

WATER RELATIONS AND NET PHOTOSYNTHESIS OF ARECANUT PALMS AFFECTED WITH YELLOW LEAF DISEASE *

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Abstract: Water relations and net photosynthesis in apparently healthy palms and palms affected with yellow leaf disease are studied. Stomatal resistance was significantly higher in the outer and middle leaves of diseased palms, while transpiration rate and photosynthetic rate were significantly lower. Chlorophyll content was also significantly reduced. Net photosynthetic rate was reduced with increasing light intensities, beyond saturating levels. CO_2 assimilation was saturated at photosynthetically active radiation of $800 \mu\text{mol m}^{-2} \text{s}^{-1}$ in healthy palms and $500 \mu\text{mol m}^{-2} \text{s}^{-1}$ in diseased palms. Total sugars and starch accumulated in leaves of diseased palms despite impaired photosynthetic machinery. Leaf water potential and turgor potential were significantly higher while osmotic potential was lower in the leaves of diseased palms. Evidence is presented to show that disease leads to stomatal closure independent of water deficit.

Keywords: *Areca catechu*, Yellow leaf disease, MLO's, Photosynthesis, Water relations.

The arecanut palm (*Areca catechu* Linn.) is an important plantation crop of commercial value in India. Yellow leaf disease (YLD) is a serious malady of arecanut which is prevalent in parts of Kerala and Karnataka in southern India (Nayar, 1976). Possible involvement of mycoplasma like organisms (MLOs) in causing YLD was reported (CPCRI, 1988; Nayar and Seliskar, 1978). Several plant 'yellows' type diseases caused by MLOs lead to stomatal closure with increased stomatal resistance and water potential (Matteoni and Sinclair, 1983). Abnormal stomatal opening with reduced stomatal resistance and water potential have also been reported in the case of root (wilt) disease of coconut (Rajagopal, *et al* 1986,1987), a MLO's caused disease. In this paper, water relation components, net photosynthetic rate, chlorophylls, sugars and starch in diseased palms were determined and compared with apparently healthy palms since information about the effects of YLD on these parameters is lacking.

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MATERIALS AND METHODS

Host Plant: 3-4 year old and 18-20 year old arecanut palms (*Areca catechu* Linn.) affected with yellow leaf disease (disease index score 20-23 (George *et al.* 1980) as well as apparently healthy palms in a farmer's field at Sullia, Karnataka and healthy palms raised at the CPCRI Farm, Vittal (12° 25' N and 75° 42' E, 91m above sea level) were used in the present study. All experimental palms were planted at a spacing of 2.7 x 2.7m. Each palm was fertilized annually with N 100 g, P 40 g and K 140 g and 12 kg each of green leaf and cattle manure. Adequate drainage was provided during rainy season with drainage channels of 90-115 cm. depth to drain excess water. Each palm received 175 liters of water in summer months once in a week.

Method of sampling: The method of leaf sampling was based upon the phylotaxy of palms. Beginning with the fully opened inner most (youngest) which numbered one, each leaf was numbered in radial spiral down to the crown. Three leaflets were sampled from the middle portion of the leaves numbered: 1 (first leaf), 3 (middle leaf) and 6 (outer leaf) in young palms and 1,4,9 in adult palms respectively, representing the spirally arranged whorls of fronds. All measurements were determined according to method described by Rajagopal *et al.* (1987) during September - October between 10.00-12.00 Indian Standard Time (IST) on clear days. The number of palms studied in each case is presented in tables with statistical analysis.

Determination of stomatal resistance (rs), stomatal conductance (cs), transpiration (E), photosynthesis (PN, and intercellular carbondioxide (ci) rs, cs, E, PN and Ci were measured with a Licor-6200 portable photosynthesis system with one litre chamber enclosing up to 20 cm² leaf area. The instantaneous water use efficiency (WUE) was calculated as the ratio of the PN to the E. During observations, the photosynthetically active radiation (PAR) ranged between 515-1200 $\mu\text{mol m}^{-2} \text{s}^{-1}$, air temperature (T_{air}) between 32.54 and 33.02° C, leaf temperature (T_l) between 29.9° C and 33.0° C, relative humidity (RH) between 58.4 and 62.8, vapour pressure between 2.81-3.08 kpa, vapour pressure deficit between 1.84-2.34 kpa and External Carbondioxide (CO₂ Ext) 317-325 ppm.

