EARLY DIAGNOSTIC TECHNIQUES ON THE ROOT (WILT) DISEASE OF COCONUT IN INDIA

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Root (wilt) disease of coconut palms (*Cocos nucifera* L.) is characterized by serologically positive reaction and relatively low stomatal resistance. As a means to detect the incipient infection of coconut palms by the root (wilt) pathogen, a comparative study was made between the serological test using the cross absorption technique and the physiological test based on the determination of stomatal diffusive resistance. These tests could detect affected palms 6-20 months earlier than the actual manifestation of the visual symptom, namely, flaccidity of leaves.

INTRODUCTION

The coconut palms in Kerala, India are affected by a serious malady characterized by predominant wilting of the crown and described as root (wilt) disease, also associated as root decay (Anonymous 1981). The disease is spread over 410,000 ha in eight districts of Central and Southern Kerala with yield loss of 900 million nuts. Recent studies on the etiology of the disease have implicated mycoplasma-like organisms (MLO) (Solomon et al. 1983b). Another widely known disease caused by MLO is the lethal yellowing disease of coconuts in Jamaica (Basham and Eskafi 1980).

The initial diagnostic symptom of the root (wilt) disease of coconut is the flaccidity or ribbing of leaflets (Radha and Lal 1972). Yellowing and necrosis are other features commonly associated with the disease. These symptoms have been reported even in young coconuts (Anonymous 1981). The time lag between the infection and manifestation of symptoms varies considerably (Nagaraj and Menon 1956, Shantha et al. 1964, and Mathen et al. 1976). Hence, some of the healthy-looking, designated as "apparently healthy," palms in the disease-affected areas are likely to harbor latent infection.

Diagnostic tests have been developed for the early detection of the disease based on either differential enzyme activity (Joseph and Shantha 1963), or extraction of biologically active constituents in EDTA (Dwivedi et al. 1977). However, these methods have had little success. Recently, a sero-diagnostic test developed by Solomon et al. (1983a) has been proven to be more sensitive in detecting the disease fairly ahead of the manifestation of its visual symptoms. Reports of Matteoni and Sinclair (1981 and 1983) clearly indicate that the changes in stomatal resistance can be effectively used to detect diseases caused by MLO. The association of MLO with the root (wilt) disease has been reported (Solomon et al. 1983b).

This paper reports the attempts made to develop a rapid physiological test as an additional tool to detect the latent infection in diseased palms (Rajagopal et al. 1986), and compare the same with the serological test.

MATERIALS AND METHODS

Coconut palms (*Cocos nucifera* L. var. West Coast Tall) planted in 1976 in the CPCRI farm formed the experimental material. They were raised under a square 7.5-m spacing in randomized block design. The usual agronomic and cultural practices were followed using 500 g urea, 320 g P_2O_5, and 1200 g K_2O per tree per year in split doses. There were four plots for healthy palms, while diseased palms were selected from five plots. The number of palms taken per plot varied (Tables 1 to 3).

Serological test:

Agar double diffusion test was used as described by Solomon et al. (1983a). Antigen was prepared by homogenizing tissues from the spindle leaf of root (wilt) diseased palms. Antiserum was made by immunizing female albino rabbits intramuscularly with purified preparation emulsified with Freund's incomplete adjuvant. The test was performed on mi-

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Plot No.	No. of Palms Observed	Serological Reaction	Stomatal Resistance sec.cm^{-1}
A. Healthy palms*			
1	13	Negative	11.91 (± 0.98)**
2	8	-do-	10.56 (± 1.13)
3	10	-do-	15.30 (± 1.44)
4	7	-do-	13.00 (± 1.06)
B. Diseased palms			
1	15	Positive	1.78 (± 0.14)
2	34	-do-	3.30 (± 0.22)
3	12	-do-	3.92 (± 0.63)
4	9	-do-	4.06 (± 0.59)
5	5	-do-	2.09 (± 0.19)

*Healthy samples were examined from disease-free area as described by Rajagopal et al. (1986).
**Mean standard error (S.E.).

TABLE 1. Comparison between serological reaction and stomatal resistance in the healthy and diseased coconut palms

Group No.*	Number of Palms Observed	Serological Reaction	Stomatal Resistance sec.cm^{-1}	Remarks
1	35	Negative	12.90 ± 0.86	Healthy
2	28	Positive	4.15 ± 0.72	Suspects
3	10	Positive	11.75 ± 1.06	Suspects
4	13	Negative	4.89 ± 0.33	Suspects

*Based on response of coconut palms to diagnostic tests.

