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Abnormal Stomatal Opening in Coconut Palms Affected with Root (Wilt) Disease

V. RAJAGOPAL,[1] K. D. PATIL AND B. SUMATHYKUTTYAMMA

Central Plantation Crops Research Institute, Regional Station, Kayangulam 690 533, Kerala, India

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ABSTRACT

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Studies on the stomatal regulation in the root (wilt) affected coconut palms (*Cocos nucifera* L.) revealed that the diseased palms had low stomatal resistance compared to the healthy palms, irrespective of their age. The same trend was observed whether the determinations were made at different times of the day (6-18 h) or under irrigated and unirrigated conditions or in different seasons ('dry' and 'wet'). Thus, the stomatal regulation was significantly impaired in the diseased palms resulting in excessive water loss compared to the healthy palms. Results are discussed with the available literature on other similar disease caused by fungi, bacteria and mycoplasma-like organisms in different plants.

Key words—Cocos nucifera L., Stomatal resistance, Root (wilt) disease.

Correspondence to: Central Plantation Crops Research Institute, Regional Station, Kayangulam 690 533, Kerala, India.

INTRODUCTION

The main external symptom of the root (wilt) disease in coconut is the flaccidity of leaves which, in the early stages appear as flattening and bending of leaflets, leading to ribbing at later stages (Radha and Lal, 1972). This flaccidity symptom appears like the 'wilting' caused in plants under conditions of water stress. There is evidence to show that the disease symptom occurs in all soil types and seasons and also under water-logged conditions (Anonymous, 1981). Root (wilt) is a non-lethal disease, but debilitating in nature.

In general, organisms associated with 'wilt' diseases in plants include *Verticillium dahliae* (Alexander and Hall, 1974), *Fusarium oxysporum lycopersci* (Duniway, 1971*a*), *Ceratocystis ulmi* (Mac Hardy and Beckman, 1973), *Pseudomonas solanacearum* (Husain and Kelman, 1958) and *Xanthomonas campestris* (Sutton and Williams, 1970). Either vascular occlusions or the toxins produced by the organism were shown to be the causes for the wilt disease (Mac Hardy, Hall, and Busch, 1974); Dimond, 1966; Rai and Strobel, 1969). These observations were strengthened by the studies on stomatal resistance and leaf water potential changes in wilted and wilt-free leaves of *Chrysanthemum* (Hall, Ali, and Busch, 1975; Mac Hardy, Busch, and Hall, 1976), tomato etc. (Duniway, 1971*a*).

In the case of root (wilt) disease of coconut the association of any fungus or bacterium has been ruled out (Anonymous, 1981). Recently, Solomon, Govindankutty, and Nienhaus

[1] Present address: CPCRI, Kudlu Post, Kasaragod 670 124, Kerala, India.

