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CHANGES IN THE PHYSIOLOGICAL PERFORMANCE OF LEAF SCORCH DECLINE (LSD) AFFECTED COCONUT (*COCOS NUCIFERA*) PALMS

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SUMMARY

Leaf scorch decline (LSD) is a disorder of coconut (*Cocos nucifera*) palms in Sri Lanka. The etiology of the disorder is not yet known. This study aimed to compare and quantify physiological and biochemical characters of LSD-affected palms with healthy palms. Thirty-year-old apparently healthy and LSD-affected (mild, moderate and severe) palms (variety *typica*) were compared at Bandirippuwa Estate, Coconut Research Institute, Sri Lanka. In LSD-affected coconut palms, the functional leaf area of the canopy, rates of photosynthesis and transpiration, leaf water potential, leaf chlorophyll and Zn contents were appreciably reduced with the development of the disorder, whereas stomatal diffusive resistances and abscisic acid concentrations in the xylem sap increased. The volume of inflorescence sap collected from LSD-affected palms was reduced compared to healthy palms, but the composition of the sap remained unaltered. Since altered stomatal behaviour might affect gas exchange and related processes, such as water movement, photosynthesis and the assimilate partitioning pattern, these results support the hypothesis that stomatal closure in LSD-affected palms may influence LSD symptoms. However, more research is needed to come up with conclusive evidence of its cause.

INTRODUCTION

The coconut industry has great economic importance and offers a broad range of dietary and foreign exchange income and employment opportunities in Sri Lanka. The coconut palm is prone to several diseases and disorders that cause a substantial loss of crop. Leaf scorch decline (LSD) is regarded as one such disorder of the coconut palm, the cause of which is unknown (Humphries, 1970; Rajapakse and Fernando, 1995). LSD has affected coconut plantations to an appreciable degree; it reduces the yield within 2–3 years and kills the palm in 5–10 years. The consequent loss to the coconut industry can be considerable. The most striking visible symptom of LSD-affected palm is the scorching of leaflets, starting from the tip and advancing towards the midrib of the frond, accompanied by a slight curling, and progressing from the lower to the middle whorl fronds. Young fronds in the upper whorl (1–5) do not show any scorching symptoms, even in advanced LSD. Tapering of the trunk, shorter fronds, fewer and smaller inflorescences, reduced nut set and yield, elongated nuts and a decaying root system are the other features in severely affected plants compared to healthy coconut palms (Peries, 1968; Mahindapala and Chandrasena, 1975; Ranasinghe *et al.*, 2002).

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Extensive research work on the differentiation and behaviour of stomata, production of stress hormones, synthesis of chlorophyll and polyphenols and properties of phloem sap has supported the hypothesis of pathogenicity in lethal yellowing-affected coconut palms (Leon *et al.*, 1996). Development of LSD symptoms also presumably involves several physiological and biochemical changes in affected palms. The study of these changes would therefore contribute to the understanding of the mode of action of this disorder, and this could eventually be useful in identifying the cause and for developing control measures.

The aim of this study was to quantify the alterations in leaf area, stomatal regulation, gas exchange, water relations and biochemical and nutritional aspects of leaf scorch decline-affected coconut palms in comparison with healthy (symptomless) palms.

MATERIALS AND METHODS

Plant material and growth conditions

The study was performed in naturally LSD-affected, 30-year-old tall coconut palms (*Cocos nucifera* var. *typica*) at Bandirippuwa Estate, Coconut Research Institute, Lunuwila (lat. 7°20′N, long. 79°53′E). The palms were subjected to uniform fertilizer treatment and cultural practices as recommended by the Coconut Research Institute. Ten examples of mild, moderate and severe LSD-affected palms were selected for the study. Severity of the LSD (mild, moderate or severe) was determined according to the development of visual symptoms as follows.

