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Incidence of fungal species associated with leaf rot disease of coconut palms in relation to weather and the stage of lesion development

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Summary

Leaf rot disease (LRD) of coconut occurs in Kerala State, India, and is generally severe during the monsoon, a time of high rainfall and r.h. and low maximum temperature. The pathogenic fungi *Colletotrichum gloeosporioides, Exserohilum rostratum, Gliocladium vermoeseni, Fusarium solani, F. moniliforme* var. *intermedium, Thielaviopsis paradoxa* and *Rhizoctonia solani* were among 14 species isolated from spindles of palms with LRD in multiple samplings of two experiments, each lasting 1 year. Co-occurrence of fungi was observed in 70–76% of spindles although leaf pieces yielded mostly individual fungi irrespective of the stage of lesion development. *C. gloeosporioides* occurred most frequently on early lesions. The incidence of *C. gloeosporioides* was most strongly correlated with rainfall and r.h. and, negatively, with maximum temperature and hours of sunshine. It was the fungus isolated most commonly during the monsoon and occurred on early lesions more frequently than on advanced lesions (determined in one experiment). *C. gloeosporioides* is thus implicated as the principal pathogen involved in initiation of the disease during monsoons. Its relatively low incidence in the dry season suggests a quiescent phase between periods of parasitic activity. *E. rostratum* was isolated less frequently and its incidence was less strongly or consistently correlated with weather or with the stage of lesion development. *Fusarium* spp. and *R. solani* were associated with dryer weather and higher temperatures. The *Fusarium* spp. were the fungi isolated most commonly during part of the dry season (January–March) and occurred more on early than on advanced lesions at one sample (January) when they may have been primary disease agents. *R. solani* was also more frequent on advanced lesions and may be principally a secondary coloniser of established lesions. The incidence of other fungi, which occurred less frequently and more sporadically, was not associated with weather or consistently with different stages of lesion development.

Key words: Coconut, leaf rot disease, fungal complex, *Colletotrichum gloeosporioides, Exserohilum rostratum, Fusarium* spp., weather, epidemiology

Introduction

The coconut root (wilt) disease, a debilitating disease thought to be caused by a phytoplasma (Solomon, Govindankutty & Nienhaus, 1983), is prevalent in southern districts of Kerala State, India. The diseased palms are affected extensively by leaf rot disease (LRD), bringing about a sharp deterioration of the palms (Menon & Nair, 1951; Srinivasan, 1991). LRD is caused by a complex of fungi. Srinivasan & Gunasekaran (1993) described the species composition of LRD. Fungi isolated from diseased palms and shown to be pathogenic are listed in Table 1.

Table 1. *Fungi isolated from spindle leaves of coconut with leaf rot disease*

[a]*Colletotrichum gloeosporiodes*
[a]*Exserohilum rostratum*
[a]*Gliocladium vermoeseni*
[b]*Fusarium solani*
[b]*F. moniliforme* var. *intermedium*
[c]*Cylindrocladium scoparium*
[d]*Thielaviopsis paradoxa*
[d]*Rhizoctonia solani*
[d]*Mortierella elongata*
[d]*Curvularia* sp.
Acremonium sp.
Thielavia microspora
T. terricola
Chaetomium brasiliense

Fungi shown to be pathogenic on coconut spindles:
[a]Srinivasan & Gunasekaran (1994)
[b]Srinivasan & Gunasekaran (1996*a*)
[c]Srinivasan & Gunasekaran (1995*a*)
[d]Srinivasan & Gunasekaran (1995*b*)

LRD usually affects the emerging spindle leaves; contraction of the disease by successively emerging spindles is possible (Joseph & Rawther, 1991). Srinivasan & Gunasekeran (1992) described symptom appearance and the extent of disease expression in different leaf whorls of the crowns of affected palms. Early symptoms of the disease are necrotic, angular lesions in the spindle leaves; these coalesce and result in severe rotting of the young tissues. Hence the pathogenic activity is intense in the spindle leaves and infection here leads to most of the damage to the expanding leaves.

LRD is generally severe during monsoon periods, May/June to November/December (Menon & Nair, 1951; Mathai, 1980), with high humidity and low temperatures favouring disease (Radha, Sukumaran & Prasannakumari, 1961; Radha & Lal, 1968). Knowledge of weather factors that affect the natural prevalence of individual pathogens is lacking. As the disease is caused by a fungal complex and the recurrence of infections in palms is common, monitoring the natural incidence of various fungi in different months of the year is appropriate. The influence of climatic factors on the population dynamics of foliar pathogens is a widely known phenomenon (Blakeman, 1993). Hence the objective of the present study was to examine the incidence and seek evidence of successions of LRD fungi in relation to weather factors and to assess their relative importance in different conditions.

Materials and Methods

At a representative site, at Kayangulam, Kerala State, with a history of endemic LRD, diseased spindle leaves (the youngest emerging leaves) were detached from coconut palms and taken intact to the laboratory. Microscopic examination of leaf scrapings showed different pathogens of leaf rot (Srinivasan *et al.*, 1995). For the present study, the fungi were isolated on agar media. Leaflets were excised from the spindles and pieces cut randomly from them. Two experiments were conducted, each of 1 year's duration.

Experiment 1

Samples were taken irrespective of disease severity and the margins of lesions cut into small pieces of approximately 0.5 cm^2. Twenty palms per month were sampled (January–December) and from each spindle 25 leaf pieces were taken.

Experiment 2

Samples were taken from early and advanced lesions on leaflets of the same spindle. Leaf pieces were cut as described above. In this experiment, 10 palms were sampled per month and from each spindle 15 leaf pieces were used for each category (early or advanced) of lesion. Early and advanced lesions were used to confirm the succession of fungal incidence in relation to weather.

Isolation and identification

Pieces of leaf were surface-sterilised by immersion in 0.1% mercuric chloride solution for 1 min followed by triple rinsing in sterile distilled water. The leaf pieces were then transferred aseptically onto potato dextrose agar at five pieces per Petri dish (9 cm diameter) and incubated at 30 ± 1°C for 7 days. The fungi were identified and enumerated using colony and spore characteristics in comparison with standard isolates from the International Mycological Institute, England.

Fungal frequency

The number of leaf pieces from which each fungus was recovered was recorded and percentage recoveries from individual spindles in each month was calculated. In Expt 2, the frequencies were calculated separately for early and advanced lesions. Overall incidence of individual fungi as well as co-occurrences on palms in different months were also calculated for both experiments.