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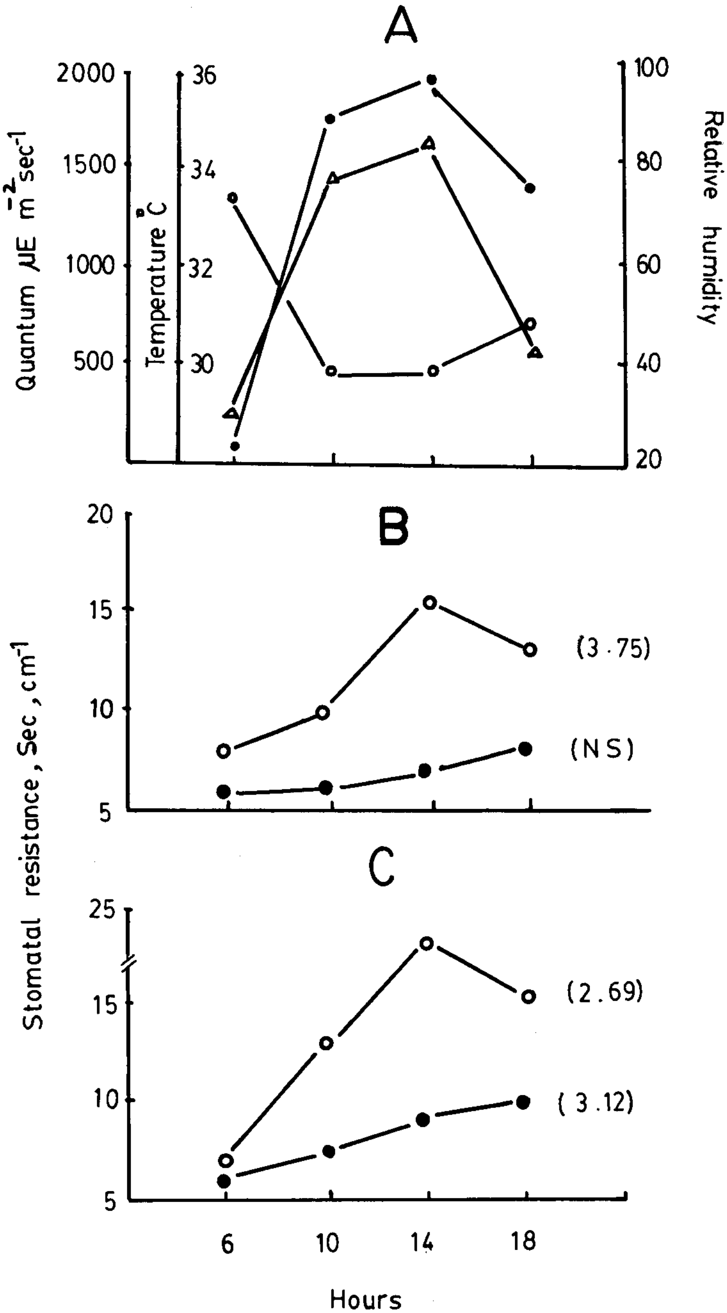

FIG. 1. Daytime fluctuations in agroclimatic conditions and stomatal resistance in coconut garden. (A) Agroclimatic parameters were recorded at the experimental site. -●- light, -▲- temperature, -o- relative humidity. Stomatal resistance was measured on (B) first and (C) middle leaves of -o- apparently healthy and -●- diseased palms. Values are means of nine palms. C.D. at 1% level are indicated in parenthesis.

(1983) had observed under the electron microscope the presence of mycoplasma-like organisms (MLOs) in the sieve elements of phloem tissues in the root tips, leaf petiole and inflorescence axis of root (wilt) diseased palms, while they were totally absent in healthy tissues. Plant species affected by MLOs were characterized by yellows disease, including lethal yellowing disease of coconut, associated with stomatal closure i.e. high diffusive resistance (Matteoni and Sinclair, 1983). Though variations in stomatal frequency, but not stomatal index, was noticed between healthy and diseased palms (Mathew, 1981), a clear understanding of the processes involved in or leading to the characteristic flaccidity symptom is lacking. There was poor uptake of water through the roots of diseased palms (Rajagopal, Mathew, Patil, and Abraham, 1986), but a thorough insight into aspects associated with water loss was not reported. Hence, the main objective of the present investigation was to study the stomatal regulation in the healthy and diseased palms and to examine the differences in wilt symptoms caused by different organisms.

MATERIALS AND METHODS

Coconut palms (*Cocos nucifera* L. var. West Coast Tall) raised in the Institute Farm at Kayangulam (disease-affected area) and at Kasaragod (disease-free area) were used for the experiments. Palms were maintained with the usual cultural and agronomic practices, which included spacing and fertilizer dose of 500 : 320 : 1200 grams of N : P : K per palm per year. In one of the experiments, palms receiving weekly irrigation during summer months and those maintained under rainfed conditions were also taken up for comparison.

The stomatal resistance of the first fully opened leaf and the middle leaf (10th to 14th depending on the age of the palm) were determined with Li-Cor 1600 Steady State Porometer (Lamda Instruments, Nebraska, U.S.A.) as described earlier (Rajagopal, Mathew, and Varkey, 1982). The first leaf was selected because it normally does not exhibit external symptoms of the disease, whereas the middle leaf was selected as it exhibits the typical flaccidity symptom of the disease. Briefly, the methods consisted of determinations of diffusive resistance in two leaflets on the abaxial surface (hypostomatic type) of the lamina at five positions each along the leaflet on either side of the midrib. Prior to each measurement, the microclimatic conditions like light, temperature and relative humidity near the palm were also recorded. Stomatal resistance was expressed as $s\ cm^{-1}$. The number of palms studied in each case is indicated under Tables and Figures with statistical analysis.

