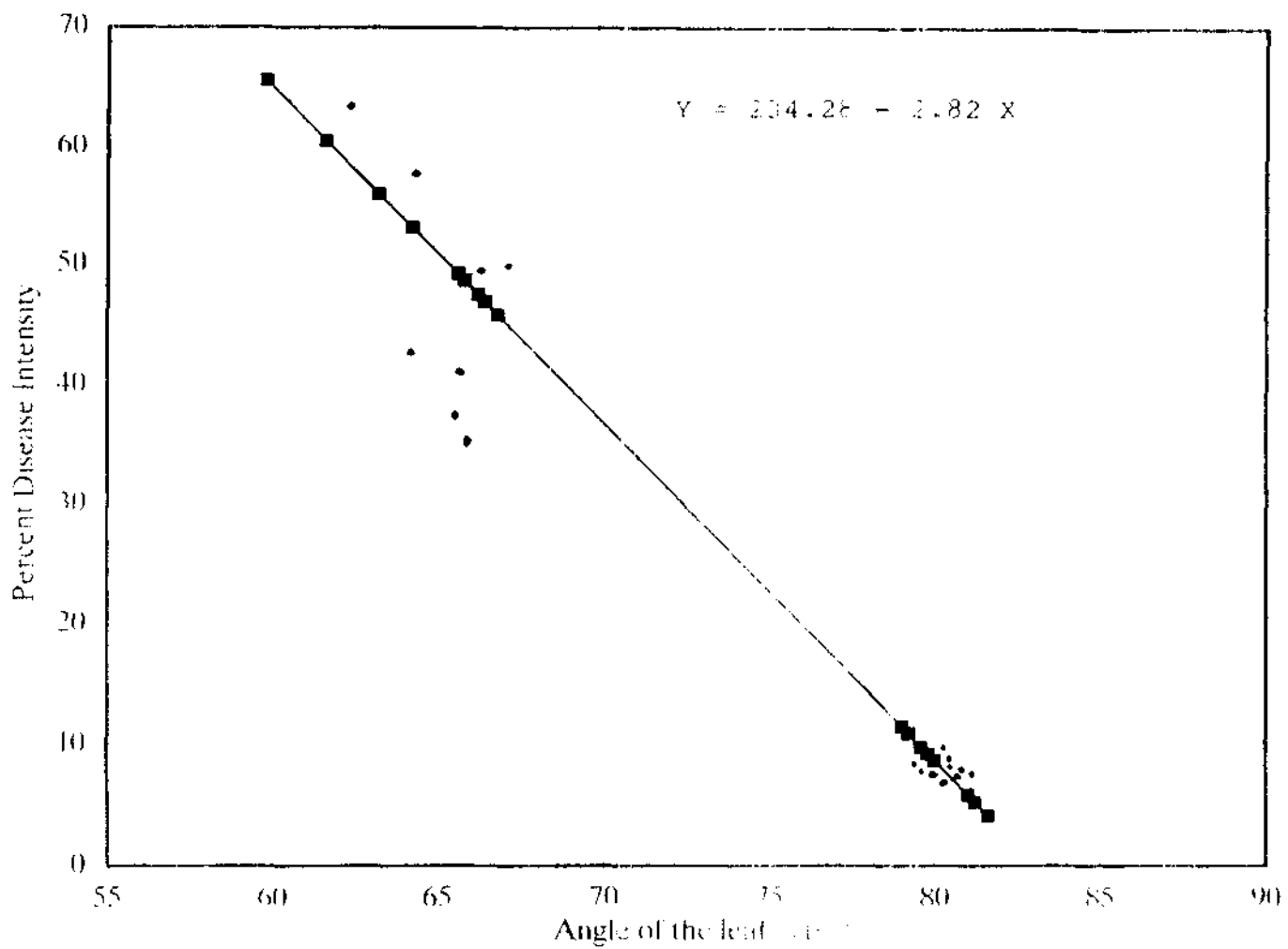
Significantly low intensity of the disease (6.7 to 9.9%) was observed (Table 27) in the varieties having erect leaves (Erectophile) with more angle of  $79.0^{\circ}$  to  $81.6^{\circ}$  as compared to varieties having drooping leaves (i.e.) planophile (32.7 to 72.7%) with less angle of  $57.1^{\circ}$  to  $66.2^{\circ}$  (Table 20). Significant negative correla-

tion was observed between leaf angle and disease intensity. With every one degree increase in the angle of the leaves, the disease intensity decreased by 2.82 per cent (Fig.5). The low disease intensity in the varieties having erect leaves might be due to non-retention of enough surface moisture for longer periods which is unfavourable for the spores to germinate and cause infection. In drooping leaves, the scope for retention of more surface moisture for longer periods is greater. The low intensity of rust disease in wheat varieties having erect leaves has been reported by Kaur and Dhillon (1985). According to Ou (1985), the leaves of rice plants become soft and drooping with low silica content resulting in high disease intensity. In the present studies higher silica content observed in erect leaves of ring spot resistant varieties (Table 30 and 31) could also be one of the reasons for low incidence of the disease in erect leaved varieties.

In view of the above, sugarcane varieties with erect leaves could be used in the breeding programmes for developing ring spot resistant varieties.

**5.6.4 Moisture stress and water stagnation on disease intensity**

The effect of moisture stress and water stagnation on the intensity of the disease was studied in



**Fig.5: RELATIONSHIP BETWEEN PER CENT DISEASE INTENSITY AND ANGLE OF THE LEAF IN DIFFERENT SUGARCANE VARIETIES/CLONES**

comparison with normal irrigation, in different varieties. Significant increase in disease intensity was observed in all the varieties both under moisture stress and water stagnation as compared to normal irrigation conditions, except in the clone 84A155 (Table 29 and Fig. 6). The mean PDI under normal irrigation condition was 31.7 while it was 40.2 and 39.5 under moisture stress and water stagnation respectively. This situation was also observed in cultivators' fields during the survey, especially in the coastal districts of Andhra Pradesh. Lack of adequate moisture and water stagnation are two extreme conditions under which there is severe stress on the plant parts leading to debilitated condition of the host. It is therefore not surprising that in either case, there was significant increase in disease intensity compared to a normal irrigated crop. High incidence of ring spot was reported earlier under moisture stress (Ricaud, 1969 and Echavez Badel, 1990) and water stagnation (Butler, 1918; Vander bijl, 1921; Caminha, 1936; Echavez Badel, 1990) conditions. Increased incidence of Pythium root rot and pine apple disease (Ceratocystis paradoxa) under water stagnation (Agnihotri, 1990) and wilt disease (Cephalosporium sacchari) under moisture stress (Sarma, 1976) have also been reported in sugarcane.

At present, soil application and foliar sprays of potassium are being recommended for rainfed sugarcane to

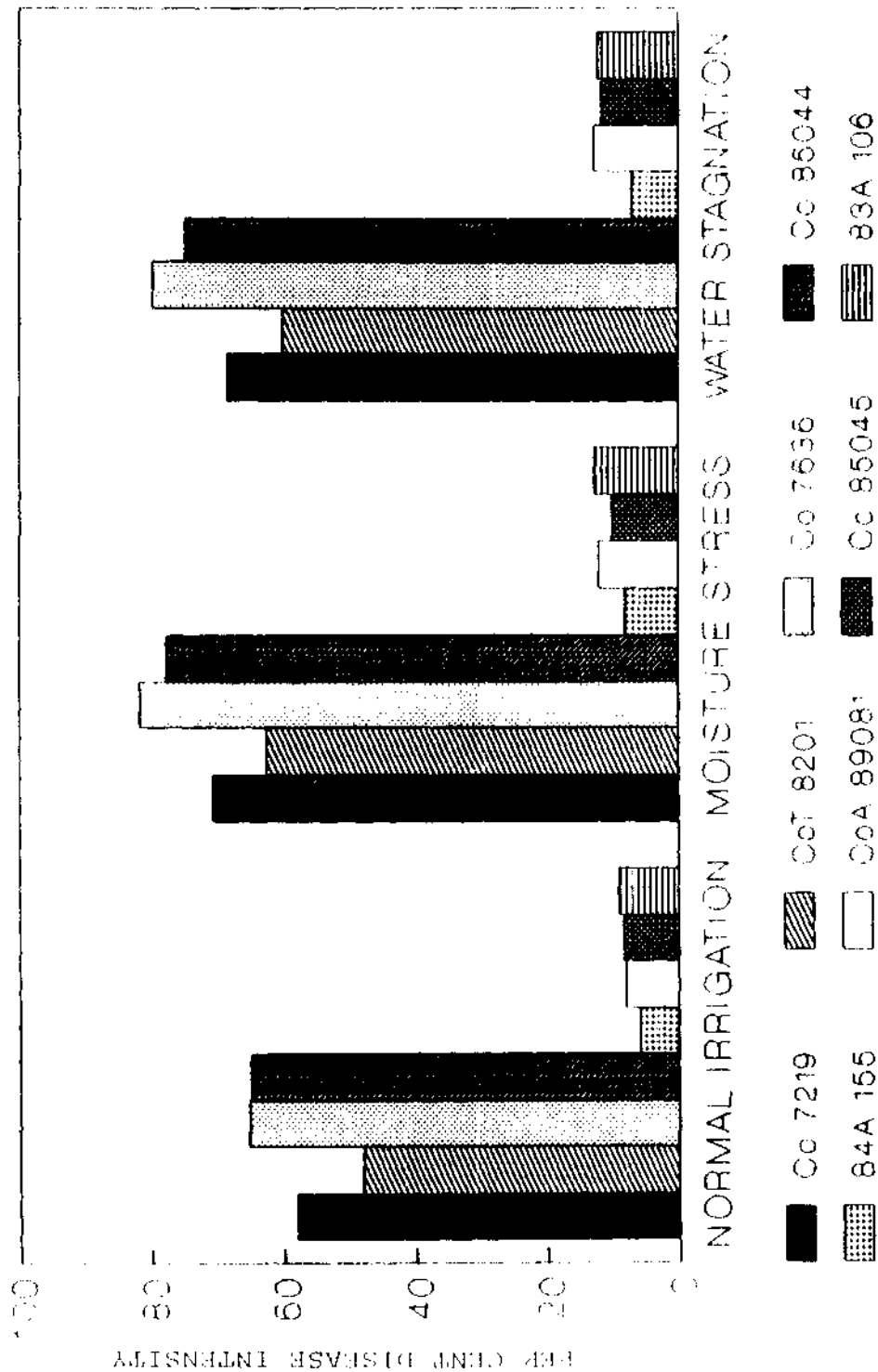
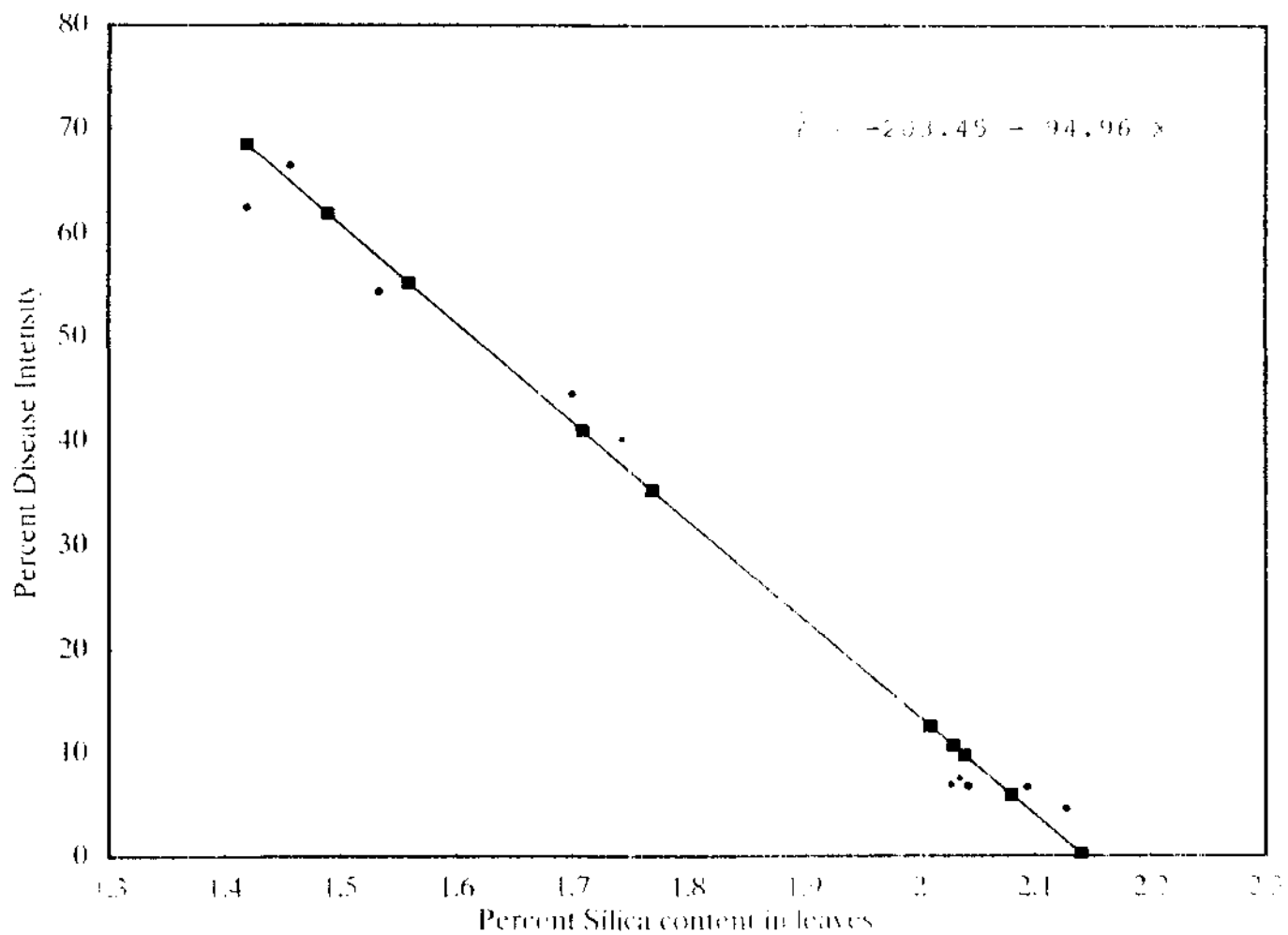