Weather data

Meteorological data (monthly total rainfall, mean r.h., mean max. and min. temperatures, and hours of sunshine) were recorded.

Statistical analysis

Frequencies of fungi, as percentages and after transformation to arcsines, were analysed by analysis of variance. Only untransformed data are presented because the same levels of significance resulted from analyses of these and of transformed percentages. Correlation coefficients (r) were used to investigate the relationship between weather factors and the frequency and overall incidence of the fungi.

Results

Weather

In each experiment, there was little or no rainfall from January to April or in December (Fig. 1). Moderate to heavy rain with occasional lulls occurred from May/June to November

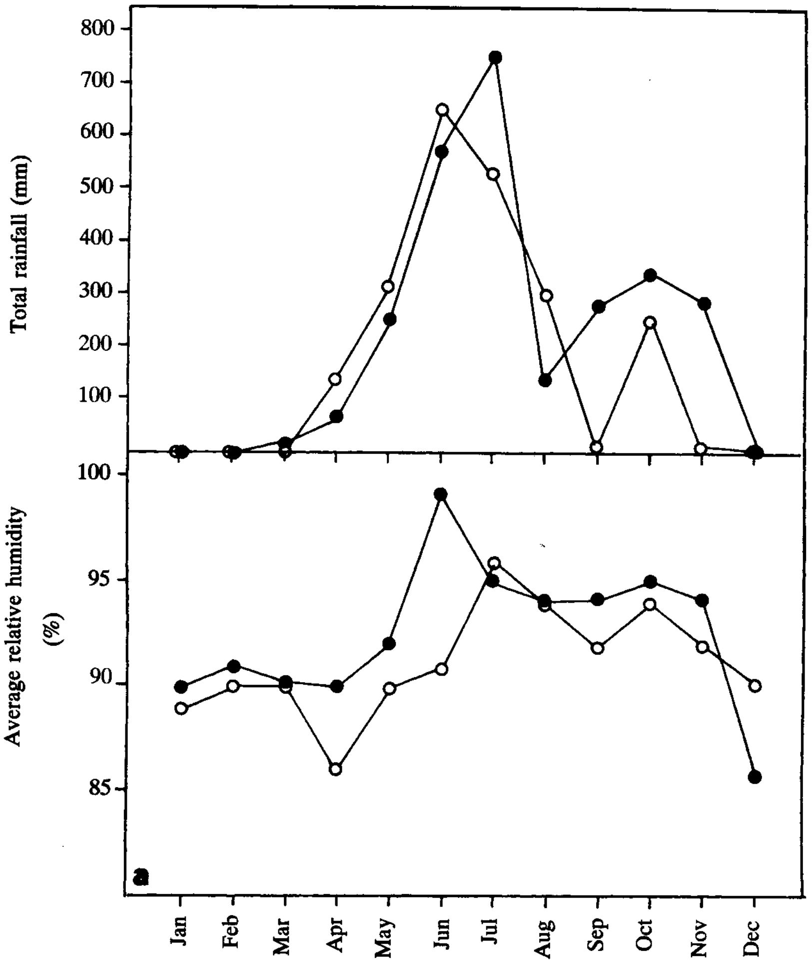


Fig. 1. Monthly weather data. ○———○, Expt 1; ●———●, Expt 2.

depending on the vigour of the monsoons. During monsoon periods the r.h. was higher, and sunshine hours fewer, almost corresponding to the rainfall pattern. The daily mean max. temperature was higher (above 32°C) from January to April/May, in summer. The min. temperature, usually lower in winter (December-January/February), was lower during Expt 2 than Expt 1.

Fungal isolations

Fungi known to cause LRD of coconut as well as certain other endophytic fungi were recorded from newly emerging diseased spindles (see Table 1). In Expt 1, the overall incidence of these fungi ranged from 27.8% of leaf pieces from spindles (July) to 80.2% (January). In Expt 2, the recovery from advanced lesions (36.7–87.3% per monthly sample) was relatively greater than from early lesions (17.3–58.0%). However, the mean incidence of recovery for both experiments was similar (46–47%).

In the following results for the occurrence of individual species on leaf pieces from spindles, the two species of *Fusarium* have been combined. Isolations of *Curvularia* sp.,

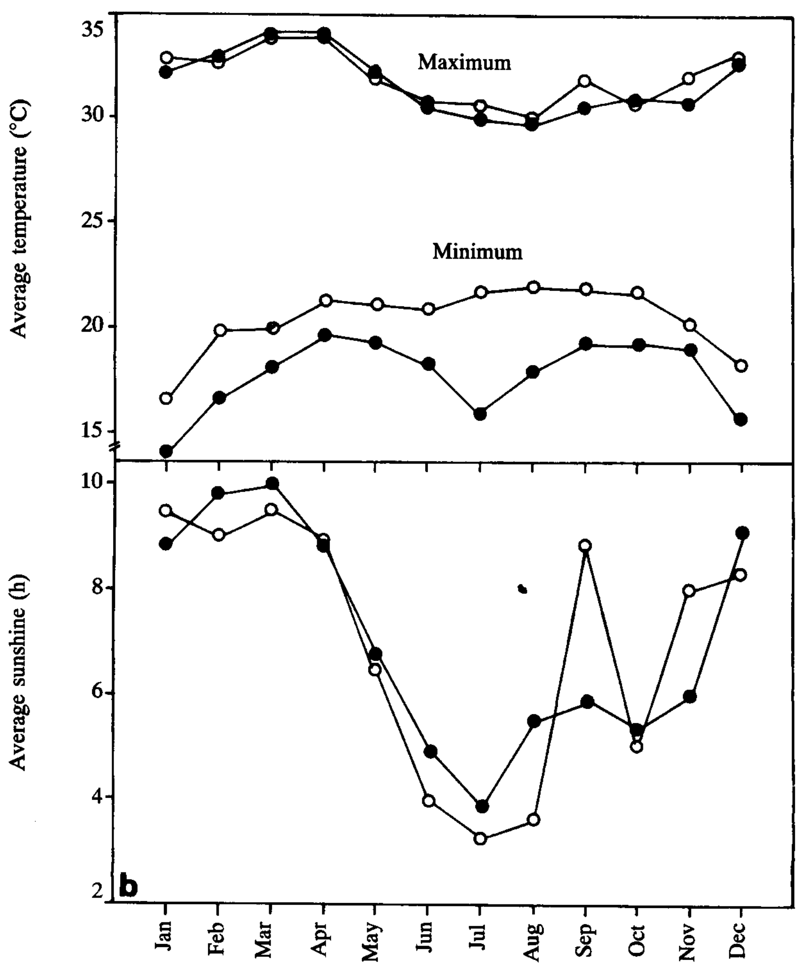


Fig. 1 continued

Acremonium sp., *Thielavia* spp. and *C. brasiliense* were infrequent and inconsistent and they are also grouped as one category.