RESULTS

When the determinations on the stomatal resistance were made at different times of the day, i.e. from 6 h to 18 h, the leaves of 7-year-old apparently healthy palms clearly showed an increase in the leaf diffusive resistance with increase in light, temperature and low relative humidity (Fig. 1). On the other hand, the stomata of diseased palms remained open through most of the day and thus resulted in higher loss of moisture (ranging from 4·0 to 6·15 $\mu g\ cm^{-2}\ s^{-1}$) compared to healthy palms (1·30 to 1·85 $\mu g\ cm^{-2}\ s^{-1}$). Even the adult palms, 30- to 35-year-old, from the disease free zone and the apparently healthy palms from the disease tract had higher diffusive resistance than the diseased palms (Fig. 2).

When the palms irrigated during summer were compared with unirrigated, once again the diseased palms consistently showed lower stomatal resistance under both the conditions than the apparently healthy palms (Table 1). Though irrigated, the apparently healthy palms showed stomatal resistance somewhat similar to those under unirrigated obviously due to the atmospheric drought that prevailed during the period.

When we studied these changes during the ‘wet’ season a somewhat different picture emerged. Leaf diffusive resistance was considerably lower in the apparently healthy palms during the ‘wet’ season compared to the ‘dry’ season (Table 2). Diseased palms exhibited still lower resistance than during the ‘dry’ season. However, it should be noted that the difference

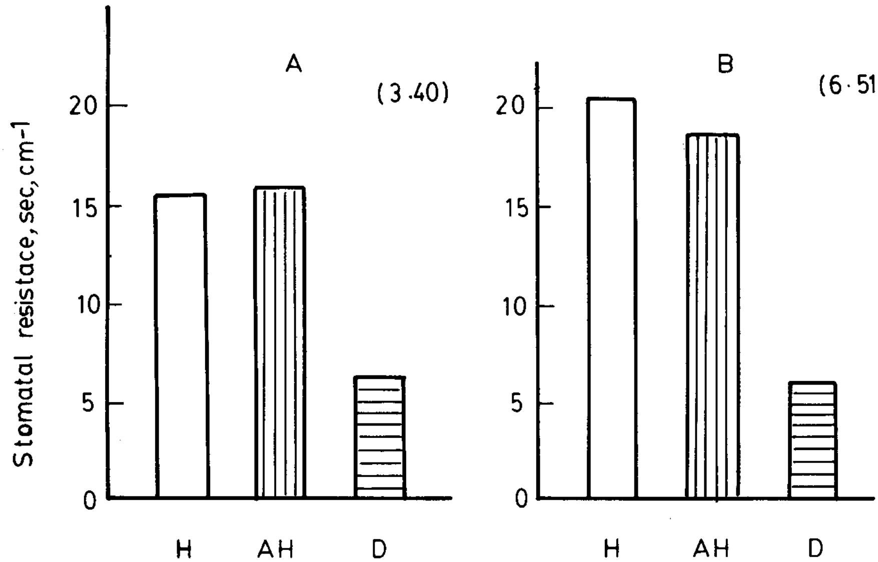

FIG. 2. Stomatal resistance in the (A) first and (B) middle leaves of healthy (H), apparently healthy (AH) and diseased (D) palms. Healthy palms were selected from a disease-free area and the latter two categories from a disease prevalent area. Values are means of 13 palms. C.D. at 1% level are indicated in parenthesis.

TABLE 1. *Stomatal resistance in the leaves of apparently healthy and diseased adult palms under irrigated and unirrigated conditions*
Values are means of 10 palms.

Treatment (A)	Palm condition (B)	Leaf position (C) First (stomatal resistance s cm^{-1})	Middle
Unirrigated	Apparently healthy	19·07	23·03
	Diseased	5·38	5·88
Irrigated[a]	Apparently healthy	17·16	20·89
	Diseased	5·33	6·74

C.D. at 1%: A = n.s. B = 2·81 C = n.s.

[a] A set of palms received summer irrigation at the rate of 250 dm^3 per palm per week. Measurements were taken when the soil moisture content was 7–8% on dry weight basis (at 30 cm and 60 cm depths), while in the unirrigated plot it was 2·0–2·3%.

in stomatal resistance between the apparently healthy and diseased palms was more discernible during the 'dry' than during the 'wet' season.

