VEGETATIVE, REPRODUCTIVE AND PHYSIOLOGICAL CHANGES IN COCONUT PALMS AFFECTED BY COCONUT RAPID DECLINE

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ABSTRACT

Coconut Rapid Decline (CRD) is a disorder of unknown etiology affecting coconut palms (*Cocos nucifera* L.) in Sri Lanka. The objective of this study was to quantify vegetative, reproductive and physiological characters, and to determine the pattern of water transport in CRD-affected palms and compare with those of apparently healthy palms.

The study was conducted on fifteen-year-old apparently healthy (S0) and CRD-affected coconut palms (var. Typica), in the incipient (S1), moderate (S2) and severe (S3)) stages of decline, in Makandura Seed Garden (MSG) of the Coconut Research Institute, Sri Lanka. The leaf canopy, trunk, unopened spadix and nut characters, total chlorophyll content, and the rate of transpiration, stomatal diffusive resistance, and xylem transport pattern were the parameters determined and compared.

CRD reduced the number of fronds in the canopy by 32%, trunk girth by 24%, and leaf chlorophyll content by 12%, and increased stomatal diffusive resistance three-fold. There was a concomitant reduction in the size of the inflorescence (by 35%), number of female flowers produced (by 71%), nut yield (by 54%) and copra weight of nuts (by 44%) indicating the destructive nature of the disorder on the productivity of affected palms. In the CRD-affected palms there was an obstruction to the transport of water from roots to the canopy, and water was retained in the roots.

Key words: *Cocos nucifera,* Coconut Rapid Decline, diseases of unknown etiology, frond drooping, trunk tapering

INTRODUCTION

Coconut Rapid Decline (CRD) is a disorder of unknown etiology recently recognized in Sri Lanka. The disorder generally affects coconut palms between 15 and 35 years of age. Generally, a reduction in yield is evident 6-8 months after the appearance of leaf symptoms and the palm dies within 2-3 years (Ranasinghe *et al.*, 2002).

Drooping of the middle and lower whorl of green fronds is the most characteristic visual symptom in the early stages of the disorder. These drooping fronds dry up rapidly leaving only a few erect fronds, of the upper whorl, on the crown. As the disorder progresses the number of healthy fronds in the canopy keeps decreasing, the leaves turn pale green and emerging fronds become shorter, causing a drastic reduction in the size of the crown. In the severe stage, scorching of the tips of leaflets of the few remaining erect fronds can be observed. Finally, the dried fronds drop off leaving a crownless coconut trunk. With the initial drooping of fronds, the trunk begins to taper and the internode length progressively decreases. As a result, the emerging inflorescences are bunched together, giving a 'rosette' like appearance to the crown. Inflorescences without any nuts (empty bunches) are also a common symptom of moderate to severely affected palms (Fig 1).

The effect of CRD on plant characters has not been quantitatively expressed. Nor have studies been carried out on water transport to the canopy and other reproductive parts of the palm which, are of primary importance in investigations of a disorder such as CRD. Martin *et al.* (1982) showed that the absorption and transport of water and the root activity of crops could be studied using the non-radio active tracer, Lithium. Pinto (1998) has tested this technique at the Coconut Research Institute and developed an effective method to use lithium as a tracer in root studies of coconut. The objectives of this study are therefore to (a) quantify the vegetative, reproductive and physiological characters of, and (b) determine the pattern of water transport in, CRD affected palms and to compare with those of healthy palms.

MATERIALS AND METHODS

Plant Material

Fifteen-year-old, CRD-affected coconut palms (*Cocos nucifera* L. var. Typica) in Makandura Seed Garden (MSG) of the Coconut Research Institute were used for the study. The 'affected' palms were grouped into three stages of decline: incipient (S1), moderate (S2) and severe (S3). Twelve apparently healthy palms (S0) and 24 affected palms of each stage S1, S2 and S3 were used in the study.

