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Research Articles

INFLUENCE OF WEATHER VARIABLES ON THE CONTENT AND COMPOSITION OF LEAF SURFACE WAX IN COCONUT *

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ABSTRACT

The epicuticular wax content (ECW) was estimated in rainfed coconut genotypes namely, West Coast Tall (WCT), WCT x Chowghat Orange Dwarf (COD) and COD x WCT between October, 1987 and August, 1988, with simultaneous measurement of weather parameters like light, temperature and relative humidity. The weather data revealed the prevalence of atmospheric and soil drought between January and May, 1988. The ECW content was higher during drought than during the non-stress periods. Thin layer chromatographic separation of the wax components indicated their qualitative differences at different seasons and among coconut genotypes.

INTRODUCTION

Leaf cuticle plays a major role in limiting water loss from leaves. Epicuticular wax (ECW) is an important factor for reducing transpiration during periods of water stress (Skoss, 1955; Hall and Jones, 1961). The development of ECW during moisture stress varies in different crops and depends on environmental factors like high radiant energy and low humidity (Baker, 1974). In soybean accumulation of ECW was associated with water stress (Clark and Levitt, 1956). A similar report on stress-induced increase in ECW was made in sorghum (Ebercon *et al.* 1977), barley (Giese, 1975) and oat (Bengtson *et al.* 1978). ECW content declines with the onset of rainfall (Mayeux and Jordan, 1987). Unlike annual crops, the report on ECW of perennial tree crops is limited (Balasimha *et al.* 1985; Mohammed *et al.* 1986 and Rajagopal *et al.* 1989, 1990a and b).

The coconut palm experiences moisture stress during summer months (January to May) under rainfed conditions. Rajagopal *et al.* (1989) have reported the critical soil moisture level for stress in coconut palms, while Kasturi Bai *et al.* (1988) have studied the impact of weather variables on stomatal resistance in two coconut genotypes. Although an earlier report by Kurup *et al.* (1993) revealed the seasonal changes in leaf water potential in a tall and two hybrids of coconut, there was no indication on the relative changes in ECW, which is an essential component directly related with drought resistance of crop plants. Hence, the present paper reports the quantitative and qualitative changes in ECW during the non-stress and stress periods in relation to prevailing weather conditions namely light, temperature, relative humidity and rainfall.

MATERIALS AND METHODS

Growing conditions

Twenty two years old coconut *(Cocos nucifera L.)* palms comprising local tall (West Coast Tall, WCT) and two hybrids WCT x Chowghat Orange Dwarf (COD) and COD x WCT maintained by the Agronomy division formed the experimental material and were described earlier (Kurup *et al.* 1993). The palms

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are grown in red sandy loam soil under rainfed conditions with three fertilizer levels, laid out in a randomized block design. There are three replications of six palms each and the palms were planted at a spacing of 7.5 x 7.5m. For the present study, palms under one fertilizer level namely 1000g N, 100g P_2O_5 and 2000g K_2O palm^{-1} year^{-1} applied in split doses ($^1/_3$ during May-June and $^2/_3$ in October) were taken up. The experiments were conducted between October, 1987 and August, 1988.

Observations on weather variables

Weather parameters like light, temperature and relative humidity were measured in the vicinity of experimental palms between 1100 and 1300 h for five consecutive days in a month using the portable steady state porometer (Li-Cor 1600, USA), as described earlier (Kasturi Bai *et al.* 1988). Data on rainfall and pan evaporation were collected from the meteorological station of the institute, adjacent to the experimental site.