Fig. 6: DISEASE INTENSITY IN DIFFERENT SUGARCANE VARIETIES/CLONES UNDER NORMAL IRRIGATION, MOISTURE STRESS AND WATER STAGNATION

tide over long spells of drought (RARS, Anakapalle, 1992). This recommendation would be useful in the present context also since potassium is found to confer resistance to ring spot. In addition to this potassium also maintains the vigour of the crop (RARS, Anakapalle, 1992). As far as water logged conditions are concerned community efforts have to be encouraged for providing proper drainage to minimise the incidence of the disease.

#### 5.6.5 Effect of silica content of leaves on disease intensity

The silica content (Tables 30 and 31) in the leaves of susceptible varieties/clones was observed to be low (1.42 to 1.71%) as compared to resistant varieties/clones (2.01 to 2.14%). The higher silica content in resistant varieties/clones correlated with low disease intensity of 4.6 to 7.8 per cent while low silica content in susceptible varieties resulted in higher disease intensity of 40.1 to 66.2 per cent. With every 0.1 per cent increase in the silica content of leaves the disease intensity decreased by 9.5 per cent (Fig.7). Similar observations were made by Nonaka et al. (1958) with stem rot of paddy (L. salvinii), Leusch and Buche nauer (1984) with glume blotch of wheat (L. nodorum) and Egan (1989) with brown stripe of sugarcane (Cochliobolus stenospilus). The role of silica content in offering resistance to blast



**Fig.7: RELATIONSHIP BETWEEN PER CENT DISEASE INTENSITY AND SILICA CONTENT IN LEAVES OF DIFFERENT SUGARCANE VARIETIES/CLONES**

disease of rice has been extensively studied (Hemmi, 1933; Suzuki, 1934; Ishizuka and Hayakawa, 1951; Ou, 1985). The reduced ring spot intensity in resistant varieties/clones of sugarcane might be due to the following reasons.

- a) The ring spot pathogen was reported to penetrate the leaf tissues directly to cause infection (Bulter, 1918). The increased silica content in resistant varieties might have offered resistance for the direct penetration of the pathogen.
- b) With increase in the silica content the leaves become erect and are subject to less infection. This was suggested by Ou (1985) working with rice. This appears to be a possible reason since the resistant varieties/clones of sugarcane tested, possessed erect leaves.

#### 5.7 VARIATION IN RING SPOT PATHOGEN

Work on the variability of the pathogen causing ring spot disease has not been attempted by earlier workers. The behaviour of six isolates of the ring spot pathogen, from different varieties and locations of coastal Andhra and Telangana was studied for their cultural, morphological and pathological characters.

No appreciable differences were observed either in cultural characters or spore dimensions (asexual and

sexual) of the six isolates studied (Table 32), although there was slight variation in the nature of spotting. The spore measurements fell within the range reported by Abbott (1964).

Variation in pathogenicity among the isolates was also not observed in the present study. Even among the fifteen varieties tested, no single variety has shown differential reaction and all the isolates behaved similarly in their virulence pattern (Table 33). Kaiser et al. (1979) reported variation in growth rate, sporulation and colony characters of isolates of L. bicolor causing leaf scorch in sugarcane. There appears to be no other report on the variability of L. bicolor and L. taiwanensis in sugarcane. However, several workers have conducted studies on the variability of Leptosphaeria spp. in different crops. Variation in the virulence pattern of isolates of L. nodorum was reported by Ruffy et al. (1981) and Allingham and Jackson (1981) in case of wheat. But Scott and Benedikz (1983) while studying the behaviour of isolates of L. nodorum and L. avenaria on different varieties of wheat, found no consistent differences in type or degree of symptoms or in the reaction pattern. Similarly, Humpherson Jones and Ainsworth (1984) detected no variation in strains of L. maculans on oil seed rape.

It is therefore reasonable to presume that strains exhibiting pathological specialisation or isolates

differing in aggressiveness in the ring spot inciting fungus do not seem to exist atleast in coastal Andhra Pradesh and Telangana.

5.8       ROLE OF SOIL, HOST DEBRIS, CANE SETTS, IRRIGATION WATER AND LEAF CONTACT ON THE SPREAD OF THE DISEASE

The mode of transmission of ring spot disease has hitherto not been clearly established. In the present study among the six agencies tested (Table 34), contact of diseased leaf with healthy leaf followed by water spray was found to be the most effective means of spread (53.3 to 64.9 PDI). The efficacy of this method may be due to availability of abundant inoculum close to the leaf and concurrent availability of moist conditions provided by water spray. The conidia are liberated as a cirrus in a gelatinous mass from the moist pycnidia and are aided by the water spray for dispersal. Abbott (1964) opined that transmission of ring spot was apparently by wind or rain borne spores. Lo (1961) also stated that wind blown rain and dew are indispensable for the dissemination of conidia of Stagnospora sacchari (L. bicolor). The conidia could not be trapped even on very windy days when there was no rain or dew. Dispersal of pycnidiospores of L. nodorum in wheat (Rapilly and Skajennikoff, 1974; Griffith and Hann, 1976; Royle et al., 1987) and L. maculans in rape

(Nyvall, 1979; Sundarmadi and Wallace, 1984) by rain splash has been reported earlier.

Diseased host debris, preserved dry for six months and spread at the base of the clumps, produced the disease (20.7 to 29.7%), while the debris that was incorporated in the soil failed to do so (Table 34). The failure of transmission through the debris incorporated in the soil might be due to greater biodegradation and non-exposure to the beating rain and wind. The host debris that was spread above the soil was more exposed to the splashing rain during October-November enabling the production and liberation of conidia to come in contact with lower leaves. Transmission of ring spot disease through infected old dead leaves with the aid of rain splashes was reported by Jones (1967). The effective role of infected leaf debris in the spread of Leptosphaeria spp. on several other crops has been well established. Transmission of L. nodorum in wheat (Holmes and Colhoun, 1975; Mehta, 1975; Sanderson and Hampton, 1978; Ruffy et al., 1981) and Magnaporthe salvini in rice (Bockus et al., 1979; Singh, 1973) through infected straw was reported. Stubble debris of rape from previous season was reported to be the main source of black leg infection (Mc Gee, 1977; Sundarmadi and Wallace, 1984; Gladders and Musa, 1980).

In the present study, it was observed that cane setts with infected leaf tissue, soil adjacent to a highly affected clump, or irrigation water failed to spread the disease. Eventhough the conidia are washed on to the soil from leaves, it has not been possible to obtain infection from the soil. Probable reason for lack of any incidence in this treatment could be the non-availability of sufficient viable inoculum. Matsumoto et al. (1955) from Taiwan also had similar experience while working with L. bicolor in sugarcane. The failure of transmission of the disease through irrigation water might possibly be due to the conidia germinating long before reaching the host leaf. The failure of sett transmission even when there was infected leaf tissue adhering to the seed piece might be due to the pathogen being unable to reach and infect the emerging young shoot. The failure of sett transmission of L. sacchari (Abbot, 1964); L. bicolor (Matsumoto et al., 1955) and Mycovellosiella koepkei (Ricaud and Autrey, 1989) was reported in sugarcane.

The results indicate that the infected debris of the previous season present on the soil surface serves as an important source of primary inoculum and the infected leaves help in the secondary spread of the disease during rainy weather as obtained in the results of the present study.

## 5.9 HOST RANGE

Twenty plant species belonging to both monocots and dicots were tested to study the host range of the pathogen under artificial inoculation. Out of them, only Saccharum spontaneum (Rellu and SES.594) developed symptoms of ring spot (Table 35). Symptoms of ring spot were also noticed on S. spontaneum (Rellu and SES.594) under natural conditions at Anakapalle, but the size of the spots was smaller compared to that on S. officinarum. Similar observations were also made by Butler (1918). Misra et al. (1971) from Bihar observed L. sacchari on Saccharum munja Roxb. (Tiger grass) for the first time. S. munja is considered to be genetically close to S. spontaneum. Hudson (1960) from Jamaica stated that L. sacchari has not been noticed on any host other than Saccharum spp. However L. bicolor in sugarcane also occurred naturally on Miscanthus sinensis and on artificial inoculation on Sorghum vulgare in Taiwan (Lo et al., 1953). This pathogen also occurred naturally on S. spontaneum in Philippines, and under artificial inoculated conditions on S. spontaneum, Miscanthus sinensis, Imperata cylindrica and Sorghum halepense (Exconde, 1963). Subsistence of L. bicolor on S. spontaneum was also reported by Sampang et al. (1980) from Philippines. Contrary to this, Kaiser et al. (1979) from Kenya reported that L. bicolor was pathogenic to several sugarcane cultivars, but not or

only weakly so to napier grass (Pennisetum purpureum), maize, rice and sorghum.

The results of the present study indicate that S. spontaneum acts as a collateral host for L. sacchari. The implication of these observations is that S. spontaneum harbours the pathogen and aids in the long term persistence of the disease even in the absence of susceptible cane varieties. Since S. spontaneum is a widespread perennial weed in many sugarcane growing areas, systematic eradication of this weed might be of some help in reducing the severity of ring spot.

#### 5.10 REACTION OF SUGARCANE VARIETIES/CLONES TO RING SPOT DISEASE

The ultimate economic tool for the field control of diseases is release of varieties with high level of genetic resistance to specific diseases (Stevenson, 1965; Sturgess, 1980). Routine selection for disease resistance and evaluation of available germplasm is rather a compulsion even when the disease appears to be at a low ebb. Evolution of resistant varieties for the control of ring spot disease has been recommended by several workers (Cook, 1925; Bourne, 1934; Nyvall, 1979). In the present studies several popular and promising varieties/clones were tested against the disease (Tables 36 to 39).