- *Mild LSD* – Only the lower whorl of fronds is affected. No tapering of the trunk. No reduction in yield.
- *Moderate LSD* – Lower and middle whorls of fronds are affected. Tapering of the trunk and reduction in coconut yield have started.
- *Severe LSD* – Only a few short, green fronds (8–12) remain in the canopy. Tapered trunk, short or no inflorescence, very low nut set and nut yield.

Apparently healthy palms were used as the controls and these palms remained unaffected even after the completion of the experiment. The design was a completely randomized single tree plots with 10 replicates per treatment.

Data collection

The data collection and leaf sampling were conducted when the soil was at field capacity to avoid possible confusion between the symptoms due to LSD and those due to water stress.

Determination of the leaf area (green), stomatal density and epidermal cell area

The green area of each leaf (frond) was estimated using the method described by Jayasekara and Mathes (1992) and, by addition, the total canopy area of a coconut palm was determined.

To determine stomatal density and epidermal cell areas, epidermal impressions of the leaves were made using a method similar to that described by Ranasinghe and Taylor (1996). A thin layer of nail varnish was applied to the lower surface of the leaf, allowed to dry for 20–30 min and then gently peeled off with forceps, placed on a microscope slide, and covered with a cover slip. The number of stomata per field was counted using a light microscope with ten replicas per leaf. From the data, stomatal density (SD, number of stomata mm^{-2}) was calculated. Since the epidermal cells of coconut leaves were approximately rectangular in shape, length and width of cells were measured using a calibrated eyepiece graticule and epidermal cell area was calculated by length × width.

Determination of physiological parameters

Measurements were taken on the ninth leaf (taking the youngest open leaf as 1 and counting downwards) during the period of 09.00 hours to 11.30 hours under a clear sky with full sun (PAR 1200–1400 μmol cm^{-2} s^{-1}).

Photosynthesis, transpiration, stomatal diffusive resistance and leaf water potential

The rate of photosynthesis was measured using an LI-6200 Portable Photosynthesis System (LI-COR Inc., Lincoln, Nebraska, USA). Rate of transpiration and stomatal diffusive resistance were measured using an LI-1600 Steady State Porometer (LI-COR Inc., Lincoln, Nebraska, USA). Leaf water potential was measured using a plant water status console model 3005 (Soil Moisture Equipment, USA; Scholandar *et al.* 1965).

Determination of biochemical parameters

Four leaflets from the mid-portion of the ninth leaf were detached during the period of 09.00 hours to 11.30 hours, kept on ice immediately and used in biochemical analysis.

Leaf chlorophyll content

A leaf was cut into small pieces and 0.100 g of sample was homogenized with 5.0 ml of 80 % acetone using an Ultra Turrax T-25 (F. G. Bode & Co. GmbH, West Germany) for 1 min. During crushing, the sample tubes were kept on ice to prevent a temperature increase within the test tube and evaporation of acetone. The crushed samples were centrifuged for 10 min at 3500 rpm, the supernatant containing chlorophyll was measured for absorbance at 645, 652 and 663 nm wavelengths and Chlorophyll *a*, Chlorophyll *b* and total chlorophyll contents were calculated according to Arnon (1949).

Leaf abscisic acid (ABA) content

ABA concentration in the xylem sap was determined using a radio immunoassay (RIA). The monoclonal antibody used, which is specific for (+)-ABA (MAC252), was obtained from Dr S. A. Quarrie, John Innes Institute, UK. Leaflets were sampled, immediately frozen in liquid nitrogen and stored at –20 °C. Xylem sap was collected as detailed by Trejo and Davies (1991), using the midrib of leaflets, cut in small sections, and placed in 10 ml syringe barrels. The syringe barrels were then placed above

micro-centrifuge tubes that were fitted inside centrifuge containers (Hermle Z 360 K, Germany). Centrifugation was for 20 min at 9000 rpm and 4 °C. Sap was collected in the tubes and 50 µl portions were analysed directly for ABA.