Quantitative estimation of epicuticular wax content (ECW)

The ECW was extracted from the first (from top downwards) and sixth leaf of three palms per genotype following the method of Ebercon *et al.* (1977), adopted earlier for coconut (Rajagopal *et al.* 1989). Segments of 3 x 1 cm were cut from the leaflets and 20 such segments were plunged into a beaker containing 15 ml chloroform and vigorously shaken for 15 to 20 seconds and decanted. This method extracts the wax from both the surface of leaflets. The extract was evaporated under vacuum. Five ml of potassium dichromate reagent was added to the dry sample. The samples were placed in boiling water for 30 minutes. After cooling, the volume was made up to 17 ml with distilled water and the colour developed was read at 590 nm on a Perkin Elmer Spectrophotometer. Internal wax is used as standard and the values are expressed in µg cm^{-2}.

Qualitative analysis of ECW

The composition of ECW in coconut was qualitatively analysed by thin layer chromatography (TLC) (Voleti and Rajagopal, 1991). For TLC separation, leaflets from the first and sixth leaf positions were taken and cut into small pieces and 10gm of such leaf tissues were taken for extraction. The leaf cuttings were stirred in 20ml chloroform for 20 to 30 seconds and decanted. The extract was concentrated to 1ml and aliquots were used for chromatographic separation.

TLC plates (20 x 20 cm) were coated with silica gel G (1 mm thickness), on which 100 µg samples were loaded. Two solvent systems were employed for the separation of wax composition. Solvent system I consisted of benzene and acetic acid (100:10), while the solvent system II comprised petroleum ether, toluene, diethylether, hexanol and methanol (140:30:30:10).

Plates were run to 10 cm in solvent system I, taken out and dried at room temperature and again run in solvent system II in the same direction. The spots were detected by placing the plate in a chromatographic chamber saturated with iodine vapour (Barrett, 1962). The brown spots were marked and compared with durum wheat leaf wax. The Rf values were recorded for comparison.

RESULTS

The weather parameters showed variations during the experimental period ng from October 1987 to August 1988 1). The mean light intensity during October nd November ranged between 600 and 900 μE $m^{-2}s^{-1}$ and increased sharply between January and May with peak value of 1850 μE $m^{-2}s^{-1}$, followed by a marked fall in August. ambient temperatures rose from 28°C in ober to 34.5°C in April and declined to 29°C in August. The relative humidity was 82 per cent in October, and dropped to 45 per cent in April and again rose to 86 per cent in August. The rainfall recorded was 100.5 mm during October and November, while January to May recorded a meagre rain fall of 24.9 mm. The al amount of precipitation between June and gust was 1550 mm. Based on the weather parameters, the period between October and December could be considered as pre-stress, while the months between January and May is a period of moisture stress; the months between June and August constituted post-stress period.

The ECW content was estimated at the above distinct stages in the tall cultivar and hybrids. In all the three, the first leaf had higher ECW during pre-stress and showed marginal change during stress period (Fig. 2). In contrast, the wax content of sixth leaf exhibited a sharp increase during stress in all the types, with WCT x COD having higher increase than the other two. There was rapid decline in ECW in both the leaves of the three genotypes during post-stress period. From the Table 1, it is clear that the ECW was highly significant (1% level) for stage and leaf position. The interaction between genotype, stage and leaf position were also significant at 1 per cent level, while that between the genotype and stage showed significance only at 5 per cent level. There was no significant difference in the interaction between the genotype and leaf position.