Examination of data in Table 36, where disease severity over a period of eight months is presented, shows the influence of time on the intensity of the disease. Five varieties/clones viz., CoA 89C81, 84A155, 86A444, Co 85045 and 83A106 recorded less than 10 per cent incidence even six months after inoculation. These varieties/clones have longer incubation period and slow lesion development as in the case of wheat infected by L. nodorum (Cunfer et al., 1988). The disease intensity increased substantially during 2nd (October), 3rd (November) and 4th (December) months after inoculation (MAI). It might be due to intermittent rains received during October and November (Table 3 at para 3.1). Further increase in disease intensity was observed in the 5th (January) and 6th (February) MAI, but it was only marginal. This increase might be due to dew fall received during the period. The disease intensity tended to decrease from 7th (March) and 8th (April) MAI (Fig. 8) which might be attributed to the rise in temperatures. Similar observations were reported by North (1923). The results (Table 36) show that the minimum lag period between inoculation and final estimation should be six months.

Out of sixty varieties/clones tested by adult cane inoculation method, seven reacted as resistant, two as moderately resistant, seventeen as moderately susceptible, twenty one as susceptible and the remaining

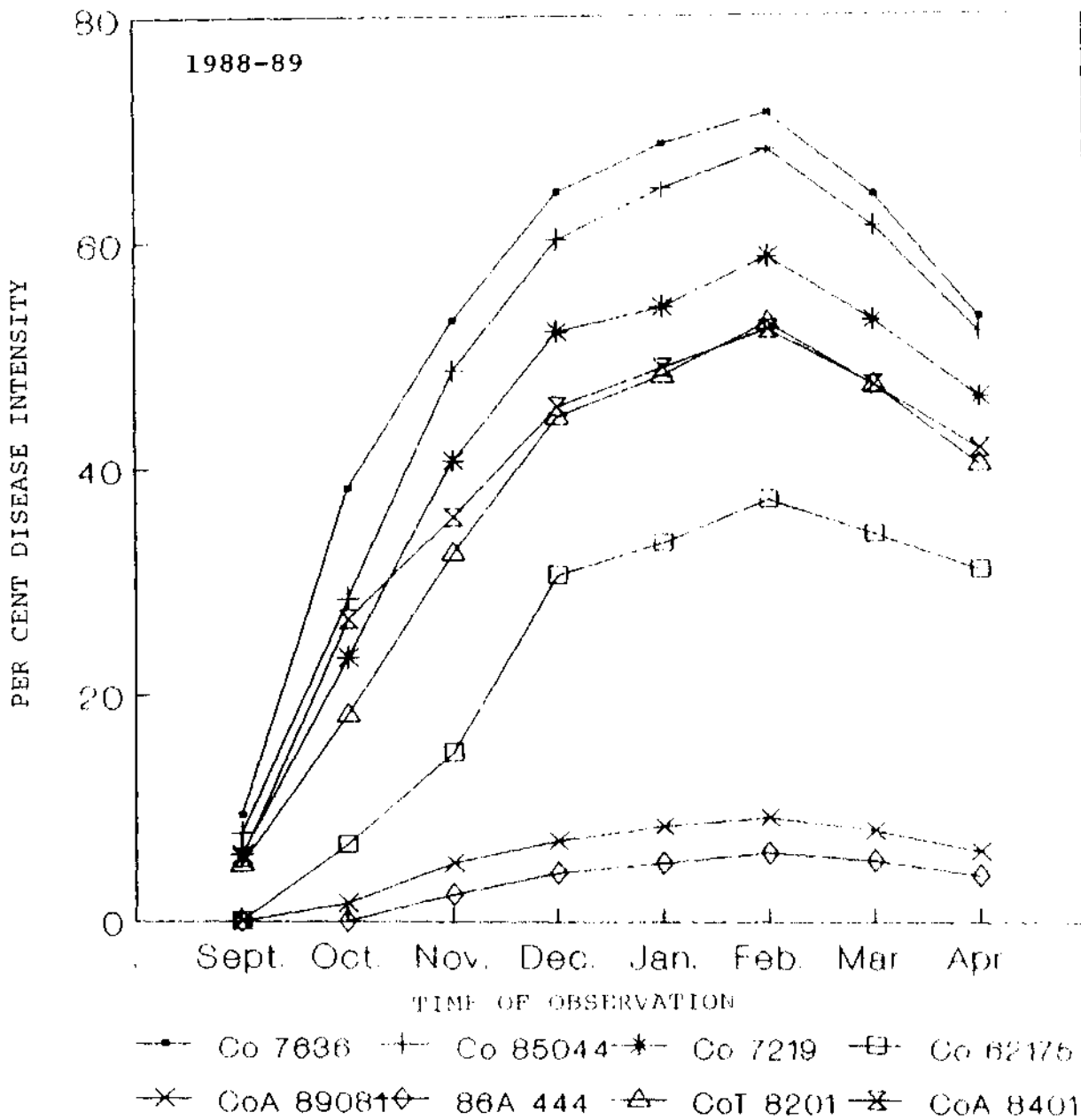


Fig.8: REACTION OF DIFFERENT SUGARCANE VARIETIES/CLONES TO RING SPOT DISEASE 1 TO 8 MONTHS AFTER INOCULATION

nine as highly susceptible (Table 37). Among the important commercial varieties CoA 89081 showed resistant reaction while Co 7219, CoT 8201, Co 7706, CoA 7602, Co 6907, CoA 8401, Co 8013, CoC 671, Co 975, Co 8021, CoA 88081, CoA 89085 showed susceptible to highly susceptible reaction to the disease. These susceptible varieties occupy large areas in the cane growing districts of coastal Andhra Pradesh and also exhibit high disease incidence even under natural conditions. Since most of these varieties possess good yield and quality attributes, they should be grown with utmost vigilance by taking up timely spraying of foliar fungicides. The reaction of sugarcane varieties to ring spot disease was reported by a few workers. Varieties PoJ2825 and 2878 were found resistant in Cuba (Bruner, 1940) while an important commercial variety Fl08 was highly susceptible to the disease in Taiwan (Matsumoto, 1952). Varieties Co 453, Co 617, Co 838, S345/52 (Sandhu et al., 1963), Mauritius 16 (Dutta, 1969), Co 313, Co 1138 and B.0.17 (Singh and Pavgi, 1989) reacted as susceptible while B.0. 32, Co 245, Co 312, Co 314, Co 416, Co 445 and Co 1007 reacted as resistant (Singh and Pavgi, 1969) under Indian conditions.

Forty varieties/clones which were screened by adult cane inoculation were also evaluated by Rajoong method (Table 38). More than 90 per cent of the varieties/clones showed similar type of reaction in both

these methods (Table 39 and Fig.9). But the FDI was slightly more in Rajoong method. It may be due to Juvenile susceptibility. Since the difference in PDI between the two methods was marginal, Rajoong method which cuts short the testing period by 3 months, has been preferred. Rapid screening of sugarcane varieties/clones to red rot using Rajoongs has been recommended by Prasada Rao (1984).

#### 5.11 CHEMICAL CONTROL

Out of the thirteen fungicides tested in vitro (Tables 40 and 41) Bavistin (10 ppm), Topsin-M (20 ppm), Calixin (50 ppm), Captaf (200 ppm), Dithane M-45 (200 ppm), Blitox (200 ppm), Bayletor (200 ppm), Dithane Z-78 (500 ppm) and Chlorothalonil (500 ppm) were found effective. Of these fungicides, six were selected for field use, based on their availability and economics. In the field tests three sprays of Captaf (0.3%), Blitox (0.4%), Bavistin (0.1%), Dithane M-45 (0.3%) and Topsin-M (0.1%) at monthly intervals, starting from disease initiation, significantly reduced the disease intensity in the variety Co 7219 (Table 43 and Fig.10) with slight increase in yield and significant increase in juice quality. Effectiveness of chlorothalonil against L. nodorum (Eynard and Shepard, 1990); Carbendazim and Thiophanate methyl against Magnaporthe salvinii (Ramsingh et al., 1988) in vitro was reported earlier. In the past,

