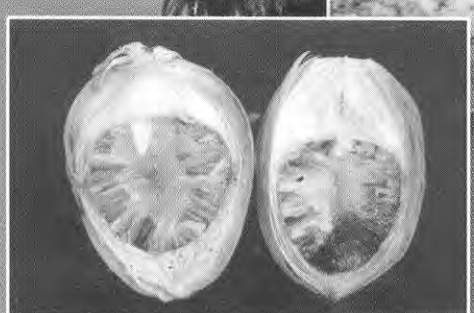
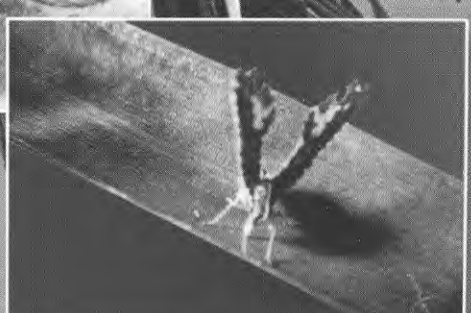
ARECANUT YELLOW LEAF DISEASE





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CENTRAL PLANTATION CROPS RESEARCH INSTITUTE
(Indian Council of Agricultural Research)
KASARAGOD 671 124 KERALA, INDIA

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CPCRI

ARECANUT YELLOW LEAF DISEASE

Editors
K.U.K. NAMPOOTHIRI
K.N. PONNAMMA
P. CHOWDAPPA

CENTRAL PLANTATION CROPS RESEARCH INSTITUTE
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KASARAGOD- 671 124, KERALA, INDIA

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K.U.K.Nampoothiri
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Central Plantation Crops Research Institute
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FOREWORD

Yellow leaf disease is one of the serious maladies hampering arecanut production especially along the West Coast of India. The disease, reported in 1914, reached epidemic proportion in Kerala and parts of Karnataka. The disease incidence is very high in Kerala affecting 233 million palms. A total of 7,04,140 palms were affected with an estimated loss of 508 tonnes of chali in Karnataka.

Research on yellow leaf disease initiated in the late 1950's at Central Arecanut Research Station, Palode (Kerala) was further intensified by Central Plantation Crops Research Institute after its establishment under Indian Council of Agricultural Research, New Delhi. As the etiology of the disease was a matter of dispute for a long time, the role of several factors (edaphic and biological) was investigated and Phytoplasma was conclusively established as the causal agent.

Identification of elite palms in 'hotspot' areas for using in resistance breeding programme and the sustained yield obtained through disease management are very significant steps. Though considerable research information on yellow leaf disease was generated, compilation of such information has not yet been undertaken. I must therefore congratulate Central Plantation Crops Research Institute, Kasaragod for taking the initiative to compile the information so far gathered for the benefit of area growers, students, researchers and planners.

Dr. R.N. PAL
Deputy Director General (Horticulture)
Indian Council of Agricultural Research
New Delhi - 110 001

PREFACE

Arecanut (*Areca catechu* L), an important and highly remunerative plantation crop in the humid tropics of India, is grown in 2,54,000 ha with an annual production of 3,33,000 tonnes. The nuts of arecanut are mainly used for masticatory purpose and in Hindu Socio-religious functions in India. The country reached self-sufficiency in arecanut production during 1974-75. Although arecanut does not have very many alternate uses and export potential value, area under the crop is rapidly expanding due to the attractive market price and resultant increase in income per unit area. Research on arecanut palm is being carried out only in India. Yellow leaf disease is one of the serious production constraints in Kerala, parts of Karnataka, Tamil Nadu and Maharashtra. The comprehensive survey indicated that total number of diseasd palms is 233 million in Kerala amounting to 36 per cent of the areca palms. In Karnataka, 7,69,140 palms are affected with an estimated yield loss of 508 tonnes of chali. The research work done, so far, on yellow leaf disease is presented in this volume particularly highlighting the major research achievements, problems and perspectives.

We are grateful to the Contributors of various chapters in this compilation without whose co-operation the venture would not have been possible. We are also thankful to Miss S. Sujatha, Dr. N. Saraswathy and Dr. Ravi Bhat for going through the manuscript and offering critical comments wherever needed and to Mr. S. Amaranarayana for word processing and image editing. Our thanks are due to M/s Codeword Printers, Mangalore for thier cooperation in bringing out this volume in time. We record our thanks to Indian Council of Agricultural Research, New Delhi for financial assistance.

EDITORS

CONTRIBUTORS

1. Mr. K.J. Abraham
   Naduvileparambil House
   Puthuppally PO
   Kottayam, Kerala

2. Dr. R.Chandra Mohanan
   Senior Scientist
   CPCRI Regional Station
   Kayangulam-690 533, Kerala

3. Dr. P.Chowdappa
   Senior Scientist
   CPCRI Regional Station
   Vittal-574 243, Karnataka

4. Dr. E.V.Daniel
   Senior Scientist (Retired)
   CPCRI Regional Station
   Vittal-574 243, Karnataka

5. Dr. C.K.Mathai
   Senior Scientist (Retired)
   Indian Institute of Horticultural Research
   Hessarghatta Lake PO
   Bangalore-560 016, Karnataka

6. Dr. A.A. Mohammad Sayed
   Scientist (Senior Scale)(Retired)
   CPCRI Research Centre
   Kannara-680 652, Kerala

7. Dr. K.U.K. Nampoothiri
   Director
   Central Plantation Crops Research Institute
   Kasaragod-671 124, Kerala

8. Dr (Mrs) K.N.Ponnamma
   Senior Scientist
   National Research Centre for Oil Palm
   Palode-695 562, Kerala

9. Dr R.S.N.Pillai
   Senior Scientist
   National Research Centre for Oil Palm
   Palode-695 562, Kerala

10. Mr P.S.Ravindran
Senior Scientist
National Research Centre for Oil Palm
Palode-695 562, Kerala

11. Mr.T.S.S.Rawther
Principal Scientist (Retired)
Salamath
Poozhikode
Kudasanad-689 512, Kerala

12. Dr. R.Radhakrishnan Nair
Senior Scientist
Central Tuber Crops Research Institute
Sreekariyam-695 017,
Thiruvananthapuram, Kerala

13. Mr P. L. Ramanandan
Scientist S-2 (Retired)
CPCRI, Kasaragod-671 124
Kerala, India

14. Dr. J.J. Solomon
Principal Scientist
CPCRI Regional Station
Kayangulam-690 533
Kerala, India

15. Dr. N. Srinivasan
Senior Scientist
CPCRI Regional Station
Kayangulam-690 533
Kerala, India

16. Dr. P. Sundararaju
Senior Scientist
National Research Centre for Banana
44, Ramalingam Nagar
South Extension
Trichy-630 017, Tamil Nadu, India

CONTENTS

ABBREVIATIONS

A	Photosynthesis
ABA	Absicic acid
ATCC	American type culture collection
AFLP	Amplified fragment length polymorphism
ATP	Adenosine triose phosphate
Ci	Internal carbon dioxide
CO_2	Carbon dioxide
CPCRI	Central Plantation Crops Research Institute
DAPI	4, 6 - Diamidino - 2 - phenylindole
DNA	Deoxyribonucleic acid
E	Transpiration
ECW	Epicuticular wax
EM	Electron microscopy
Fo	Initial fluorescence
Fv	Variable fluorescence
Fm	Maximum fluorescence
Fv/Fm	Ratio of variable to maximum fluorescence
gs	Stomatal conductance
gm	Mesophyll conductance
LM	Light microscopy
MLO's	Mycoplasma like organisms
NAA	Napathelene acetic acid
OTC	Oxytetracycline
O_2	Oxygen
PAR	Photosynthetically active radiation
PCR	Polymerase chain reaction
Pi	Ortho phosphate
PSII	Photosynthetic system II
Q_A	Primary Electron Acceptor of PSII
RNA	Ribo nucleic acid
rs	Stomatal resistance
UAS	University of Agricultural Sciences
VPD	Vapor pressure deficit
ψ	Water potential
YLD	Yellow leaf disease

1
ORIGIN, DISTRIBUTION AND SPREAD

T.S.S.RAWTHER

Yellow Leaf Disease (YLD) is the most serious malady affecting the arecanut palm. The earliest information on YLD is found in the publication "Diseases of coconut palm" (Varghese, 1934). The disease is commonly known as "Kattuveezcha" or Chovakedu in Malayalam (Nambiar,1949) and "Chandiroga" or "Arasinaroga in Kannada (Dastagir, 1963, 1965). The disease was first reported in 1914 from Muvattupuzha, Meenachil and Chalakudi areas of Central Kerala succeeding a heavy flood (Nambiar, 1949; Nambiar and Sreenivasan, 1951). Nambiar (1949) also observed that the disease had some similarities with the 'root and leaf disease' of coconut prevalent in those areas.

Thorough and systematic observations were made on the pattern and spread of the disease in the seedlings planted during 1961 in virgin soil reclaimed from forest at Central Plantation Crops Research Institute, Research Centre, Palode, a predominantly diseased area. The seedlings began to manifest typical symptoms of the disease in 1968. The pattern of spread of the disease from each focus of infection in the farm comprising of 8 ha was studied by plotting the appearance of infection on a map. By 1971, within a period of 4 years from the appearance of the first symptoms, about 80 per cent of the palms in the garden became diseased which indicates the rapidity of the spread of infection (Rawther and Abraham, 1972). Though the spread was rapid, it did not follow any definite pattern. The disease appeared in small foci. The disease became epidemic following heavy winds indicating the possibility of causal organisms or agents being dispersed by wind. The disease incidence appeared to be more in low lying plots where water table was high during rainy season. Usually seedlings planted in the affected soil, exhibited the symptoms after three years of planting.

A preliminary survey conducted during 1959-60 showed that the disease had spread to all parts of Kerala with a maximum incidence of 90 per cent in Quilon district (Anon., 1960). Later, occurrence of disease was also noticed from the central regions of Bombay, parts of Karnataka and Tamil Nadu (Menon, 1963). A comprehensive survey undertaken in 1976 in arecanut growing areas of Kerala revealed that the malady is prevalent in almost all the districts of Kerala (Table 1). In Kerala, the total number of diseased palms was estimated to be 233 million. On an average 36 per cent of the areca palms in Kerala was found to be affected with disease. The disease was very severe in four districts such as Thiruvananthapuram (72%), Kollam (75%), Kottayam (94%) and Idukki (97%). Though highest percentage of diseased palms (about 95%) were found in Kottayam and Idukki districts, their population comprised less than 20 per cent of the total palms in the state. But, Kollam district had the maximum number of diseased palms (about 40 million). The percentage of infection in Kozhikode, Malappuram

Table1. Distribution of yellow leaf disease in Kerala

District	Area under arecanut ('000ha)	Percentage of area affected by YLD
Kannur	16.58	1.20
Kozhikode	8.10	0.70
Malappuram	15.50	NA
Palaghat	3.70	NA
Thrissur	15.10	6.30
Idukki	1.70	97.00
Ernakulam	7.80	34.10
Kottayam	5.40	94.30
Alappuzha	5.10	NA
Kollam	9.20	75.40
Thiruvananthapuram	4.50	71.80
Total	[illegible]	[illegible]

and Kannur districts was negligible. Generally, the disease incidence was higher in the southern districts than in northern part (George, Nayar and Rawther, 1982, unpublished).

A systematic survey conducted in Kasaragod and Kannur districts revealed that Kasaragod taluk was found to be relatively free from yellow leaf disease (Anon., 1988). In Hosdurg taluk, 2,508 palms were affected in five villages. The total production of arecanut in the district was estimated at 19,017 tonnes of chali and the estimated loss due to YLD was 3.1 tonnes. A recent survey conducted in Kasaragod and Kannur districts revealed that a total of 4618 palms in Kasaragod and 16,71,243 palms in Kannur district were affected by YLD resulting in a loss of 2.8 tonnes and 1,365 tonnes of chali, respectively (Anon., 1992).

A garden to garden survey conducted in Karnataka involving CPCRI, University of Agricultural Sciences (UAS), Bangalore, Department of Horticulture and Department of Agriculture,

Table 2. Occurrence and distribution of yellow leaf disease of arecanut in Karnataka

District	Healthy	Diseased	Loss in yield (tonnes)
Dakshina Kannada	1,89,93,425	12,483	6.99
Udupi	25,43,357	544	0.29
Kodagu	10,07,436	2,25,937	7.9
Chickmagalur	1,13,42,343	5,15,269	404.6
Shimoga	1,59,02,476	1,92,590	86.8
Uttara Kannada	1,53,17,445	2,102	1.7
Total	6,51,06,482	9,48,925	508.28

Government of Karnataka during 1989 and1990 revealed that disease is prevalent in all arecanut growing districts (Table.2). A total of 12,483 palms in Dakshina Kannada, 544 in Udupi, 2,25,937 in Kodagu, 5,15,260 in Chickmagalur, 1,92,590 in Shimoga and 2,102 in Uttara Kannada districts were affected by YLD, resulting in a loss of 508. 3 tonnes of chali.

Apart from taking a heavy toll of the palms every year, the disease rendered arecanut cultivation uneconomical to the farmers due to reduced yield. So, it is necessary to identify fresh incidence of the disease in YLD affected belt as well as in surrounding areas and to eradicate such palms in time to prevent further spread of the disease.

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2
SYMPTOMATOLOGY

R.R.NAIR AND T.S.S RAWTHER

The symptom expression of yellow leaf disease is well pronounced soon after the monsoon, when maximum temperature is 30°C –32°C, nights are cool and wind currents mild to heavy. Paradoxically, with the rise in temperature, the symptom expression is reduced. The intensity of yellowing of the leaves is minimum in May i.e., before the onset of South-West monsoon and maximum in August i.e., mid-monsoon (Nayar, 1976).

Nambiar (1949) recorded characteristic symptoms of the disease as yellowing of the leaves and shedding of both mature and immature nuts. According to Menon (1963), the first visible signs are translucent spots, 1-3 mm in diameter on the growing spindle. Brown necrotic streaks running parallel to the lamina are present in the unfolded leaves. As the leaves develop, yellowing starts from the tip of leaflets, gradually extending to the middle of the lamina. The chlorosis could be distinguished from the physiological yellowing by the abrupt demarcation between the green and yellow regions in the diseased leaf (Fig.1). Subsequent studies have

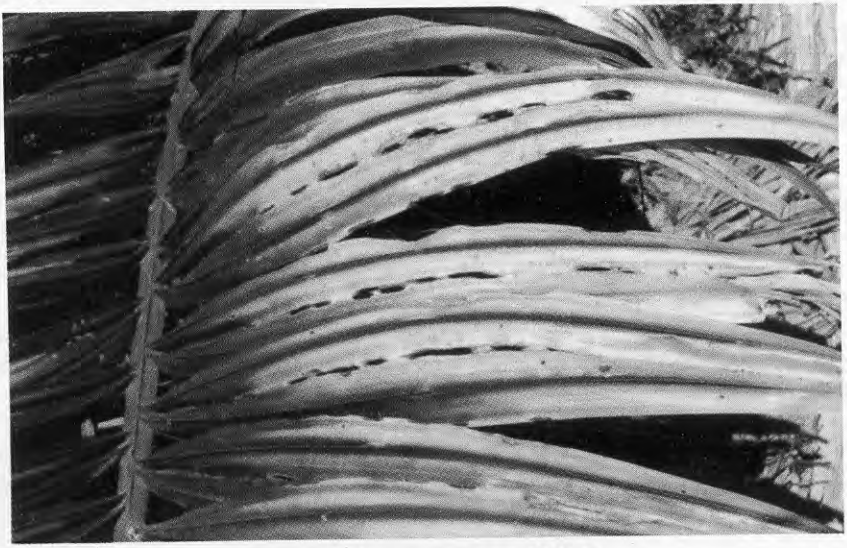

Fig. 1 Symptoms of YLD

shown that the first visible symptom is yellowing starting from the tips of leaflets in two to three leaves of the outermost whorl (Rawther, 1976). The disease may affect one or two leaflets in any part of the crown or the entire foliage. Tips of the chlorotic leaves eventually dry up. In advanced stage, leaves are reduced in size, become stiff and pointed, closely bunched and abnormally puckered. Ultimately the crown falls off leaving a bare trunk (Nayar and Selsikar, 1978).

The root system of the palm is also affected. The lateral roots are not produced as profusely as in healthy palms. Tips and absorbing regions of young roots turn dark and gradually rot (Table 1 and Fig.2). The endosperm of the diseased nuts becomes blackish and soft, which render them unsuitable for consumption (Fig.3). Though endosperm discoloration is associated with foliar yellowing in majority of cases, palms exhibiting foliar yellowing sometimes produce normal nuts. Further, all the nuts produced in a bunch of a diseased palm may not show kernel discoloration (Rawther, 1976). Palms with normal green foliage standing among diseased ones are also observed to produce nuts with blackened kernel (Table. 2)

Table 1. Root decay in YLD affected palms.