Depending on the leaf positions, the

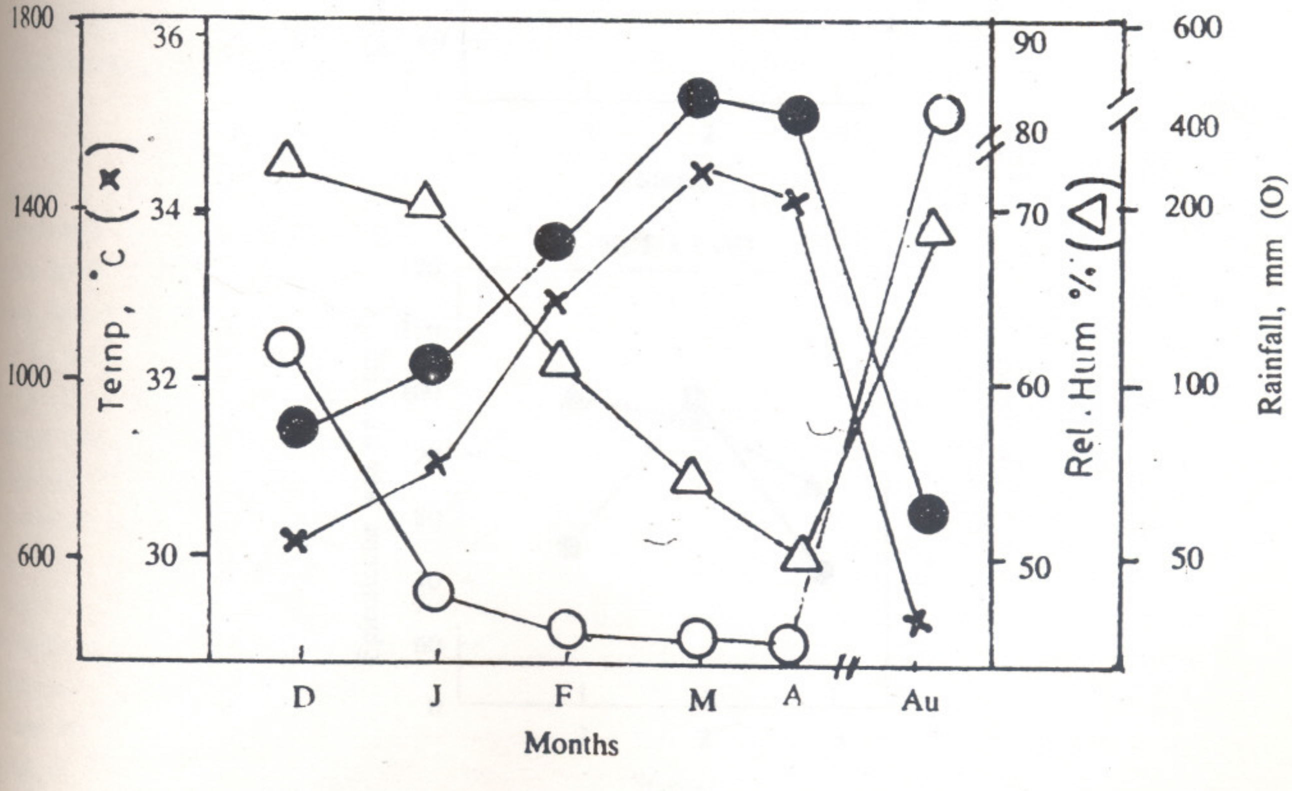

Fig. 1. **Weather parameters during the experimental period**

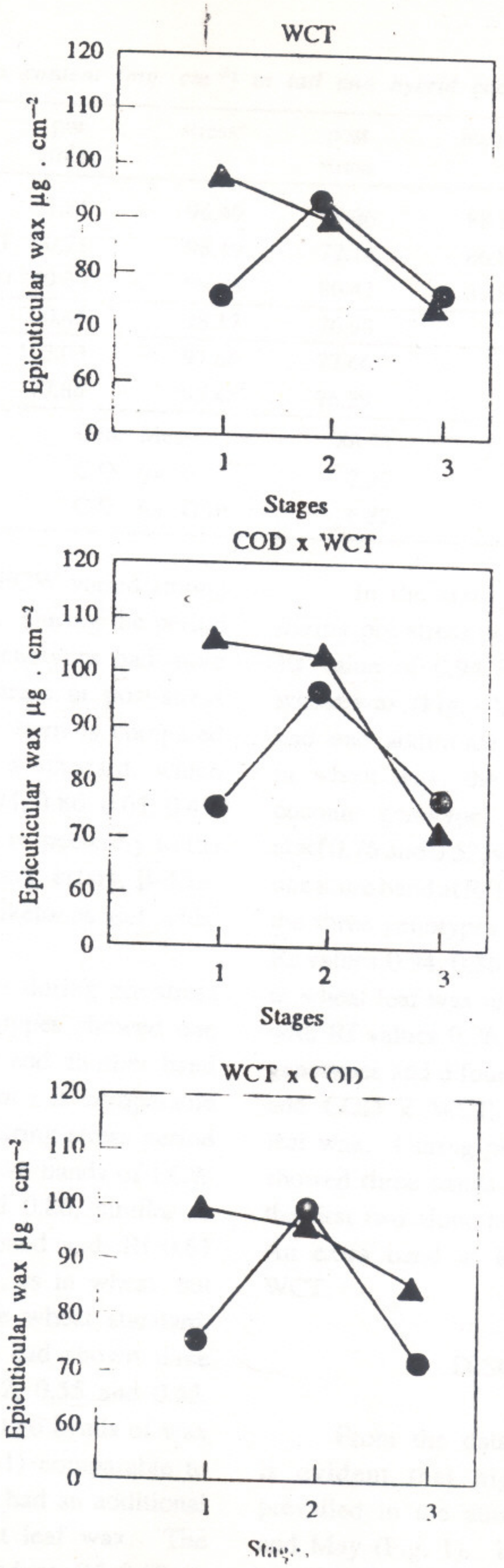

Fig. 2. Epicuticular wax content in coconut genotypes during stress development

▲—▲ 1st Leaf ●—● 6th Leaf

1. Pre-stress 2. Stress 3. Post-stress

le I. *Epicuticular wax content (mg. cm^{-2}) in tall and hybrid coconut*

		pre stress	stress	post stress	mean	Position 1	Position 6
Genotype	WCT	91.81	96.49	78.36	88.89	91.67	86.11
	COD x WCT	90.21	98.19	72.14	86.85	91.63	82.07
	WCT x COD	89.33	99.83	80.42	89.86	95.25	84.47
	Mean	90.45	98.17	76.98			
Position	1	103.03	97.86	77.66			
	6	77.88	98.48	76.29			
S.E./plot	4.11	Gen. Mean		88.53	C.V (%)		4.64
C.D. for S	2.79	C.D. for P		2.27	C.D. for GS		4.83
C.D. for SP	3.94	C.D. for GSP		6.82			

qualitative development of ECW varied among the tall cultivar and hybrids. During the period of moisture stress all the genotypes had more bands than either the pre-stress or post-stress periods. The ECW band pattern is compared with durum wheat leaf as a standard, which had following Rf values : 0.94, 0.86, 0.61, 0.49, 0.31 and 0.12 corresponding respectively to the wax components hydrocarbons, esters, β-diketones, alcohols, hydroxy β-diketones and acids.

The ECW components during pre-stress period in all the three genotypes showed one band with Rf value of 0.94 and another band with Rf 0.72, the former alone is comparable with wheat wax (Fig. 3). During stress period all the three genotypes had two bands of ECW with Rf values of 0.94 and 0.86, similar to wheat wax. An additional band with Rf 0.61 occurred in both the hybrids, as in wheat, but absent in WCT. Unlike the wheat standard, the three coconut genotypes had shown three bands with Rf values of 0.72, 0.55 and 0.52. Post-stress period resulted in two bands of wax component (Rf 0.94 and 0.31) comparable to wheat leaf wax. WCT alone had an additional band at Rf 0.12 as in wheat leaf wax. The wax component that appeared at Rf 0.52 in all the three genotypes is not identifiable in wheat.