Expt 1

C. gloeosporioides, E. rostratum, G. vermoeseni and *Fusarium* spp. were isolated at different frequencies from leaf pieces in different months (Fig. 2). *C. gloeosporioides* was most frequent during June-November with highest incidence in June. Isolations of *E. rostratum* were fewer, increasing gradually but erratically between March and November. *G. vermoeseni* was recorded in all months except November, with no obvious monthly pattern of incidence. *Fusarium* spp. were most frequent from January to May. *T. paradoxa* was recorded in most months, with a peak in January. *C. scoparium, R. solani, M. elongata* and other fungi were infrequent, with little variation among months, except that peaks for *M. elongata* occurred in March and May.

Expt 2

Occurrence of *C. gloeosporioides* was low from January-April, increasing to more than 50% between May and July and thereafter remaining the most commonly detected species

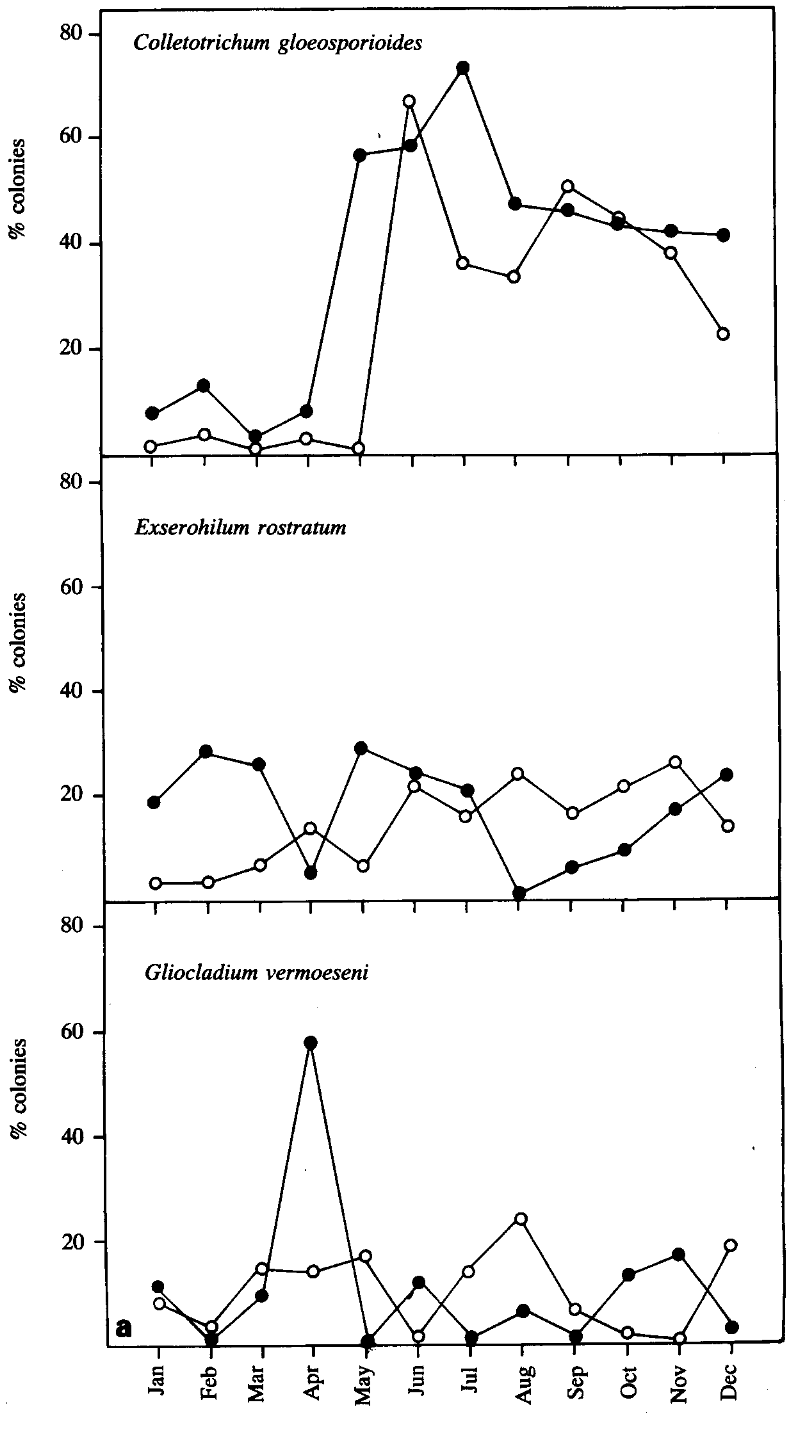
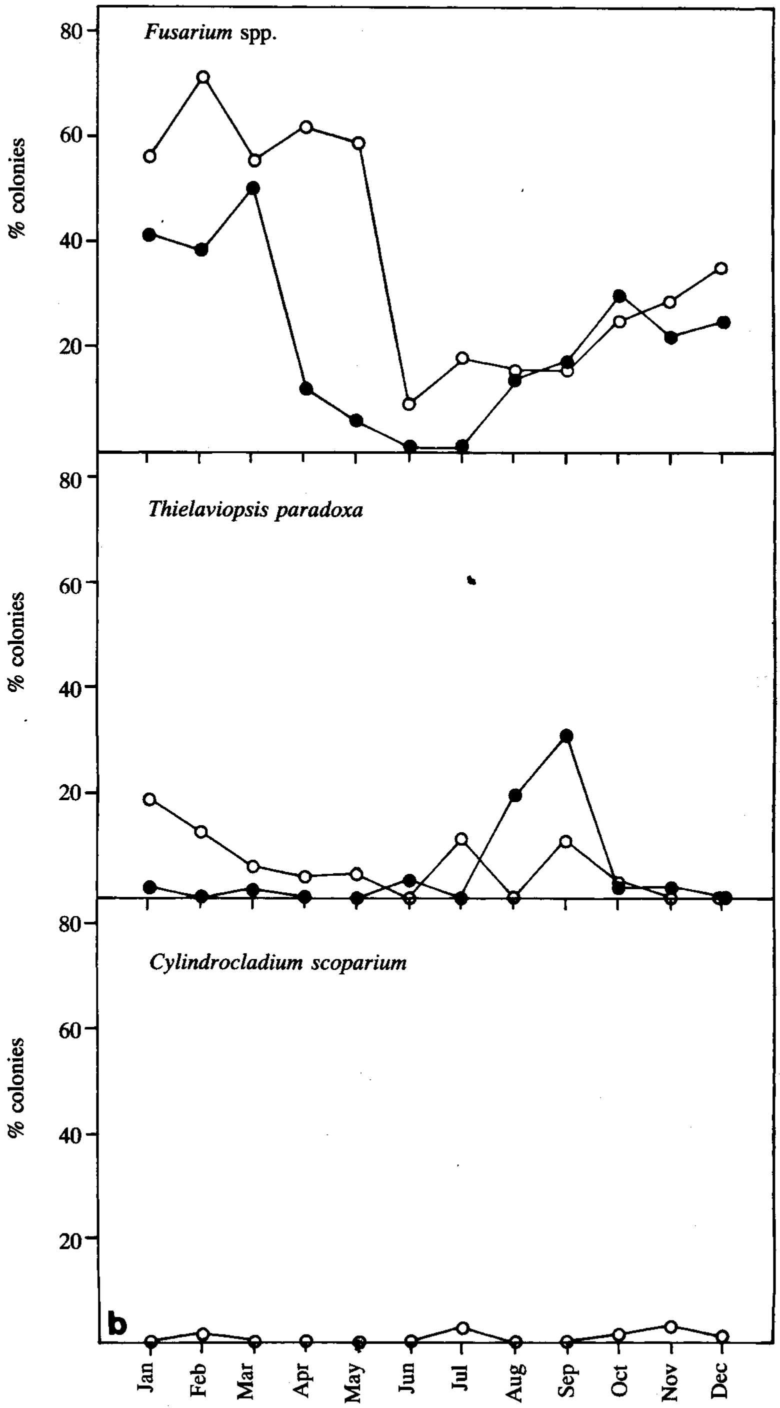