Condition of the palm	Number of main roots examined in a sector of the bole	Percentage of healthy main roots in the sector	Percentage of decayed main roots in the sector	Average number of lateral roots per main root
Healthy	134	97.20	2.50	23
Disease early	120	72.82	27.20	12
Disease advanced	130	28.90	71.10	4

Table 2. Relationship between kernel discoloration and foliar yellowing

Foliar condition of the palm	Percentage of palms showing kernel discoloration	Percentage of nuts showing kernel discoloration
Yellowing	70	88
Normal	30	60

Fig. 2 Root decay

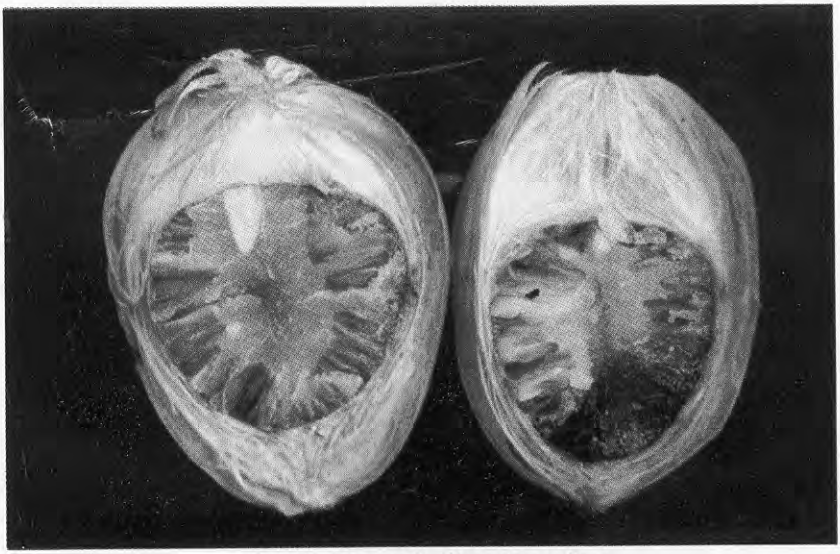

Fig. 3 Kernel discoloration

As the disease advances, the girth of the crown gradually tapers. The internodal length of the affected stem reduces due to reduction in normal growth. The yield of the affected palms is reduced to the extent of 50 per cent over a period of three years. This is mainly due to the reduction in the inflorescence production as well as number of nuts (Table 3).

Table 3. Effect of YLD on yield and leaf fall

Parameter	Before disease expression	After disease manifestation	Percentage of reduction
Average no. of nuts	4433	2138	51.79
Leaf fall / year	8	7.68	4
Disease index	-	8.75	NA

A formula for quantifying the disease intensity was developed after studying the association of the various symptoms in more than 2000 palms (George *et al.*, 1980). Due weightage was given to foliar yellowing, necrosis and reduction in the size of the whole crown.

$$I \text{ (intensity)} = \frac{(Y+N+R)}{L} \times 10$$

Where Y and N are the sum of grade points for yellowing and necrosis, L is 50 per cent of the number of leaves on the crown and R is the grade point for reduction in size of the crown for the whole palm.

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3.1

ETIOLOGY – FUNGI, BACTERIA AND VIRUS

N. SRINIVASAN

The etiology of yellow leaf disease of arecanut was a matter of dispute for a long time. Experimental evidence to rule out the involvement of fungi and bacteria in causing this disease is discussed in this chapter.

Fungi

Khandige *et al.* (1957) first recorded the association of fungi with YLD. So far 54 different species of fungi are recorded from different parts of the disease affected palms. A number of fungi such as *Cercospora arecae, Exosporium arecae, Leptosphaerea* sp., *Diplodia* sp., *Phyllosticta* sp., *Dimerosporina* sp., and *Trametes corrugata* were isolated from the diseased leaves (Menon, 1959: Anon., 1963). Roots of diseased palms yielded *Trichoderma* sp., *Pestalotia* sp., *Aspergillus* sp., *Penicillium* sp., *Fusarium* sp., *Acremonium* sp., and *Colletotrichum* sp., which were not pathogenic on inoculation to seedlings (Anon., 1963: Anon., 1976). Species of *Pythium* and *Phytophthora* were isolated from the roots of disease affected palms using selective media (Rawther, 1982 – Personal Communication). All the inoculation experiments with frequently occurring fungi yielded only negative results. None of the species tried was able to produce the typical disease symptoms.

Bacteria

Srivastava *et al.* (1970) reported bacterial streaming associated with YLD affected roots. Out of two bacterial isolates recovered from roots, one was tentatively identified as *Pseudomonas sp.* Later reports (Anon., 1974) revealed that samples from different regions exhibited bacterial streaming in the order of 70 per cent from samples of Palode (diseased) and 20 per cent from samples of Mohitnagar (healthy); but no bacterial streaming from samples of Vittal (healthy). Three genera of bacteria viz., *Bacillus, Xanthomonas* and *Serratia* isolated from areca roots were reported to cause symptoms like discoloration with water soaked areas, when inoculated to areca seedlings and cow pea (Anon., 1963). Subsequently, pathogenicity of bacteria could not be proved when inoculated on to 18 month old seedlings with isolates from areca roots. According to Bopaiah (1979), the gram-positive bacteria appeared more (70-80%) than gram negative ones (15-30%) in the root region of healthy areca palms. In diseased gardens, the bacterial population has been reported to be more by 31 per cent than in healthy gardens(Anon., 1964). In mixed-cropped garden, no consistent bacterial population was recorded and organic recycling in such farming systems did neither increase the yield of arecanut nor reduce the

yellow leaf symptoms (Rawther *et al.*, 1979). The bacterial counts in root region of diseased palms are found to be more than in the healthy palms but the qualitative difference is unclear (Bopaiah, 1982).

Virus

Paper chromatographic studies (Menon, 1961) indicated that some proteins or their sub-units were present in diseased areca palms which were absent in the healthy ones. Serological investigation with crude arecanut antigen and disease specific rabbit antiserum showed that there was precipitation reaction, indicating antibody formation. Menon (1960) therefore suggested the possibility of virus or virus-like organisms being involved in the disease. Menon (1963) transmitted yellow leaf disease to indicator plants viz., *Jatropha curcas, Canavalia ensiformis* and *Vinca rosea* using partially clarified sap by mechanical inoculation using carborandum as abrasive.

Mites

Khandige *et al.* (1957) reported association of mites with the yellow leaf. Menon (1960) distinguished the yellowing caused by mite from the foliar yellowing due to yellow leaf disease. The foregoing account ruled out the involvement of fungi, bacteria and virus in causation of yellow leaf disease.

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3.2
ETIOLOGY – NEMATODES

P. SUNDARARAJU

The association of nematodes with arecanut was first reported by Nair in 1964. He observed *Meloidogyne javanica*, *Helicotylenchus sp.* and *Tylenchorhynchus sp.* from the root zone of YLD affected palms at Palode, Kerala. Weischer (1967) recorded seven genera of plant parasitic nematodes from a few soil samples collected from the root zone of healthy and YLD affected palms, but population of any of these and the incidence of the disease was not correlated. However, he suggested intensive studies on the role of nematodes in causation of the disease.

Swamy and Parvatha Reddy (1969) reported the occurrence of *Aphelenchus* sp. and *Helicotylenchus* sp. from arecanut rhizosphere in Mysore. Kumar *et al.* (1971) recorded the presence of *Hemicriconemoides gaddi, Hemicriconemoides sp. Pratylenchus coffee, Radopholus similis* and *Tylenchorhynchus sp.* from soil samples collected from root region of arecanut in the coffee tracts of South India. Detailed and systematic studies on phytoparasitic nematodes of arecanut were undertaken at Central Plantation Crops Research Institute, Regional Station, Kayangulam since 1974. The role of plant parasitic nematodes in the etiology of yellow leaf disease was studied through survey, population dynamics, pathogenicity, control and varietal screening.

Survey

Extensive surveys were conducted during 1974 to 1979 in arecanut growing areas of Kerala, Karnataka and Tamil Nadu with special reference to the incidence of yellow leaf disease. A total of 435 soil and 822 root samples were collected from healthy and YLD affected arecanut gardens and assessed for nematode populations. Out of 435 soil samples assessed, 314 were from Kerala, 106 from Karnataka and 15 from Tamil Nadu. Twenty eight genera of plant parasitic nematodes were recorded. *Rotylenchus reniformis* with largest number (4800/250cc) was found in 66 per cent of samples analysed. It was seen that phytoparasitic nematodes such as *R. reniformis, Helicotylenchus dihystera, Radopholus similis, Hemicriconemoides mangiferae, Caloosia longicaudata* and *Hoplolaimus seinhorsti* were recorded in maximum number of soil samples from all the three states (Sundararaju and Koshy, 1982 a).

The burrowing nematode, *Radopholus similis* was obtained from maximum number of root samples (250/822). Except for *R. similis* the occurrence of nematodes belonging to other genera which were in small numbers in the root zone of healthy and diseased palms may not be of any significance. They may probably be feeding on other herbaceous weeds growing in the root zone of arecanut gardens.

It is evident from Table.1 that there is wide spread occurrence of *R. similis* in all soil types, but the percentage occurrence was more in sandy loam soil (44.5) followed by red loam (42.0) and laterite (31.0) (Koshy *et al.*, 1978; Sundararaju and Koshy, 1982 a). Samples from the low lying sandy loam areas yielded more *R. similis* than in elevated. This may be due to the availability of better soil moisture condition in low-lying areas. Obviously, *R. similis* prefers loose, well drained soil and the situation is in agreement with findings of O'Bannon and Tomerlin (1971) and Tomerlin and O'Bannon (1974) who reported that spread of decline symptoms on citrus was more severe in well drained deep sandy soils, compared to moderate symptoms of infection in other soil types.

Table 1. Occurrence of *R. similis* in different soil types with reference to health of the arecanut palm

Soil type	Healthy Y/T	Apparently healthy Y/T	Diseased Y/T	Total
Sandy loam	6/19 (31.6)*	14/36(39.0)	21/37(57.0)	41/92(44.5)
Laterite	38/110(34.5)	39/114(34.2)	83/295(28.0)	160/519(30.5)
Alluvial	8/31(25.8)	13/37(35.0)	13/44(30.0)	34/112(30.0)
Clayey	3/14(21.4)	3/36(8.3)	5/8(27.7)	11/58(16.0)
Red loam	2/14(14.3)	5/8(62.5)	6/9(66.6)	13/31(42.0)
Total	57/188(30.0)	74/231(32.0)	128/403(32.0)	259/822(31.5)

$$Y/T = \frac{\text{Number of samples yielded } R.\ similis}{\text{Total number of samples collected.}}$$

* Figures in Parenthesis indicate the percentage

The percentage occurrence of *R. similis* was 32 in the yellow leaf disease affected tracts against 30 in the healthy arecanut tracts. Again the incidence was 25.3 per cent and 37.5 per cent, respectively in pure crop of arecanut and arecanut inter cropped with banana. Banana as an intercrop is known to favour the incidence of *R. similis* in arecanut. Hence, growing banana as an intercrop in arecanut gardens infested with *R. similis* may not be a desirable practice unless nematode free planting materials of both banana and arecanut are used (Koshy *et al.*, 1976). Koshy and Sosamma (1977) studied the population fluctuation of *R. similis* in arecanut roots and recorded maximum population during September to November and minimum or even nil during March to July.

Survey was conducted again in November 1980 in healthy and disease affected arecanut gardens in Koppa, Sringeri, Sullia, Thirthahalli, Narasimharajapura and Somavarpet taluks in Karnataka State. Root populations were assessed and 7/17 (41%) from healthy and

16/20 (80%) from disease affected areca palms yielded *R. similis* with a maximum of 440 per gram of root in disease affected palms against 48 in healthy palms. Nematode populations were assessed in soil and *R. similis* was recorded from 7/17 samples collected from healthy against 14/20 from yellow leaf disease affected areca palms.

Pathogenicity

Pathogenicity of *R. similis* on arecanut seedlings was studied by using different levels of inoculum (10, 100, 1,000 and 10,000 per plant) to gather information on the role of this nematode in causing damage, expressed in terms of plant growth parameters. Inoculation of the burrowing nematodes isolated from arecanut root showing lesions and rotting were inoculated on to healthy arecanut seedlings grown in sterile soil contained in pots. Inoculated seedlings exhibited typical orange coloured, elongated cortical lesions separate from one another initially on young, fleshy roots, which later coalesced and caused extensive root rotting. They also showed reduced vigour, height (44%), girth (37%) and number of leaves (43%) as compared to the uninoculated plants over a period of two years. This established the pathogenicity of the nematode on arecanut (Koshy *et al.*, 1976).

The pathogenicity of the burrowing nematode on areca seedlings was again studied with different levels of inoculum collected from arecanut palms under pot condition. Significant reduction was recorded in length, and weight of shoot and root, number of leaves and collar girth of inoculated plants compared to uninoculated ones. The percentage reduction over control was as high as 30, 53, 31, 57, 31 and 41 for shoot length, shoot weight, root length, root weight, number of leaves and collar girth respectively with an initial inoculum level of 10,000 nematodes per plant over a period of three years. This experiment established the potential of the burrowing nematode as a pathogen of arecanut (Koshy and Sundararaju, 1981). A fungus, *Cylindrocarpon obtusisporum* was isolated from lesions produced by *R. similis* on arecanut roots (Sundararaju and Koshy, 1982 b)

Histopathology of *Radopholus similis* infested arecanut roots

Examination of longitudinal and transverse sections of *R. similis* infested roots revealed that there was considerable damage to root tissues. Longitudinal burrows developed underneath the outer cortical cell layers and nematodes as well as their eggs could be located in these cell layers. Nematodes were also seen in both inter and intra-cellular positions although intercellular orientation was more common.

In no case the nematodes were seen intruding stellar tissues. Necrotic changes occurred around the head of the nematodes and the burrows harbouring them. The feeding of nematode disintegrated the cytoplasm and cell wall of the host, and coalescence of these led to the formation of cavities or burrows in which the nematodes bred and multiplied (Sundararaju, 1984).

Control

Effect of different nematicides and neem oil cake to control *Radopholus similis* in YLD affected arecanut palms: An experiment to study the effect of different nematicides and neem oil cake to control *Radopholus similis* and amelioration of YLD symptoms in yellow leaf disease affected arecanut palms was initiated in 1976. Three nematicides viz., fensulfothion @ 50 g a.i./palm, aldicarb @ 10 g a.i./palm, DBCP @ 10 ml a.i./palm and neem cake @ 1.5 kg/palm were applied thrice in a year against *R. similis* on arecanut palms affected with YLD. Initial nematode populations were assessed every year during October when nematode population was at maximum. Nematode population was reduced considerably after application of nematicides compared to control palms. In the second year (1977) the nematode population in palms treated with fensulfothion, aldicarb, DBCP and neem cake was significantly less than in control. However, better yield response was noticed in palms under fensulfothion and neem cake treatments. During 1978 also, significant reduction in the nematode population was observed and in the succeeding years (1979 and 1980), no *R. similis* could be recovered from the treated palms whereas the untreated palms recorded 205 to 425 nematodes per 25 g of roots (Table.3). Analysis of the results with respect to disease indices of arecanut palms in different treatments showed that disease indices (Table.2) did not differ significantly between treatments. Considerable reduction of disease indices was noticed in the palms treated with fensulfothion and aldicarb compared to untreated (control) palms. Decrease in disease incidence and increase in yield was observed in all treatments compared to control in the fifth year. While, comparing the severity of disease symptoms before and after application of nematicides, a gradual decrease in disease incidence was seen in treated palms as compared to that in untreated palms (Sundararaju, 1984).

Table 2. Effect of different nematicides and neem oil cake in the control of *Radopholus similis* in YLD affected arecanut palms.

Treatments	Dosage	Pre-treatment disease index	Post-treatment disease index in different years					Mean
			1976	1977	1978	1979	1980	
Aldicarb	@ 10 g a.i./palm	37.80	28.80	29.80	24.80	24.20	21.00	25.72
DBCP	@ 10 ml a.i./palm	33.60	30.40	26.20	27.20	30.80	34.20	23.72
Fensulfothion	@ 50 g a.i.	28.00	22.60	20.80	21.60	24.80	21.20	22.20
Neem oil cake	@1.5 kg/palm	35.40	31.80	30.40	23.80	23.40	27.00	27.28
Control		25.20	33.60	35.20	36.20	37.20	43.00	37.04
CD at 5 %		NS	NS	NS	NS	NS	NS	

Table 3. Effect of different nematicides and neem cake on the control of *Radopholus similis* in YLD affected arecanut palms: (a) Nematode population in the roots during different years.