Measurement of vegetative and reproductive characters

The numbers of fronds in the canopy, nuts per bunch and female flowers produced per inflorescence were counted. The trunk girth at the base of the canopy, and at 30 cm below the base, length and circumference of the most mature unopened spadix, and length and circumference of mature coconuts were measured using a measuring tape. The weight of the nut and fruit components: husk, shell, kernel, and volume of nut water were recorded.

Determination of transpiration and stomatal diffusive resistance

The rate of transpiration and diffusive resistance were measured on the central leaflets on both sides of the ninth frond (counting the youngest fully open leaf as one), between 9.00 and 13.00 hours, with full sun, using the LI-1600 Steady Porometer (LI-COR Inc, Lincoln, USA).

Estimation of total chlorophyll content

The central leaflets on both sides of ninth frond were sampled on ice, cut into small pieces, and homogenized in Acetone using an electric crusher (Ultra turrax T-25, GMBH, West Germany). While homogenizing the sample tubes were kept on ice to prevent the temperature within the test tube increasing and evaporating the Acetone. The crushed samples were centrifuged at 3000 rpm for five minutes and the absorbance was measured at 645 nm and 663 nm using UV/VIS spectrophotometer (Shimadzu UV 160 – A, Japan.). Total chlorophyll content was calculated according to Arnon (1949).

Determination of the pattern of water transport from the root system to the canopy

Lithium was used as a non-radio active tracer to determine the pattern of water transport as described by Martin *et al.* (1982) and adopted for coconut by Pinto (1998). Six each of apparently healthy and CRD-affected (incipient stage) palms were root fed with 100 ml of 1% Lithium Chloride solution. Three days after treatment application, leaflets of the ninth frond, five month-old developing nuts, and healthy roots nearest to the lithium treated root were sampled. Nut water of harvested nuts was immediately frozen at –20 °C. The leaflets (without mid ribs), pieces of husks and roots were oven dried at 80 °C for three days and powdered (1 mm sieve size) using a grinder. One gram each of the powdered samples was shaken for two hours in 20.0 ml of de-ionized water, and filtered using Whatman No. 42 paper. The lithium content in the filtrate and in defrosted nut water was determined using an Atomic Absorption Spectrophotometer (GBC, Australia).

Analysis of data

Data was analyzed by ANOVA, using the GLM procedure of SAS software. Least Square Means (LSMs) were calculated and pair-wise t tests were performed to compare the LSMs.

(a) (b) (c) (d)

Figure 1: Different stages in the progression of Coconut Rapid Decline - (a) healthy palm (no visible symptoms of decline) (b) incipient stage, (c) moderate stage, and (d) severe stage.

RESULTS

Leaf canopy and trunk characters

The number of fronds in CRD-affected palms, in all stages of decline, was significantly less than in healthy palms (Table 1). Trunk girth at the base of the crown and 30 cm below the base was also significantly less in CRD-affected palms. The girth at the base of the crown of moderately and severely affected palms was significantly less than that of CRD affected palms at the incipient stage (Table 1).

Table 1: Number of fronds/palm, girth of trunk (at the base of the crown and 30 cm below) of healthy palms (S0) and CRD-affected palms: Incipient (S1), Moderate (S2) and Severe (S3).

Stage of CRD	Number of fronds /palm	Trunk girth (cm)	
		At base of the crown	At 30 cm below the crown
S0	31 a	84.94 a	82.90 a
S1	20 b	67.14 b	66.95 b
S2	20 b	65.01 c	67.41 b
S3	20 b	61.83 c	62.77 c

Values with the same letters, within a column, are not significantly different (*** $p < 0.001$)

Nut and inflorescence characters

Numbers of nuts per bunch, and female flowers produced per inflorescence, and length and circumference of unopened spadix in CRD-affected palms were significantly less than in healthy palms. However, there was no significant difference in these characters between the stages of decline, except with the number of nuts per bunch where S3 palms yielded significantly less than S1 and S2 palms (Table 2).

The nut weight in CRD-affected palms, in all stages of decline, was significantly less than in healthy palms. However, in the length and circumference of the nuts a significant reduction was noted only in S3 palms; there was no difference between S0, S1 and S2 palms (Table 3).