Fig. 2. Frequency of isolation of fungi in different months. ○———○, Expt 1; ●———●, Expt 2. The computation is based on total colonies enumerated from leaf pieces in each month. In Expt 2, the mean of early and advanced lesions was used for the computation. *Cylindrocladium scoparium* was not detected during Expt 2. In Expt 1, SED (88 df) = 4.9; in Expt 2, SED (77 df) = 2.4.



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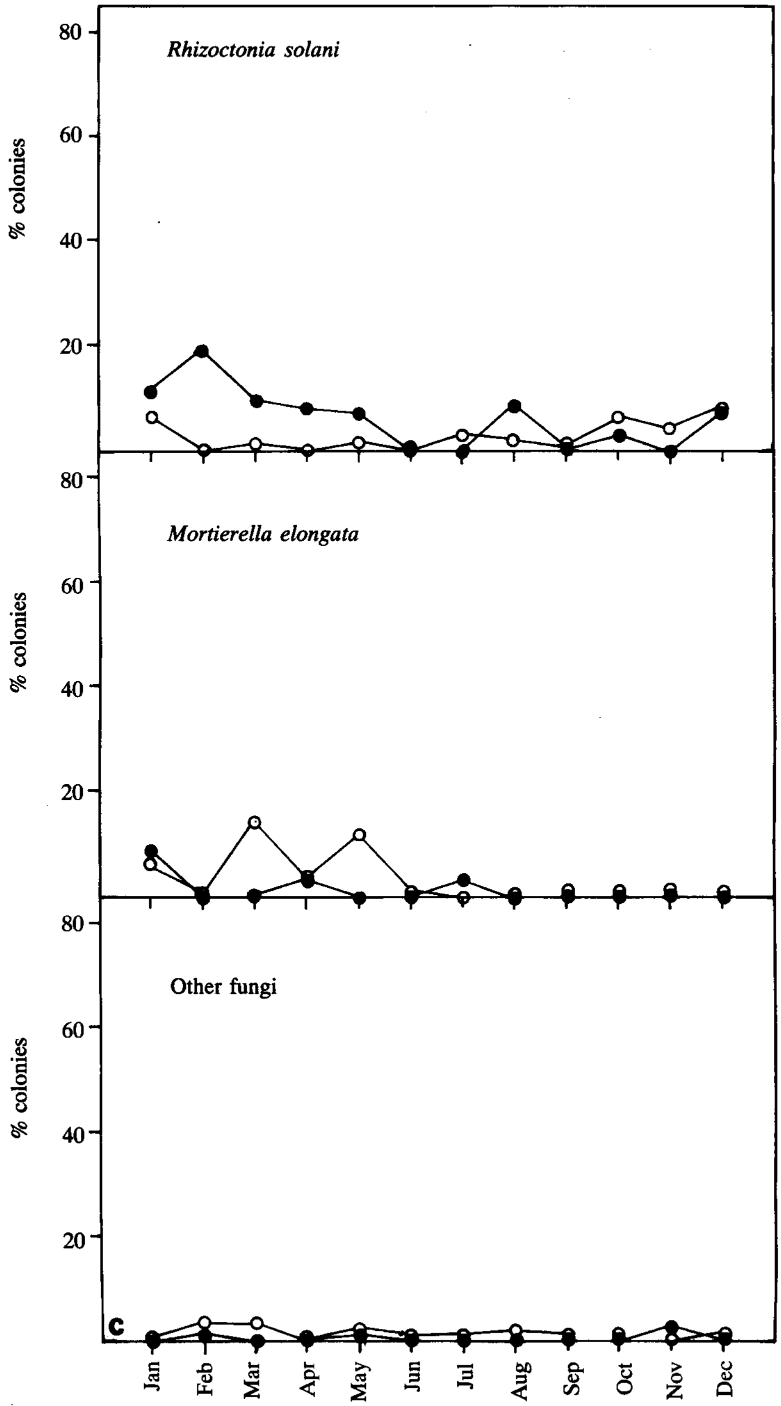