Treatments	Dosage	Initial population	Nematode population in 25g of roots in different years				
			1976	1977	1978	1979	1980
Aldicarb	@ 10 g a.i./palm	1525 *	180	48	15	0	0
DBCP	@ 10 ml a.i./palm	1350	152	4	0	0	0
Fensulfothion	@ 50 g a.i./palm	865	125	7	5	0	0
Neem cake	@ 1.5 kg/palm	242	120	135	50	0	0
Control		625	648	380	425	472	205

*Mean of five replication

Table 4. Effect of application of various nematicides on population build up of *Radopholus similis* and incidence of YLD symptoms on arecanut seedlings in field

Nematicide	Dosage	Population in 25 g roots Initial population				Percentage of disease incidence	
		1980	1981	1982	1983	1982	1983
Aldicarb	@ 3 g a.i./plant	40	30	0	12	12.5	12.5
Carbofuran	@ 3 g a.i./plant	165	68	45	75	50.0	50.0
Phenamiphos	@ 3 g a.i./plant	215	85	48	13	62.5	50.0
Phorate	@ 3 g a.i./plant	318	110	55	21	25.0	25.0
DBCP	@3ml a.i./plant	195	116	80	31	25.0	25.0
Inoculation with nematodes (Infested roots)	10,000 nematodes per seedling	28	168	345	525	62.5	62.5
Inoculation with axenic culture of *R. similis* population	"	126	372	590	625	12.5	25.0
Control		95	285	275	252	25.0	66.6

Effect of application of various nematicides on population build up of *Radopholus similis* and incidence of YLD symptoms on arecanut seedlings in field: The data on nematode population recorded in the roots in arecanut seedlings at the commencement of experiment and thereafter during 1980-83 as well as on expression of YLD symptoms on arecanut seedlings are summarized in Table.4. There was considerable reduction in the nematode

population in nematicide treated palms but the differences between treatments were not significant.

Seedlings belonging to all treatments had expressed YLD symptoms. Maximum percentage of disease incidence occurred in untreated control palms (66.6%), closely followed by seedlings inoculated with nematodes (62.5%). In general, the percentage incidence of disease was comparatively less in treated palms than in untreated control or on inoculation. The percentage incidence of YLD symptoms was significantly less in aldicarb treatment compared to other treatments. Sundararaju (1984) reported that aldicarb was superior to all other nematicides tested, because it had controlled nematode population significantly with least percentage of disease incidence in seedlings (12.5%).

In conclusion, it could be said that though there is no direct role of nematodes as an etiological agent in the YLD; nematode control in arecanut is very important as nematodes debilitate the palms.

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3.3
ETIOLOGY - PHYTOPLASMA

K N PONNAMMA AND J J SOLOMON

Phytoplasma, earlier known as Mycoplasma-like organisms (MLOs), were implicated in the etiology of YLD by various workers. Nayar (1971) reported culturing of Phytoplasma from leaf bits collected from yellow leaf diseased palms from Kerala. Numerous umboid colonies were produced in solid plate transfers from liquid cultures. These colonies were similar to those of other Phytoplasma colonies. However, the cultures have neither been deposited with the American Type Culture Collection Centre(ATCC) nor with any other repository for confirmation. Selsikar and Wilson (1981) opined that further investigation is needed before conclusions are drawn. Inoculation trials with few arecanut seedlings grown under a glass house produced some yellowing symptoms, but these data were too meagre to confirm that Phytoplasma was responsible for YLD. Cross inoculation with rabbit antiserum using double gel diffusion techniques showed that the areca antigen reacted against sandal spike specific rabbit antiserum (Nayar, 1971). Further electron microscopic studies (Nayar and Selsikar, 1978; Selsikar and Wilson, 1981; Anon., 1983) showed the presence of Phytoplasma in the young sieve elements of YLD affected arecanut palms from Kerala and Karnataka.

In order to confirm the above reports and to establish the constant association of the organism with the disease, efforts were made to identify the ideal plant part, which harbours the organism (Phytoplasma) in higher concentration. Eight diseased palms with YLD symptoms from South Kerala and four healthy palms from a disease free area in Dakshina Kannada district of Karnataka were subjected to destructive sampling and segments of apical meristem, petiole of developing leaves, rachilla of tender inflorescence, spear leaf, mature leaf with symptoms and freshly emerging roots (close to the tip region) were sampled.

Ultra-thin sections of sieve tubes revealed the presence of Phytoplasma in tissues of all the diseased palms (Fig.1). However, such bodies were totally absent in healthy palms studied. Neither the protozoan flagellate nor any other sub-microscopic agents were observed in the tissue . The organisms were found in increasing numbers in the juvenile tissues in the following order; apical meristem, root tip, rachilla, petioles of developing leaves and spear leaf. Phytoplasmas in fewer numbers and lacking internal contents, possibly degenerated forms, were observed in the yellow mature leaves. Only a few sieve tubes in a vascular patch contained the organism. The concentration of Phytoplasma were much higher in YLD affected arecanut palms than that in root (wilt) affected coconut palms (Anon., 1983, 1985).

The organisms were bound by triple layered membranes, having less dense fibrillar nuclear area and peripherally distributed ribosomes constituting the electron dense portion. The pleomorphic forms, often assumed sites close to the walls of the sieve tube, were also found

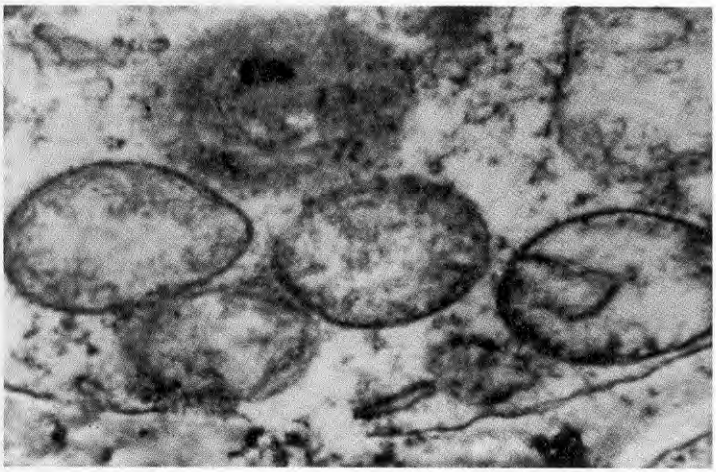

Fig. 1 Ultra thin sections of sieve tubes of diseased palms showing Phytoplasma

traversing the sieve pores. Forms ranging from spherical, oval and elongated budded structures were observed. In general, the size of the organism ranged between 250-500 nm. Elementary bodies with electron dense contents were rarely noticed. Root tips and rachilla of juvenile inflorescence were found to be the ideal plant part for locating Phytoplasmas. A total of 27 diseased palms with different intensities of disease and twenty-five healthy palms from various locations in Kerala were studied. Phytoplasmas were observed in root/rachilla tissues of all the palms examined. A significant observation was the recording of hyper infection of the Phytoplasma by tailed bacteriophages (Anon., 1983). Cole *et al.* (1973) had recorded "classic" tailed bacteriophage in the Morocco strain of *Spiroplasma citri*. The exact role of bacteriophages infecting the Phytoplasma is still not clear.

Field observations showed that in majority of yellow leaf diseased areca palms, the symptoms get masked during January-August. This normally happens when the leaves with symptoms are shed and the freshly emerging leaves in the canopy remain green. Three yellow leaf diseased palms which showed symptoms during September-October, 1986 and in which the symptoms were masked subsequently during 1987 were chosen for this study. For a comparison, three other palms in which symptoms were observed throughout the year were also included. Rachilla of tender inflorescence from these six diseased palms was sampled out at bi-monthly intervals both during the symptom expression and symptom masked periods. Phytoplasmas were observed in palms of both the categories throughout the year. Structural alterations such as elaboration of the membrane system and necrosed phloem cells with nacreous cell walls were often observed in the palms with masked symptoms. The constant presence of the organisms in these palms during the symptom masked period indicated that once the palm gets diseased it continued to have the organism irrespective of the symptoms. The expression of foliar symptoms may be due to a great extent influenced by certain environmental factors (Solomon, 1991). Assumption of Nayar (1971) that the remission noticed during summer months may be due to high temperature prevailing during the period appear to be correct.

As electron microscopy is laborious and time consuming, light microscopic techniques using certain histological dyes have been standardized. Dienes' staining and fluorochromes such as DAPI and Hoechst 33258 were found useful in detection of Phytoplasma infection in YLD affected areca palms (Anon., 1985). Histological staining techniques employing Dienes', Aniline blue, Gram's stain and Thionine – Acridine orange combinations were tried for the detection of Phytoplasmal infection in tissues of diseased palms. The Dienes' staining technique which was developed as a specific stain for Phytoplasmas (Deelay *et al.*, 1979) was used to screen the symptomatic palms at 0.2 per cent concentration. Positive staining reaction (as blue areas in the phloem) indicative of the presence of Phytoplasma infection could be observed in all the tested samples of disease affected palms, while such reaction was conspicuously absent in tissues of healthy palms. In transmission studies using the plant hopper, *Proutista moesta* (Fig.2) and the dodder Laurel (*Cassaytha filiformis*), the suspected samples were subjected to light microscopic studies using Dienes'stain. The gram staining technique for detection of Phytoplasmas in tissues of YLD affected palms and Thionine-Acridine orange stain were also found useful to detect Phytoplasmal presence in sieve tubes (Solomon, 1991).

Fig. 2 Proutista moesta, a vector of YLD

The identification of yellow leaf diseased areca palms based on foliar symptoms especially in the early stage of disease is not always very reliable. In some palms, the symptoms get masked during certain periods of the year. Hence, attempts were made to develop a reliable and rapid sero-diagnostic method similar to one standardized for detecting root (wilt) disease condition (Solomon,1991). The antiserum produced, on testing in double diffusion test, gave only a faint reaction against samples from diseased palms.

A more sensitive and rapid sero-diagnostic technique such as immuno-osmophoresis was also attempted on microscope slides. The tissue extracts from yellow leaf diseased palms on immuno-osmophoresis produced a single precipitin line, midway between the antigen and antiserum wells, while no such precipitin line was observed against tissue extracts from healthy

areca palms (Solomon, 1991). Preparation of high tittered antiserum and standardization of ELISA should be taken up as future lines of investigation.

The constant association of Phytoplasma with the disease warranted search for insect vector(s) that transmits the disease. The involvement of insect in transmission of the disease is evident from an experiment in which areca seedlings, protected from aerial vectors, grown in insect proof cages did not contract the disease as against seedlings grown in open field (Ponnamma,1994). Similarly, seedlings protected by fortnightly spraying of insecticides were free of the disease. In an observational trial, the reduction in rate of disease incidence by insecticide treatments and the higher incidence in untreated border seedlings revealed that an insect vector transmits YLD pathogen. The decreased rate of spread of the disease among seedlings given foliar treatments of insecticides suggested that a leaf-feeding insect spreads the disease. A correlation between the population level of *P. moesta* and YLD incidence indicated the role of *P. moesta* as the probable vector.

Inventory of putative vectors through various trapping aids and direct examination of over 300 areca seedlings for a period of two years revealed the consistent presence of *P. moesta* on areca palms. Survey of representative gardens in Karnataka and Kerala clearly established that the disease does not occur independent of the presence of the insect. This fulfills one of the requirements enunciated by Leach (1940) for an insect to be implicated as a vector of any disease. This led to the logical study to ascertain whether the insect can acquire Phytoplasmas while feeding on diseased palms.

EM examination of the ultrathin sections of salivary glands revealed the presence of Phytoplasma in the salivary glands of plant hoppers which were offered acquisition and incubation periods of over 30 days on the foliage of YLD affected areca palms. However, such bodies were neither observed in the salivary glands of the laboratory-reared insects nor in insects with acquisition and incubation period of less than 30 days. The organisms were confined to the acini of the salivary glands. The pleomorphic bodies had triple layered unit membrane and contained ribosomes and reticulated DNA strands. Electron dense elementary bodies were also at times found in the acini. This observation indicated the capability of this insect to acquire the organism, sustain its multiplication and possibly act as a vector in transmitting the disease (Ponnamma *et al.*, 1991).

In order to provide adequate proof for insect transmission of the disease, the disease was to be produced on experimental areca seedlings under controlled conditions by inoculating with infective plant hoppers (with sufficient checks) as per the requirement put forward by Leach (1940). Transmission of YLD from diseased to healthy areca seedlings, using the plant hopper, *Proutista moesta* was done at controlled conditions. In the first set, out of six seedlings which received infective insects, three showed characteristic foliar yellowing symptom of YLD, twenty one months after the initiation of inoculation and the rest after 32 months. Root tissues from seedlings showed positive reaction to Diene's stain. Electron microscopic examination of ultra thin sections of root apices revealed the presence of Phytoplasmas in the sieve tube of one of the

seedlings. Phytoplasmas were observed in five out of six *P. moesta* inoculated seedlings between 21-32 months after the start of inoculation. All the control seedlings remained healthy (without any foliar symptoms) and the tissues from the roots showed negative results for LM and EM studies.

In the second set of 15 seedlings, foliar yellowing appeared for the first time on five seedlings, twenty two months after the initiation of inoculation. After twenty seven months, a total of eight seedlings showed foliar yellowing. Phytoplasmas were located in samples from five seedlings under EM. All the control seedlings were free of any foliar symptoms and the 8 samples from these were free of Phytoplasmas. The above results are the direct evidences on the role of *P. moesta* as a vector of YLD of arecanut (Ponnamma, 1994). Earlier studies have proved that *Carvalhoia arecae* is not a potential vector (Jacob, 1990).

Experimental transmission from diseased to healthy areca seedlings through dodder laurel (*Cassytha filiformis*) was attempted. Dodder laurel was established on twenty naturally infected (3-4 year old) areca palms. New shoots of the dodder laurel were trailed on to twenty-eight healthy areca seedlings raised under insect-proof conditions. Out of these, four exhibited YLD symptoms. Five more seedlings also showed symptoms like marginal yellowing and reduced size of the crown. EM examination of the recipient palms showed Phytoplasmas in three out of four areca seedlings with foliar symptoms. In the fourth seedling, sieve tubes showed structural alterations that are often observed in association with Phytoplasma infection. The presence of Phytoplasma in the source palm connecting dodder laurel strands and in the recipient palms conclusively established the transmission of the disease (Solomon, 1991).

Nayar (1971) reported the remission of symptoms of YLD by oxytetracycline treatment (OTC). Two field experiments were conducted, one at Palode and another at Peechi, using OTC, Hostacycline, Ledermycin, Neomycin, Penicillin and Gentamycin. The antibiotics were injected into experimental areca palms using the 'pneumatic pressure injection device'. Injections were performed at the bole region of the palms at a pressure of 4 kg/cm^2. A higher number of palms treated with OTC, Hostacycline, Ledermycin, Neomycin and Gentamycin showed improvement over the pre-treatment condition proving the Phytoplasma static effect of the antibiotics while Penicillin and distilled water treated palms deteriorated over the pre-treatment condition, thereby establishing the Phytoplasmal etiology of the disease (Solomon, 1991).

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4

PHYSIOLOGY

P. CHOWDAPPA, E.V. DANIEL, D. BALASIMHA AND C.K.MATHAI

The arecanut palms exhibit very clear symptoms of YLD during 'wet' season (August-October). In a majority of the diseased palms, the symptoms begin disappearing well before the onset of 'dry' season and remain symptomless during 'dry' season (December-May).

Weather variables on symptom expression

Environmental variables, therefore, play an important role in manifestation of visible symptoms in the yellow leaf affected arecanut palms (Chowdappa *et al.*, 1995). A high evaporative demand in the atmosphere existed during 'dry' (December-May) period as indicated by high PAR, temperature and VPD masking the disease symptoms, while during 'wet' season (August-October), the weather parameters showed differences (Table.1). There was no soil moisture stress during both the periods as the palms were irrigated, although atmospheric drought occurred during May. Similarly, the controlling influence of environmental variables, particularly temperature on the growth of *Spiroplasma citri in vitro* is well-documented (Chen and Davis, 1979). According to them, the optimal temperature for growth of *S. citri* ranged from 28°C to 33°C and practically no growth occurred at 37°C. This optimal temperature was found to be favourable for the expression of stubborn disease symptoms in citrus (Bove and Sailard, 1979). McCoy (1979) reported, by electron microscopy, the complete degeneration of safflower phyllody Phytoplasma in periwinkle during the course of heat treatment. Probably the higher temperature recorded in both locations during 'dry' season may have a detrimental effect on the organisms resulting in a temporary remission of symptoms (Chowdappa *et al.*, 1995).