Standard ABA samples were included in each assay for the construction of the standard curve. The incubation procedures, and the generation of standard curves as well as the calculation of the ABA concentration in the samples were as described by Quarrie *et al.* (1988). The validation of RIA, for use with unpurified xylem sap of coconut was confirmed by a dilution/spike recovery test for non-specific interference (Jones, 1987).

Determination of micro nutrient content of leaves

Leaf samples were collected from Bandirippuwa Estate (BE), Walpita Estate (WE) and Poththukulam Research Station (PRS) for this analysis. Six middle leaflets from the fourteenth frond of LSD-affected and apparently healthy palms were sampled and oven dried at 70 °C for 24 hours. Fe, Mn, Cu and Zn contents of the powdered leaf samples were analysed according to Cuniff (1999).

Determination of yield and biochemical composition of the inflorescence sap

The sap was collected employing the tapping technique that has been traditionally evolved over centuries, but with some modifications as described by Nathanael (1966). Only the healthy, mild LSD and moderate LSD-affected palms were used in this study since severe LSD palms rarely produce spadices that can be tapped to obtain the sap. A thin slice of the spadix was pared transversely twice a day, morning (07.00 hours–09.00 hours) and evening (16.00 hours–18.00 hours), and the collected fresh sap yield of each palm was measured. Samples were collected from each palm at 3-monthly intervals to measure the osmotic potential and sugar contents. The fresh sap, collected into 1.5 ml eppendorf tubes, immediately after slicing, was frozen at −20 °C until analysis.

Estimation of osmolality of the sap

The osmotic potential of 10 µl of thawed sap was measured with a Wescor 5500 vapour pressure osmometer (Wescor Inc 84321, Utah, USA) calibrated with 290 and 1000 mmol kg^{-1} NaCl solutions.

Estimation of sugar content in the sap

Sap was diluted 100 times, purified using a sep-pak cartridge and analysed for total sugars using a high performance liquid chromatography (HPLC) system (Waters, USA) with a sugar-pak column.

Statistical analysis of data

The data were analysed using the SAS statistical package, with one-way ANOVA.

Table 1. Variation in green leaf area, number of leaves, stomatal density (SD) and epidermal cell area (ECA) of healthy, mild, moderate and severe LSD coconut palms. *s.e.* is the standard error of the mean.

Status of palm	Area per leaf (m^2)	No. of leaves ($palm^{-1}$)	SD (mm^{-2})	ECA (μm^2)
Healthy	5.15	30.7	168	681
Mild LSD	4.5	30.3	168	659
Moderate LSD	3.83	25.3	162	652
Severe LSD	1.75	17.5	163	670
s.e.	0.21	0.79	2.0	17.0

Table 2. Variation in leaf chlorophyll *a* (Ch*a*), chlorophyll *b* (Ch*b*), total chlorophyll (Ch) content and abscisic acid (ABA) concentrationin the xylem sap of healthy, mild, moderate and severe LSD coconut palms. *s.e.* is the standard error of the mean.

Status of palm	Ch-*a* (mg g^{-1} fresh weight)	Ch-*b* (mg g^{-1} fresh weight)	Total Ch (mg g^{-1} fresh weight)	ABA (ng ml^{-1})
Healthy	1.59	0.66	2.25	17.52
Mild LSD	1.33	0.57	1.90	26.49
Moderate LSD	1.37	0.59	1.96	36.35
Severe LSD	1.12	0.45	1.57	33.80
s.e.	0.04	0.02	0.05	2.01

RESULTS

Leaf area, stomatal density and epidermal cell area

There was a significant reduction in area per leaf and the number of leaves in the canopy in moderate and severe LSD-affected palms compared with healthy palms. The reduction in leaf area at the severe LSD stage was 66 % in comparison with healthy palms (Table 1). No significant differences were observed in stomatal density and leaf epidermal cell area between healthy and LSD-affected (mild, moderate, severe) palms (Table 1).