Determination of water potential (ψ_w), Osmotic potential (π), and Turgor potential (P): ψ_w was determined on excised leaves using a Scholander's pressure chamber (soil moisture equipment, USA) according to the method described by Milburn and Zimmermann (1977). Leaf sap for π determination was obtained according to the method described for hard material like coconut by Slavik (1974). The leaflets were cut into small segments and immediately plunged into liquid nitrogen. After a few minutes of thawing, sap was obtained using screw type metal hand press. A

10 - μ l aliquot of the expressed sap was absorbed on to a filter paper disc and osmotic potential was measured with vapour pressure osmometer model 5100 (Wescor, USA). The instrument was calibrated with standard sodium chloride solutions before initiation of the experiment. Turgor potential was calculated from the values of ψ_w and π using the formula $P = \psi_w - \pi$ according to Slavik (1974).

Determination of total sugars and starch: Leaf discs of 0.5 cm diameter were punched throughout the length of leaflets and 2 g (fresh weight) pooled samples were extracted three times in 25 ml 80% aq. ethanol at 80°C for 15 min. The pooled extracts were concentrated to dryness and extracted thrice in a total volume of 10 ml distilled water. The extract was fractionated into cationic, anionic and neutral fractions by passing through columns (2 g wet gel) of Dowex - 50 (H⁺) and Dowex -1 (H-co⁻) in sequence. The soluble sugars present in the neutral fraction was estimated by the phenol-sulphuric acid method (Dubois, *et al* 1956) with glucose as standard. The alcohol insoluble residue containing carbohydrates and starch was dried to constant weight at 60° C and powdered. 50 mg of the sample was digested thrice in 10 ml 36% Perchloric acid with grinding at room temperature (28 ± 1° C) for 4 hr. filtered and made up to 50 ml with distilled water. An aliquot of extract was used to estimate the hydrolysed starch by phenol-sulphuric acid method with starch (Sigma, USA) as standard (Dubois *et al* 1956).

Chlorophyll estimation: 100 mg of pooled leaf tissue was homogenized and extracted with 85% cold aq. acetone and made upto 25 ml after filtering through Whatman No. 1 filter paper. All the operations were carried out at 4° C in a dark room. The samples were read at 663 nm, 646 nm and 470 nm and chlorophyll a, chlorophyll b and carotenoids were estimated according to Lichtenthaler and Wellburn (1983).

RESULTS AND DISCUSSION

The effect of YLD on the rs and E of young and adult palms are shown in Table 1 and 2 respectively. In young palms, rs increased by 55.55% and 72.53% in the middle and outer leaves respectively, over controls. The diseased young palms showed 34.96% and 57.98% reduction in E in the middle and outer leaves respectively, over that of the apparently healthy palms. Ci was higher in the first, middle and outer leaves of the diseased young and adult palms than in the controls. In adult palms, rs was higher by 19.71% and 46.06% in the middle and outer leaves. While E was decreased by 5.34% and 33.16%. Changes in ψ_w , π and P is presented in Table 1. ψ_w increased in the first, middle and outer whorls of the diseased palms while π and P were decreased in the middle and outer leaves compared to the values in similar leaves of the apparently healthy palms. Total chlorophyll content of middle and outer

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leaves of the diseased palms was reduced by 8.5% and 82.87% respectively compared to the apparently healthy controls (Table 3). Diseased palms showed 5.7% and 83.06% reduction in chlorophyll a content in the middle and outer leaves respectively compared to controls. Chlorophyll b and Carotenoid contents decreased also in the middle and outer leaves over that of apparently healthy palms.

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Table 1. Photosynthetic characteristics and water relations in healthy, apparently healthy and yellow leaf disease affected leaves of young arecanut palms.