Table 1. Mean microclimatic variables recorded in the vicinity of palms

Location	Season	PAR ($\mu mol\ m^{-2}\ s^{-1}$)	T air (°C)	VPD (Kpa)	Visual symptoms
Sullia	Dry	1651	39.19	3.72	Absent
	Wet	1168	33.11	2.69	Present
Palode	Dry	1550	34.80	3.37	Absent
	Wet	1180	29.20	1.86	Present
Vittal	Dry	1609	37.07	3.24	Absent
	Wet	1128	32.67	2.85	Absent

Water relations and stomatal responses

The effect of water-relations on Phytoplasma induced diseases has not been well understood, even though it has a profound effect on the physical environment within the sieve tubes. The leaf samples of the affected palms showed that the moisture touches the lowest level in June, the month of symptom emergence, by 59.10 per cent. The corresponding value in healthy leaves was 70.84 per cent. Root samples of the above groups did not vary much (Yadava *et al.*, 1972).

A hole made at the base of the trunk of affected palms allows a viscous dark liquid to come out and such palms recoup temporarily from this malady. The affected palms showed a high leaf sap acidity (pH) of 3.29 as compared to 4.63 of healthy ones. Chowdappa *et al.* (1995) reported that the diseased palms had higher stomatal resistance (*rs*) and lower transpiration (E) than apparently healthy palms in the 'wet' season. The data are presented in Tables 2 and 3.

Table 2. Water relation components in the leaves of healthy, apparently healthy and YLD affected arecanut palms during 'wet' season

	Parameter	Values
1.	**Stomatal resistance (S cm^{-1})**	
	Healthy	2.14
	Apparently healthy	3.15
	Diseased	5.84
2.	**ECW(mg cm^{-2})**	
	Healthy	17.22
	Apparently healthy	21.10
	Diseased	26.74
3.	**Water potential (Mpa)**	
	Healthy	-1.14
	Apparently healthy	-1.11
	Diseased	-0.81
4.	**Osmotic potential (Mpa)**	
	Healthy	-1.65
	Apparently healthy	-1.85
	Diseased	-2.40
5.	**Turgor potential**	
	Healthy	0.73
	Apparently healthy	0.76
	Diseased	1.90

Table 3. Net CO_2 assimilation rate (A), stomatal conductance (*gs*), intercellular carbondioxide (Ci), Transpiration rate (E) in the leaves of apparently healthy and yellow leaf diseased palms during 'wet' season

Parameter	Apparently healthy	Diseased
A(μmol m^{-2} s^{-1})	5.9	1.2
gs(mol m^{-2} s^{-1})	0.5	0.2
Ci (ppm)	266.4	289.1
E (mmol m^{-2} s^{-1})	4.5	2.5
A/*gs*	15.8	6.6
A/Ci	0.02	0.0038
A/E	1.6	0.6

There was significant linear relationship between E and *rs*. The trend was similar irrespective of the palm's condition and season as evident from pooled data (Fig.1). The water potential values of apparently healthy palms was –1.11 Mpa, whereas diseased palms showed higher values (-0.81). When determinations on *rs* were made at different times of the day i.e., from 8th to 16th hour, the leaves of apparently healthy palms showed an increase in *rs* with increase in light, temperature and VPD (Fig.2). Even though, the diseased palms followed similar diurnal patterns, the stomata remained closed through most of the day and thus resulted

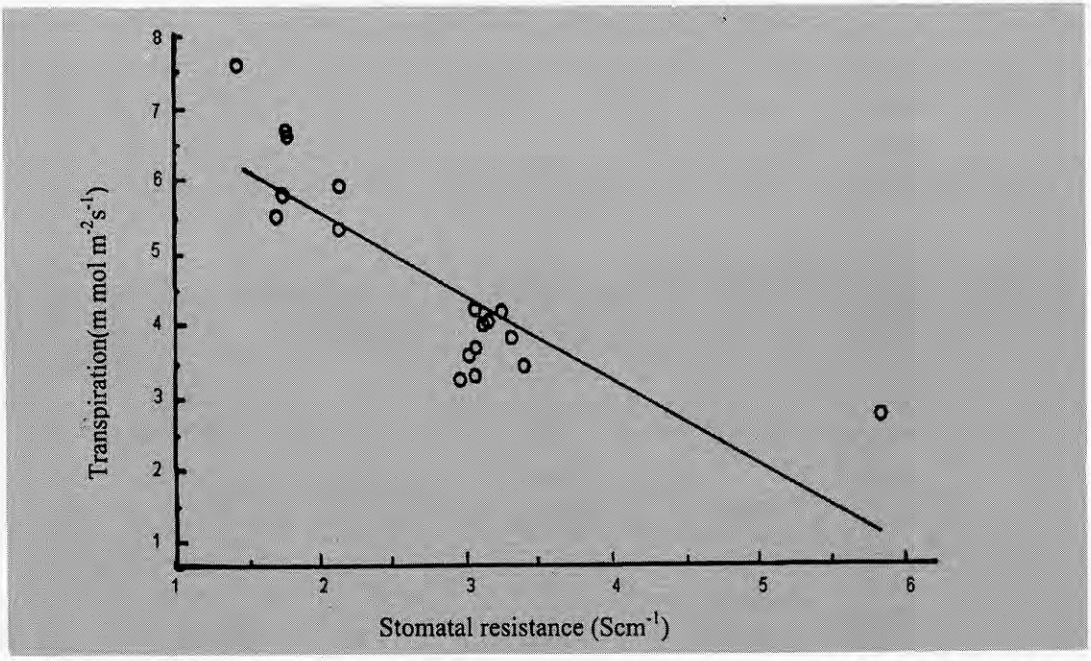

Fig. 1 Relationship of transpiration to stomatal resistance; pooled data from all categories and seasons. Y = 7.849–1.159x, R^2=–0.695, P<0.01.

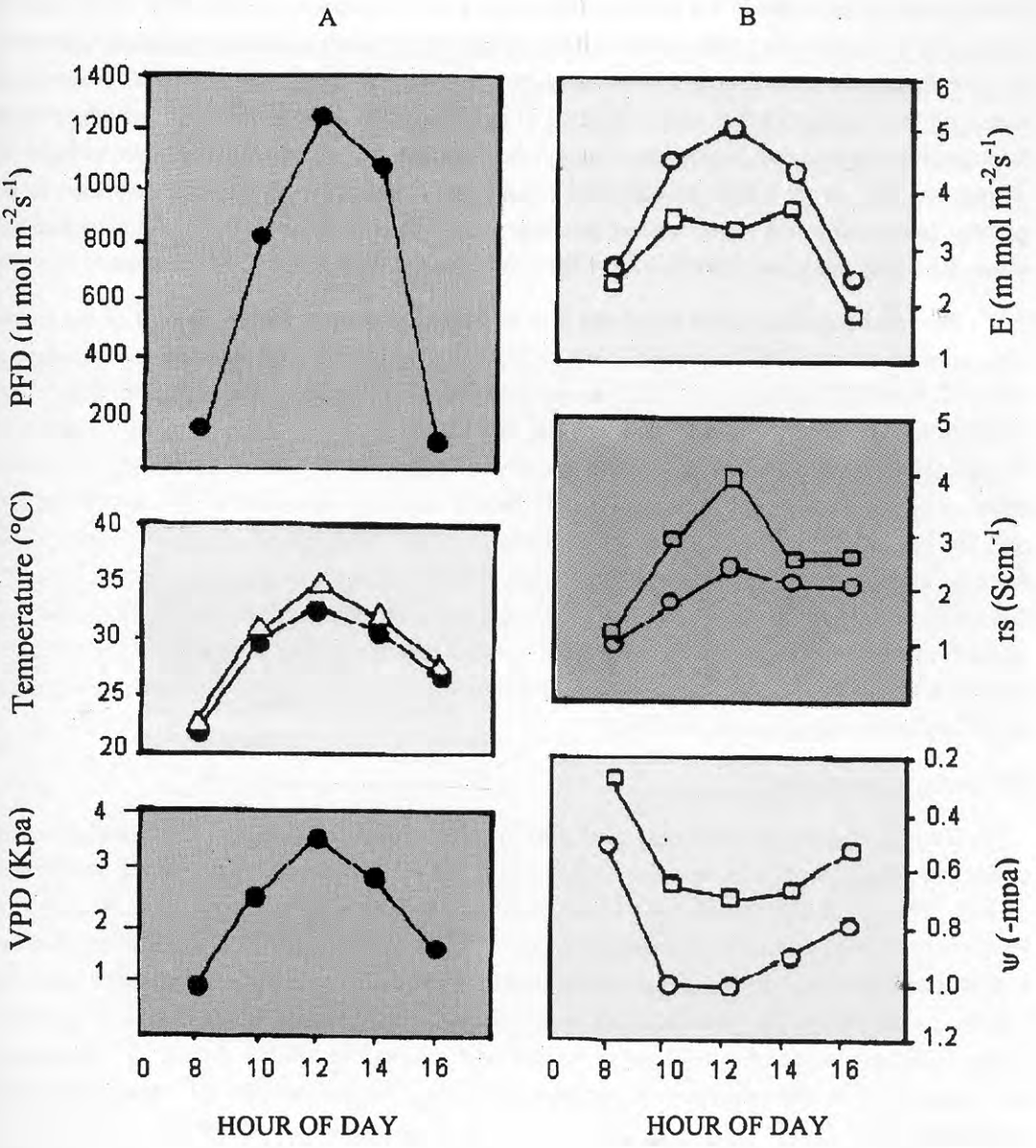

Fig. 2. ***A) Day time fluctuations in environment variables, (● - ●) recorded in the vicinity of palms and leaf temperature (Δ - Δ) during the 'wet' season.***

B) Day time fluctuations in water potential, stomatal resistance and transpiration rate in the leaves of outer whorl in the 'wet' season; apparently healthy palms (0-0) and diseased palms (□-□).

in accumulation of water in the leaves of diseased palms irrespective of the hour of the day as compared to apparently healthy palms. The differences in water relation components between apparently healthy and diseased palms were not observed during the 'dry' season. From these observations, it appears that water balance of healthy palms was maintained in both seasons through effective stomatal regulation. Though the diseased palms maintained the water balance during the 'dry' season through stomatal regulation, the regulatory mechanism seems to be greatly impaired in the 'wet' season resulting in a higher Ψ and *rs*, thus indicating that the diseased palms could be identified from the healthy palms only in the 'wet' season.

Stomatal regulation was impaired due to infection irrespective of the age of the palms (Chowdappa *et al.*, 1993). Indeed, Nair (1976) reported various abnormalities in epidermal cells and stomatal apparatus of YLD affected palms although leaf thickness did not vary much between healthy and diseased palms. Abnormal stomatal closure, as a distinctive feature of Phytoplasma caused yellow diseases, have been reported in *Ulmus americana, Fraxinus americana, Catharanthus roseus,* x-disease in *Prunus virgiana,* corn stunt in *Zea mays* (Matteoni and Sinclair, 1983) and lethal yellowing of coconut (Mc Donough and Zimmermann, 1979). Arntzen *et al* . (1973) demonstrated that *Helminthosporium* toxin inhibited light induced K^+ uptake by guard cells that close the stomata similar to the mode of action of ABA. A similar kind of mechanism might be operating in the leaves of YLD affected palms, though the nature of toxin is yet to be identified. Toxins have been implicated in yellow diseases of the periwinkle caused by *Spiroplasma citri* (Daniels, 1979).

Osmotic adjustment

Various organic compounds and ions are known to accumulate in plantation crops decreasing leaf osmotic potential in response to water deficits (Rajagopal and Balasimha, 1994). The metabolic adaptations in arecanut palms due to yellow leaf disease caused by Phytoplasma were studied (Chowdappa *et al.*, 1993). The leaves of diseased palms showed higher water potential and turgor potential, but lower osmotic potential compared to leaves of healthy palms (Table 2). The decrease in osmotic potential at full turgor in leaves of diseased palms is accounted for by increase in sugars and aminoacids (Table 6 and 7). Evidences for increase in the concentration of sugars and aminoacids for osmotic adjustment has been recorded.

Leaf waxes

The epicuticular wax (ECW), which is easily extracted with organic solvents, forms the outer most layer of the leaf cuticle. Various functional and adaptational roles have been ascribed for the leaf surface lipids. Most important function among these is the ability to prevent water loss through cuticular transpiration to withstand water losses. In YLD affected palms, ECW content was significantly higher than those of apparently healthy and healthy palms (Table 2), resulting in low transpiration rate (Table 3) and the accumulation of higher water content .

Photosynthesis

The diseased palms exhibited lower photosynthesis (A) and higher internal carbon dioxide (Ci) with significant reduction in stomatal conductance (*gs*) (Table.3). When determinations on A were made at different times of the day, i.e., from 8 to 16 hours, the leaves of apparently healthy palms clearly showed an increase in A with increase in PAR, temperature and VPD upto 12 hrs and then declined (Fig.3). On the other hand, the diseased palms exhibited decrease in A throughout the day. The *gs* decreased more in the diseased palms than apparently healthy palms at any given time. The diseased palms had higher levels of Ci, irrespective of the hour of

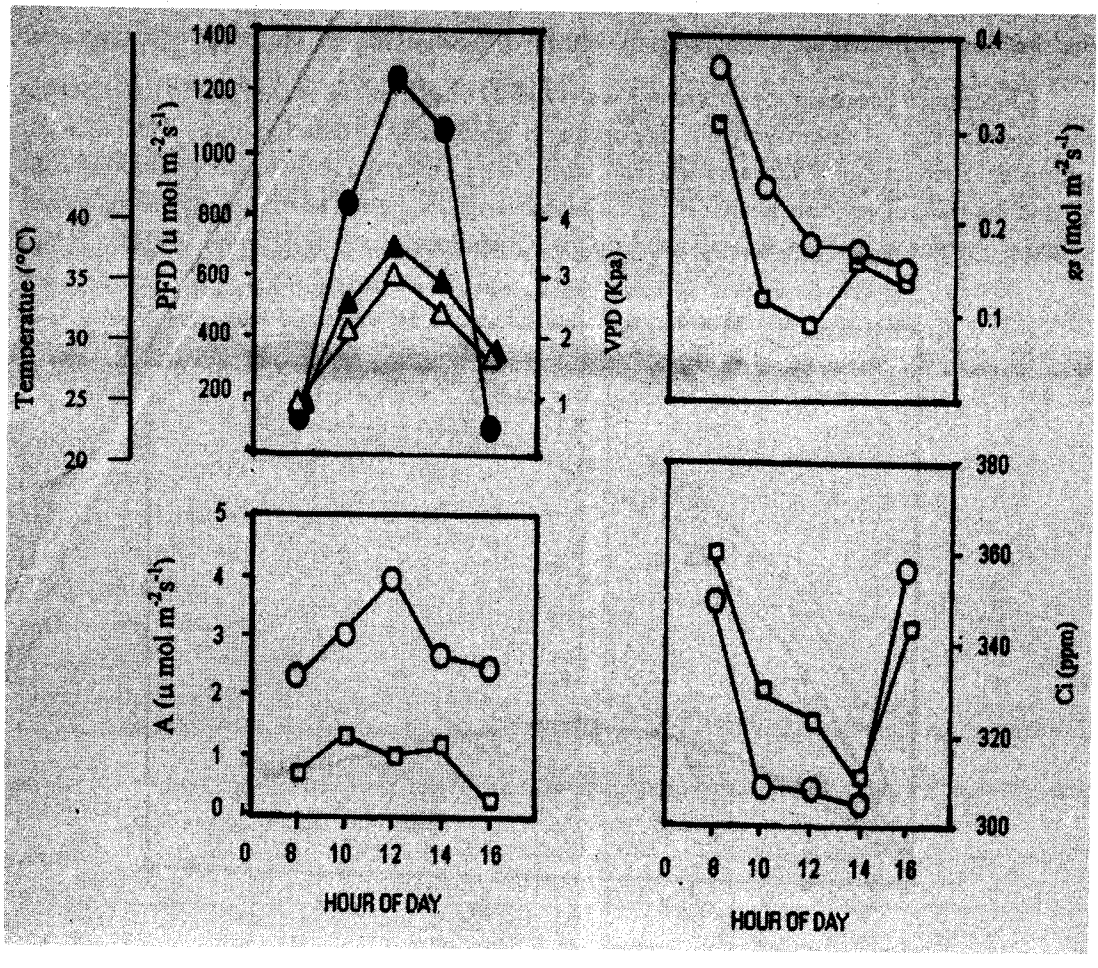

Fig. 3. Day time fluctuations in environment variable recorded in the vicinity of palms: photon flux density (• - •), temperature (Δ - Δ) and VPD (▲ - ▲), and A, gs and Ci in the leaves of apparently healthy palms (0-0) and YLD affected palms (□-□).

the day as compared to apparently healthy palms. The ratio of A to gs was significantly lowered in the diseased palms which implied that mesophyll factors were more affected than stomatal factors. The reductions in the A/E ratio, probably coupled with the lack of strong feed back control of *gs* by *gm* (mesophyll conductance) lead to the impairment of stomatal regulation (Chowdappa and Balasimha, 1992). Net photosynthetic rate was reduced with increasing light intensities, beyond saturation level (Fig. 4). CO_2 assimilation was saturated at PAR of 800 mol m^{-2} s^{-1} in healthy palms compared to 500 mol m^{-2} s^{-1} in the diseased palms. This could be attributed to photoinhibition due to reduction in carotenoid pigments (Table.4) which could have caused light stress at higher irradiances in the diseased palms. Under these circumstances, the quantity of light absorbed could easily exceed the capacity of chloroplasts. Excess light may cause photo-inhibitory damages to photosynthetic apparatus due to destruction of excitation energy dissipating mechanisms, which is probably mediated by carotenoid pigments.