Table 2: Number of nuts per bunch, female flowers produced per inflorescence, and length and circumference of unopened spadix of healthy palms (S0) and CRD-affected palms: Incipient (S1), Moderate (S2) and Severe (S3).

Stage of CRD	No. of nuts per bunch	No. of female flowers produced per inflorescence	Unopened spadix characters (cm)	
			Length	Circumference
S0	8 a	35 a	94.44 a	25.17 a
S1	5 b	13 b	62.70 b	16.22 b
S2	4 b	10 b	63.58 b	16.89 b
S3	2 c	7 b	58.73 b	16.31 b

Values with the same letters, within a column, are not significantly different (*** $p < 0.001$).

Table 3: Weight per nut, and length and circumference of the nuts of healthy palms (S0) and CRD-affected palms: Incipient (S1), Moderate (S2) and Severe (S3).

Stage of CRD	Weight per nut (Kg)	Nut dimensions (cm)	
		Length	Circumference
S0	1.736 a	31.34 a	53.58 a
S1	1.130 b	29.42 a	50.86 a
S2	1.059 b	25.43 a	44.45 a
S3	1.027 b	20.52 b	35.91 b

Values with the same letters, within a column, are not significantly different (** $p < 0.01$).

Table 4: Weight of fruit components: Husked nut, fresh and dry husk and shell of healthy palms (S0) and CRD-affected palms: Incipient (S1), Moderate (S2) and Severe (S3).

Stage of CRD	Husked nut weight (g)	Husk weight (g)		Shell weight (g)
		Fresh	Dry	
S0	750 a	908 a	359 a	218 a
S1	439 b	735 b	269 b	142 b
S2	465 b	590 c	261 b	151 b
S3	424 b	600 c	243 b	163 b

Values with the same letters, within a column, are not significantly different (** $p < 0.01$)

The weight of a husked nut, its husk (dry), shell, and kernel (fresh and dry), and the volume of nut water in CRD-affected palms were significantly less than in healthy palms. However, there was no significant difference in these characters between the stages of decline (Table 4 and 5).

Table 5: Weight of solid endosperm (kernel) and volume of liquid endosperm (nut water) of healthy palms (S0) and CRD-affected palms: Incipient (S1), Moderate (S2) and Severe (S3).

Stage of CRD	Kernel weight (g)		Volume of nut water (ml)
	Fresh	Dry.	
S0	373 a	206 a	225.42 a
S1	223 b	109 b	73.67 b
S2	236 b	122 b	78.09 b
S3	206 b	117 b	57.10 b

Values with the same letters, within a column, are not significantly different (** $p < 0.01$).

Physiological parameters

The rate of transpiration of healthy palms (S0) was significantly higher than in CRD-affected palms, in all stages of decline, S1, S2 and S3; and the transpiration rate in S1 and S2 palms was *significantly* higher than in S3 palms (Table 6). This pattern was reversed in respect of stomatal diffusive resistance, and the differences were statistically significant. The stomatal resistance of the palms at the incipient stage (S1) and of healthy palms was not significantly different, but with the decline advancing S2 and S3 palms recorded a significantly higher stomatal resistance than in healthy and S1 palms (Table 6). Leaf chlorophyll content of healthy palms was also significantly higher than in CRD-affected palms in all stages of decline; there was, however, no significant difference between S1, S2 and S3 palms (Table. 6).

Table 6: Rate of transpiration ($\mu g\ cm^{-2}\ s^{-1}$), stomatal diffusive resistance ($s\ cm^{-1}$) and total chlorophyll content (mg/g fresh weight) of healthy palms (S0) and of CRD-affected palms: Incipient (S1), Moderate (S2) and Severe (S3).

Stage of CRD	Transpiration	Stomatal diffusive resistance	Chlorophyll content
S0	2.73 a	5.30 a	3.13 a
S1	2.11 b	11.43 ab	2.73 b
S2	1:54 b	15.71 bc	2.81 b
S3	1.48 c	21.47 c	2.68 b

Values with the same letters, within a column, are not significantly different (** $p < 0.01$).