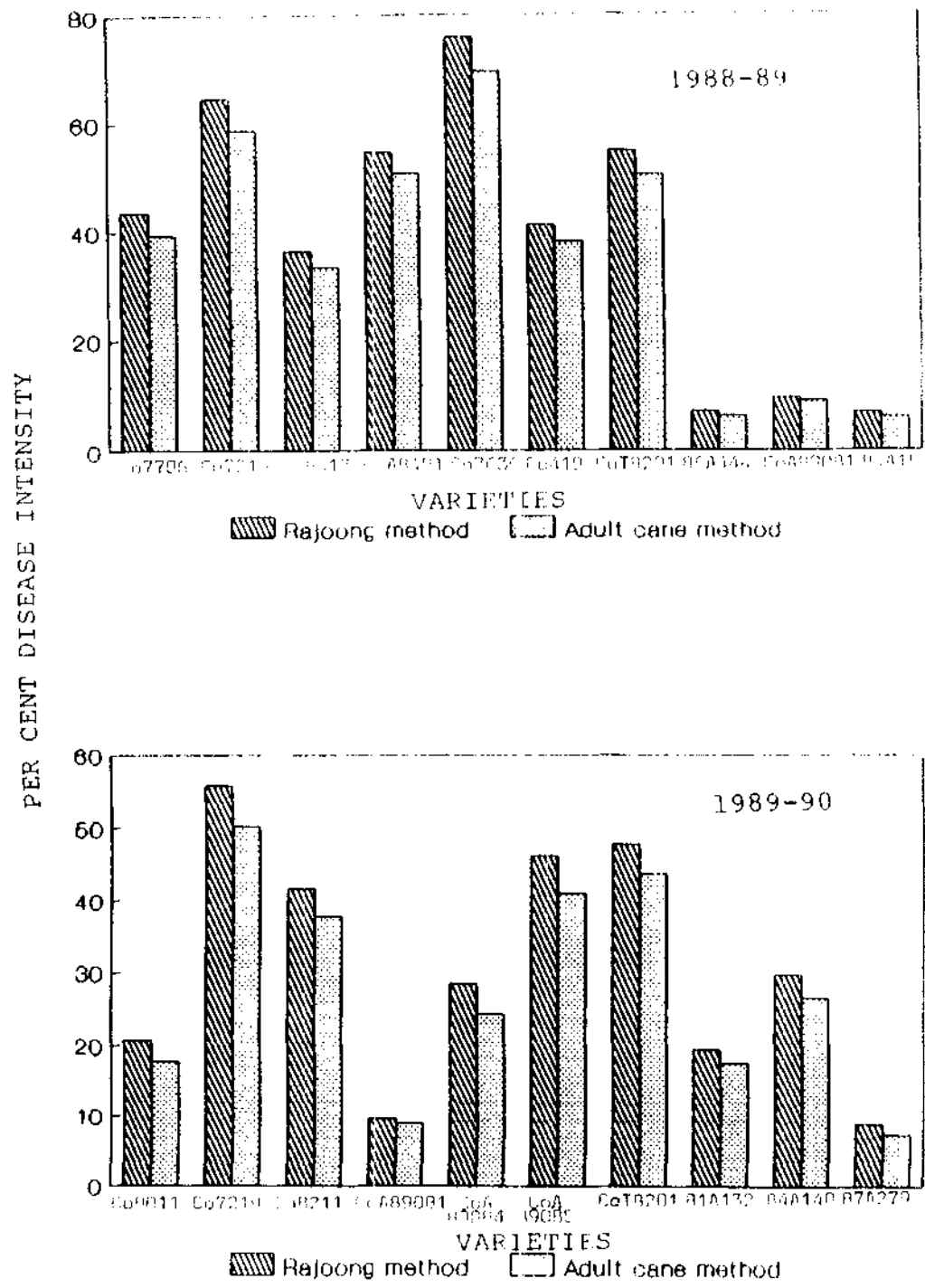


Fig.9: PER CENT DISEASE INTENSITY IN DIFFERENT VARIETIES/CLONES IN RAJOONG AND ADULT CANE METHODS OF INOCULATION

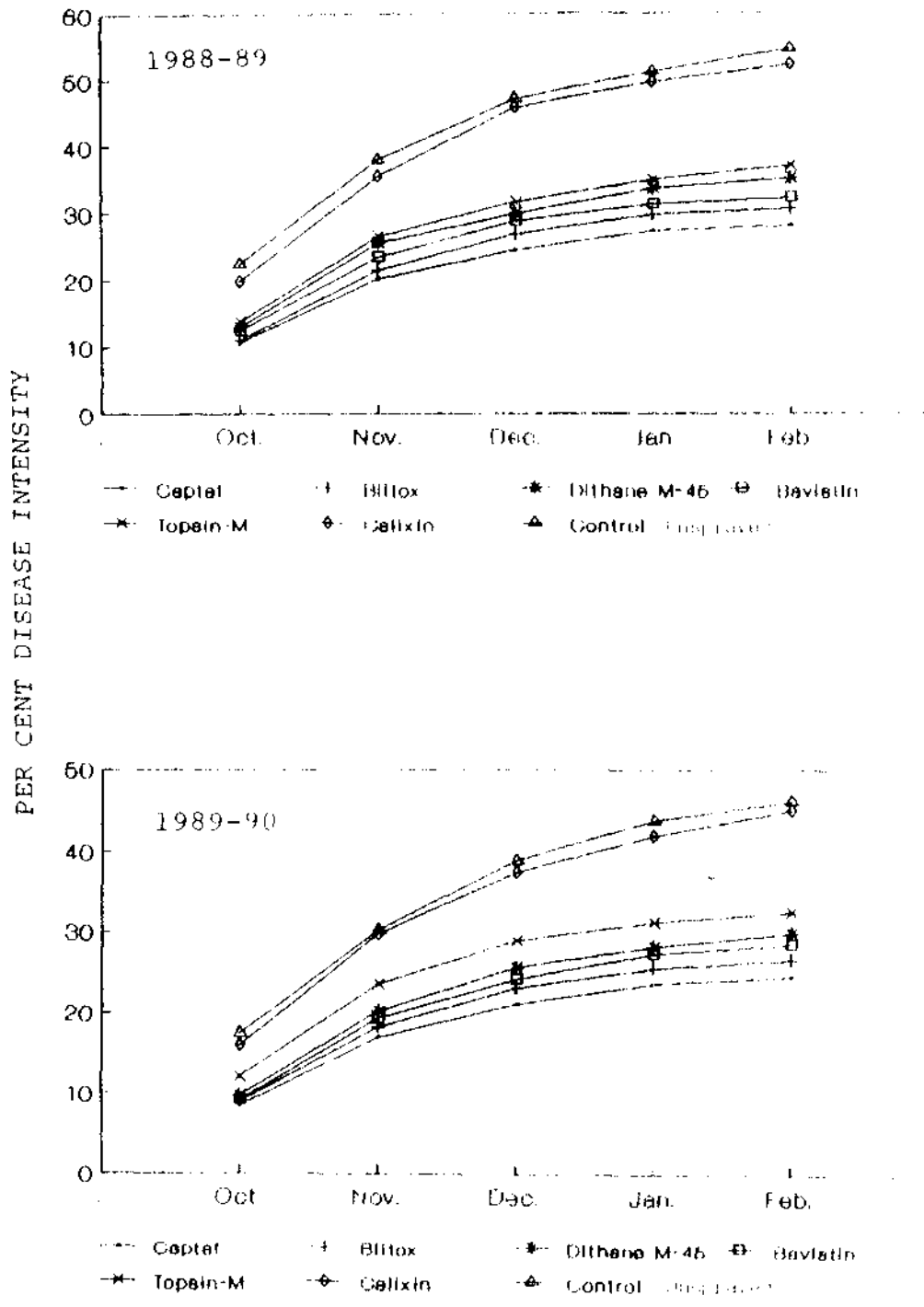


Fig.10: EFFECT OF DIFFERENT FUNGICIDES ON THE INTENSITY OF RING SPOT DISEASE IN SUGARCANE CULTIVAR Co 7219

several workers studied the effect of foliar sprays with systemic and non-systemic fungicides on several foliar diseases of sugarcane and diseases incited by Leptosphaeria spp. in different crops. Among the attempts made on sugarcane diseases Bordeaux mixture (1%) on L. sacchari, (Govindaswamy and Alagia Nagalingam, 1981), Dithane Z-78 on L. taiwanensis (Leu and Hsieh, 1969) and copper oxychloride against Cochliobolus stenospilus (Ahmed et al., 1976), Mycovellosiella koepkei (Prakasam and Satyanarayana, 1969) and Bipolaris sacchari (Kumaraswamy and Urs, 1978) were observed to be effective in reducing the disease intensity. Reduction in the intensity of glume blotch of wheat with increased yields was observed by spraying Maneb and Benomyl (Spiertz, 1973; Jacobsen, 1977), Calixin and Topsin-M (Oppitz and Hoerer, 1978), Dithane M-45 (Barros et al., 1984), Captafol (Zwartz, 1979; Mielke and Ahlf, 1985) and Bayleton (Christ and Frank, 1989). The effectiveness of Carbendazim and Topsin-M in reducing stem rot (Magnaporthe salvini) of rice and increasing yields was observed by Li et al. (1984), Ramsingh et al. (1988), Shahjahan et al. (1988), while in rape, Carbendazim and Calixin effectively reduced the intensity of black leg (Humpherson Jones and Burchill, 1982).

There was no significant increase in yield (Fig. 11) and its parameters like length and diameter of the

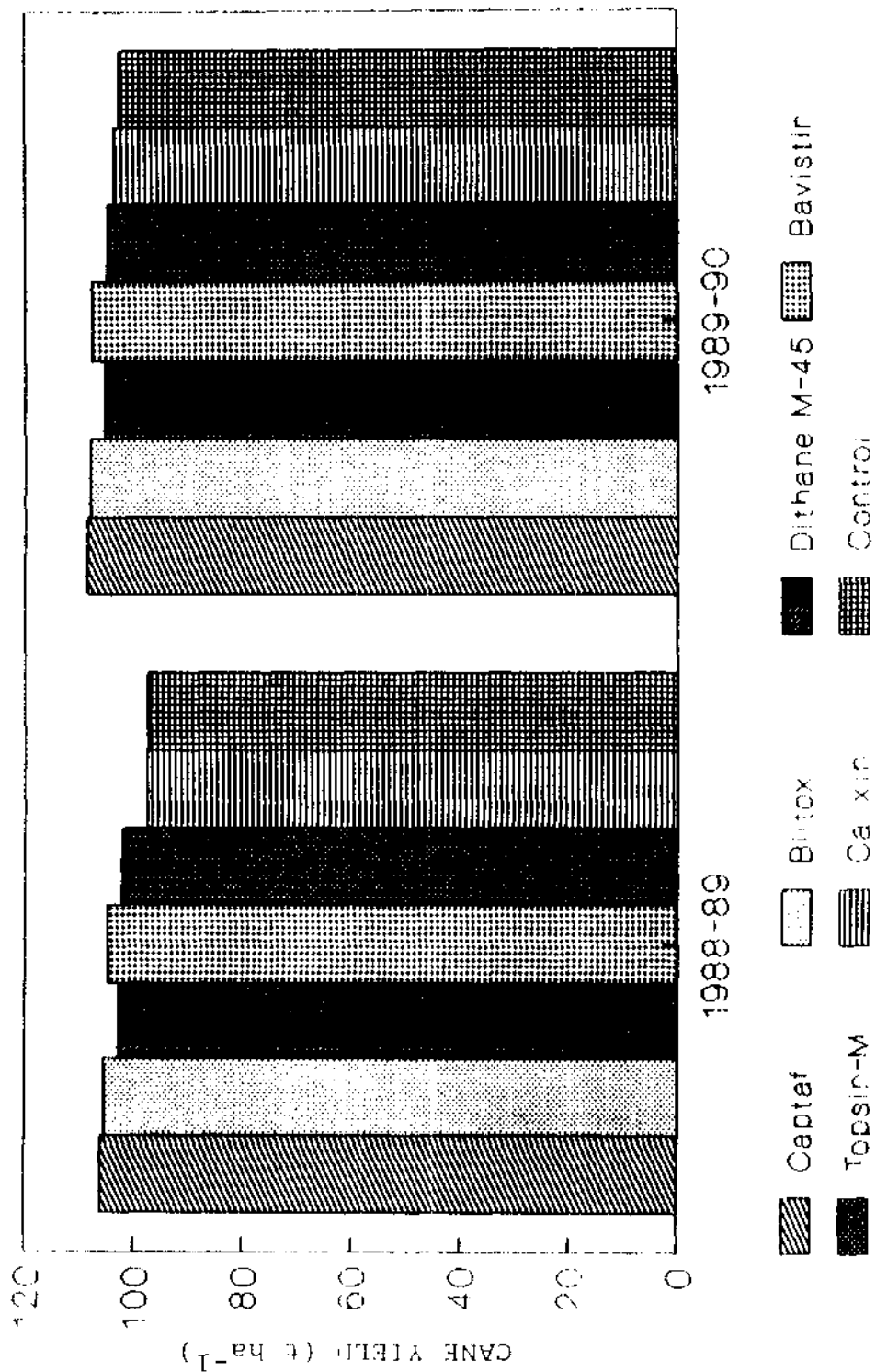


Fig.11: EFFECT OF DIFFERENT FUNGICIDES ON CANE YIELD IN SUGARCANE CULTIVAR Co 7219

cane in the cultivar Co 7219 by spraying fungicides (Table 44). As the disease intensity was observed to be fast and progressive at the fag end of the growth phase, it might not have affected the growth parameters like length and diameter of the cane. Peak intensity during the maturity phase might have affected the yield parameters to a certain extent resulting in slight reduction in cane yield. However, the sucrose was significantly improved (Fig. 12) along with other juice quality parameters in all the treatments as compared to untreated control (Table 45). The maximum disease intensity during the maturity phase (November to January) must have affected the photosynthesis and thereby reduced sugar accumulation in the plant. Similar results were obtained with yellow spot disease of sugarcane by Prakasam and Satyanarayana (1969) and Ricaud et al. (1980) where copper oxychloride (0.4%) and Benomyl (0.1%) were used as foliar sprays.

The studies on field testing of fungicides revealed that spraying Captaf (0.3%), Blitox (0.4%), Bavistin (0.1%), Dithane M-45 (0.3%) or Topsin-M (0.1%) thrice at monthly interval not only reduced the disease intensity significantly but also significantly increased the juice quality coupled with slight increase in yield. Eventhough Calixin was not useful in reducing disease intensity and increasing the cane yield, it significantly improved the juice quality which might be due to the