Chlorophyll fluorescence characters were widely used to monitor changes in the activities and organization of photosynthetic apparatus in abiotically stressed plants. Fluorescence parameters such as initial fluorescence (Fo), variable fluorescence (Fv), maximum fluorescence (F_M) and the ratio of variable to maximum fluorescence (F_V/F_M) were used to study the functional activities of chloroplast in YLD affected palms (Chowdappa and Balasimha, 1994). Initial

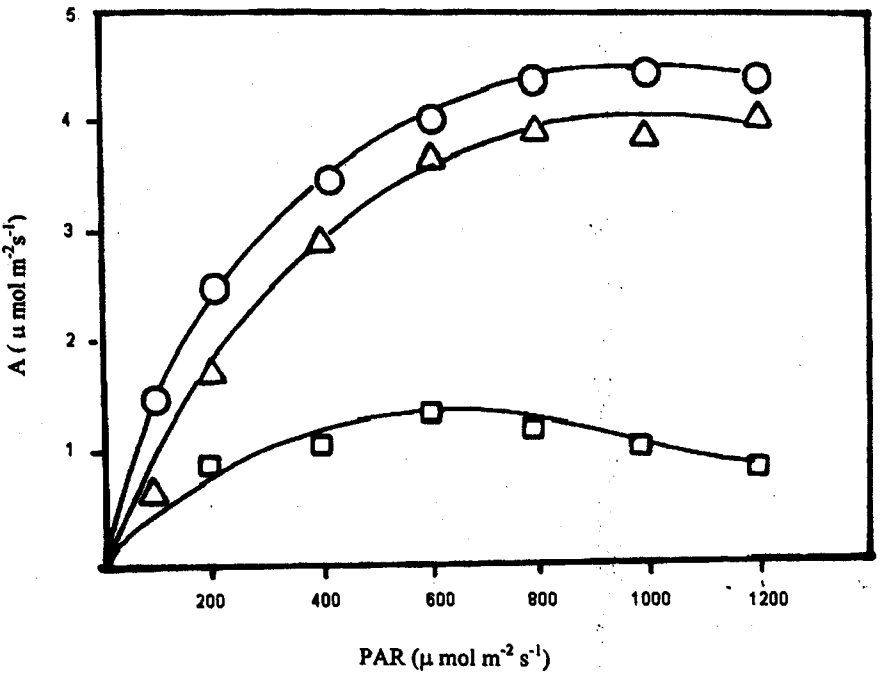

Fig. 4. Relationship between photosynthesis and photosynthetically active radiation (PAR) in healthy (0-0), apparently healthy (Δ - Δ), and YLD affected palms (□ -□)

fluorescence (Fo) values were not affected in the leaves of diseased palms compared to apparently healthy palms, presumably reflecting normality at the PS II pigment arrangement (Table.5). The reduced F_M values in induced chlorophyll fluorescence of the leaves of the diseased palms, suggesting the inhibition of Q_A reduction, a primary electron acceptor of PSII. The potential photochemical efficiency of PSII and quantum yield of photosynthetic 0_2 evolution was reduced in the leaves of diseased palms as evidenced by the decrease in F_V/F_M ratio. The decrease in fluorescence indices in the leaves of diseased palms was concurrent with a reduction in chlorophyll and carotenoid pigments (Table 4). These changes result in reduction of carboxylation efficiency (Chowdappa and Balasimha, 1992).

Development of yellowing symptom

Mc Coy (1979) reported that loss of thylakolids and chlorophylls of chloroplasts along with development of plastoglobules containing dissolved carotenoids is the major phenomenon for development of typical yellowing symptom in 'yellows' diseases of Phytoplasmal etiology. Srinivasan (1982 a) recorded the association of a deranged chlorophyllase – chlorophyll system with yellow leaf affected areca palms. In the diseased palm, activity of chlorophyllase was

Table 4. Total chlorophylls, chlorophyll a and b, chlorophyll a/b ratio and carotenoids in yellow leaf disease affected palms during 'wet' season

Parameter	Healthy	Apparently healthy	Diseased
Total chlorophylls (mg/g fresh weight)	2.82	3.61	0.65
Chlorophyll a (mg/g fresh weight)	2.03	2.47	0.42
Chlorophyll b (mg/g fresh weight)	0.79	1.14	0.23
Chlorophyll a/b ratio	2.60	2.29	2.21
Carotenoids (mg/g fresh weight)	0.65	0.79	0.16

Table 5. Chlorophyll fluorescence induction characteristics of apparently healthy and yellow leaf diseased palms during 'wet' season.

Parameter	Apparently healthy	Diseased
F_o	678	604
F_M	3225	1401
F_V	2549	837
Fv/F_M	0.789	0.544

enhanced and concomitantly the pigment chlorophyll declined. Changes occurred in major plant pigments due to the disease and yellow leaf appeared in plant yellows. Changes in foliar-assimilatory pigments have been compared with degree of damage to host tissue. Under such circumstances, the chlorophyll destruction had primary relation with the degree of expression of yellow leaf syndromes; consequently, the pigment changes were apparently related with diagnostic symptoms of yellow leaf disease (Srinivasan, 1982 b). Thus, the phenomenon of foliar yellowing was most probably due to loss of chlorophylls and carotenoids mediated by impairment of phloem functions (Chowdappa *et al.*, 1993).

Carbohydrate metabolism

A higher accumulation of carbohydrates in the leaf tissues of the diseased palms points to the affected carbohydrate metabolism (Yadava *et al.*, 1972). Abnormal accumulation of starch and sugars is one of the prominent features of Phytoplasma affected plants. Nair (1976) reported phloem necrosis in YLD affected arecanut palms. Due to this, sugar translocation might have been disrupted resulting in the accumulation of sugars and starch in the diseased palms (Chowdappa *et al.*, 1993). Although total sugars, reducing sugars and starch increased in the diseased palms (Table.6), no qualitative differences were found in soluble sugar fractions. The impeded removal of these assimilates in the diseased palms following phloem interruption could reduce A (Table 3) probably due to an orthophosphate (Pi) limitation.

Nucleic acid metabolism

There was no significant difference in leaf DNA and RNA contents as well as DNA/RNA ratio between the healthy and diseased palms (Table 7), indicating that these quantitative characters may not be useful as diagnostic criteria.

Aminoacid metabolism

Nair and Aravindakshan (1971) studied the aminoacid make-up in healthy and yellow leaf affected palm parts. They reported that, in the leaves, the disease markedly increased the contents of cystine and methionine, threonine was sharply reduced, phenylalanine and alanine were less. In the inflorescence of the diseased palm cystine, glutamic acid and serine contents

Table 6. Sugars, reducing sugars and starch in healthy, apparently healthy and yellow leaf diseased palms during 'wet' season.

Parameter	Healthy	Apparently healthy	Diseased
Total sugars (mg/ g fresh weight)	15.01	18.74	30.21
Reducing sugars (mg/ g fresh weight)	9.77	7.85	23.29
Starch (mg/ g fresh weight)	46.69	40.05	52.34

were higher whereas lysine, asparagine, arginine, methionine and hydroxyproline were lower. In the roots, organic acids such as maleic, tartaric, oxalic, succinic and tannic were increased by the disease, as were the protein contents in the leaves and inflorescence, chlorophyll content was much reduced but pH of sap was not much affected by the disease.

Lysine and arginine contents of leaves progressively increased with advancement of disease. The aminoacids, serine and glutamic acids were absent in leaves while they were present in large quantities in inflorescence tissues. Serine, arginine and threonine declined in stem tissues with advancement of disease. Proline, cystine and histidine totally disappeared from roots on infection. One of the prominent features of YLD is an abnormal accumulation of arginine and other total aminoacids (Table 7) similar to other yellows diseases. Arginine dehydrolase pathway has been suggested as the major energy source for the growth of the organism in some non-fermentative Phytoplasmas (Schmike *et al.*, 1966). In the case of YLD affected arecanut palms, the presence of high levels of arginine in leaves might provide a constant energy supply in the form of ATP for the growth of Phytoplasma by entering the dehydrolase pathway and favour growth of the causal agent and further decay of the diseased palm.

Protein metabolism

Difference in protein concentration and electrophoretic banding patterns have been studied in several yellows diseases (McCoy, 1979). Although, some differences in protein concentration and profiles are discernible, they have not been sufficient to serve as a means of diagnosis or as a possible early detection technique. No significant differences in protein content (Table 7) and electrophoretic protein-banding pattern were found between the healthy and yellow leaf diseased palms.

Table 7. Total proteins, aminoacids and arginine content in the yellow leaf disease affected arecanut palms during 'wet' season.

Parameter	Apparently healthy	Diseased
Total proteins (mg/ g fresh weight)	31.74	27.39
Total aminoacids (µg/ g fresh weight)	395	683
Arginine (µg/ g fresh weight)	45	201
Total nucleic acids (mg/ g fresh weight)	5.58	5.61
DNA (mg/ g fresh weight)	1.94	1.81
RNA (mg/ g fresh weight)	3.64	2.79
DNA/ RNA ratio	0.53	0.48

Isozyme pattern

When peroxidase activity of the leaf discs of apparently healthy and diseased palms were studied during months of May, February and September, the peroxidase activity did not differ significantly between different categories at any season (Table 8). On electrophoresis, under alkaline pH conditions, no change in peroxidase isozyme pattern among the categories as well as during the seasons was noticed (Fig.5). These results indicate that the peroxidase isozyme pattern cannot be used as a marker for the early diagnosis of the disease.

Table 8. Phenols, sterols and peroxidase activity in the yellow leaf disease affected arecanut palms during 'wet' season.

Parameter	Apparently healthy	Diseased
Total phenols (µg/ g fresh weight)	579	605
Ortho dihydric phenols (µg/ g fresh weight)	195	294
Total sterols (µg/ g fresh weight)	1138	1081
Peroxidase activity (A420/min/mg/protein)	3.16	4.02

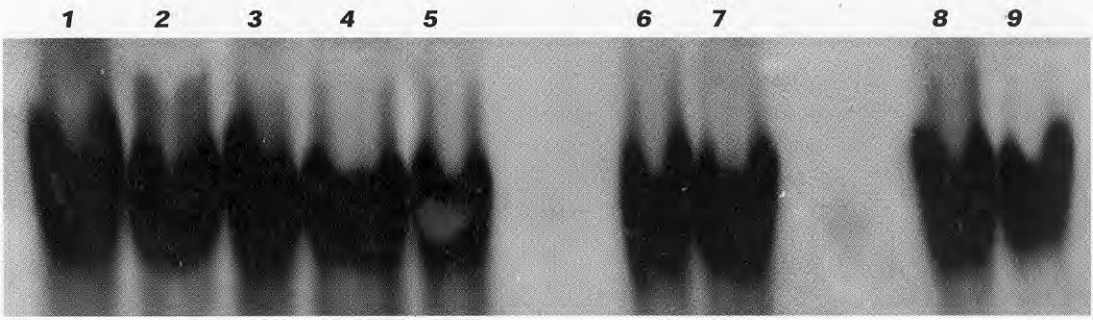

Fig. 5. Gels stained for isozyme patterns of peroxidase in leaves of healthy (Lane 1,2,3,4,5), apparently healthy (Lane 6, 7) and yellow leaf diseased palms (Lane 8,9).

Phenol metabolism

Biochemical studies in leaf tissue during symptom development indicated that phenol metabolism is altered and lead to an accumulation of ortho dihydroxy phenols during 'wet' season (Table 8). However, leaf phenolics did not differ qualitatively due to disease contraction. Srinivasan(1982 c) reported derangement in activity of oxidative enzymes-polyphenol oxidase, peroxidase, catalase and ascorbic acid oxidase in yellow leaf affected areca palms.

Hormonal imbalance

Accumulation of ABA has been reported in Phytoplasma caused lethal yellowing affected coconut palms (Leon *et al.*, 1993), in which the stomatal regulation is similar to one observed in YLD of arecanut. Although, the mechanism responsible for non-stomatal limitation of the photosynthesis in YLD affected arecanut palms is not well understood, it is surmised that the inhibition of photosynthesis may be due to either hormonal imbalance or toxins. However, accumulation of cytokinin in the inflorescence of the diseased palms has been reported (Anon., 1988).

Sterol metabolism

Sterol content was significantly reduced in leaves of the diseased palms (Table 8). The lower content of sterol in diseased palms might be possibly due to the higher rate of utilization by Phytoplasma associated with disease for their growth and multiplication. This observation assumes significance in the light of the finding that sterols are important constituents in culture media for culturing of *Spiroplalsma citri* associated with citrus stubborn disease (Bove and Saillard, 1979).

Mineral metabolism

Although leaf tissue of YLD affected palms had higher amount of N, P and K, typical YLD symptoms could not be produced in pot culture experiments with deficiency of N and K (Nayar, 1976). The foliar spray of N, P and Mn increased their concentration in leaf tissue, but no effect on symptom expression. Mohapatra *et al* (1976) reported that, except P, all other elements are sufficient in diseased leaf tissue. The low content of Mg in leaves of diseased palms is attributed to high CaO/MgO ratio. As the Mg is key component of chlorophyll, application of $MgSo_4$ to the basins of palms has been suggested.

Amelioration of disease by chemicals

Effect of foliar application of urea, diammonium phosphate and manganese sulphate on the condition of diseased palms was studied (Anon., 1982). The disease index indicated a general decline in the disease conditions of the palm. However, yield did not show any definite trend. Leaf analysis showed that nitrogen content increased by seven percent in the leaves of diseased palms that received urea application and the level of Mn by 100 percent, which received manganese sulphate. Consequently, Fe/Mn ratio was lowered in the treated palms.

Phosphorous application had no effect on the level of P in the leaves. Foliar spraying and root feeding of ascorbic acid, NAA and phenols (catechol and caffeic acid) did not show any ameliorative effect on YLD (Anon., 1983).

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5
HISTOPATHOLOGY

R.S.N.PILLAI

Research work on histo-pathological aspects in relation to yellow leaf disease of arecanut was initiated during 1961-62 in the erstwhile Regional Arecanut Research Station, Palode. It consisted of cyto-anatomical studies of the leaf, flower, nut, stem and root of disease affected palms in order to find out the possible structural changes in the palm due to the incidence of the disease (Anon., 1962).

The material concerned was fixed in FAA and dehydrated in ethyl alcohol-butanol series, cleared in xylol and embedded in paraffin. Sections of all parts except fruits, were cut at 8 μ and fruits at 20-25 μ with rotary and freezing microtomes. Staining was done with safranin and fast green for anatomical studies and with haematoxylin for nuclear studies. Iodine stain was used in fruit sections.

Observations made on different plant parts revealed that in chlorotic leaves, there were degeneration and clumping of chloroplasts and in some cases the palisade was found to be completely filled with a dark-brown and rust coloured substances. The presence of these substances prevented a more detailed study of the cell contents. Apposition of cell wall was found to be a common feature in phloem cells. Extensive degeneration of the embryosac, which is responsible for the flowers failing to develop beyond a stage and for their shedding, was evident. The vascular connections in the nut were found blocked and the endosperm cells degenerated. Vascular elements in the stem showed degeneration and blackening. The vascular bundles and the adjoining parenchymatous ground tissue were found to be filled with discoloured masses. The tracheal elements and the parenchymatous connections to the lateral roots were blocked with black pigments. The xylem elements were compressed and the cell walls were thinner than that of the healthy tissues. Endodermis and phloem strands abutting on the pericycle showed multi-nucleate condition. Apposition was found to be a common feature in the phloem in this case also (Nair, 1969).