DISCUSSION

That the root (wilt)-affected coconut palms failed to close their stomata in response to soil and atmospheric drought, as against apparently healthy palms which conserved moisture by effectively regulating the stomata is evident (Table 1). Thus, the stomatal regulation was adversely affected due to the disease irrespective of the age of the palm, time of the day and

TABLE 2. *Stomatal resistance in the leaves of apparently healthy and diseased young palms during the 'dry' (March) and 'wet' (September) season*
Values are means of nine palms.

Season[a] (A)	Palm condition (B)	Leaf position (C)	
		First	Middle
		(stomatal resistance s cm^{-1})	
Dry	Apparently healthy	14·33	16·53
	Diseased	4·52	7·95
Wet	Apparently healthy	1·82	1·82
	Diseased	1·58	1·47
	C.D. at 1%: A = 1·83	B = 1·83	C = 2.59

[a] Changes in the agroclimatic factors (light, temperature and relative humidity) were recorded near the experimental palms, with the Steady State Porometer.

season (Figs 1, 2 and Table 2). From these observations it appears that damage was caused to the stomatal apparatus of diseased palms, though the nature of damage is not clear. These resulted in altered water potential which was of the order of −1·5 to −1·6 MPa in the leaves of healthy palms as against −1·9 to −2·1 MPa in those of diseased palms (Rajagopal, Sumathykuttyamma, and Patil, 198[illegible]).

The controlling influence of stomata on the water balance of coconut palms was reported earlier (Milburn and Zimmermann, 1977). The authors suggested that the water balance of palms was more protected in the 'dry' than in the 'wet' season, as was also indicated by the healthy palms in the present study. In the diseased palms, however, the stomatal regulation was affected in both the seasons, though with greater magnitude in the 'dry' than in the 'wet' season. It was also obvious that of all the climatic factors, relative humidity differences between the two seasons (37·4% and 52·4% in the 'dry' and 'wet' seasons respectively) influenced greatly the stomatal regulation, as also reported by Schultz, Lange, Evenari, Kappen, and Bushbom (1974). From the foregoing, it could be inferred that the healthy palms develop a protective mechanism to regulate their water balance through an effective stomatal system, while this protective mechanism seems to have been greatly impaired in the palms affected by the root (wilt) disease.

In 'wilt' caused by either *Verticillium* sp. or *Fusarium* sp. the vascular occlusion was observed (Mac Hardy *et al.*, 1974; Dimond, 1966). This implied that wilt symptoms appeared due to blockage in the movement of water through the xylem vessels. Further evidence was obtained through determinations of stomatal resistance which was relatively high in wilt affected leaves (Hall *et al.*, 1975; Mac Hardy *et al.*, 1976; Duniway, 1971*a*), thus indicating that loss of water was not the cause for wilts. Bacterial wilts like the one by *Corynebacterium sepedonicum* in potato differed from the above in that the toxins produced by the organism in the host tissues altered the membrane structures of cells, thereby affecting both absorption and retention of water which led to wilting (Rai and Strobel, 1969).

Abnormal stomatal opening is characteristic of certain diseases like canker disease of peach and almond caused by *Fusicoccum amygdali* (Ballio, D'Alessio, Randazzo, Bottalico, Graniti, Sparapano, Bosnar, Casinovi, and Gribanovski-Sassu, 1976), potato blight by *Phytophthora infestans* (Farrell, Preece, and Wren, 1969), barley leaf blotch by *Rhynco-sporium secalis* (Ayres, 1972), rust in bean by *Uromyces phaseoli* (Duniway and Durbin,

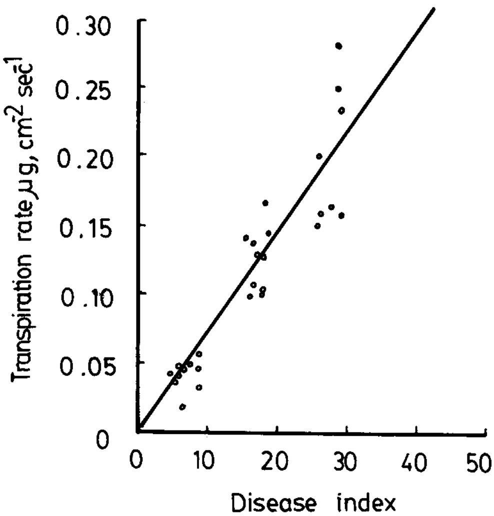

FIG. 3. Relationship between disease index and transpiration rate in coconut palms.