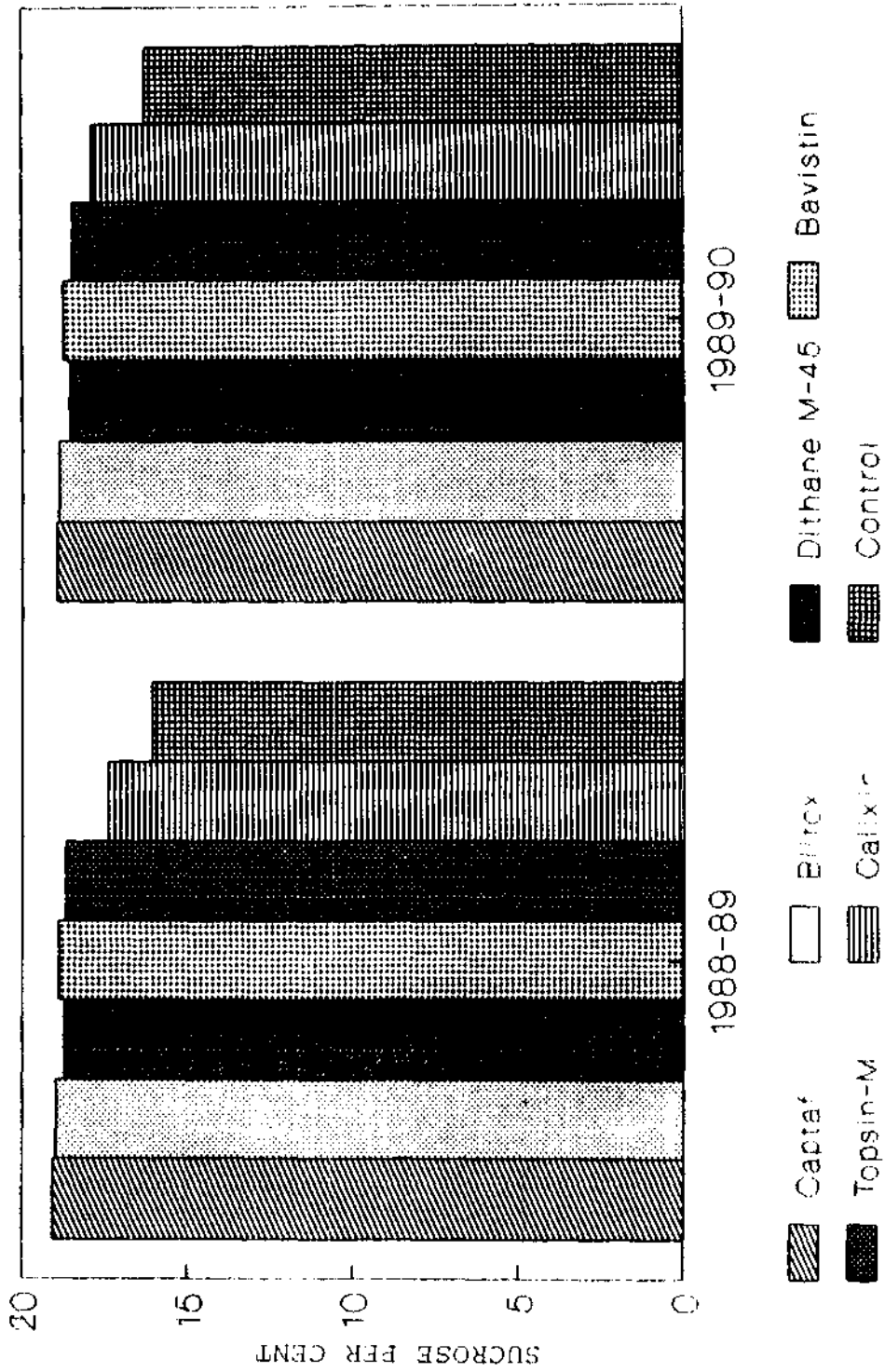


Fig.12: EFFECT OF DIFFERENT FUNGICIDES ON PER CENT JUICE SUCROSE IN THE SUGARCANE CULTIVAR CO 7219

dehydrating effect of the fungicide. Similar observations were also made with rust on sugarcane (RARS, Anakapalle, 1979). Future studies on this aspect are needed.

## 5.12 LOSSES DUE TO RING SPOT DISEASE IN DIFFERENT SUGARCANE VARIETIES/CLONES

### 5.12.1 Loss of chlorophyll due to the disease

The study revealed that chlorophyll a and b contents decreased in unsprayed canes over captaf sprayed canes in all the test varieties. In the resistant varieties/clones there was not much variation in chlorophyll a (1.37 to 5.25%) and b (2.84 to 8.41%) between sprayed and unsprayed canes (Table 46). In susceptible varieties the per cent reduction in chlorophyll a and b in unsprayed canes ranged from 39.91 to 52.33 and 28.19 to 49.11 respectively. Highly significant positive correlation was also observed between PDI and reduction in chlorophyll a and b in susceptible varieties (Table 47). This clearly indicates the sensitivity of these varieties to ring spot with respect to chlorophyll content. Though the contents of both chlorophyll a and chlorophyll b were reduced in the leaves of unsprayed canes, the reduction of chlorophyll a was more as compared to chlorophyll b. The loss of chlorophyll indirectly affects the net photosynthetic activity of the plant. The loss of overall chlorophyll content

with reduced photosynthetic activity in fungal leaf spot diseases of sugarcane has been reported (Agnihotri, 1990). Loss of chlorophyll due to L. bicolor in sugarcane (Exconde, 1963; Sampang and Reyes, 1985), L. glumarum in wheat (Karjalainen and Karjalainen, 1990) have been reported.

#### 5.12.2 Loss in length of millable cane, diameter and weight of cane

The loss due to ring spot on length, diameter and weight of cane was studied in 20 sugarcane varieties/clones under sprayed and unsprayed conditions. There is significant reduction in cane length, diameter of the cane as also cane weight in unsprayed canes over sprayed ones (Tables 50 to 53). In the present study, the per cent loss in cane weight was found to be negligible (0.00 to 0.78%) in varieties/clones (84A155, 86A444, 83A106, CoA 89081, Co 85045) which recorded less than 10 PDI, while it was more (7.21 to 11.85%) in four varieties viz., (CoA 8401, Co 7219, Co 7636, Co 85044) which recorded 50 PDI and more. With every one unit increase in PDI value, the reduction in length, diameter and weight of cane increased by 0.14 cm, 0.17 cm and 0.17 kg respectively (Table 54 and Fig. 13).

The quantitative estimation of losses due to ring spot disease by controlling the disease with fungicides

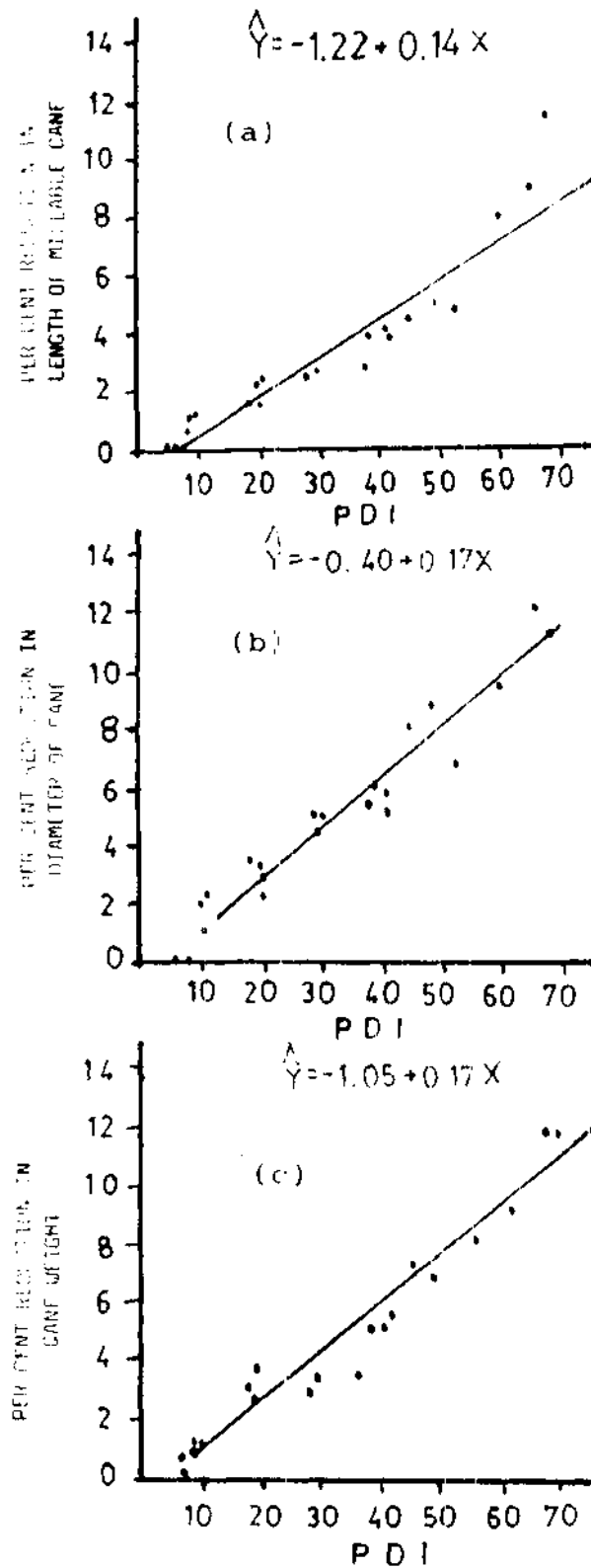


Fig.13: RELATIONSHIP BETWEEN PDI IN UNSPRAYED CANE AND PER CENT REDUCTION IN (a) LENGTH OF MILLABLE CANE (b) DIAMETER AND (c) CANE WEIGHT IN UNSPRAYED CANE OVER SPRAYED CANE

for effective comparison, has not been reported previously. Butler (1918) opined that no considerable damage was caused by ring spot disease inspite of severe attacks in Burma on thick canes than on thin canes. North (1923) from Australia and Altson (1926) from Guiana reported severe out breaks of ring spot. Appreciable damage in susceptible varieties from Cuba (Bourne, 1934; Bruner, 1940), Guiana (Altson, 1926) and Madagascar (Moreau, 1949) and slight damage from Venezula (Muller, 1941) have been reported. According to Agnihotri (1990) the losses caused by ring spot have not been worked out, because it was presumed that the losses were almost negligible and the disease, by and large, was considered of minor importance but opined that it could be dangerous. Cane yield reductions upto 10 to 30 per cent due to leaf blight caused by L. taiwanensis (Yen, 1964; Leu and Hsieh (1969) and 17 to 25 per cent due to leaf scorch caused by L. bicolor (Lo and Ling, 1950; Exconde, 1963; Sampang and Reyes, 1980) were reported. Marked reduction in cane length, diameter as well as number of green leaves due to leaf scorch resulting in reduction of cane yield of 32.8 per cent was reported by Sampang and Reyes (1985). Yield reductions due to Leptosphaeria infection in other crops like wheat (Wafford and Whitbread, 1978; Luz, 1984; Roux, 1984; Karjalainen and Karjalainen, 1990) and rice (Paracer and Luthra, 1944 and Rangaswamy, 1979) have been well documented.

### 5.12.3 Loss in quality of cane juice

Several qualitative characteristics of cane juice are affected as a result of the disease in 20 sugarcane varieties/clones tested. There was a fall in total solids, sucrose, juice purity and pH while an increase was noticed with respect to glucose and electrical conductivity of juice (Table 55). With every one unit increase in PDI value the reduction in total solids, sucrose content, purity and acidity of juice increased by 0.27, 0.33, 0.07 and 0.003 units respectively. Similarly, the increase in glucose and E.C. was found to have increased by 0.004 and 0.015 units respectively with every one unit increase in PDI value (Table 63 and Fig.14) Yen (1964) and Leu and Hsieh (1969) reported loss of sucrose in juice due to L. taiwanensis. A reduction of 10-36 per cent in juice sucrose (Lo and Ling, 1950; Exconde, 1963; Sampang and Reyes, 1985) and 3.7 to 16.3 per cent in total solids (Chu and Tsai, 1952) has been reported due to infection by L. bicolor in sugarcane. Reduction in juice sucrose and purity due to yellow spot has been recorded by Prakasam and Satyanarayana (1969). Reduction in juice sucrose, total solids, juice purity and pH and increase in glucose and electrical conductivity of juice due to redrot (Omkar Singh and Waraitch, 1977; Achutarama Rao and Satyanarayana, 1983) and leaf scald (Satyanarayana, 1985) have been reported. In the present study, a maximum reduction