Nayar (1968) observed multi-nucleate cells, deranged tissue differentiation and palisade cells blocked with dark brown pigments in various stages of degeneration in leaves of affected palms. Further extensive degeneration in the phloem of stem and leaves of the affected palms was observed. Medullary rays were found disturbed and accumulation of starch grains was observed indicating impaired translocation. Nair (1976) found that the diseased leaves possessed smaller epidermal cells, stomatal pores and midrib parenchyma cells. Blocking of xylem vessels of the older leaves of diseased palms, degeneration of the cortex and presence of tyloses in the xylem were also noticed in the diseased roots.

For anatomical studies, fresh materials of the roots, stem and leaves were fixed in FAA and preserved in 50 per cent alcohol. The epidermal patterns in diseased and healthy palms

were studied from leaf samples collected from palms under identical conditions. Leaf samples were drawn from the central portion of the newly opened leaf. Epidermal peelings were taken from the base, middle and tip portions of diseased and healthy samples. In all cases, free hand sections were taken and stained with Haematoxylin, Safranin and Fast Green. For recording observations, 24 or more sections were randomly selected. Diseased and healthy arecanut palms were cut to size and suitable bits of comparable portions were sectioned for the stem anatomy. A sector of the bole at 45^0 of diseased and healthy palms were exposed for the collection of root samples. For pollen studies, male flowers of healthy and diseased palms were collected in the morning hours and slide prepared by staining the grains in acetocarmine. For pollen germination studies, the hanging drop technique was followed using 0.5 per cent sucrose and 0.1 per cent agar medium. Germination counts were taken after 30 minutes. Results obtained on the different aspects are summarized below.

Leaf

No appreciable difference was observed in the thickness of the cutinised layer on both adaxial and abaxial surfaces of the lamina between healthy and diseased palms in the initial and middle stages of attack. Diseased epidermal cells were devoid of cell contents and hence not turgid, the nuclei were deeply stained than those of healthy. Slight increase in the size of epidermal cells was noticed in diseased leaf, as compared to that of healthy. The size of stomatal apparatus was greater in healthy palms. Similarly, the maximum size of guard cells was also more in case of healthy. In general,slight reduction was observed in the diseased palms with respect to the development of stomatal apparatus. Both the number of epidermal cells and number of stomata were highly variable in their distribution and were found to be more in diseased samples with a concomitant reduction in size which is attributed to the enhanced rate of division of the upper epidermal cells resulting in slight downward curling of leaf-lets. No correlation was arrived at between stomata and epidermal cells. The stomatal index was lower in diseased palms which might probably be due to the break down of the normal metabolism of the epidermal cells by the influence of the yellow leaf disease. Size of stomatal opening was very low in diseased leaves. Yellow leaf diseased palms showed a more rapid collapse of stomata. The collapsed stomata had irregular shape with degenerated and deformed guard cells. The stomata as a whole were shrunk very much. Also the stomatal pore was plugged with some discolored crystal-like exudate. Such stomata did not take stain and appeared yellowish. Stomatal collapse has been considered as an index for loss of vitality and vigour of epidermal cells. The spongy mesophyll tissue was almost devoid of cell contents and in advanced stage of disease complete shattering of chloroplasts was observed.

A slight increase in the size of xylem vessels was observed in the case of leaf samples. Another most important difference seen in the diseased palms was the presence of a particular kind of outgrowth of different numbers ranging from 0 to 9 in the xylem vessels. These outgrowths appeared to be spherical filled with a colorless fluid and sometimes with a prominent nucleus. The percentage of xylem vessels blocked by the outgrowth was 35. The diameter of these structures varied from 3 μ to 64 μ. It was suggested that this is a special characteristic of

diseased palms, which appears probably during the initial stages of attack in the midribs of leaflets and enlarge as the disease advanced. This very special feature can be taken as an index in distinguishing infected palms from healthy ones. Occurrence of such structures in large numbers might contribute to the slight enlargement of xylem vessels. Phloem bundles of diseased leaves in the mid-rib region revealed a significant increase in their size. Another noteworthy feature was the presence of a small degree of blocking with some dark pigment like particles in the phloem bundles. The extent of blocking was found to be 4.7 per cent. Other differences noticed in diseased palms were: phloem cells were thinner, sometimes irregular, not turgid, exhibited minute outgrowths and was very narrow. The parenchyma sheath in the mid-rib region showed a slight reduction in the size of cells in diseased palms.

Leaf anatomical studies in general revealed that the disease has profound influence on various leaf-parts creating abrasions like reduction in vigour and growth of cells, degeneration and disorganization or clumping of chloroplasts, blocking of vascular elements, disorganization of nuclei and cell contents, proliferation and apposition of phloem.

Inflorescence

A comparative anatomical study of different parts of healthy and YLD affected arecanut inflorescence revealed degeneration, disorganization and occasional plugging of vascular elements with some discolored particles, in the case of diseased materials.

Nut

Healthy and diseased nuts at different stages of maturity, along with the diseased and fallen nuts were subjected to anatomical studies. The important deviations noted when compared to healthy were I) degeneration and malformation of embryosac 2) degeneration and under development of endosperm 3) discoloration (blackening) and softening of the endosperm and 4) degeneration and blocking of vascular elements with black pigment especially near calyx region.

Stem

The anatomical differences observed in the case of diseased stems were 1) apposition and degeneration of the phloem bundles 2) the presence of a discolored mass blocking vascular bundles 3) clear disorganization of ground tissue and 4) thinner parenchymatous cell walls.

Root

The important differences seen in the diseased roots from the healthy ones were 1) the frequency in the formation of lateral roots was reduced considerably 2) the root tips with the growing regions became black and decayed 3) exodermal cortex were mostly in degenerated form and could be seen as dark masses of cells 4) clear disintegration of vascular elements especially, phloem bundles 5) phloem cells were comparatively smaller with thin cell walls and 6) blocking of phloem cells to some extent.

Mean values obtained for the various leaf characters considered in the study are presented in Table 1.

Table.1 Anatomical features of leaves of arecanut palms affected with yellow leaf disease.

Characters	Healthy	Diseased
No.of epidermal cells/ unit area	107	118
Area of epidermal cells	671.01 μ	627.88 μ
No. stomata/unit area	21	25
Area of guard cells	625.97 μ	604.50 μ
Area of stomatal pore	119 sq μ	85 sq μ
Size of stomatal apparatus	4151 sq μ	4060 sq μ
Stomatal index	12.5	10.2
Stomatal collapse (%)	-	6.67
Diameter of xylem vessels	44.50 μ	45.35 μ
No. cellular outgrowths in xylem (Range)	-	1-9
Diameter of cellular outgrowths (Range)	-	3-64 μ
Blocking of xylem (%)	-	35
Size of phloem cells	122.3 μ	101.92 μ
Blocking of phloem cell (%)	-	4.7

Pillai and Rawther (1983) while studying the anatomy of YLD affected arecanut roots in comparison with that of the healthy ones, observed lateral and linear proliferation of phloem tubes in diseased palms. Around 60 per cent of the roots showed the presence of spherical or sub-spherical ingrowths of varying sizes within the xylem vessels similar to tyloses. In older roots, these projections were seen completely blocking the lumen of the xylem vessels.

Ultra-structural studies

An attempt was made to study the ultra-structural changes in yellow leaf diseased areca palms to understand the host - parasite interaction. Tissue samples from apparently healthy palms and palms with different disease intensity were studied. Segments of tender and mature leaves and roots were mainly examined. One of the prominent changes observed in tissues of diseased palms is an elaboration of the membrane system. Invagination of plasmalemma into the cell lumen enclosing empty vesicles was often observed. These structures were interpreted as paramural bodies. Such bodies are generally observed in plants under stress condition and also as a pathological symptom associated with microbial infection. Empty vesicles were very prominent in mature tissues where the cytoplasm was found adhering to the sieve tube wall. Aggregation of endoplasmic reticulum in stacks was observed adjacent to sieve tube wall. The number of cell organelles such as mitochondria and rough endoplasmic reticulum per sieve tube in palms in the early stage of disease was found to be much more than that is normally

observed in healthy palms. Electron dense positively stained "cuneate structures" occurring in aggregates and surrounded by a double layered membrane were frequently observed in sieve tubes. Such structures although are common in monocotyledons especially in palms (Parthasarathy, 1974 b, c and d), the frequency of occurrence and the number per cell were very high. Free lying "cuneate structures" were also observed. It is interesting to note that Parthasarathy (1974 a) observed such structures in lethal yellowing affected coconut palms in Jamaica. Both these diseases are known to be induced by Phytoplasmas. The plastids also contained starch grains and crystalline inclusions. The accumulation of starch grain in plastids of rachilla and leaves indicate derangement in translocation of synthesized food materials. Some of the sieve tubes were also filled with fibrilar material, which could be P-protein – a normal host component.

The sieve pores in roots and rachilla tissues were often lined with callose. Such deposition of unusually large amount of callose, a product of wound reaction, was predominantly observed in tissues of diseased plants. The protophloem elements in diseased palms were generally found to be crushed, necrotic and occluded with electron dense contents. The changes recorded may be affecting the functional capability of phloem in transport of synthesized food material from source to "sink region".

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6
VARIETAL REACTION

P. S. RAVINDRAN, K.U.K. NAMPOOTHIRI, R.S.N. PILLAI,
R. CHANDRA MOHANAN AND A.A.MOHAMMED SAYED

Yellow leaf disease, a malady of Phytoplasmal etiology, is not amenable to control by conventional plant protection measures (Ponnamma *et al.*, 1997). As management practices did not yield any positive results, the only other practical solution available for controlling this malady is to evolve resistant/tolerant varieties. During the past three decades, considerable work has been done on this aspect.

Screening of varieties and hybrids

In a multi location trial conducted during 1970's, six promising cultivars such as VTL-3 (released as Mangala) , VTL-11(released as Sumangala), VTL-12, VTL-13, VTL-17(released as Sreemangala) and Mohitnagar with South Kanara local as check were evaluated and the results indicated that all of them were susceptible (Table 1). The disease index varied from 6.7

Table 1. Reaction of promising cultivars to yellow leaf disease.

Cultivar	Disease index
South Kanara	34.6
Mohitnagar	25.4
VTL-3	6.7
VTL-11	24.6
VTL-12	22.1
VTL-13	24.3
VTL-17	21.9

(VTL-3) to 34.6 (South Kanara). Nampoothiri (1982) reported that 52 arecanut collections derived from both exotic and indigenous sources also succumbed to YLD with varying degrees of intensity. Further, large scale screening of germplasm collection/ varietal hybrids, hybrids produced from disease escapes, inter-se/selfed progenies of different collections involving 88 different cross combinations comprising of 2,328 palms during 1976-1993 (Table 2) were undertaken in YLD affected belt. All of them were highly susceptible and 18 genotypes showed less than 25 per cent of disease incidence (Table 3). The succeeding account reveals the results of these experiments.

The 21 diallel cross combinations planted at CPCRI, Palode in 1976 have contracted the disease within a period of three years .The disease incidence varied from 63.9 to 100 per cent (Table 4). Maximum incidence was noticed in VTL-3 x VTL-13, VTL-11 x VTL-13,

Table 2. Screening of hybrids / *Inter-se* materials against yellow leaf disease.

Year of planting	Varietal hybrids	Hybrids involving disease escapes	Inter-se	Self	Total
1976	6	-	-	-	6
1981	-	6	-	1	7
1983	9	-	-	-	9
1984	8	-	-	-	8
1985	-	7	1	-	8
1986	3	3	-	1	7
1986	1	6	-	-	7
1988	3	-	5	-	8
1989	2	-	5	-	7
1990	5	-	2	-	7
1991	4	-	3	-	7
1993	-	-	6	1	7
Total	41	22	22	3	88

Table 3. Reaction of varieties/ hybrids screened against YLD from 1976-1993

Varieties/ Hybrids	Total screened	Disease incidence (%)			
		100	50-99	25-49	Below 25
Varietal hybrids	41	23	9	0	9
Hybrids involving tolerant palms	22	11	4	4	3
Inter-se	22	11	2	4	5
Self	3	0	1	1	1
Total	88	45	16	9	18

VTL-11 x Thirthahalli, VTL-13 x VTL-17 and VTL -l7 x Thirthahalli (100%) and minimum in VTL 12 X Thirthahalli (63.9 %). The hybrid combinations between Hirehalli dwarf mutant and promising cultivars (VTL-3, VTL-11, VTL-13, Mohitnagar and Thirthahalli) planted in 1976 exhibited certain degree of tolerance in the initial years (Anon., 1981). However, all succumbed to YLD within a period of 6-8 years (Table 5). The disease incidence was highest in Thirthahalli x Dwarf (62.9 %) and least in Dwarf x VTL-11 (18.1%).

The most promising results were obtained from the trial laid out in 1981 at CPCRI Research Centre, Palode with field tolerant palms. Even though all the progenies of Saigon x Mangala

Table 4. Reaction of diallel cross combinations to yellow leaf disease.

Hybrids	No. of palms tested	No. of palms diseased	Per cent of incidence	Mean disease index
VTL-3 x VTL-11	12	11	91.7	25.90
VTL-3 x VTL-12	15	10	66.7	14.60
VTL-3 x VTL-13	8	8	100	21.40
VTL-3 x VTL-17	11	10	90.9	20.10
VTL-3 x Mohitnagar	13	10	76.6	11.80
VTL-3 x Thirthahalli	15	13	86.7	20.40
VTL-11 x VTL-12	12	10	83.3	18.50
VTL-11 x VTL-13	12	12	100	23.40
VTL-11 x VTL-17	14	13	92.9	17.60
VTL-11 x Mohitnagar	10	7	70.00	13.00
VTL-11 x Thirthahalli	20	20	100	25.60
VTL-12 x VTL-13	23	19	82.6	21.70
VTL-12 x VTL-17	11	10	90.9	21.40
VTL-12 x Mohitnagar	18	16	88.90	24.40
VTL-12 x Thirthahalli	11	7	63.90	15.30
VTL-13 x VTL-17	13	13	100	31.30
VTL-13 x Mohitnagar	8	6	75.00	8.70
VTL-13 x Thirthahalli	19	17	94.10	23.00
VTL-17 x Mohitnagar	12	10	83.30	16.90
VTL-17 x Thirthahalli	2	2	100.	32.50
Mohitnagar x Thirthahalli	17	13	84.60	21.20

Table 5. Incidence of yellow leaf disease on arecanut hybrids after 8years of planting (planted in1984).

Hybrids	No. palms	No. palms contracted disease	Disease incidence (%)	Mean disease index
Mangala x Hirehalli dwarf	21	9	42.8	9.3
VTL-13 x Hirehalli dwarf	30	14	46.6	15.4
Mohitnagar x Hirehalli dwarf	27	11	40.7	15.5
Thirthahalli x Hirehalli dwarf	27	17	62.9	23.8
Hirehalli dwarf x VTL-11	11	2	18.1	15.0
Mangala	16	7	43.7	21.6
Hirehalli dwarf	31	16	51.6	10.3

did not show resistance to the disease, hybrids between two palms No. 300 (Saigon) and No.125 (Mangala) exhibited high level of tolerance. The disease index in this combination was only 2.8 per cent (averaged over nine years) with an average yield of 9.19 kg/palm/year (Table 6). One of the palms belonging to this combination did not show any symptom even after 13 years. Since the yield of this palm was also high (14.3 kg), it can be considered as a very promising breeding material and can be successfully used in future breeding programme for YLD tolerance.

A field trial involving nine varietal hybrids and Mangala and South Kanara as control initiated during 1984 at CPCRI Research Centre, Palode indicated that all of them contracted the disease within a period of three years except VTL-12 x South Kanara combination (Table 7). Later, this combination also succumbed to the disease. Another experiment laid out at Kannara

Table 6. Reaction of hybrids, planted in 1981,to yellow leaf disease and their yield potential.