TABLE 2. Variations in serological reaction and stomatal resistance in coconut palms devoid of foliar symptoms, (i.e. "apparently healthy") of the root (wilt) disease

croslides layered with 1% melted oxide agar in 0.005M phosphate buffered saline with pH of 7.4. The precipitin line formed in between the antigen and antiserum well is considered as a positive reaction, while its absence indicates a negative reaction.

Physiological test:

The stomatal resistance of first fully open leaf was determined with Li-Cor 1600 Steady State Porometer (Lamda Instruments, Nebraska, U.S.A.) as described by Rajagopal et al. 1986). Low diffusive resistance (abnormal opening of stomata with excessive transpiration rate), which is the characteristic feature of the disease, is taken as a useful indicator to identify the nature of palms. Determination of stomatal resistance between 10.00h and 11.30h during the dry season was found to be the most appropriate time to distinguish the healthy and the diseased palms.

Experiments were carried out between 1983 and 1986. Diagnostic tests were performed at frequent intervals (2-4 months), and repetition of tests was undertaken whenever confirmation was needed. Quarterly observations on the disease index based on the flaccidity, yellowing, and necrosis were recorded following the disease scoring method of George and Radha (1973).

RESULTS

Coconut palms from a healthy area showed serologically negative reaction (Fig. 1) and high stomatal resistance, ranging from 10.5 to 15.3 sec.cm^{-1}, whereas the root (wilt) diseased palms had positive reaction to the serological test and low diffusive resistance, ranging from 1.78 to 4.06 sec.cm^{-1} (Table 1).

No. of Palms	Palm Condition during Diagnostic Tests				Visual Symptoms until 1986
	Visual Symptoms	Serological Reaction	Stomatal Resistance sec.cm^{-1}	Remarks	
12	Present	Positive	2.87 ± 0.32	Diseased	
5	Absent	Positive	10.69 ± 0.98	Suspects*	29 (with symptoms)
3	Absent	Negative	1.38 ± 0.09	Suspects*	3 (dead)
26	Absent	Positive	1.99 ± 0.18	Suspects*	2 (no symptoms)
9	Absent	Negative	9.76 ± 1.06	Healthy*	3 (with symptoms)**
					6 (no symptoms)

*Palms monitored for expression of foliar symptoms.
**Symptom expression preceded by response to the tests.

TABLE 3. Comparison between the diagnostic tests performed in 1983 and the development of foliar symptoms of the root (wilt) disease until 1986 in 55 coconut palms

When the two tests were carried out on palms devoid of foliar symptoms, that is, apparently healthy palms occurring in the disease tracts, an interesting trend was noticed. The responses to the two tests could be arranged into four groups irrespective of the plots. From Table 2, only 35 out of 86 palms were truly healthy, as characterized by negative serological reaction and high stomatal resistance, while the rest of the palms could be considered as disease "suspects" with typical responses to the two tests, either singly or in combination. These palms were kept for monitoring the foliar symptoms.

In a single plot in July 1983, there were 12 palms with clear disease symptoms, as supported also by the two tests, while 43 palms were considered as "apparently healthy" by virtue of the absence of foliar symptoms (Table 3). However, only nine out of 43 palms turned out to be really healthy, with the remaining 34 palms showing response to one or both tests. Thus, these palms were designated as disease "suspects." Frequent observations of these palms revealed that by December 1986, 29 palms exhibited the foliar symptoms, while three palms died due to attack by red palm weevil or bud rot. Only two palms failed to show the symptoms. Surprisingly, out of the nine healthy palms, three showed reactions similar to disease "suspects" by May 1984, and the foliar symptoms appeared by January 1986. Likewise, out of seven "suspected" palms in May 1984 in an adjoining plot, three expressed the symptoms in November 1984 and two each in March 1985 and December 1986.

DISCUSSION

The positive reaction to the serological test by the typical diseased palms or "suspects" conforms with earlier findings (Solomon et al. 1983a). The same palms had low leaf diffusive resistance, a significant feature of the root (wilt) disease (Rajagopal et al. 1986). It is clear that the palms show differential responses to the two tests in time and space, but repeated determinations help in establishing the nature of palms. It is apparent from Table 3 that in less than three years, the number of diseased palms more than doubled in the plot. Thus, the detection tests which could predict the disease much earlier are of practical significance.