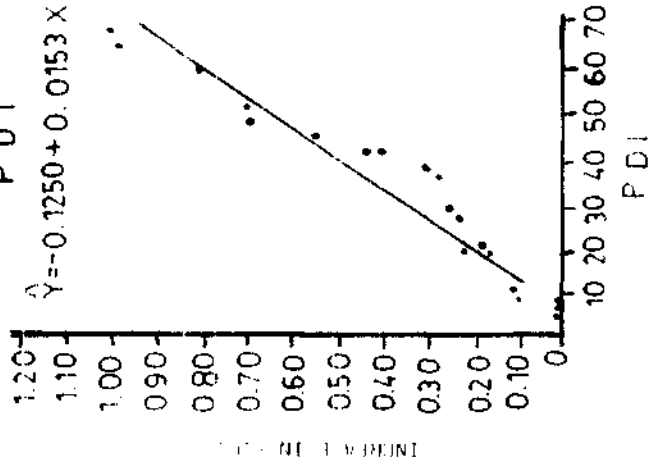
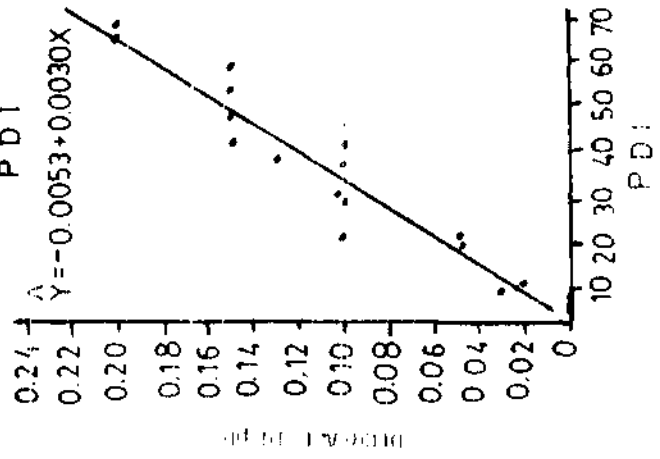
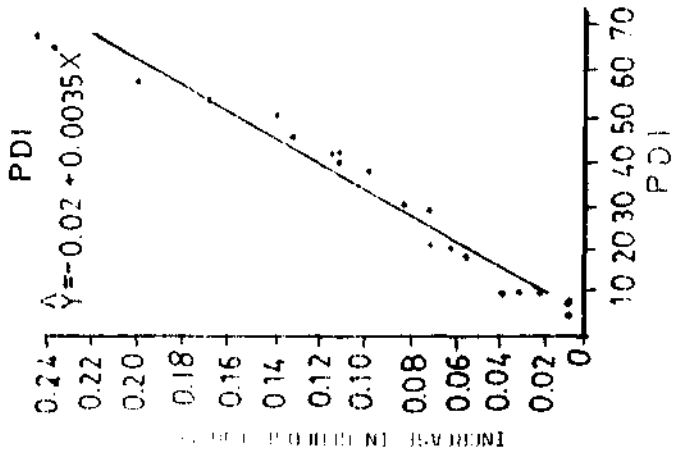
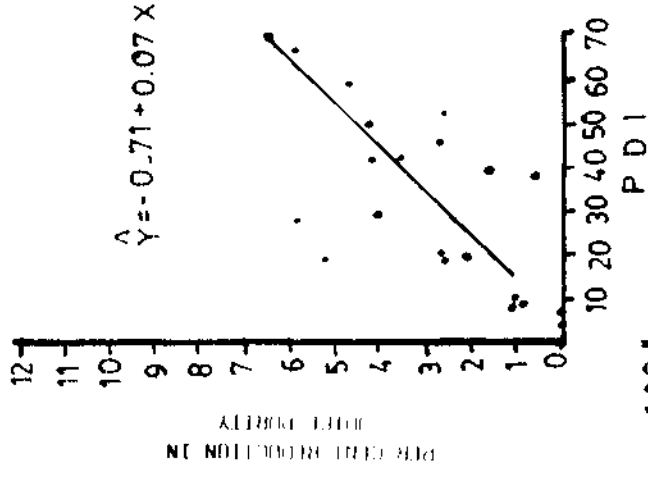
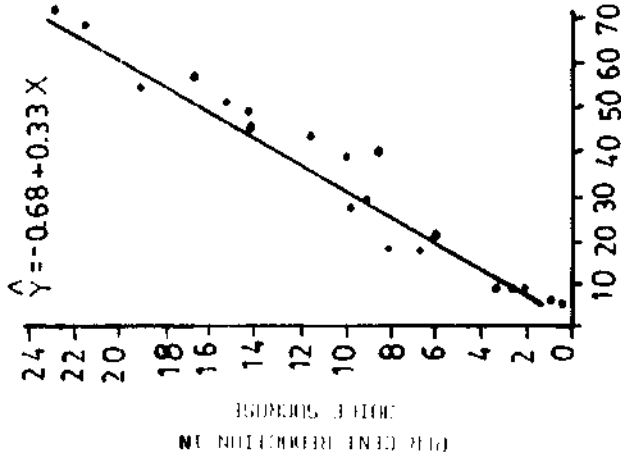
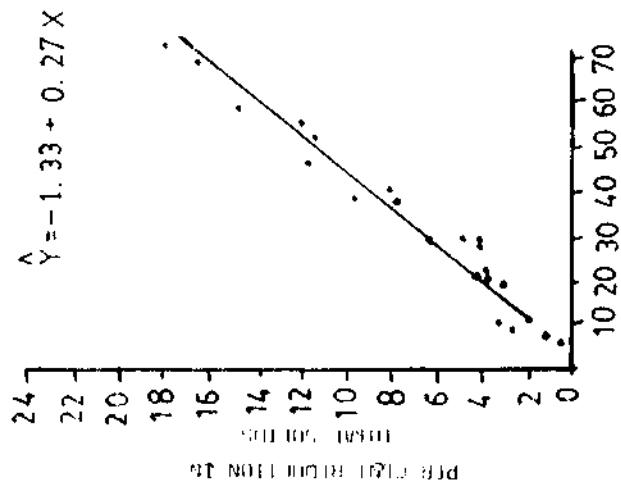


Fig. 14--THE RELATIONSHIP BETWEEN PDI IN UNSPRAYED CANE AND CERTAIN QUALITATIVE CHARACTERS OF CANE JUICE AS REDUCTION/INCREASE IN UNSPRAYED OVER SPRAYED.

of 22.88 per cent in juice sucrose in Co 7636 was observed (Table 55), indicating the adverse effect of the disease on recovery of sugar or jaggery.

There was progressive reduction in cane weight and juice sucrose due to ring spot infection in all the test varieties/clones in maturity phase particularly in susceptible varieties (Table 65 and Fig.16). This reduction might be due to progressive increase in the disease intensity from the starting of maturity phase (November) till harvest in February (Table 64 and Fig.15). The losses in cane weight and juice quality might be attributed to reduced photosynthetic activity towards the lag end of growth phase and entire maturity phase. Reduction in yields due to loss of chlorophyll and reduced photosynthesis in many fungal leaf spot diseases of crops have been reported (Agrios, 1978). Loss in yield due to reduced green leaf area in ring spot (Singh, 1978) and leaf scorch (Exconde, 1963; Sampang and Reyes, 1985) of sugarcane and glume blotch of wheat (Karjalainen and Karjalainen, 1990) has also been recorded.

As the disease causes losses in yield and juice quality, both the farmer and sugar factory management incur losses when susceptible varieties like Co 7219, Co 975 and CoT 8201 are grown in large areas. As the cane price is fixed in a factory based on the sugar recovery, the cultivators incur loss from ring spot both due to low