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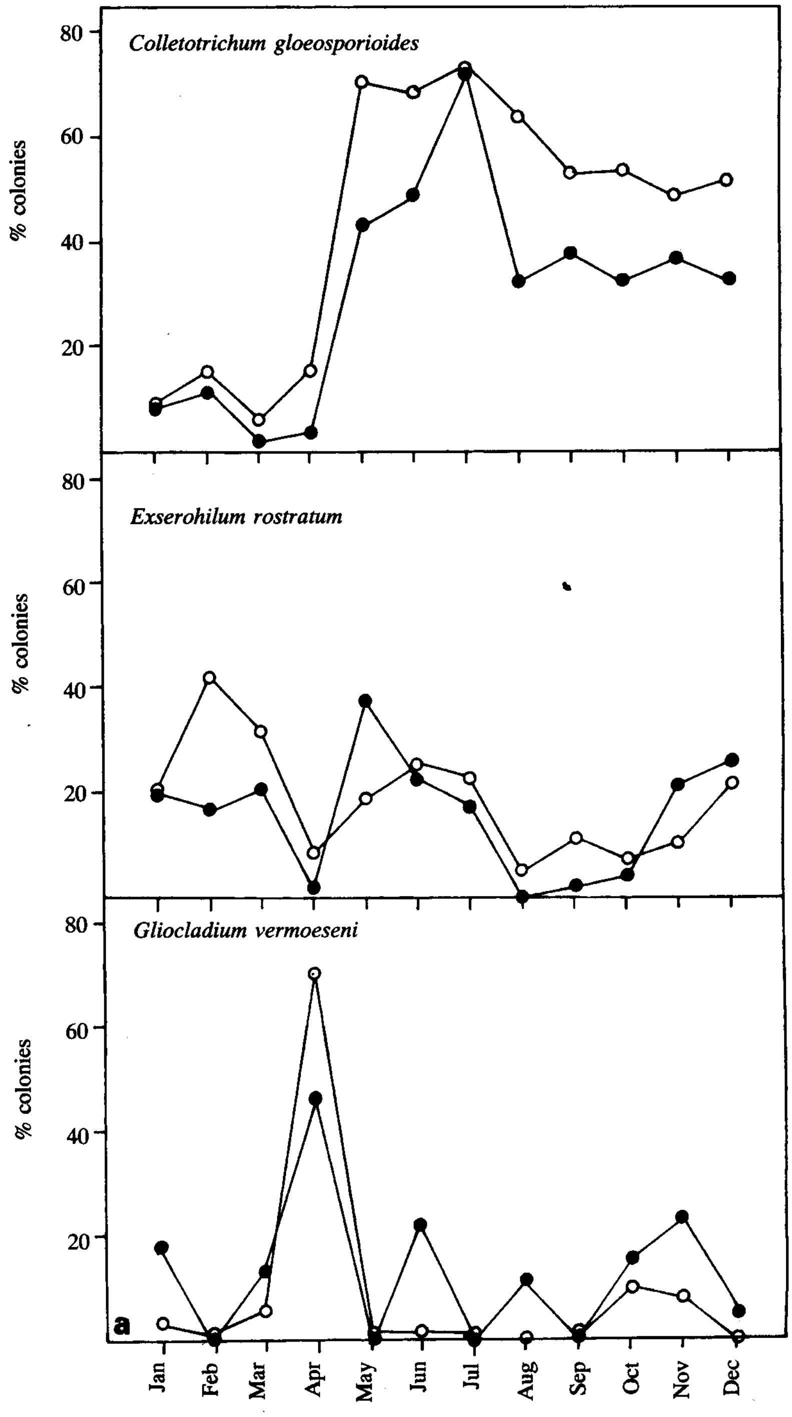


Fig. 3. Frequency of isolation of fungi from early (○———○) and advanced (●———●) lesions in Expt 2. *Cylindrocladium scoparium* was not detected. For fungus × lesion type interaction, SED (77 df) = 3.3; for fungus × month interaction, SED (77 df) = 8.1.