Distribution of lithium in different parts of the palm

The Li concentration in the leaves, husks and nut water of CRD-affected palms (incipient stage) was lower than in healthy palms, whilst the reverse was true of the concentration in the roots. The decrease in Li concentration was highest in leaves and lowest in nut water (Fig. 2).

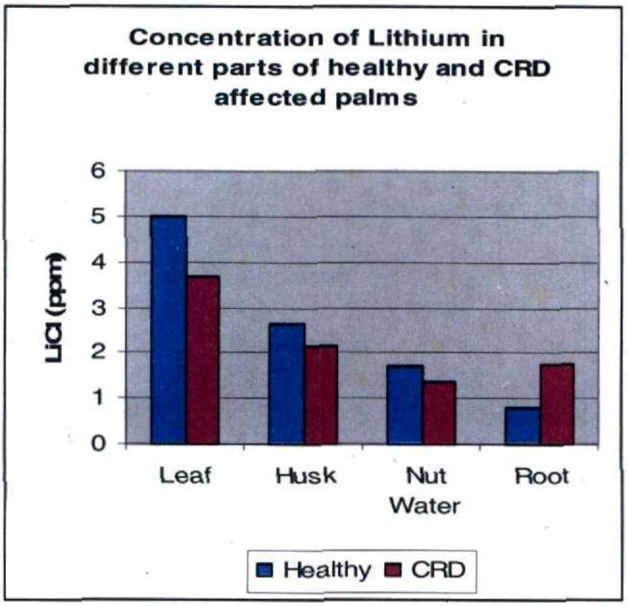

Figure 2: **Concentration of Lithium in different parts of healthy and CRD-affected palms**

DISCUSSION

The vegetative growth, yield, nut size and fruit components, gas exchange capacity and water transport in palms subject to CRD, irrespective of its severity, are appreciably different from that of apparently healthy coconut palms. The marked reduction in the leaf canopy and the consequent reduction of its capacity to intercept light, appears in turn to reduce the quantum of assimilates partitioned to developing reproductive organs and other vegetative structures, and ultimately lower the total plant productivity, as described by Taiz and Zeiger, (1991). In this context it is relevant to note that although there was a progressive tapering of the trunk in CRD-affected palms, dissections did not reveal any abnormalities in the internal tissues of the trunk, at any stage of the decline (Ranasinghe *et al.*, 2002).

Abnormal stomatal closure in CRD-affected palms may be one of the factors that kill these palms, because significantly increased stomatal resistance affects other processes such as photosynthesis and water and nutrient transport. The decrease in xylem transport to the canopy in CRD-affected palms, as shown by lithium tracer studies, could be mainly attributed to the abnormal stomatal closure. This is supported by the evidence of increasing stomatal resistance with the progression of the disorder. A similar decrease in stomatal conductance (increase in stomatal resistance) coinciding with the appearance of foliar symptoms was a consistent symptom of ash yellows in *Fraxinus americana,* of corn stunt in maize and of lethal yellowing in *Cocos nucifera*, all of which, are diseases caused by phytoplasma (Matteoni and Sinclair, 1983; Leon *et al.*, 1996; Martinez *et al.*, 2000). The pattern of lithium distribution in CRD-affected coconut palms (Figure 2), also suggests some obstruction to water transport from roots to canopy, and retention of water in the roots of affected palms. The study also revealed that the water transport least affected was to the developing nuts where there is a high demand for water due to high metabolic activities (Bai and Shivasankar, 1999). Therefore, it is of utmost importance to determine the factors which obstruct xylem transport and cause abnormal stomatal closure in CRD-affected palms as this phenomenon may be responsible for the disorder symptoms.

Finally, it can be concluded that there are significant differences in vegetative, reproductive and physiological aspects between CRD-affected and healthy palms. The quantification of CRD symptoms in the present study would be useful in formulating an index for grading the severity of the disorder. It also raises the possibility of using gas exchange parameters viz., transpiration and stomatal diffusive resistance for the early diagnosis of the disorder.

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