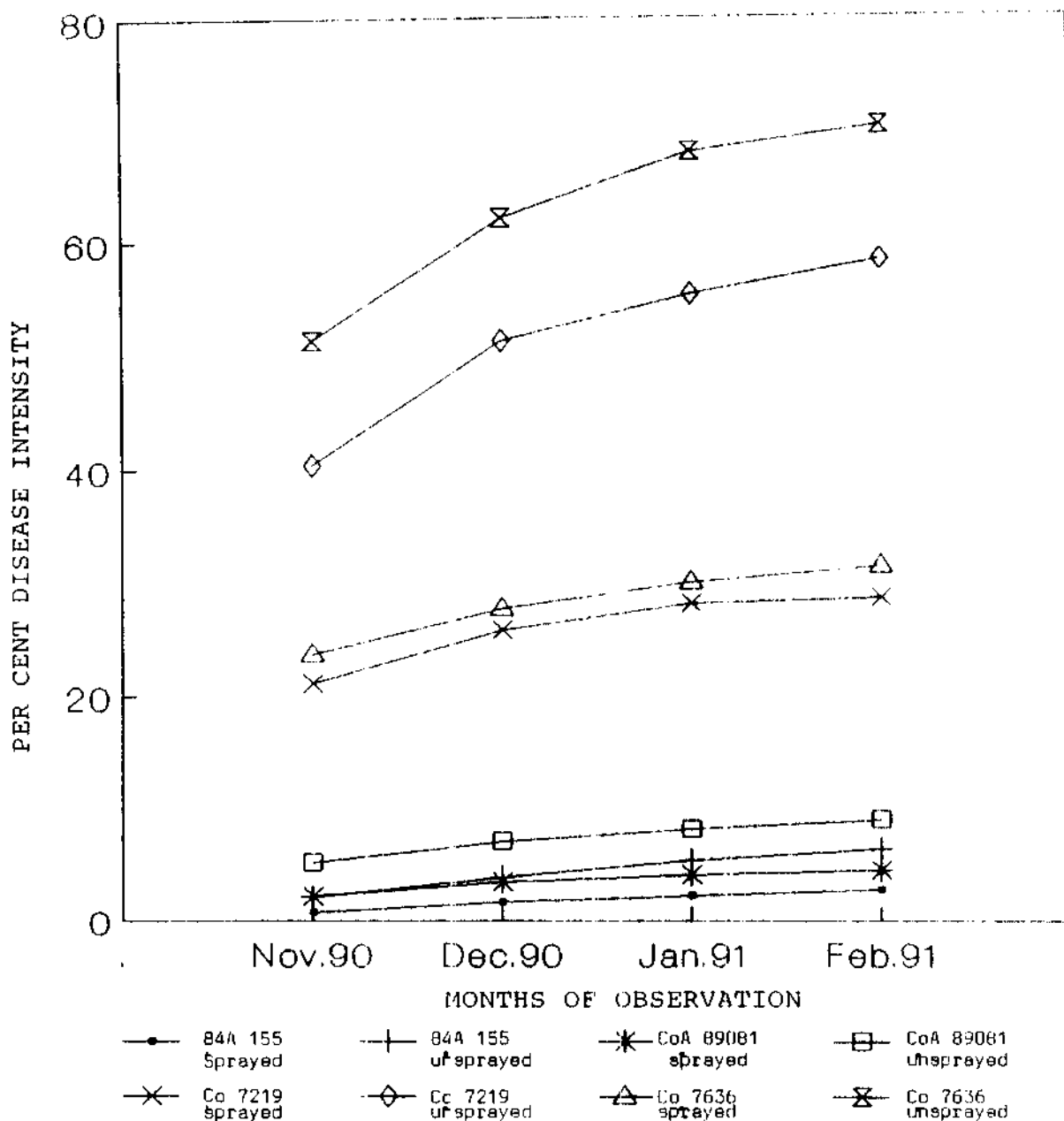


Fig.15: PROGRESS OF DISEASE INTENSITY IN MATURITY PHASE OF THE CROP

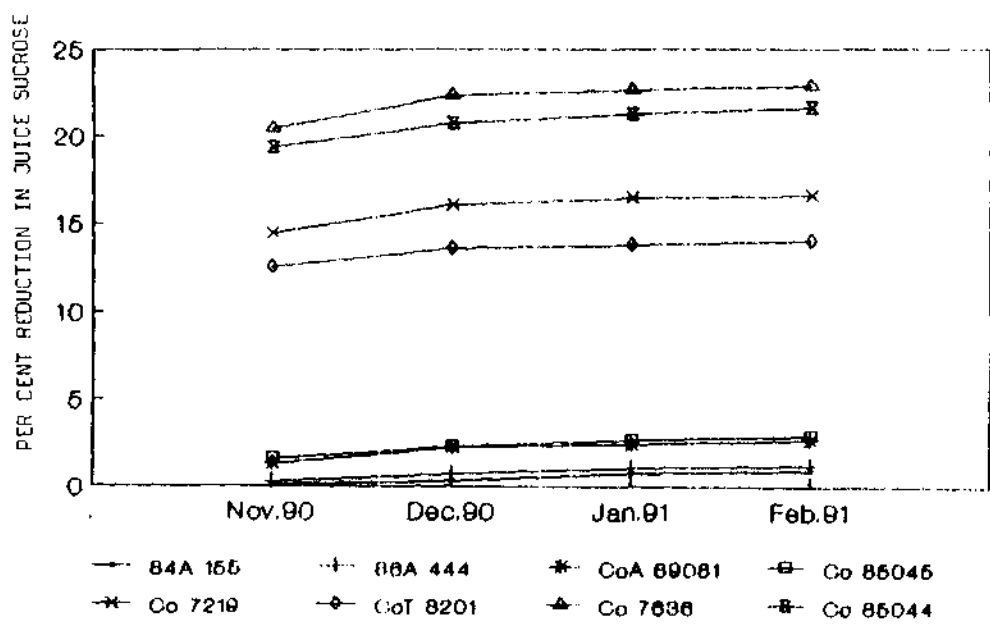
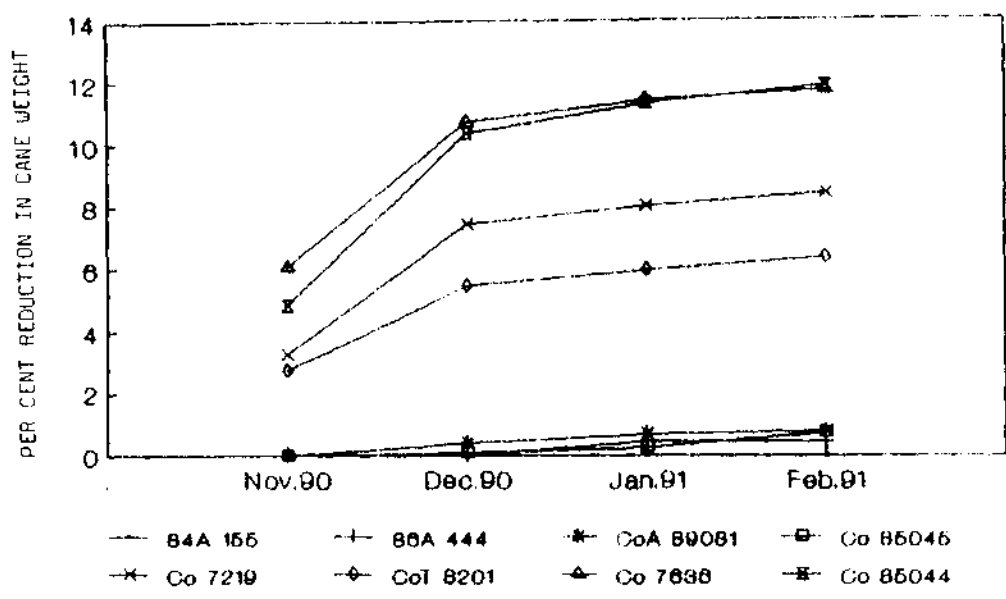


Fig.16: RATE OF REDUCTION IN CANE WEIGHT AND JUICE SUCROSE IN MATURITY PHASE DUE TO RING SPOT DISEASE IN DIFFERENT VARIETIES/CLONES (1990-91)

tonnage of cane and low cane price paid per tonne due to loss in sugar recovery.

Future studies on development of synthetic media for production of perithecia and ascospores, mechanism of disease resistance, disease cycle, role of perithecia/ascospores in the production and perpetuation of the disease, effect of different levels of N and K fertilizers on disease intensity in different resistant and susceptible cultivars, nature of inheritance of disease resistance, effect of disease on jaggery yield and quality are required to have a much better understanding of ring spot disease of sugarcane.

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# SUMMARY

## CHAPTER VI

### SUMMARY

Ring spot of sugarcane caused by Leptosphaeria sacchari van Breda de Hann, considered a disease of minor importance, started appearing in severe form since 1985-86 after the introduction of varieties like Co 7219, CoT 8201, Co 975 and CoP 8001 on commercial scale in Andhra Pradesh, India. The endemic and serious nature of the disease in most of the cane growing areas of the state particularly in coastal districts of Andhra Pradesh prompted taking up investigations reported in this thesis at Regional Agricultural Research Station, Anakapalle situated in North Coastal Andhra Pradesh. The results of these investigations are summarised below.

Development of symptoms in nature was studied on four popular varieties of sugarcane viz., Co 7219, CoT 8201, CoA 89081 and Co 85045. The symptoms of the disease were observed only on the lamina region but not on leaf sheath and cane stalk. The spots are oval to irregular with reddish brown margin and with straw coloured centre in which perithecia developed as black dots. The spots coalesced resulting in drying of large part of the leaf followed by premature senescence. In Co 7219 and CoT 8201, except 3 to 4 leaves at the top, the remaining foliage dried up at the time of harvest during February.

In CoA 89081 and Co 85045 only few spots were produced and no premature senescence was noticed. The time of appearance of the disease and shape and size of the spots varied among varieties.

Isolation from immature spots yielded Nigrospora sacchari, Curvularia lunata, Phyllosticta saccharicola and Bipolaris sacchari. Isolations from mature spots did not yield perithecia but produced pycnidia and pycnidiospores of Phyllosticta saccharicola abundantly. Both single ascospores and pycnidiospores yielded only pycnidia one month after inoculation on different media. Perithecia and ascospores were however produced in abundance on sterile host leaf bits of susceptible varieties Co 7219, Co 7636 and CoT 8201 inoculated with ascospores from mature spots when vitamins and sucrose were added and incubated at 24°C and 26°C and exposed to alternate light and darkness for 12 hours each for 18-21 days. Following perithecial production, pycnidia of P. saccharicola developed on these leaf bits three weeks later. The measurements of fruiting bodies and spores produced both sexually and asexually in nature as well as in culture agreed with those of L. sacchari and P. saccharicola respectively as given in CMI descriptions.

Pathogenicity tests conducted with L. sacchari and P. saccharicola on Co 7219, CoT 8201 and Co 7636 resulted in clear development of typical ring spot lesions

while other fungal isolates did not produce typical symptoms.

Out of several media tested, corn meal agar and bean meal agar were found good for mycelial growth of L. sacchari whereas PDA + cane leaf extract and OMA + cane leaf extract were better for both growth and sporulation of P. saccharicola.

For estimation of disease intensity, a 0-9 scale was developed based on the per cent green leaf area affected.

Survey in the three regions of the state of Andhra Pradesh indicated that the disease intensity was severe in coastal Andhra Pradesh and Telangana but negligible in Rayalaseema particularly in Renigunta factory area. The disease intensity was observed to be more under stress conditions particularly in clay loam soils where high doses of nitrogen fertilizers were applied. Ratoon crops in most of the areas recorded more disease intensity compared to plant crop.

Among the four successful inoculation techniques tested, spraying conidial suspension on the leaves was preferred as it was found to be easy and simple to operate.

Out of five screening methods tried, Rajoung method of screening was preferred as it was rapid, reliable and saves two months time compared to other methods.

The disease intensity increased progressively and significantly with increased level of nitrogen from 0 to 112 and 112 to 224 kg ha<sup>-1</sup>. No significant increase in the intensity was observed from 224 to 336 kg ha<sup>-1</sup>. Application of phosphorus at 100 kg ha<sup>-1</sup> did not influence the disease severity, while potassium at the same level reduced the disease incidence significantly. The PDI exhibited highly significant positive correlation with per cent leaf nitrogen and highly significant negative correlation with per cent sheath potassium.

Studies indicated that age of the crop had no effect on disease intensity.

Highly significant negative correlation was observed between PDI and angle of the leaf, and silica content of the leaf.

Irrespective of the variety, the disease intensity was noted to be more under moisture stress (7.8 to 81.7%) and water stagnation (8.1 to 79.8%) compared to normal irrigation (6.1 to 65.2%).

Six isolates of P. saccharicola (L. sacchari) from different varieties and locations did not show any

variation in their cultural, morphological and pathological characters.

The disease was observed to spread mainly through contact of diseased leaf with healthy leaf and infected host debris on the surface of soil at the base of the clumps. Soil, irrigation water, cane setts with infected leaf tissues did not aid in the spread of the disease.

In the host range studies, out of twenty plant species inoculated only one viz., Saccharum spontaneum (Rellu and SES.594) took infection and produced typical ring spot symptoms.

For assessing varietal reaction, it was found desirable that final disease assessment be made six months after inoculation.

Out of several varieties/clones of sugarcane screened by adult cane and Rajoong method of inoculation, 8 varieties/clones viz., Co 85045, CoA 89081, 83A106, 84A155, 86A444, Co 85032, 87A142 and 87A279 were found resistant and fourteen important commercial varieties viz., Co 7219, CoT 8201, CoA 8401, Co 7706, Co 419, CoA 7602, Co 62175, Co 6907, Co 8013, CoC 671, Co 975, Co 8021 and CoA 89085 were found susceptible to highly susceptible. Screening by Rajoong method of inoculation was preferred as it is rapid and reliable.

Bavistin, Topsin-M and Calixin at 10 to 50 ppm; Captaf, Dithane M-45, Blitox, Captafol and Bayleton at 200 ppm and Dithane 2-78 and chlorothalonil at 500 ppm were effective against P. saccharicola in vitro. Out of the six fungicides tested in vivo for their efficacy in controlling the disease on the susceptible variety Co 7219, five fungicides viz., Captaf (0.3%), Blitox (0.4%), Bavistin (0.1%), Dithane M-45 (0.3%) and Topsin-M (0.1%) not only significantly reduced the disease intensity but also significantly increased the juice quality. Spraying with fungicides did not affect the yield parameters significantly.

Significant reduction in the chlorophyll a and b contents due to the disease was observed in all the susceptible varieties tested. Reduction was more in case of chlorophyll a than that of chlorophyll b.

Ring spot infection was found to adversely affect both quantitative and qualitative characters in twenty varieties/clones studied. There was significant reduction in length of millable cane, diameter and weight of cane in unsprayed canes compared to captaf sprayed canes. With every one unit increase in PDI, reduction in length of millable cane, diameter and weight of cane increased by 0.14 cm, 0.17 cm and 0.17 kg respectively. There was a fall in total solids, sucrose, purity and pH of juice while there was an increase in glucose and electrical

conductivity of juices of unsprayed canes. With every one unit increase in PDI value, the reduction in total solids, sucrose content, purity and pH of juice increased by 0.27, 0.33, 0.07 and 0.003 units respectively. Similarly the increase in glucose and E.C. increased by 0.004 and 0.015 units respectively with every one unit increase in PDI value.

With progressive increase in disease intensity, there was progressive reduction in cane weight and juice quality in all the test varieties, especially in the susceptible ones.

The present study revealed that incidence of ring spot disease of sugarcane was severe in coastal Andhra Pradesh and Telangana and negligible in Rayalaseema. The intensity of the disease was noted to be more under high nitrogen fertilization, moisture stress and water stagnation. The disease caused loss, both in cane weight and juice quality in different sugarcane varieties. The intensity of the disease and losses could be reduced by application of recommended dose of N fertilizer, application of potassium at  $100 \text{ kg ha}^{-1}$  in drought prone areas and providing proper drainage in the fields. Spraying of captaf (0.3%) or Blitox (0.4%) or Bavistin (0.1%) thrice at monthly intervals from the initiation of the disease reduced the disease incidence and increased juice quality.

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## LITERATURE CITED

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\* Original not seen

# APPENDICES

Appendix A: Reduction in length of millable cane in unsprayed and capital sprayed canes of different varieties/clones.