Chlorophyll and abscisic acid (ABA) content

The contents of chlorophyll *a* and total chlorophyll in LSD-affected palms started to decline as the symptoms first appeared and decreased further at later stages. In the severe LSD stage, chlorophyll contents were significantly lower than in mild and moderate LSD-affected palms. However, the content of chlorophyll *b* was reduced only at the severe LSD stage compared with healthy and early stages of LSD (Table 2).

The concentration of ABA in xylem extracts increased with 'decline' progression. The increase was significant in moderate and severe LSD palms compared with healthy palms, and increased steadily to double the original value (Table 2).

Gas exchange and water relations parameters

The average rate of photosynthesis of healthy coconut palms was 9.76 µmol m^{-2} s^{-1}. The rate remained high in mild and moderate LSD palms but fell significantly at

Table 3. Variation in rate of photosynthesis, stomatal diffusive resistance, rate of transpiration and leaf water potential of healthy, mild, moderate and severe LSD coconut palms.

Status of palm	Rate of photosynthesis ($\mu mol\ m^{-2}\ s^{-1}$)	Stomatal diffusive resistance ($s\ cm^{-1}$)	Rate of transpiration ($\mu g\ cm^{-2}\ s^{-1}$)	Leaf water potential (MPa)
Healthy	9.76	3.24	7.43	−1.26
Mild LSD	8.82	4.55	6.17	−1.39
Moderate LSD	9.41	6.70	4.45	−1.51
Severe LSD	5.77	7.90	3.85	−1.72
s.e.	0.36	0.30	0.19	0.29

severe LSD stages to 5.77 $\mu mol\ m^{-2}\ s^{-1}$ (Table 3). The stomatal diffusive resistance increased steadily with LSD symptom development. The increase was significant in moderate and severe LSD palms when compared with healthy and mild LSD palms (Table 3). The transpiration rate of LSD-affected palms was significantly lower than for healthy palms. The rate started to fall as symptoms first appeared and decreased to approximately 48 % of its original at the severe stage (Table 3). No change occurred in the water potential of leaves in mild LSD palms, but it significantly decreased in moderate and severe LSD palms compared with healthy ones (Table 3).

Leaf micronutrient levels

Leaf Fe, Mn and Cu contents of LSD-affected palms were similar to those of healthy palms in all three locations (BE, WE and PRS). However, there was a significant reduction in the leaf Zn level of LSD-affected palms compared to healthy at BE, PRS and WE (Table 4).

Yield, osmolality and sugar content of inflorescence sap

When unopened spadices of healthy, mild LSD and moderate LSD palms were tapped, the total annual yield of the sap was significantly reduced with the development of the LSD disorder. The reductions were 24 % in mild LSD and 49 % in moderate LSD palms compared to healthy ones. The osmolality and total sugar contents of the sap were not altered due to LSD (Table 5).

DISCUSSION

In LSD-affected coconut palms, the functional leaf area of the canopy, rate of photosynthesis, rate of transpiration, leaf water potential, leaf chlorophyll and Zn contents were appreciably reduced with the development of the disorder. Furthermore, the stomatal resistance and ABA concentration in the xylem sap were increased with its progression. However, the frequency of stomatal differentiation, leaf epidermal cell expansion, and content of some essential micronutrients (Fe, Mn and Cu) were unaffected by the disorder. In the inflorescence sap, LSD reduced the yield without altering the sugar and other solutes.

Table 4. Variation in micronutrient contents (ppm) in healthy, mild, moderate and severe LSD coconut palms at Bandirippuwa Estate (BE), Walpita Estate (WE) and Poththukulam Research Station (PRS).