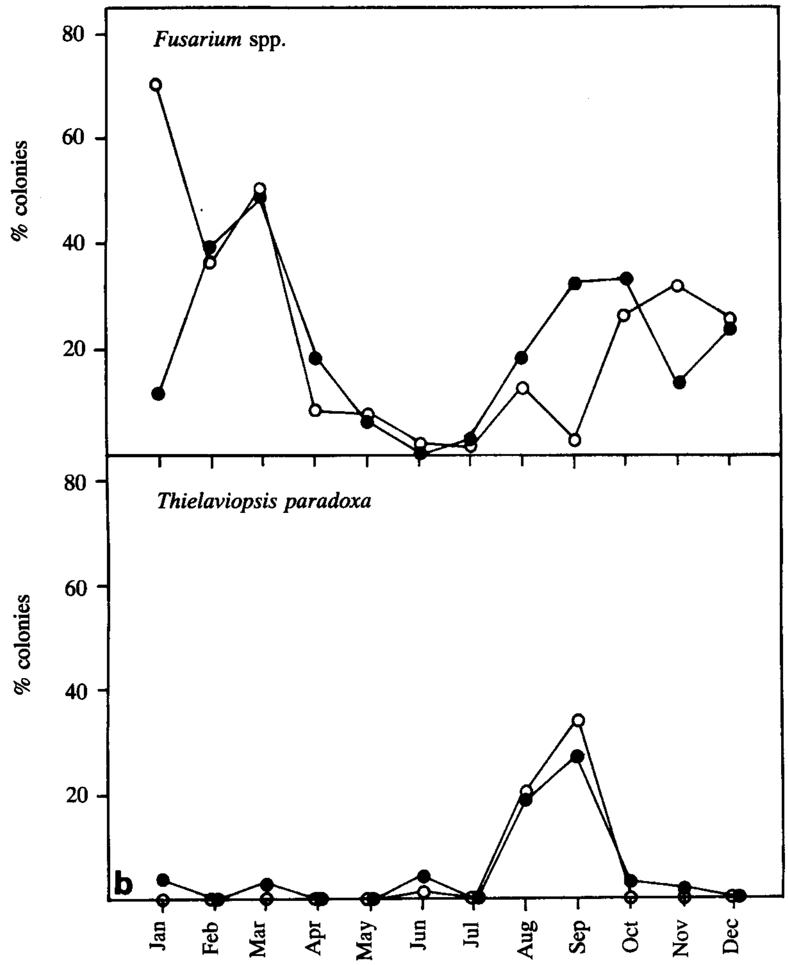
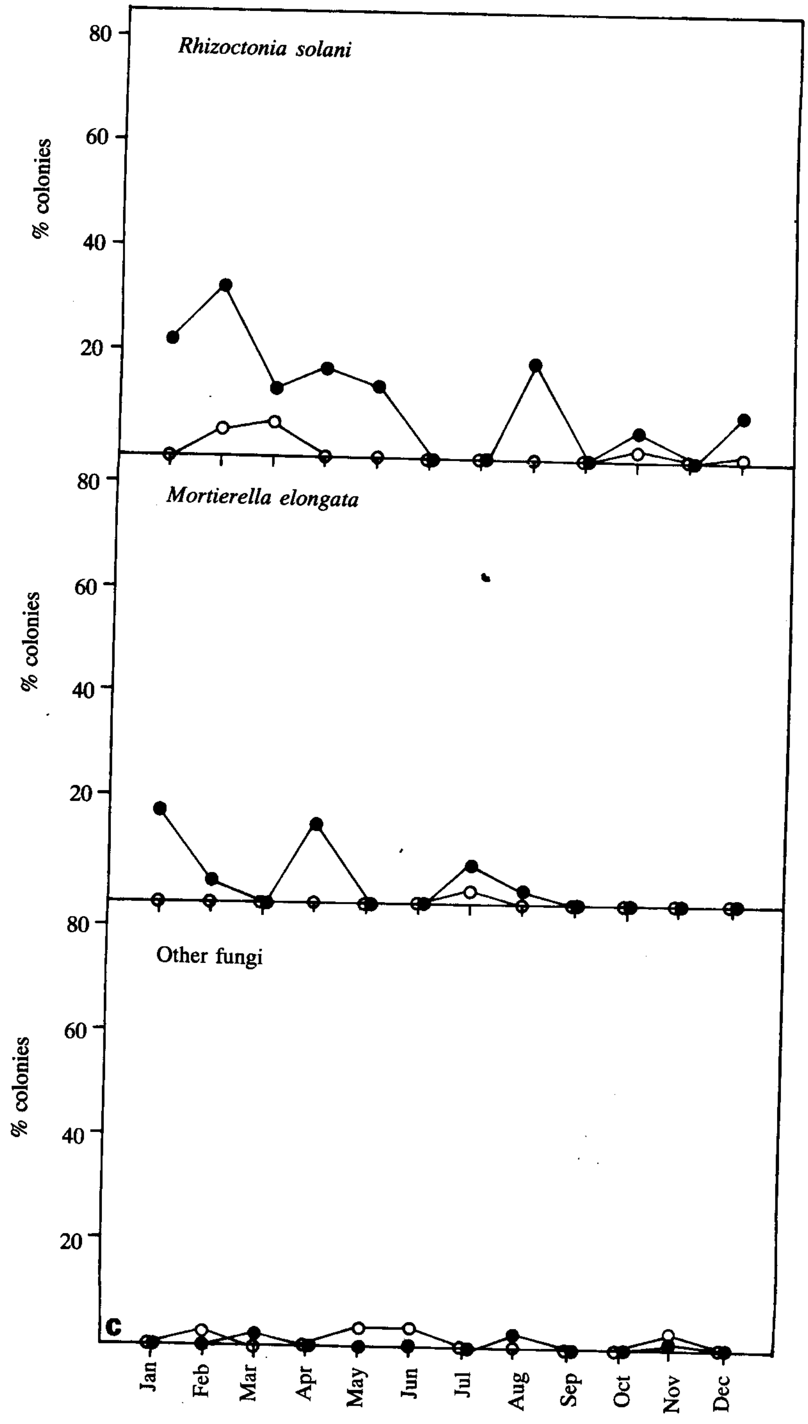


Fig. 3 continued

until December (Fig. 2). *E. rostratum* was less frequent than *C. gloeosporioides* from April–December, with periodic fluctuations through the year. *G. vermoeseni* was isolated in all months expect February and May, with a peak in April. *Fusarium* spp. were most frequent from January–March, similar to the situation in Expt 1. *T. paradoxa* was recorded at low levels in all months except August and September, and *C. scoparium* was not detected. *R. solani* was observed from January–May and at lower frequencies in other months. *M. elongata* and other fungi were also less frequently associated with the disease.

Early and advanced lesions

C. gloeosporioides was recorded from more early than advanced lesions throughout the year, except January and July (Fig. 3). Monthly fluctuations in incidence were similar for the two types of lesions. *E. rostratum* was more frequent on early lesions in February and March but differences were small in other months. *G. vermoeseni* was generally isolated more often from advanced lesions except in April. *Fusarium* spp. were more frequent on early lesions in