Parameter/ leaf position	Healthy			Apparently Healthy			Diseased			LSD	
	1	3	6	1	3	6	1	3	6	Category	Leaf position
Photosynthesis, PN ($\mu\text{mol m}^{-2} \text{ s}^{-1}$)	4.56 ^a	4.00	4.00	6.12	5.35	3.85	4.05	2.85	1.11	0.87**	0.87**
							(-37.00) ^b	(-46.72)	(-71.16)		
Stomatal conductance, G_s (cm^{-1})	0.39	0.50	0.43	0.72	0.73	0.52	1.07	0.53	0.18	0.17**	0.17**
							(+48.60)	(-27.39)	(-65.38)		
PN/ G_s ratio	11.69	8.00	10.07	8.50	7.32	7.40	3.78	5.37	6.16		
							(-55.52)	(-20.03)	(-16.75)		
Stomatal resistance, r_s (Scm^{-1})	3.47	2.82	2.63	1.78	1.76	2.56	1.22	3.96	9.32	1.35**	1.35**
							(-31.46)	(+55.55)	(+72.53)		
Transpiration E ($\text{mmol m}^{-2} \text{ sec}^{-1}$)	3.73	4.62	4.52	4.46	4.55	3.38	5.33	2.96	1.42	0.73*	0.73**
							(+16.32)	(-34.96)	(-57.98)		
Intercellular carbon dioxide, C_i (ppm)	247.03	264.63	255.63	264.59	265.70	276.19	284.07	277.61	278.86	14.61**	NS
							(+ 7.36)	(+ 4.48)	(+ 0.96)		
Water potential, (MPa)	-1.11	-1.11	-1.14	-1.17	-1.18	-1.09	-0.88	-0.62	-0.50	0.82**	0.82**
							(+14.78)	(+47.45)	(+54.12)		
Osmotic potential (MPa)	-1.65	-1.68	-1.55	-1.79	-1.72	-1.85	-1.40	-1.75	-2.40	NS	0.19**
							(+21.78)	(-1.74)	(-29.72)		

Parameter/ leaf position	Healthy			Apparently Healthy			Diseased			LSD	
	1	3	6	1	3	6	1	3	6	Category	Leaf position
Turgor potential, P	0.57	0.55	0.73	0.61	0.53	0.76	0.53	1.13	1.90	0.25**	0.25**
							(+13.00)	(+113.20)	(+150.00)		
Water Use Efficiency, WUE	1.37	1.01	1.12	1.42	1.24	1.60	0.76	1.00	1.01	0.25**	NS

a Each value is the mean of ten palms

b Values in parentheses show percentage increase (+) or decrease (-) over apparently healthy palms

** or

* denotes significant at P = 0.01 or P = 0.05; NS = Not Significant

Table 2. Photosynthetic characteristics and water potential in healthy, apparently healthy and yellow leaf disease affected leaves of adult arecanut palms.

Parameter/ leaf position	Healthy			Apparently Healthy			Diseased			LSD**	
	1	4	9	1	4	9	1	4	9	Category	Leaf position
Photosynthesis, PN ($\mu\text{mol m}^{-2} \text{ sec}^{-1}$)	4.45 ^a	6.02	3.57	5.86	4.60	5.42	4.28	1.60	0.56	0.56	
								(-7.5) ^b	(-29.96)	(-61.72)	
Stomatal conductance, G_s (cm^{-1})	0.61	0.80	0.52	0.77	0.75	0.48	1.19	0.77	0.26	NS	0.12*
							(+54.54)	(-2.66)	(-45.83)		
PN/ G_s ratio	7.36	7.52	6.86	7.61	7.81	10.00	4.55	5.55	6.15		
							(-40.21)	(-28.93)	(-38.5)		
Stomatal resistance, r_s (Scm^{-1})	1.78	1.44	2.14	1.75	1.71	3.15	1.17	2.13	5.84	0.52	0.52
							(-33.14)	(+19.71)	(+46.06)		
Transpiration, E ($\text{mmol m}^{-2} \text{ sec}^{-1}$)	6.60	7.58	5.92	5.81	5.51	4.04	6.68	5.32	2.70	0.57	0.57
							(+13.02)	(-5.34)	(-33.16)		

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Parameter	LSD**										
	Healthy			Apparently Healthy			Diseased			Category	Leaf position
leaf position	1	4	9	1	4	9	1	4	9		
Inter-cellular carbon dioxide, CO_2 ppm	274.84	267.67	276.75	271.53	268.46	255.97	279.70	277.30	284.53	7.17	NS
							(+29.67)	(+3.27)	(+11.15)		
Water potential, w (MPa)	-1.26	-1.21	-1.14	-1.21	1.20	1.13	-1.15	-0.95	-0.81	0.06	0.06

a Each value is the mean of twenty palms

b Values in parentheses show percentage increase (+) or decrease (-) over apparently healthy palms

** Significant at P = 0.01, NS = Not Significant

Table 3. Total chlorophylls, chlorophylls a and b, chlorophylls a/b ratio and carotenoids in yellow leaf disease affected leaves of adult arecanut palms.