1971), which reflect on the high rate of transpiration. For instance, the leaf diffusive resistance of bean infected by *U. phaseoli* was only 8 s cm^{-1}, whereas in a healthy leaf it was 50 s cm^{-1} (Duniway and Durbin, 1971). Though caused by MLO, root (wilt) diseased palms also exhibited lower stomatal resistance (ranging from 1·5 to 6·8 s cm^{-1}) as compared to healthy leaves (13·5 to 22·0 s cm^{-1}). A relationship between the disease index and transpiration rate was plotted in Fig. 3. It is evident that with the advancement of disease there is greater disturbance in stomatal regulation resulting ultimately in excessive transpiration. A similar relationship between the severity of visual symptoms and high stomatal resistance was reported in some of the MLO-caused yellows diseases (Matteoni and Sinclair, 1983). The possibility of employing the stomatal diffusion resistance measurements for early detection of yellows disease in periwinkle and white ash prior to visual symptom expression was highlighted by Matteoni and Sinclair (1981) and also in root (wilt) diseased coconut palms (Rajagopal *et al.*, unpublished).

The trend in stomatal behaviour observed in the present study is somewhat similar to that with fusicoccin, a phytotoxic terpenoid glucoside produced by the fungus *Fusicoccum amygdali* in almond. Besides causing wilting of leaves and necrotic lesions in many plant species, fusicoccin was shown to favour opening of stomata both in light and dark, thus resulting in enhanced rate of transpiration (Chain, Mantle, and Milborrow, 1971; Heichel and Turner, 1972). Based on the response to two cultivars of almond (resistant and susceptible to the fungus) to fusicoccin treatment, Turner and Graniti (1976) suggested that the toxin moves to the leaves and induces K^+ accumulation and turgor changes that open the stomata. A similar mechanism might be operating in the leaves of root (wilt) diseased palms although the nature of toxin produced is yet to be investigated. Toxins have been implicated in yellows disease of periwinkle caused by *Spiroplasma citri* (Daniels, 1979). It is evident that MLOs produce metabolites that influence overall stomatal regulation—either their closure as in yellows diseases of *Ulmus americana, Fraxinus americana, Catharanthus roseus,* x-disease in *Prunus virginiana* and corn stunt in *Zea mays* (Matteoni and Sinclair, 1983) or stomatal opening as in the present study. Thus, the only 'wilt' disease where MLO is associated is root (wilt) of coconut and it is the first report of abnormal opening of stomata in an MLO disease.

The involvement of a fusicoccin-like substance in root (wilt) disease is strengthened by a preliminary study with a histological staining technique for locating K^+ in guard cells under the fluorescent microscope, which revealed a relatively high content of K^+ in leaves with the flaccidity symptom (Rajagopal and Govindankutty, unpublished). The fact that the impairment of stomatal regulation occurs in leaves even before wilting symptoms are expressed (Rajagopal *et al.*, unpublished), indicates that the metabolites produced by the organism might influence the water relation aspects. For instance, the leaf water potential was reduced more than 50% in the spindle leaf (unopened) of diseased palms compared to healthy, although the distinguishable wilting symptom appear only when the leaf reaches the middle whorl (Rajagopal *et al.*, 1987).

Excessive transpirational loss of water in the diseased palms is not replenished adequately, since the uptake of water by the roots of diseased palms is significantly affected (Rajagopal *et al.*, 1986). Thus, there is an imbalance in the water economy of diseased palms, ultimately leading to an irreversible flaccidity symptom. This contrasts with other wilt diseases described above, wherein vascular occlusion or toxins affect the movement of water towards the leaves, but not transpiration, leading to wilt symptoms. The uniqueness of root (wilt) disease thus seems to lie in the impairment caused to both the facets of water transport namely, absorption and transpiration leading to internal water stress, the degree of which depend on the intensity of the disease. In conclusion, the overall disturbance in the water relations precede the flaccidity symptom in root (wilt) diseased coconut palms.

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