Hybrids	Mean no. of nuts/ palm	Fresh weight of ripe nuts (Kg)	Disease index
300 Saigon x 108 Mangala	159*	5.43	2.3
300 Saigon x 125 Mangala	251	9.19	2.8
28 Aryankavu x 115 Mangala	192	4.97	4.9
159 Peechi x 122 Mangala	83	2.87	3.6
105 Mangala x 299 Saigon	206	6.04	4.7
105 Mangala x 108 Mangala	123	3.57	8.1
28 Aryankavu x 28 Aryankavu	107	2.81	9.5
SE	28.8	1.00	1.1
CD (5%)	88.7	3.10	3.5

* Mean for 9 years (1985-1993)

Table 7. Reaction of varietal hybrids, planted in 1984, to yellow leaf disease

Cross combination	No.of palms	No.of palms diseased	Percentage[1] of disease incidence	Mean disease index
Hirehalli dwarf x VTL-3	27	2	7.4	4.0
VTL-3 x Hirehalli dwarf	27	1	3.7	5.0
South Kannara x VTL-12	26	3	11.5	1.3
VTL-12 x South Kannara	27	0	0	0*
VTL-12 x VTL-3	16	2	12.5	7.5
VTL-12 X Hirehalli dwarf	25	4	16.0	2.5
VTL-12 x Sreevardhan	20	2	10.0	17.5
VTL-3 x South Kannara	19	1	5.3	17.0
Hirehalli xVTL-17	27	2	7.4	3.5
Mangala	25	4	16.0	11.0
South Kannara	27	2	7.5	17.5

[1] Data recorded in 1987 * Succumbed to disease in 1990

in 1986 involving hybrids from disease escapes and Mangala and South Kanara local as check showed that more than 50 per cent of palms in all combinations were susceptible to the disease (Table 8). The hybrids 96-M x 260 and 172 x 71 M gave satisfactory yield of 5.42 and 6.15 kg nut weight respectively with 217 and 268 mean number of nuts. All other combinations were poor yielding with a mean weight of 2.38 kg and less than 80 nuts/palm.

Table 8. Reaction of hybrids derived from disease escapes to yellow leaf disease.

Cross combinations	Percentage of disease incidence	Mean disease index	Mean no. of nuts	Mean yield (Ripe fresh nut in Kg)
8 M x 25 A	91.60	32.40	75	1.978
31 M x 260	75.00	18.80	79	2.260
96 M x 260	83.30	22.70	217	5.418
414 x 34 M	95.80	32.90	28	0.758
172 x 71 M	95.80	28.90	268	6.151
149 X 177	100	40.70	40	1.143
Hirehalli dwarf x VTL 13	50.00	5.70	35	1.400
Mangala	54.10	10.50	79	2.381
South Kanara	95.80	29.80	14	0.462

Among the exotic types and species planted in 1968 at Kannara, only two genotypes (Indonesian II and British Solomon Islands I) have remained disease free. In the experiment in which "true" Mangala and segregants were planted to study the intensity of YLD, 24 per cent segregants contracted the disease in the fourth year after planting compared to four per cent in true Mangala (Anon., 1993). Even after18 years of field experimentation, none of the hybrids or varieties were completely resistant.

Field tolerant elite palms

As no variety or hybrid was found to be disease tolerant, it was appropriate to search for field tolerant elite palms in 'hot spot' of YLD affected areas in Kerala State.

The criteria for selection of elite disease escape palms were :

- More than 90 per cent of the surrounding palms should be affected by YLD.
- The palms should have a minimum age of 20 years
- Disease free nature of palms should be confirmed by light microscopic tests.
- Selected palms should yield a minimum of 200 nuts/palm/year under neglected conditions (Farmers neglect the gardens when YLD incidence is very high)
- Selected palms should be generally free from major pests and diseases.

From an intensive survey in 'hot spot' areas conducted during 1985-1987 in 13 districts of Kerala involving 1,32,750 palms, 70 healthy/disease escapes were identified. They were further subjected to light microscopic examination and six of them were disease free (Table 9). A recent survey conducted in 1998 led to identification of five disease free elite palms in Thrissur and 10 palms in Ernakulam districts. Further, an arecanut garden consisting

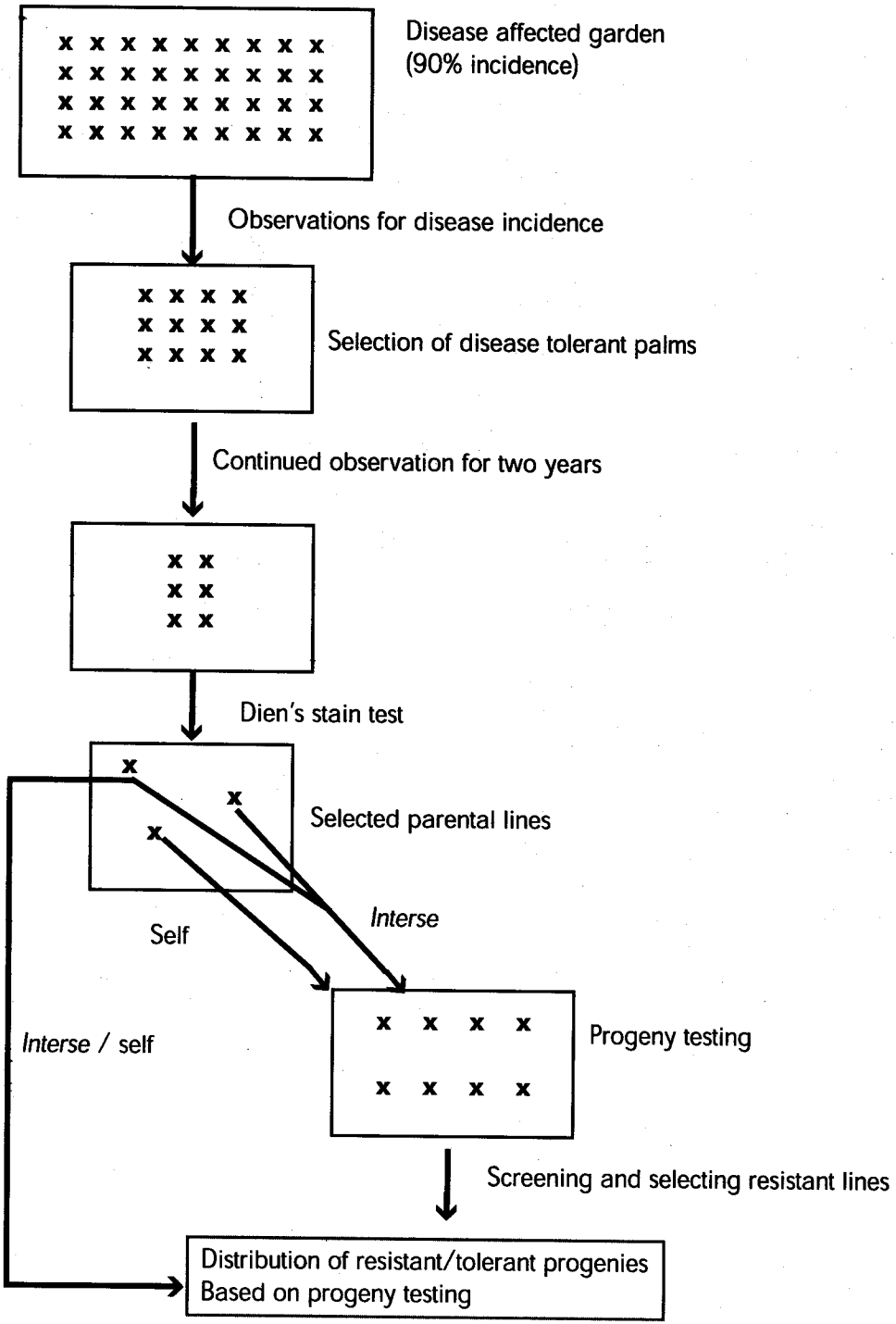
Table 9. Details of 'hot spot' survey conducted in 13 districts of Kerala

District	Year of Survey	No. gardens visited	Total no. of palms	No. of disease free palms	No. of tolerant palms identified
Thiruvanathapuram	1985	28	12,678	0	—
Kollam	1985				
Pathinamthitta	1985				
Kottayam	1986	13	11,994	3	1
Alappuzha	1986	7	17,857	2	0
Ernakulam	1986	29	12,071	3	0
Idukki	1986	20	14,433	5	0
Thrissur	1986	13	11,310	11	4
Palaghat	1986	11	11,875	1	0
Malappuram	1986	10	8,967	0	0
Kozhikode	1986	10	8,950	36	0
Wynad	1986	8	9,615	0	0
Kannur	1987	25	13,000	9	1
Total		174	1,32,750	70	6

of 52 palms, raised by the farmer using the seed nuts collected from a single YLD free high yielding palm of about 30 years old, occurring in middle of all other YLD affected palms, was also identified in the 'hot spots' of Ernakulam district during the survey. These 52 palms are now more than 20 years old. Though they were raised from open pollinated seed nuts of YLD symptom free elite palm, none of these palms were showing symptoms of YLD except three palms exhibiting kernel discolouration. Thus, there were 49 palms (second generation) without any symptoms of YLD. None of the palms exhibited any YLD symptom so far. All the 67 palms (52 palms of second generation and 15 disease escapes from Ernakulam and Thrissur districts) were subjected to histological staining using Diene's stain (Deelay *et al.*,1979). Based on reaction to Diene's stain, the disease escapes identified in 'hot spots' were categorised into healthy and infected palms. The 10 YLD symptom free elite palms in Ernakulam district were negative in their reaction to Diene's stain. Out of the 5 palms identified in Thrissur district, one palm was negative to staining reaction. Among the 52 second generation YLD free palms in Ernakulam district, 33 palms exhibited negative reaction to Diene's stain. Of these 33 palms, 24 palms were identified and marked as elite palms based on yield evaluation. Thus, 34 palms in Ernakulam district and one palm in Thrissur district were selected as YLD free elite palms in Kerala state for production of seed nuts by selfing and *inter-se* mating. These palms would be monitored every year for disease expression and disease free palms will be confirmed by light microscopic technique.

Seedlings raised from open pollinated seed nuts of second generation YLD free elite palms identified in Ernakulam district are being evaluated for their reaction to YLD by interplanting them in arecanut gardens with more than 90 per cent of YLD affected palms. Selfing of inflorescences of these 24 second generation elite palms is also in progress. A schematic diagram depicting breeding for YLD tolerant arecanut palms is given in Fig.1 (Chandra Mohanan and Nampoothiri, 2000).

Fig. 1 Evolving YLD tolerant arecanut lines



Future strategy

Identification of disease resistant/tolerant palms and screening of these progenies should be a continuous process in the search for tolerant genotypes for YLD. As breeding for resistance in tree crops like arecanut is a slow process, early predictive tests for resistance are needed to accelerate the breeding process. Assessing resistance to YLD using biotechnological tools can be tried to shorten the breeding cycle. Screening the materials against the disease with dodder and insect vector and early disease detection aids using ELISA would accelerate the identification of resistance. PCR based molecular fingerprints can be exploited for determination of genetic diversity in arecanut germplasm and tagging resistant genes. Pathogen derived resistance mechanisms need to be employed to produce transgenics to resolve this stalemate.

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7

SOIL AND NUTRITION MANAGEMENT

P. L RAMANANDAN AND K. J. ABRAHAM

Among different factors associated with yellow leaf disease of arecanut, soil health and balanced nutrition are profoundly important as they are assumed to influence the disease incidence either directly or indirectly. Menon (1960, 1961) suggested that lack of balanced nutrition and improper cultivation practices made the palms susceptible to the disease. Holmes (1964) observed that yellow leaf disease was due to deficiency or toxicity of either manganese or iron. Lal *et al* (1964) suggested a nutritional approach to the problem in addition to provision of proper drainage. There was also a report that application of fertilizers improved the condition of diseased palms (Anon., 1967). So, numerous field and laboratory studies related to soil and nutrient management practices and their role in the control of this malady were being investigated at different locations.

Major and micronutrients

Trials conducted at Central Plantation Crops Research Institute, Research Centre, Palode to find out the role of major nutrients on the incidence of yellow leaf disease revealed that yellowing symptoms were not reproduced with nutrient cultures devoid of nutrient element. However, the symptoms of yellow leaf disease were developed when healthy seedlings were kept in balanced nutrient solutions containing diseased sap (Anon., 1960). Another set of pot culture studies to investigate the role of major nutrient deficiencies in the development of the disease did not produce any symptoms typical of this malady(Yadava *et al.*, 1972).

The first report on nutritional aspects of this malady showed the existence of nitrogen and phosphorus deficiency in the soils of disease affected gardens. Studies in YLD affected belt of Kerala (1959-62) indicated that soils were highly acidic with pH as low as 3.8 and were deficient in all three major nutrients (Anon., 1960, 1961, 1962). Velappan (1969) also observed that the soils of yellow leaf disease affected gardens were low in pH, organic carbon, available phosphorous and magnesium. Leaves of diseased palms contained lower amount of nitrogen, phosphorus, magnesium and zinc. When seedlings grown in soils supplied with manganese, calcium, boron and zinc, the toxicity symptoms developed did not resemble those of YLD. Subsequently, leaf analysis of diseased palms at Vittal since 1969 revealed that leaf samples contained more than 3 ppm of dilute acetic acid extractable aluminium, a level, which is considered dangerous to plants. Similarly, soil in diseased tracts recorded higher contents of exchangeable aluminium (Mohapatra *et al.*, 1975).

In a comprehensive soil survey in disease affected areas in Kerala and Karnataka, the soils were found to be high in organic carbon and medium to low in available phosphorus and potassium (Table 1). The exchangeable iron, manganese, zinc and copper contents were above the level of sufficiency in these soils. It was also reported that increase in soil acidity and

Table 1. Fertility constituents of soils from YLD affected and healthy areas of Kerala and Karnataka

Constituents	Kerala State		Karnataka State	
	Apparently Healthy	Diseased	Healthy	Diseased
pH (H_2O)	5.63	5.58	6.35	6.34
pH (KCl)	7.33	4.28	5.12	5.18
O.C.(%)	0.86	0.90	1.29	1.24
Av.P $_2O_5$ (ppm)	9.11	7.62	18.15	14.0
Av. K_2O(ppm)	75.26	80.35	153.64	141.99
Ex. Ca(ppm)	197.25	185.8	706.6	665.75
Ex. Mg(ppm)	58.35	52.10	182.0	186.25
Ex. Al(ppm)	82.22	94.45	41.65	43.03
Fe^{2+} + Fe^{3+}	17.15	20.07	13.82	12.63
Ex. Mn(ppm)	8.43	8.23	14.98	15.99
Exr. Zn(ppm)	1.095	1.06	2.34	2.35
Ex. Cu(ppm)	1.98	1.98	3.98	3.44

clay content significantly increased the quantity of exchangeable aluminium in Kerala state . A sand culture experiment to investigate the role of aluminium on yellow leaf disease in 1974 revealed that addition of aluminium at 5, 10 and 20 ppm reduced the leaf size and growth of palms (Anon., 1976).

The results of trials on micronutrient toxicity were inconsistent. Deficiency of zinc showed some relationship to the disease symptoms(Velappan, 1969). Sam Raj and Pailey (1965) noticed that symptoms similar to those of the YLD were expressed by the application of boron to soil. However, subsequent studies at Vittal showed that yellowing caused by boron was different from the symptoms of yellow leaf disease. Pot culture experiments conducted from 1962 to 1974 indicated that copper, molybdenum, zinc, manganese and iron did not produce the characteristic symptoms of the disease (Anon.,1962, 1976). It is evident from Table 2 that contents of silica, phosphoric acid and potash were high in diseased samples while percentage of N and CaO were high in healthy palms (Anon., 1976). Evidence was adduced to show that the diseased palm tissue contained more water soluble and HCl soluble iron content and higher CaO/MgO ratio as compared to healthy palms. The Mg content was very low in diseased tissues bringing about a wide difference in CaO/MgO ratio, the average value for healthy and diseased leaves being 1.14 and 8.37 respectively. Leaf analysis revealed that N, K, Ca and Mn contents of healthy palms were higher than those of diseased palms (Anon., 1967). On the other hand, palms from healthy locality showed lower values for P and Mg as compared to diseased samples (Table. 3). Zn and Al contents of healthy and diseased palms did not show any appreciable difference (Anon., 1974)

Table 2. Analysis of healthy and diseased arecanut leaves collected from Palode area

Constituents (%)	Healthy	Diseased
Moisture	12.53	9.27
Ash	7.60	9.00
N	0.91	0.76
P_2O_5	0.55	0.63
K_2O	0.22	0.30
CaO	0.90	0.75
Mg	Traces	Traces
Soluble silicate	0.60	1.30
Insoluble silica	3.30	4.22

Table 3. Mineral nutrient content of arecanut leaf from healthy and yellow leaf disease affected areas

Element	Healthy area	Diseased area (Palode)	
	Vittal	Apparently healthy palms	Diseased palms
Major elements (%)			
Nitrogen	1.51	1.37	1.06
Phosphorus	0.21	0.35	0.34
Potassium	1.02	1.59	0.88
Calcium	1.14	0.72	0.64
Magnesium	0.26	0.60	0.75
Minor elements (ppm)			
Iron	440	476	328
Manganese	190	161	76
Boron	5	8	10
Copper	5	2	5
Zinc	38	33	34
Aluminium	420	442	403

Root tissues of diseased palms from Palode (Kerala) and Sullia (Karnataka) contained more aluminium and less NPK than those of healthy palms from Vittal(Anon., 1974) (Table. 4). Further analysis of leaf samples from healthy and yellow leaf disease affected arecanut palms from Karnataka and Kerala showed that except for leaf-P values, which are low in apparently healthy and diseased palms from Kerala (Table. 5), the amounts of other nutrients were above the levels of sufficiency (Anon., 1976). The role of micronutrients on the incidence of yellow

Table 4. Chemical composition of leaf and root in healthy and diseased palms

Location	N (%)		P (%)		K(%)		Al (ppm)	
	Healthy	Diseased	Healthy	Diseased	Healthy	Diseased	Healthy	Diseased
Root								
Sullia	0.546	0.392	0.003	0.025	1.185	0.900	2925	3775
Palode	0.605	0.694	0.026	0.034	0.0676	0.624	2550	9350
Vittal	0.666	—	0.075	—	2.242	—	925	—
Leaf								
Sullia	2.338	1.302	0.175	0.143	1.065	1.502	145	75
Palode	1.920	1.808	0.131	0.134	1.057	1.374	66	144
Vittal	1.946	—	0.150	—	1.065	—	235	—

Table 5. Nutrient status of healthy and YLD affected palms.