From the data, the time lag between the performance of diagnostic tests and manifestation of visual symptoms ranges from 6 to 20 months. This wide gap may be due to palm-to-palm variation not only in its response to the tests but also in the development of disease symptoms. The long duration observed in the slow development of symptoms may not be unexpected because of the perennial nature of the crop. The quick pre-visual test performed on MLO-infected white ash (*Fraxinus americana*) revealed that the stomatal resistance increased 4-6 weeks before the onset of visual symptoms (Matteoni and Sinclair 1981). Korner (1981) demonstrated that the wilt disease in apricot, a fungal disease, could be detected three months earlier using the symptomatic changes in stomatal resistance.

It may be noted that the two tests are independent,

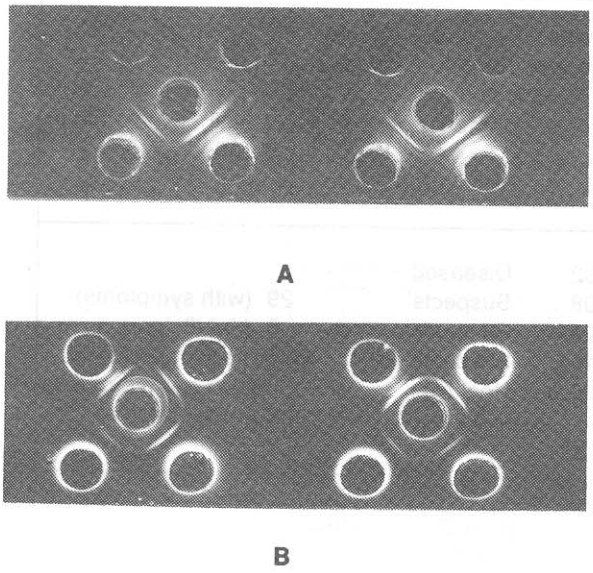

Figure 1. Photographs showing the agar double diffusion test. (a) Leaf extracts from four healthy (upper wells) and four root (wilt) diseased palms (lower wells) showing the reaction to the antiserum (centre wells). Precipitin line is absent in the upper wells. (b) Leaf extracts from four diseased palms (upper wells) and four disease "suspects" (lower wells) showing the reaction to the antiserum (centre wells). In the lower wells, the precipitin lines are of low intensity compared to upper wells. This reaction is indicative of latent infection in the palms, that is, disease suspects.

as one does not influence the other. The serological test, which is qualitative, is based on changes in nucleoproteins and is performed on the spindle leaf. The physiological test, based on stomatal regulation, is carried out on the first and middle whorl leaves. It is quantitative in nature, as exemplified by marked differences between the healthy and the diseased or suspected palms in their stomatal resistance values (Tables 1 to 3). The latter test is faster, as the trend will be known while recording the values; the serological test is time consuming. Nevertheless, the two tests can compliment each other in identifying the palms prior to symptom expression. It is rare that a comparison between unrelated tests for a complex disease in a perennial crop is reported.

The use of stomatal closure as a pre-visual detection method for some MLO diseases has been demonstrated (Matteoni and Sinclair 1983). Present data lend support to the view that stomatal resistance measurements can be effectively employed to detect the root (wilt) disease of coconut with which MLO are associated (Solomon et al. 1983b). A similar approach has been made in the other MLO-caused disease of coconut, namely, lethal yellowing in Jamaica (Bashmam and Eskafi 1980).

Solomon et al. (unpublished) reported that, as confirmed in the two tests, MLO were detected in the young leaf and root tissues of eight early diseased palms. Thus, the validity of the diagnostic tests was strengthened.

Subsequently, samples from diseased "suspects" have also been examined under the electron microscope for the MLO. As the visual symptoms appear long after infection and the detection of MLO through electron microscope is a lengthy and complicated process, the emphasis therefore is on the early diagnostic methods for routine field surveys. These methods are widely used in the hot spot survey of the disease in farmers' fields. Recent findings further revealed that measurement of leaf water potential is a useful adjunct to early diagnosis of the disease (Rajagopal and Sumathykuttyamma, unpublished). Since the flaccidity symptom is caused by changes in leaf turgor potential (Rajagopal et al. 1987), it can be concluded that the nature of "apparently healthy" palms can be ascertained through the pre-visual tests perfected in the field.

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