	BE	WE	PRS
		Zn content	
Healthy	19.5	23.2	21.5
Mild LSD	16.8	15.0	16.2
Moderate LSD	17.8	11.9	17.5
Severe LSD	na	15.6	14.8
s.e.	1.03	1.67	0.81
		Fe content	
Healthy	106.4	91.1	114.4
Mild LSD	99.5	86.7	94.3
Moderate LSD	110.3	83.5	97.3
Severe LSD	na	74.9	96.2
s.e.	5.50	2.50	5.53
		Mn content	
Healthy	52.0	126.8	85.0
Mild LSD	41.4	114.1	158.2
Moderate LSD	45.0	140.8	96.6
Severe LSD	na	95.7	85.4
s.e.	2.52	6.38	11.3
		Cu content	
Healthy	3.8	5.1	3.2
Mild LSD	3.6	5.0	2.6
Moderate LSD	3.8	5.0	2.5
Severe LSD	na	4.5	2.4
s.e.	0.10	0.10	0.12

na: not analysed.

Table 5. Variation in yield, osmolality and total sugar content of the inflorescence sap of healthy, mild and moderate LSD coconut palms.

Status of palm	Sap yield ($L\ year^{-1}$)	Osmolality ($mmol\ kg^{-1}$)	Total sugar content ($g\ 100\ ml^{-1}$)
Healthy	771	997	15.5
Mild LSD	588	930	16.3
Moderate LSD	397	1005	17.4
s.e	26.1	34.4	0.31

The reduction in canopy area in LSD-affected palms resulted in a marked reduction in the light interception by the canopy (photosynthesizing area) and consequently the total plant productivity as described by Taiz and Zeiger (1991). The reduction in coconut yield was 5–15 %, 30–40 % and 65–70 % in mild, moderate and severe LSD palms respectively, at all the sites compared with healthy palms. Stomatal regulation of a plant is mainly important in balancing the intake of CO_2 for photosynthesis and providing a pathway for water loss in transpiration (Lambers *et al.*, 1998). Although the stomatal density of the affected palms was not altered by LSD, transpiration was

reduced, even in mildly affected palms. This was similar to the observations recorded in coconut palms affected by lethal yellowing (LY) and coconut rapid decline (CRD) in which the stomatal resistance was increased with the development of visual symptoms (Leon *et al.*, 1996; Martinez *et al.*, 2000; Ranasinghe *et al.*, 2002). This altered stomatal behaviour of LSD-affected palms can potentially affect plant functions that depend on gas exchange, water and nutrient transport and photosynthesis (Oropeza *et al.*, 1991; Eskafi *et al.*, 1986). The great reduction in the rate of photosynthesis in LSD-affected palms may possibly be due to abnormal stomatal closure and hence limited CO_2 availability for photosynthesis (Leon *et al.*, 1996). The reduced rates of photosynthesis may also be explained by the loss of photosynthetic pigments. The chlorophyll contents of LSD-affected palms dropped to 84 % of the original levels (healthy stage) even at the mild-LSD stage. Similarly, in LY-affected and CRD-affected coconut palms, there was a reduction in chlorophyll content with the development of the 'disease' symptoms (Leon *et al.*, 1996; Ranasinghe *et al.*, 2002). These data suggest that the decline of the photosynthesis rate in LSD-affected palms resulted from a decrease in the chlorophyll content together with reduced availability of CO_2 due to increased stomatal resistance.

LSD-induced stomatal closure at the mild-LSD stage cannot be explained by a change in the water status of the leaves, as the leaf water potential remained the same. However, the xylem sap ABA concentration steadily increased with the appearance of LSD symptoms; in fact, there was an increase in stomatal resistance together with ABA concentration in the xylem sap, even at the mild-LSD stage, though the effect was not significant. This result suggests that ABA is promoting stomatal closure in LSD-affected palms but the involvement of changes in leaf water potential at latter stages cannot be ruled out. Numerous studies with diverse species have reported that environmental stresses such as drought, flooding and salinity can result in stomatal closure due to increased levels of ABA (Zhang *et al.*, 1987; Davies and Zhang, 1991; Hurley and Rowarth, 1999; Netting, 2000). Further evidence from studies with plants subjected to water stress suggests that the degree of stomatal closure is more directly related to increased levels of ABA in the roots and in the xylem sap than to ABA concentration in leaves (Davies and Zhang, 1991; Schurr *et al.*, 1992). Abnormal stomatal closure induced by LSD may be analogous to what happens in water-stressed plants.