Parameter and leaf position	LSD										
	Healthy			App. healthy			Diseased			Category	Leaf position
	1	4	9	1	4	9	1	4	9		
Total chlorophylls (mg g ⁻¹ fresh wt.)	2.03a	2.78	2.82	3.01	3.98	3.61	3.47	3.64	0.65	0.47**	0.47**
							(+13.00)	(-8.5)	(-82.27)		
Chlorophyll a (mg g ⁻¹ fresh wt.)	1.49	2.04	2.03	2.11	2.64	2.47	2.27	2.48	0.42	0.29**	0.29**
							(+7.58)	(-5.7)	(-83.06)		
Chlorophyll b (mg g ⁻¹ fresh wt.)	0.53	0.75	0.79	0.90	1.35	1.14	1.20	1.16	0.23	0.23**	0.20**
							(+24.36)	(-14.00)	(-80.53)		
Chlorophyll a/b ratio	2.83	2.78	2.60	2.46	2.97	2.29	2.93	2.14	2.21	0.40**	NS
							(-21.00)	(+7.9)	(-4.5)		
Carotenoids (mg g ⁻¹ fresh wt.)	0.50	0.67	0.63	0.65	0.75	0.79	0.67	0.74	0.16	0.12**	0.12*
							(+3.07)	(-1.33)	(-79.74)		

a Each value is the mean of six palms

b Values in parentheses show percentage increase (+) or decrease (-) over apparently healthy palms

** or * significant at P = 0.05; NS = Not Significant.

Total sugar content increased in the middle and outer leaves of the diseased palms by 35.93% and 37.96% respectively. Starch content increased by 33.20% and 23.48% in the middle and outer leaves respectively, over that of the controls (Table 4).

Table 4. Total sugars, reducing sugars and starch in healthy, apparently healthy and yellow leaf disease affected leaves of adult arecanut palms.

Parameter/leaf position	L.S.D							
	Healthy		App. healthy		Diseased		Category	Leaf position
	4	9	4	9	4	9		
Total sugars (mg/g-l fresh wt.)	13.31a	15.01	17.24	18.74	26.91	30.21	4.75**	NS
					(+35.93)	(+37.96)		
Reducing sugars (mg g ⁻¹ fresh wt.)	8.81	9.77	8.82	7.85	19.23	23.29	2.63**	NS
					(+118.02)	(+196.08)		
Starch (mg g ⁻¹ fresh wt.)	16.07	46.69	44.07	40.05	60.05	12.34	8.14**	NS
					(+33.20)	(+23.48)		

a Each value is the mean of six palms.

b Values in parentheses show percentage increase (+) or decrease (-) over apparently healthy palms

** or * significant at P = 0.05; NS = Not Significant

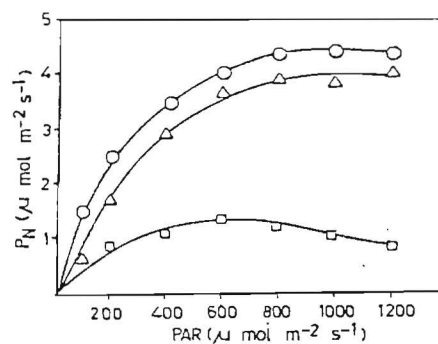


Fig. 1. Relationship between photosynthesis and photosynthetically active radiation (PAR) in healthy (O - O), apparently healthy (Δ - Δ) and YLD affected arecanut leaves (\square - \square).

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Infection by YLD significantly reduced net photosynthesis over a wide range of irradiances with maximum reduction at higher irradiances (Fig-1). In healthy palms PN saturated at $800 \mu\text{mol m}^{-2} \text{s}^{-1}$ whereas in diseased palms saturation occurred at $500 \mu\text{mol m}^{-2} \text{s}^{-1}$. The net photosynthetic rates in both apparently healthy and infected leaves are shown in Table 1 & 2. Diseased young palms showed 37.09%, 46.72% and 71.16% reduction in net PN in first, middle and outer leaves respectively, over that of controls. In diseased adult palms, PN was significantly lower by 7.5%, 29.96% and 61.72% in first, middle and outer leaves respectively than that of controls.