Parameter	Kerala		Karnataka	
	Healthy	Diseased	Healthy	Diseased
N (%)	2.54	2.45	2.88	2.79
P (%)	0.150	0.145	0.190	0.182
K (%)	0.758	0.716	0.741	0.753
Ca (%)	0.395	0.379	0.567	0.477
Mg(%)	0.221	0.219	0.254	0.225
Fe (ppm)	182.59	214.62	338.63	353.93
Mn (ppm)	190.00	180.35	98.56	87.50
Zn (ppm)	21.00	20.37	27.00	23.49
Cu (ppm)	15.20	14.25	60.35	54.20

leaf disease was not clear as disease symptoms characteristic of yellow leaf disease could not be reproduced (Anon., 1967, 1976). However, spraying of $MnSO_4$ (5 g / 5 lit of water) and $MgSO_4$ (5 g / 5 lit. of water) had improved the condition of the affected palms with fresh growth of leaves. The application of fertilizer also resulted in more number of non-chlorotic leaves in the disease affected garden (Menon and Kalayanikutty, 1961).

Water logging

Pal *et al* (1960) reported that water logging is considered as one of the pre-disposing factors in the spread of yellow leaf disease. It was reported that water table was within the root zone of palms in disease affected garden which finally leads to reduced condition during rainy season (Mohapatra, 1975; Mohapatra *et al.*, 1976). Studies on the effect of submergence on soil pH and exchangeable aluminium indicated that there was increase in pH from 5.01 to

6.08 during first 15 days of submergence and continuous submergence up to 90 days led to considerable decrease of pH (4.27). The exchangeable aluminium was inversely related to changes in soil pH (Mohapatra *et al.* 1976).

Management

Since the yellow leaf disease is not amenable to control by conventional plant protection measures, it became imperative to find out other means of containing the disease so as to obtain maximum economic returns from the affected gardens. Soil and nutrient management practices such as drainage, cropping systems, pesticide and fertilizer use are assumed to have greater influence on the disease incidence. A mixed cropping trial involving regular organic recycling in a disease affected garden indicated that there was an increase in yield with cowpea, NB 21 fodder grass and guinea grass as mixed crops in arecanut garden (Table 6). Application of higher dose of phosphorus (160 g/year/palm), dolomite (4 kg) and farm yard manure (12 kg) with irrigation had no apparent effect on disease incidence and yield of palms (Table 4) (Anon., 1977, 1980). However, in another trial at Palode, lime and phosphate application resulted in lowest disease incidence (12.5%) compared to lime (33%) and supherphosphate

Table 6. Mixed farming with fodder grass and dairy-yield and disease index of arecanut palms under the trial (Average of 1975-78)

Treatments	Disease index		Yield of nuts (kg ripe nuts)	
	With irrigation	Without irrigation	With irrigation	Without irrigation
Cowpea	53.23	56.87	13.47	12.45
NB 21	61.70	63.63	7.07	9.87
Guinea grass	63.53	64.77	11.35	11.80
No fodder (control)	60.00	65.00	10.78	14.55

Table 7. Incidence of YLD following Agrimycin, Bavistin and Temik treatments

Treatments	Disease incidence	% incidence
Agrimycin	3/17*	16
Bavistin	4/15	27
Temik	4/17	24
Agrimycin + Bavistin	5/16	31
Agrimycin + Temik	2/14	14
Bavistin + Temik	12/42	29
Agrimcin +Bavistin + Temik	6/9	75
Control	2/13	15

* Number of diseased palms / number of palms tested

Table 8. Effect of application of macro and micro nutrients on yield of arecanut

Treaments	Jayapura (Karnataka)			Annamanada, Kothattukulam & Punalur (Kerala)		
	1966-67	1968-69	% difference	1966-67	1968-69	% difference
A.NPK (140 g Ammonium sulphate, 225 g SSP and 115 g MOP	371.53*	371.59	+0.02	132.22	126.14	- 4.59
B. Treatment A+ lime (1 kg/palm)	321.83	323.24	+0.43	128.80	153.33	+ 9.04
C. Treatment B + Ferrous sulphate(57 g/palm)	369.75	392.22	+6.27	127.23	123.92	- 2.60
D. Treatment B + Sodium borate(23 g/palm)	326.75	455.61	+39.43	124.99	141.54	+ 13.24
E. Treatment B + Zinc sulphate(23 g/palm)	298.23	342.57	+14.86	111.67	129.05	+ 15.56
F. Treatment B + Manganese sulphate (68g/palm)	295.21	336.50	+5.30	135.85	112.88	- 16.90
G. Treatment B + Magnesium sulphate (68g/palm)	343.15	343.37	+0.06	131.95	128.00	- 2.99
H. Control- Healthy palms with farmers practice	460.98	520.35	+12.87	119.44	122.33	+ 2.41
I.Treatment B + Manganese sulphate (68 g/palm) + Magnesium sulphate(68 g/palm)	235.80	443.99	+55.34	138.02	133.44	- 3.31
J. Treatment I + Zinc sulphate(23 g/palm)	373.55	454.62	+21.70	149.83	157.00	+ 4.78
K. Treatment I + Sodium borate (23 g/palm)	282.27	356.26	+26.21	128.36	127.40	- 0.74
L. Treatment I + Zinc sulphate(23 g/palm) + Sodium borate(23 g/palm)	285.24	307.87	+7.93	123.62	118.37	- 4.24
M. Control – diseased palms with farmers Practice	286.04	356.32	+24.56	124.41	120.37	- 3.24

* Average number of nuts/tree/year

alone (15.4%). An integrated control trial at Palode in Kerala with the application of agrimycin, bavistin and temik alone and in combination revealed that the palms treated with agrimycin + bavistin + temik were free from YLD(Table 7).

Effect of lime and fertilizer

Soil application of NPK and lime with or without zinc sulphate resulted in significant reduction in the yellowing of the leaves in Karnataka (Dastagir, 1963). There was also better response to soil application of NPK together with lime, boron and manganese. Addition of dolamite @ 4 and 8 kg /palm/year had no significant effect on the disease condition of the palm (Anon., 1974). Mohapatra *et al* (1976) also reported that application of lime as dolamite and phosphorus had no ameliorative effect on the disease.

Effect of nutrients and irrigation

In Kerala and Karnataka, the field trials conducted during 1965-69 to find out the effect of macro and micro nutrients on disease incidence revealed that there was a general increase in yield ranging from 0.2 to 55.34 per cent in Karnataka (Table 8). However, the trend was different in Kerala. There was marginal yield increase (0.74 – 4.59 %) in eight of the thirteen treatments and there was increase in yield (2.41 – 10.04 %) in the remaining treatments. In contrast, a field trial at Palode to study the effect of nutrients and irrigation(Table 9 and 10) indicated that application of macro and micro nutrients with or without irrigation was not effective in controlling the disease (Rawther and Abraham, 1974).

Table 9. Effect of nutrients and irrigation on incidence of yellow leaf disease

Treatment	Percentage of disease incidence				
	1968	1969	1970	1971	1972
Control- No cultivation, No manuring	0.03	10.70	13.30	29.33	64.00
NPK + Irrigation	0.04	9.30	20.30	30.66	62.66
NPK without irrigation	0.02	8.00	16.20	42.66	64.00
NPK + Micronutrients with irrigation	0.04	12.00	20.30	45.33	68.00
NPK + Micronutrients without irrigation	0.00	10.70	21.10	41.33	68.00

Table 10. Effect of nutrients and irrigation on yield of YLD affected palms

Treatments	1968	1969	1970	1971	1972
Control- No cultivation, No manuring	1.13	6.95	19.80	8.79	30.70
NPK + Irrigation	88.60	278.92	208.80	154.33	208.40
NPK without irrigation	25.91	58.65	61.00	49.23	120.00
NPK + Micronutrients with irrigation	108.87	253.47	216.00	157.39	211.80
NPK + Micronutrients without irrigation	37.56	66.35	78.80	67.95	172.70

* Average number of nuts/tree

Field study carried out by University of Agricultural Science (UAS) Bangalore at Sagar in Karnataka on the effectiveness of fungicides, insecticides, drainage and nutrients in reducing the disease incidence, root drenching of emissan (5 lit./palm) and pauchamycin (5 lit./palm), soil application of NPK(140 g urea, 500 g super phosphate and 150 g MOP) + zinc (8.5 g / palm), NPK + zinc + lime (85 g / palm) and application of neem cake (5 kg / palm) were found to improve the condition of disease affected palms within one year of treatment imposition. A field study conducted at Sullia, Karnataka in 1974 indicated that addition of NPK fertilizers alone and in combination with dolamite did not help in ameliorating the disease (Anon., 1976). In a comprehensive package trial in farmer's field at Sampaje in Karnataka, application of NPK + lime, NPK + lime + boron and NPK + lime + zinc increased the yield by 20 per cent (Anon., 1983) and addition of NPK + dolamite + neem cake reduced the disease intensity.

By taking into account all the results from the earlier trials, a detailed experiment was initiated in 1982 at Palode (Kerala) with four management practices on two varieties of arecanut to evaluate their effect on the incidence of yellow leaf disease (Anon.,1991). Incidence of the disease was least in palms treated with higher dose of phosphorus application over and above the normal package. Mangala and it's segregants were superior to South Kanara local. The effect of phosphorus in reducing the incidence of yellow leaf disease was evident (Table.11)

The foregoing account indicates that the role of soil and nutrient factors in the causation of the disease is not yet established. However, from the above trials, it is apparent that soil and nutrient management had improved the condition of disease affected palms and increased the

Table 11. Effect of management practices on the incidence of yellow leaf disease

	Per cent palms affected by YLD						Yield of arecanut		
	1985	1986	1987	1988	1989	1990	1988	1989	1990
Varieties									
Mangala	2.99	2.70	5.86	2.70	4.10	20.80	4.90	3.00	3.50
S. Kanara Local	5.67	9.90	9.02	10.40	13.90	28.50	1.10	0.70	1.60
Mangala segregants	-	7.80	7.59	10.50	12.30	16.80	3.10	1.80	2.20
Management practices									
1. Normal package of practices	8.96	9.30	8.80	11.40	12.90	33.80	2.70	1.60	2.10
2. Treatment 1 + 12 kg OM	4.29	7.40	8.33	9.10	12.10	19.40	3.30	1.90	2.50
3. Treatment 1 + 120 g P	1.47	5.50	2.77	5.90	5.90	11.80	2.40	1.70	2.60
4. Treatment 1 + 12 kg OM +120 g P	2.86	7.60	10.06	7.10	12.90	23.00	3.60	2.10	2.50

yield to some extent or maintained the yield level. Thus, it is essential to follow management recommendations in order to reduce the disease incidence and to realize maximum economic returns from the affected garden.

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Epilogue

Yellow leaf disease (YLD) is the most serious problem affecting arecanut industry. The disease was first reported in 1914 in central Kerala and later, it has spread to all parts of Kerala, parts of Karnataka and Tamil Nadu. It was noticed even in central regions of Maharashtra. In Kerala, about 35.8 per cent of the area under arecanut cultivation was affected with this malady. The incidence is also very high ranging from 72 to 97 per cent. In Karnataka, the loss due to this disease was estimated to be around 508.3 tones of chali. The occurrence of the disease is noticed in isolated patches without any definite pattern. The disease is prevalent mainly in laterite soils. Arecanut is found susceptible to the disease at all stages. The diagnostic symptom of this malady appears as yellowing of leaflets at the tip in two to three leaves of outermost whorl with abrupt demarcation between yellow and green regions. The kernel discoloration and rotting of roots are also associated symptoms.

The etiology of the disease was a matter of dispute for long time. Thus, it necessitated to investigate the role of several factors assumed to be associated with the disease. Although association of various biological agents such as fungi, bacteria and nematodes with disease has been found, their role as etiological agents of the disease could not be proved. Presence of some form of protein in the diseased palms along with negative results obtained with fungi, bacteria and nematodes and non- reproduction of symptoms by deficiencies/ toxicity of macro and micronutrients indicated the possibility of virus or virus like agent as etiological agent of the disease.

Further studies by several workers involving electron microscopic examination revealed the presence of Phytoplasma in the young sieve elements of YLD affected palms and in the salivary glands of the vector, *Proutista moesta*. Light microscopic techniques using certain histological dyes (Diene's stain) and flurochromes(DAPI and Hoechst 33255) have been standardized to detect Phytoplasma. The disease has been successfully transmitted from diseased palms to healthy areca seedlings using *Proutista moesta* , a plant hopper and dodder laurel (*Cassaytha filiformis*). The remission of symptoms obtained with tetracycline group of antibiotics further lent support to Phytoplasma as etiological agents. The interrelationships among Phytoplasmas affecting arecanut, coconut and oil palm needs to be established using AFLP finger prints as these crops are commonly grown in the vicinity of each other.

Abnormal stomatal closure accompanied by higher stomatal resistance and water potential similar to other yellow diseases is a characteristic feature of the disease. Increased concentration of starch ,sugar and amino acids, particularly arginine, and leaf waxes are adaptive features of the diseased palms to maintain osmotic adjustment and to reduce the rate of transpiration. Degeneration and clumping of chloroplasts in chlorotic leaves and phloem necrosis are anatomical abnormalities associated with diseased palms.

Though, the role of soil and nutrient factors in the causation of the disease is not established, it is essential to adopt all the recommended practices, as detailed below, for reducing the disease intensity and sustaining the yield level of affected palms.

- Annual application of 200g of urea, 200g Mussorie phos and 230g muriate of potash per palm per year in two splits and an additional application of 800g Mussorie phos in the affected garden.
- Application of 12kg each of compost and green leaves per palm per year in addition to chemical fertilizers.
- Irrigation during summer months.
- Avoiding water stagnation in the garden during monsoon by providing drainage facilities.
- Growing cover crops in the garden.
- When only a few palms are affected in a garden, remove them to prevent further spread of the disease.
- Adopting need based plant protection measures against other pests and diseases.

Eradication of severely affected palms and development of cost- effective and feasible management practices are most important in controlling the disease effectively. Satellite survey of the disease affected arecanut tract using geographic information system (GIS) would be helpful in identifying the extent of spread and intensity of the disease as well as in understanding the incidence in surrounding areas. This could, then coupled with the existing information on the disease, provide more useful information. The intricacies of environmental and edaphic factors on the spread and incidence of the disease are relatively important and need to be investigated as they influence the disease incidence either directly or indirectly.

In the absence of suitable and effective control measures, the main emphasis must be on identifying the tolerant/ resistant elite palms in the 'hot spot' areas. Identification of such palms and screening their progenies should be a continuous process in the search for tolerant YLD lines. As breeding for resistance in perennial palms like arecanut is slow process, early disease detection aids using ELISA would accelerate the identification of resistance. New vistas in biotechnology like PCR based molecular markers can be exploited for determination of genetic diversity in arecanut germplasm and tagging resistant genes. Evolving field tolerant lines coupled with suitable management practices will be final solution in tackling the YLD of arecanut.

AUTHOR INDEX

SUBJECT INDEX