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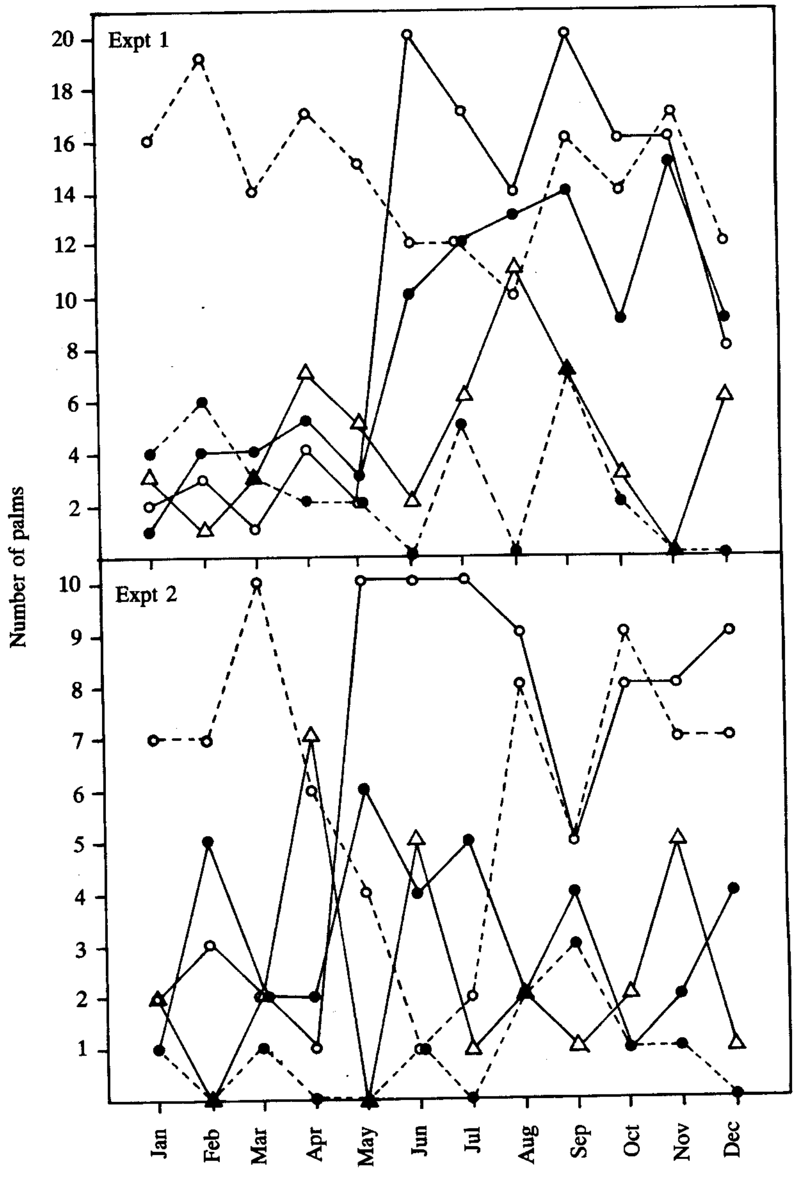

Fig. 4. Frequency of fungi detected in different months in Expt 1 (20 palms per month) and Expt 2 (10 palms per month; includes early and advanced lesions). ○———○, *Colletotrichum gloeosporioides*; ●———●, *Exserohilum rostratum*; △———△, *Gliocladium vermoeseni*; ○- - - - -○, *Fusarium* spp.; ●- - - - -● *Thielaviopsis paradoxa*. In Expt 1, SED (55 df) = 1.9; in Expt 2, SED (55 df) = 1.0.

January, but were similarly frequent on each type of lesion from February–July and thereafter generally more frequent on advanced lesions. *T. paradoxa* was similarly frequent on early and advanced lesions whilst *R. solani* and *M. elongata* were isolated more from advanced lesions.

Frequency of fungi on palms

The frequencies of some of the most common fungi were compared on palms, irrespective of the percentages of leaf pieces producing colonies (Fig. 4). In Expt 1, *C. gloeosporioides* and *E. rostratum* were recorded less frequently from January–May whereas *Fusarium* spp. were recorded more frequently in that period. In Expt 2, the greater frequency of *C. gloeosporioides* during May-December and of *Fusarium* spp. in part of the dry season (January–March) was consistent with Expt 1.

Co-occurrence of fungi

Fungi co-occurred on 70% (of 240) palms in Expt 1 and 76% (of 120) palms in Expt 2. The most frequent co-occurrences were *C. gloeosporioides* with *E. rostratum* (5% in Expt 1, 8% in Expt 2), with *Fusarium* spp. (6% in Expt 1, 10% in Expt 2) or both (15% in Expt 1, 10% in Expt 2), and *G. vermoeseni* with *Fusarium* spp. (4% in Expt 1, 6% in Expt 2). Most leaf pieces (approximately 95%) yielded only one fungus, irrespective of whether the lesion was early or advanced (data not shown).

Table 2. *Correlation coefficients for incidence of fungi (% leaf pieces of spindles from which isolated) with weather factors*

		Weather factors				
				Temperature (°C)		
Fungus	Expt	Rainfall (mm)	Relative humidity	Max.	Min.	Sunshine (h)
Colletotrichum gloeosporioides	1	0.52	0.60*	−0.71**	0.45	−0.61*
	2 E	0.74**	0.57*	−0.42	0.26	−0.85**
	2 A	0.89**	0.59*	−0.42	0.05	−0.86**
Exserohilum rostratum	1	0.38	0.53*	−0.61*	0.51	−0.59*
	2 E	−0.12	−0.21	0.10	−0.43	0.46
	2 A	0.08	−0.14	−0.14	−0.20	0.12
Gliocladium vermoeseni	1	0.00	−0.06	0.04	0.07	−0.09
	2 E	−0.21	−0.21	0.19	0.36	0.26
	2 A	−0.12	0.03	−0.14	0.29	0.13
Fusarium spp.	1	−0.54*	−0.73**	0.78**	−0.46	0.70**
	2 E	−0.62*	−0.49	0.38	−0.56*	0.65*
	2 A	−0.58*	−0.41	0.48	0.12	0.57*
Thielaviopsis paradoxa	1	−0.26	−0.08	0.24	−0.39	0.35
	2 E	0.02	0.23	0.00	0.29	−0.30
	2 A	0.02	0.29	−0.06	0.26	−0.33
Cylindrocladium scoparium	1	0.05	0.45	0.20	0.06	−0.17
	2 E	—	—	—	—	—
	2 A	—	—	—	—	—
Rhizoctonia solani	1	−0.33	0.14	0.04	−0.57*	0.11
	2 E	−0.41	−0.30	0.26	−0.05	0.58*
	2 A	−0.77**	−0.62*	0.43	−0.43	0.76**
Mortierella elongata	1	−0.14	−0.42	0.48	−0.21	0.36
	2 E	0.68**	0.23	0.00	−0.31	−0.48
	2 A	−0.26	−0.30	0.24	−0.44	0.33
Other fungi	1	−0.16	−0.05	0.16	−0.03	0.14
	2 E	0.18	0.30	−0.33	0.25	−0.13
	2 A	0.02	0.32	−0.29	0.23	−0.16

*Significant at $P \leqslant 0.05$
**Significant at $P \leqslant 0.01$
Cylindrocladium scoparium was not detected during Expt 2.
E Isolations from early lesions.
A Isolations from advanced lesions.