The diseased palms showed significantly higher r_s and ψ_w as compared to apparently healthy palms. As a result of stomatal closure, E was significantly reduced in outer and middle whorls of diseased palms. Further, stomatal regulation was impaired due to infection irrespective of the age of the palms. Similarly in American elms, white ash, chokecherry, periwinkle and coconut showing typical 'yellows' type of symptoms induced by respective MLO's agents, r_s and ψ_w were higher than in healthy controls indicating that infection caused stomatal closure (Matteoni and Sinclair 1983, McDonough and Zimmermann 1979). Induction of stomatal closure by internal factors such as ABA in response to water deficits (Walton, 1980) or high C_i (Zeiger, 1983) or toxins under the stress of microbial attack (Strobel, 1974) have been reported earlier. The leaves of first whorl of the diseased palm had stomatal opening despite higher C_i as compared to the outer leaves where r_s was higher. 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 yellows diseases of periwinkle caused by *Spiroplasma citri* (Daniels, 1979).

The net PN of diseased palms was significantly reduced by infection with MLOs associated with YLD of arecanut. The importance of stomatal closure in regulating PN due to infection in 'Yellows' diseases was revealed by earlier findings of parallel reductions of PN and E as disease developed (Matteoni and Sinclair, 1983). However, in the present study, the photosynthetic CO_2 assimilation was reduced at high C_i with decreased PN /cs ratio which implies that mesophyll factors are more affected than stomatal factors. Jacob and Udaya Kumar (1988) suggested that changes in PN /cs ratio reflects stress imposed effects on the stomatal and mesophyll factors. A decrease in PN /cs ratio leads to an increase in C_i which implies that mesophyll capacity for PN is affected than cs.

Reductions in chlorophyll content (Table 3) in the leaves of diseased palms could reduce PN by decreasing overall photophosphorylation resulting in less generation of ATP and NADPH (Hopkins and Hampton, 1969). It is obvious that the decrease

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in ATP and NADPH levels in the diseased leaves could exert major control on PN as photosynthetic reduction cycle (PCR) can not function without continued supply of these two metabolites (Robinson and Walker, 1981). Similarly reductions in chlorophylls have been reported in periwinkle (Carling and Miliken, 1977), sugarcane (Shukla, *et al.*, 1984) Sandal wood (Parthasarathi, *et al.*, 1976) and *Tephrosia purpurea* (Purohit, 1978) with their respective MLO's agents.

Accumulation of soluble sugars and starch in leaves during photosynthesis is a common phenomenon in plants either due to abiotic stress conditions such as water deficit, chilling or due to low translocation rates or low sink demand (Neales and Incoll, 1968). Nayar (1976) reported phloem necrosis in YLD affected arecanut palms. Due to this, sugar translocation might be disrupted resulting in the accumulation of sugars and starch in the diseased palms. Similar increase in starch and sugar content has been reported in brinjal little leaf (Srinivasan and Chelliah, 1980), in little leaf of cotton (Nalini and Bidari, 1985) and in *Petalium murex* phyllody (Joshy and Mishra, 1981) incited by their respective MLO's agents. The impeded removal of these assimilates in the diseased palms following phloem interruption could reduce PN, probably due to an orthophosphate (pi) limitation (Stitt and Quick, 1989).

Infection with YLD significantly reduced PN over a wide range of irradiances with maximum reduction occurring at higher irradiances could be attributed to photoinhibition due to reduction carotenoid pigments. The lower levels of carotenoid pigments 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. That excess in light may cause photo inhibitory damages to photosynthetic apparatus due to destruction of excitation energy dissipating mechanisms which is probably mediated by carotenoid pigments (Powell, 1984).

Thus the study indicates that MLOs associated with YLD include stomatal closure despite higher P, thereby reducing E significantly. The results also suggest that PN/cs ratio decreased in diseased palms leading to accumulation of C_i which implies that mesophyll factors are more affected than stomatal factors. The mesophyll factors such as reduced levels of chlorophylls, Carotenoids and increased levels of sugar and starch decreased CO_2 assimilation in diseased palms. In conclusion, the overall disturbance in the water relations and photosynthetic apparatus precede 'yellowing' symptoms in YLD affected arecanut palms.

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