Variety/ clone	Sprayed (cm)	Unsprayed (cm)	Difference (cm)	Per cent reduction in unsprayed over sprayed
C5 419	293.6	282.7	10.9	3.71
C5 975	278.2	266.4	11.8	4.24
C5 7219	312.6	288.2	24.4	7.81
C5 7636	260.6	230.1	30.5	11.71
C5 7706	282.4	271.6	10.8	3.82
C5 8205	294.8	289.7	5.1	1.73
C5 8220	291.2	284.1	7.1	2.44
C5 62175	338.2	329.1	9.1	2.69
C5 85038	288.6	280.3	8.3	2.87
C5 85044	268.4	240.8	27.6	10.28
C5 85045	276.9	275.6	1.3	0.47
C5A 7602	320.8	308.7	12.1	3.77
C5A 8401	282.4	269.1	13.3	4.71
C5A 8402	304.7	298.6	6.1	2.00
C5A 89081	302.6	299.8	2.8	0.92
C5I 8201	295.4	281.2	14.2	4.81
83A 106	284.6	281.5	3.1	1.10
83A 214	283.9	278.2	5.7	2.01
84A 155	314.4	314.2	0.2	0.000
84A 444	287.1	286.7	0.4	0.000
Mean	293.1	282.0	11.3	3.86

Appendix B: Reduction in diameter of the main stem of spruce trees over eight years of sprayed versus unsprayed different carpet rose clones

Variety/ clone	Sprayed (cm)	Unsprayed (cm)	Difference (cm)	Per cent reduction in unsprayed over sprayed
Co 419	2.68	2.51	0.17	6.34
Co 975	2.64	2.45	0.21	7.95
Co 7219	2.75	2.49	0.26	9.45
Co 7636	2.72	2.41	0.31	11.40
Co 7706	3.05	2.90	0.15	4.92
Co 8205	2.87	2.77	0.10	3.48
Co 8220	2.69	2.56	0.13	4.83
Co 62175	3.21	3.03	0.18	5.61
Co 85038	2.65	2.51	0.12	4.56
Co 85044	2.64	2.32	0.32	12.12
Co 85045	2.82	2.79	0.03	1.06
CoA 7602	2.92	2.75	0.17	5.83
CoA 8401	2.62	2.45	0.17	6.49
CoA 8402	2.70	2.61	0.09	3.33
CoA 89081	2.77	2.71	0.06	2.17
CoI 8201	2.81	2.56	0.25	8.90
83A 106	2.81	2.74	0.07	2.49
83A 214	2.74	2.68	0.06	2.19
84A 155	3.04	3.02	0.01	0.30
86A 444	2.62	2.62	0.00	0.00
Mean	2.79	2.74	0.15	5.16

Appendix C: Reduction in cane weight (kg) in unsprayed over sprayed cane and difference in cane loss (t/ha)

Variety/ clone	Sprayed (kg)	Unsprayed (kg)	Difference (kg)	Percent reduction in unsprayed over sprayed
Co 419	1.190	1.130	0.060	5.04
Co 975	1.080	1.065	0.015	1.39
Co 7219	1.425	1.305	0.120	8.42
Co 7636	1.110	0.980	0.130	11.71
Co 7706	1.380	1.310	0.070	5.07
Co 8205	1.020	0.990	0.030	2.94
Co 8220	1.340	1.303	0.037	2.76
Co 62175	1.515	1.460	0.055	3.63
Co 85038	1.260	1.215	0.045	3.57
Co 85044	1.055	0.930	0.125	11.85
Co 85045	1.035	1.028	0.007	0.68
CoA 7602	1.345	1.330	0.015	1.12
CoA 8401	1.010	0.965	0.045	4.51
CoA 8402	1.170	1.078	0.092	7.87
CoA 89081	1.255	1.265	0.010	0.78
Co1 8201	1.415	1.355	0.060	4.24
83A 106	1.075	1.025	0.050	4.66
83A 214	0.987	0.958	0.029	2.94
84A 155	1.475	1.475	0.000	0.00
86A 444	1.110	1.110	0.000	0.00
Mean	1.214	1.157	0.057	4.75

Appendix D: Reduction in total solids in juice of unsprayed over control sprayed canes of different varieties/clones

Variety/ clone	Sprayed (%)	Unsprayed (%)	Difference (%)	Per cent reduction in unsprayed over sprayed
Co 419	19.7	17.7	2.0	10.15
Co 975	18.9	16.7	2.2	11.64
Co 7219	20.8	17.6	3.2	15.39
Co 7636	20.7	16.8	3.7	18.05
Co 7706	20.5	18.8	1.7	8.29
Co 8205	19.7	18.9	0.8	3.08
Co 8220	20.1	19.3	0.8	3.98
Co 62175	18.1	17.2	0.9	4.97
Co 85038	19.8	18.8	1.0	5.05
Co 85044	19.6	16.3	3.3	16.83
Co 85045	19.8	19.5	0.3	1.51
CoA 7602	18.9	16.2	2.7	14.28
CoA 8401	20.7	17.4	3.3	15.94
CoA 8402	21.7	20.4	1.3	5.99
CoA 89081	21.9	20.6	1.3	5.93
CoI 8201	18.9	17.6	1.3	6.88
83A 106	20.2	19.6	0.6	2.97
83A 214	19.3	18.6	0.7	3.63
84A 155	21.2	21.1	0.1	0.47
86A 444	19.9	19.3	0.6	2.99
Mean	20.0	18.3	1.7	8.51

Appendix 1: Reduction in grape production in unsprayed plots  
 capital sprayed cases and difference varietal clones

Variety/ clone	Sprayed (%)	Unsprayed (%)	Difference (%)	Per cent reduction in unsprayed over sprayed
Co 419	18.75	16.27	1.89	8.21
Co 975	16.92	14.22	2.31	13.59
Co 7219	19.36	16.14	3.22	16.64
Co 7636	18.10	14.19	4.21	23.80
Co 7706	18.90	16.77	2.13	11.57
Co 8205	17.72	16.28	1.64	8.17
Co 8220	18.72	16.49	1.75	9.53
Co 62175	16.77	14.71	1.54	9.19
Co 85038	18.72	16.74	1.67	8.97
Co 85044	17.94	14.07	3.88	21.63
Co 85045	18.17	17.72	0.45	2.54
CoA 7602	16.01	13.81	2.20	13.74
CoA 8401	19.17	19.65	-3.62	-18.79
CoA 8402	19.32	18.20	1.23	6.35
CoA 89081	18.75	18.36	0.39	2.01
CoI 8201	18.34	19.65	-2.29	-12.47
83A 106	18.70	17.90	0.60	3.21
83A 214	17.02	16.00	1.03	6.05
84A 155	19.36	19.36	0.10	0.51
86A 444	17.75	17.58	0.17	0.95
Mean	18.13	16.30	1.83	10.05

Appendix U: Residue (mg) per 100 g juice per plant for 16 grape varieties and 16 grape cultivars sprayed with captan compared to canopy of different cultivars (residues)

variety/ clone	Sprayed	Unsprayed	Difference ( $\mu\text{g}$ )	Percentage reduction in unsprayed over sprayed
Co 419	92.15	91.86	0.27	0.29
Co 975	87.56	85.15	2.31	2.64
Co 7219	95.68	88.90	6.18	4.89
Co 7636	89.75	83.96	5.60	6.24
Co 7706	92.70	88.99	3.75	4.06
Co 8205	90.85	86.14	4.75	5.21
Co 8220	90.64	85.44	5.21	5.75
Co 62175	86.96	85.52	1.38	1.59
Co 85038	92.88	89.04	3.84	4.14
Co 85044	91.55	86.26	5.27	5.76
Co 85045	91.67	90.87	0.80	0.87
CoA 7602	88.45	85.25	3.20	3.62
CoA 8401	92.20	89.94	2.26	2.49
CoA 8402	91.55	89.21	2.34	2.66
CoA 89081	89.08	88.35	0.70	0.79
CoI 8201	92.66	88.90	3.76	4.06
83A 106	91.58	90.72	0.86	0.94
83A 214	88.55	86.82	1.72	1.97
84A 155	91.55	91.25	0.30	0.30
86A 444	91.55	91.08	0.48	0.50
Mean	90.85	88.17	2.67	2.91

Appendix G: Increase in chlorine content in juice of unsprayed (over control) sprayed clones of different varieties/clone

Variety/ clone	Sprayed µg/ltr	Unsprayed µg/ltr	Difference µg/ltr	Per cent. reduction in unsprayed over sprayed
Co 419	0.7	0.58	0.12	25.71
Co 975	0.45	0.56	0.11	31.11
Co 7219	0.70	0.58	0.12	28.57
Co 7636	0.71	0.59	0.12	30.61
Co 7706	0.56	0.55	0.01	25.91
Co 8205	0.52	0.55	0.03	19.23
Co 8220	0.48	0.55	0.07	14.58
Co 62175	0.42	0.52	0.10	23.81
Co 85038	0.57	0.60	0.03	19.28
Co 85044	0.36	0.60	0.24	66.67
Co 85045	0.46	0.48	0.02	4.35
CoA 7602	0.58	0.69	0.11	18.97
CoA 8401	0.58	0.59	0.01	32.11
CoA 8402	0.49	0.52	0.03	15.38
CoA 89081	0.55	0.41	0.04	10.01
CoI 8201	0.51	0.45	0.06	35.18
83A 106	0.57	0.42	0.15	26.32
83A 214	0.56	0.63	0.07	12.50
84A 155	0.44	0.45	0.01	21.21
86A 444	0.51	0.52	0.01	1.96
Mean	0.49	0.55	0.06	15.29



Appendix H: Fall in pH in juice of unprayed over control sprayed canopy of different varieties/clones

Variety/ clone	Sprayed	Unsprayed	Difference
Co 419	5.75	5.65	0.10
Co 975	5.60	5.50	0.10
Co 7219	5.20	5.05	0.15
Co 7636	5.45	5.25	0.20
Co 7706	4.75	4.65	0.10
Co 8205	5.65	5.60	0.05
Co 8220	5.25	5.15	0.10
Co 62175	5.45	5.32	0.13
Co 85038	4.75	4.65	0.10
Co 85044	5.15	4.95	0.20
Co 85045	5.40	5.40	0.00
CoA 7602	5.50	5.35	0.15
CoA 8401	5.55	5.40	0.15
CoA 8402	5.35	5.30	0.05
CoA 89081	5.55	5.52	0.03
CoI 8201	5.45	5.30	0.15
83A 106	5.20	5.18	0.02
83A 214	5.05	4.95	0.10
84A 155	5.10	5.10	0.00
86A 444	4.55	4.55	0.00
Mean	5.28	5.18	0.10

Appendix 1: Increase in Electrical Conductivity (dS/m) of unsprayed over control sprayed clones of different varieties/ clones

Variety/ clone	Sprayed	Unsprayed	Difference	Percent increase in unsprayed over sprayed
Co 419	2,762	3,104	0,342	12,38
Co 975	1,932	2,472	0,540	27,95
Co 7219	2,269	3,066	0,797	35,13
Co 7636	2,592	3,492	1,000	41,81
Co 7706	2,612	3,018	0,406	15,54
Co 8205	2,761	2,942	0,181	6,56
Co 8220	2,616	2,848	0,232	9,28
Co 62175	2,522	2,622	0,300	12,52
Co 85038	2,496	2,756	0,260	10,42
Co 85044	2,702	3,686	0,984	36,42
Co 85045	2,554	2,568	0,014	0,60
CoA 7602	2,052	2,486	0,434	21,15
CoA 8401	3,264	3,962	0,698	21,38
CoA 8402	2,722	2,926	0,204	7,50
CoA 89081	2,492	2,791	0,099	3,97
CoT 8201	2,512	3,116	0,604	24,05
83A 106	2,570	2,672	0,102	3,96
83A 214	2,592	2,598	0,006	0,21
84A 155	2,602	2,614	0,012	0,46
86A 444	2,272	2,286	0,014	0,62
Mean	2,473	2,875	0,382	15,46

VITA

I, **M.ACHUTARAMA RAO**, was born on 30th September, 1949 to **Smt. Subbalakshmi** and **Venkata Subrahmanyam** at Lolla Village, East Godavari district, Andhra Pradesh (India). I married Miss **Vijayalakshmi** on 4th February, 1973 and we have one son **M.V.S.Srikanth** and one daughter **M.V.Bhargavi**. I obtained my B.Sc.(Ag) degree in 1970 and M.Sc.(Ag) in Plant Pathology in 1971 from Andhra Pradesh Agricultural University, in First Class. I joined the A.P.Agricultural University as Research Assistant in Sugarcane Pathology at Regional Agricultural Research Station (Sugarcane Research Station), Anakapalle in April, 1975. I worked for 19 years in Sugarcane Pathology as Research Assistant and Assistant Plant Pathologist. At present I am working as Assistant Plant Pathologist (Selection Grade) in AICRP on Pepper at Regional Agricultural Research Station, Chintapalle. During January, 1987, I have joined the Ph.D. programme in Andhra Pradesh Agricultural University. I worked for my Ph.D. degree in Plant Pathology under the guidance of **Dr.M.Sugunakar Reddy**, Dean of Agriculture, A.P. Agricultural University. I have published 34 research papers in National and International Journals, three of which have secured awards. I have also published 24 popular articles in Telugu for the benefit of farmers. I was adjudged as the best Assistant Research Officer engaged in Sugarcane Research, during 1985 and was awarded **Sri C.V.Sudarsana Raju Memorial Prize**, with a gold medal and citation.