Correlations between weather and fungal isolations

Incidence of *C. gloeosporioides* was positively correlated with amount of rainfall (Expt 2 only, early or advanced lesions) and r.h., and negatively correlated with maximum temperature (Expt 1 only) and hours of sunshine (Table 2). Incidence of *E. rostratum* (Expt 1) had a weak positive correlation with r.h. and negative correlations with maximum temperature and hours of sunshine. Incidence of *Fusarium* spp. was negatively correlated with amount of rainfall and r.h. (Expt 1) and positively correlated with maximum temperature (Expt 1) and hours of sunshine. Incidence of *R. solani* (Expt 2) was negatively correlated on advanced lesions with amount of rainfall and r.h., and positively correlated with hours of sunshine.

Discussion

A complex of fungal pathogens was isolated from leaf spindles of coconut palms affected by LRD. *C. gloeosporioides, E. rostratum, G. vermoeseni* and *Fusarium* spp. were already particularly associated with disease as indicated by their frequencies of isolation (Srinivasan & Gunasekaran, 1996*b*). Fungi co-occurred on almost 75% of palms, implying that the fungal complex is the cause of disease. Single fungi were usually isolated from early lesions. Each of those fungi occurring first, and isolated singly, may have a significant, independent role in the disease. Their relative frequencies on early and advanced lesions provide evidence of which fungi are primary infection agents and which are secondary colonisers.

The occurrence of *C. gloeosporioides* during the monsoon and its correlation with weather conditions prevailing at that time (*cf.* Sutton, Gillespie & Hildebrand, 1984) suggest that it is most important in LRD. Its frequency reached a peak in June/July and it remained common during the monsoon, during which it could continue to cause damage. The south-west monsoon (May/June–September), with well distributed rainfall in normal years, provides conditions suitable for disease development from abundant inoculum production. This finding is similar to those for *Colletotrichum* spp. affecting a number of crop plants, usually involving infection of young tissues contributing to die-back or anthracnose symptoms, in which leaf wetness and r.h. were shown to be the main weather variables influencing infection. An example is infection of *Stylosanthes scabra* by *C. gloeosporioides* (Chakraborty & Billard, 1995). High r.h. can also compensate for lack of leaf-surface wetness (Chakraborty, Ratcliff & McKay, 1990); high r.h. can occur in the monsoon period even when rainfall is interrupted, providing leaf wetness for the minimum duration required for sporulation on anthracnose lesions on *S. scabra* (Lamsupastt, Chakraborty, Cameron & Adkins, 1993).

Incidence of *E. rostratum* was less closely correlated with weather conditions. In Expt 2, moderate incidence of *E. rostratum* occurred in the winter (dry) months in spite of little or no rainfall, low r.h. and low minimum temperature (December–February/March). Its presence at this time may depend on suitable microclimatic conditions after the cessation of the monsoon. An example of this is infection of rice by *Helminthosporium oryzae* (*Cochliobolus miyabeanus*); this requires high r.h. and free moisture, and successful inoculations can be achieved in the evenings, when such conditions occur (Ou, 1972). These conditions occurring in the winter may allow infection and sporulation of *E. rostratum* and other fungi.

It is possible that *C. gloeosporioides* (and perhaps also *E. rostratum*) may exist in a quiescent state during the dry period (*cf.* Jeffries, Dodd, Jeger & Plumbley, 1990); the germination of conidia of *Colletotrichum* spp. has been shown to be negligible in the absence of free surface water (Nutman & Roberts, 1960; Wastie, 1972). Dodd *et al.* (1991) observed that high r.h. (95% or more) allows conidial germination, appressorium formation and disease development in mango by *C. gloeosporioides*. Inoculum availability was, indeed, an important determinant of the number of infections in the dry season on *S. scabra* (Chakraborty & Billard, 1995). The pathogens may be vulnerable to severe desiccation leading to a rapid decline in population sizes in dry seasons and hence a limited incidence of LRD. Exposure of leaves to sunlight in such periods may also result in elevated temperatures which affect survival (Ou, 1972) or subsequent dormancy. *C. gloeosporioides* is suspected to persist in a quiescent state as subcuticular hyphae in avocado (Jeffries *et al.*, 1990). Further work is needed to determine the significance and location of quiescent infections of LRD.

Fusarium spp. occurred mainly in the dryer, hotter periods (January–May) and their incidence was negatively correlated with rainfall but positively correlated with hours of sunshine. Parallel observations have been made in very different temperature conditions. Parry (1990) observed an upsurge of pathogenic *F. culmorum* and *F. graminearum* in a dry summer in stem bases of wheat and found high temperatures favourable for both species. Bateman (1993) related the severe foot rot in a dry summer, with plants under moisture stress, to an increased incidence of *F. culmorum* and considered that plants weakened or altered by a primary coloniser (*Pseudocercosporella herpotrichoides*) were often a suitable substrate for a secondary coloniser (*Fusarium* sp.) which might infect at a distance from the original lesion and often not cause distinct symptoms itself.

The *Fusarium* spp. have been shown to be pathogenic and capable of causing extensive rotting of spindle tissues (Srinivasan & Gunasekaran, 1996*a*) even though foliar diseases caused by such fungi were scarce. Their presence throughout the year and their predominance in the dry period suggest they are potential pathogens of leaf rot, co-occurring with other fungi and perpetuating the disease in the dry period (Srinivasan & Gunasekaran, 1992). They may possibly also predispose the palms to subsequent infection by other pathogens when conditions become favourable.

Dry weather conditions favoured *R. solani*, which occurred especially in advanced lesions. This adds to the concern about the persistence of LRD in such periods. There is other evidence for *Rhizoctonia* spp., typically soil-borne pathogens, causing foliar diseases (Frisina & Benson, 1987; Litterick, McQuilken & Holmes, 1995).

G. vermoeseni, T. paradoxa and other fungi were not influenced by specific weather conditions and may have less significance in LRD, but may play some role in disease expression in certain circumstances.

The results indicate that weather, especially rainfall and r.h., influence the predominant pathogens, other than *G. vermoeseni*, associated with LRD. These fungi were found to be more associative than antagonistic to each other (Srinivasan & Gunasekaran, 1995*c*) and fluctuations in their frequency are more likely to result from weather than from antagonism (*cf.* Johnson, Mead, Cooke & Dean, 1992). Comparison of pathogenicity of leaf rot fungi revealed that *C. gloeosporioides* and *E. rostratum* may be the main pathogens of the disease (Srinivasan & Gunasekaran, 1996*c*). Their quiescence in the dry season may break with the onset of the south-west monsoon in May or June when they become aggressive in favourable weather conditions.

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