In addition to ABA, cytokinins or auxins can promote stomatal opening and override the ABA induced stomatal closure in plants (Snaith and Mansfield, 1982; Incoll and Jewer, 1987; Shashidhar *et al.*, 1996). In LSD-affected coconut palms, it is possible that stomatal closure might result from an increased supply of ABA and a reduced supply of cytokinin, as has been proposed for drought-induced stomatal closure (Davies and Zhang, 1991). However, further research is required to determine what happens to the growth promoting hormones with the development of the disorder, thus LSD can be explained further by a hormonal imbalance as proposed by Dabek and Hunt (1976).

The LSD-affected coconut palms are generally found to occur in all soil types under which coconut has been grown in Sri Lanka, irrespective of the level of management of the plantation. The investigations carried out in the past have ruled out possible involvement of major nutrient (N, P, K and Mg) deficiency (Jayasekara *et al.*, 1988) and the present study ruled out the involvement of Fe, Mn and Cu in LSD. However,

the only nutrient found to be deficient in leaves of the LSD-affected palms was Zn. Disorders such as 'little leaf' and 'rosette' of apples, peaches and pecans, resulting from growth reduction of leaves and internodes are known to be caused by Zn deficiency. Inter-veinal chloroses was showed in Zn deficient leaves of maize, sorghum, bean and fruit trees, suggesting that Zn participates in chlorophyll formation or prevents chlorophyll destruction (Vallee, 1976). These studies have further suggested that the retardation of stem growth in the absence of Zn might result partly from its apparent requirement for the production of a growth hormone, indoleacetic acid (auxin), indicating a hormonal imbalance. Many enzymes also contain tightly bound Zn that is essential for their function; considering all plant species, more than 80 such enzymes are known (Vallee, 1976; Salisbury and Ross, 1991). Hence, a reduction in enzyme activities related to growth and development of the palm, coupled with Zn deficiency may be proposed as another potential reason for altered physiological activities of LSD-affected coconut palms.

Any micro-organism that can be present in the vascular system of the palm is of special interest in research on disorders of unknown aetiology. The sugar-rich phloem is a possible niche for such organisms. The inflorescence sap of LSD-affected coconut palms, exuded from unopened spadix, showed a significant reduction in yield per palm, even at the mild-LSD stage. However, the concentration of total solids and sugars in the sap of LSD-affected palms at any stage was the same as for healthy palms. The palms with more than half the canopy affected by LSD and tapered trunk produced little or no sap. Therefore, the physical or biochemical aspects of the sap of severe-LSD palms could not be analysed in the present study. These findings are consistent with a disturbance in phloem flow in LSD-affected palms by callose formation (Taiz and Zeiger, 1991) or by involvement of phytoplasmas (McCoy *et al.*, 1976; Matteoni and Sinclair, 1983), but provide no evidence, as to its cause. Similar observations were recorded in LY-affected coconut palms and in Thanjavur wilt diseased coconut, but they also failed to come up with a conclusive evidence of its cause (Eden-Green and Waters, 1982; Vijayraghavan *et al.*, 1987).

It can be concluded that there is a significant reduction in leaf area development, gas exchange capacity, water relations, flow of sap, leaf chlorophyll and Zn contents, and an increase in the stomatal diffusive resistance and ABA concentration in the xylem sap of LSD-affected coconut palms. The variations were quantified and these values could be used as early diagnostic tools for LSD. Altered stomatal behaviour seems to affect related physiological processes of the palm. This information would also be useful in understanding the mode of action of the disorder. Future research should concentrate on detailed anatomical studies of the cellular and vascular abnormalities and the presence of subcellular pathogens. Detection of the pattern of water transport and vascular blockages to water flow and evaluation of the effect of nutrient spraying on the expression of LSD symptoms are proposed to determine the cause and to identify possible remedial measures for the disorder.

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