In the sixth leaf the ECW components during pre-stress period showed one band with Rf value of 0.94 in all the genotypes, as in wheat wax (Fig. 4), while COD x WCT alone had one additional band at Rf 0.49. Unlike in wheat wax, the leaf wax of all the three coconut genotypes had two additional bands at Rf 0.76 and 0.52, with COD x WCT possessing one more band at Rf 0.73. Under stress condition, the three genotypes had three bands each with Rf values 0.94, 0.86 and 0.49 which are present in wheat leaf wax also. Three additional bands with Rf values 0.76, 0.73 and 0.52 in the three genotypes and a fourth band at Rf 0.83 in WCT and COD x WCT are not present in wheat leaf wax. During post-stress all the genotypes showed three bands at Rf 0.94, 0.49 and 0.52, the first two alone being similar to wheat wax. An extra band at Rf 0.76 appeared only in WCT.

DISCUSSION

From the data on weather variables it is evident that high evaporative demand prevailed in the atmosphere between January and May (Fig. 1). High pan evaporation (5.5 mm day^{-1}) and rainless condition during the period indicated the atmospheric drought, which was further accentuated by soil drought with

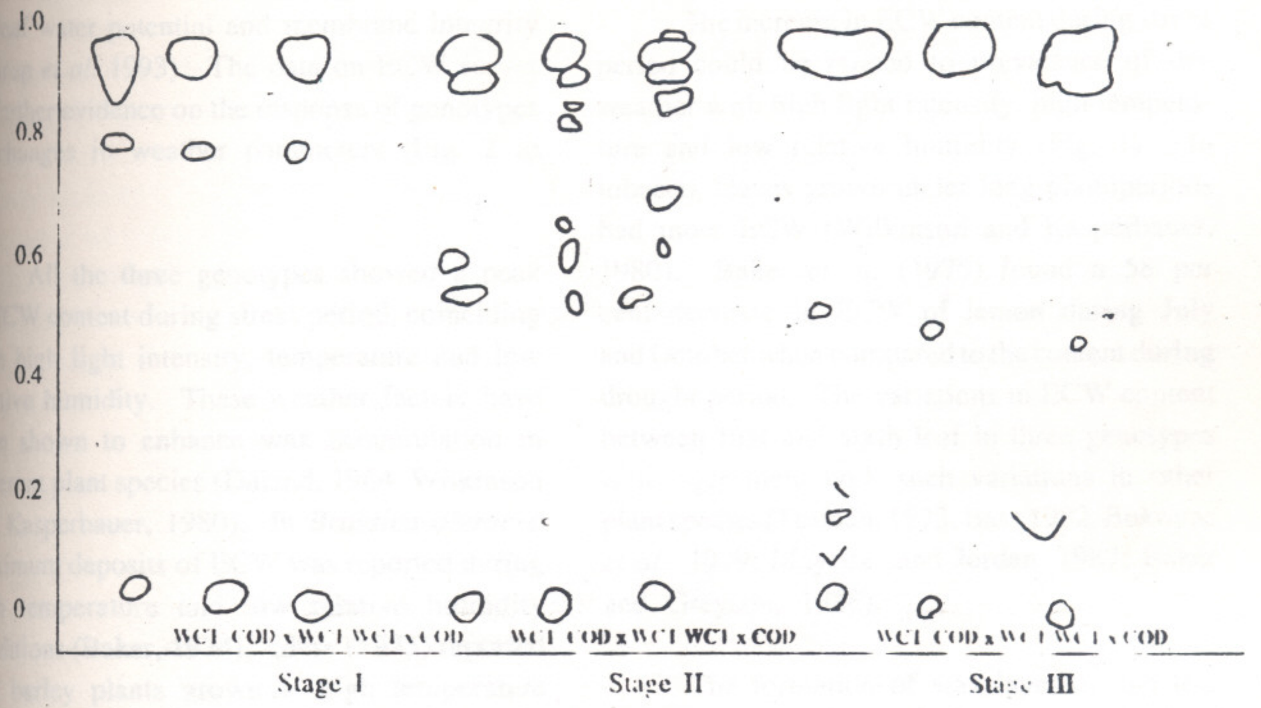

Fig. 3. Thin layer chromatography of ECW from the Ist leaf of three coconut genotypes

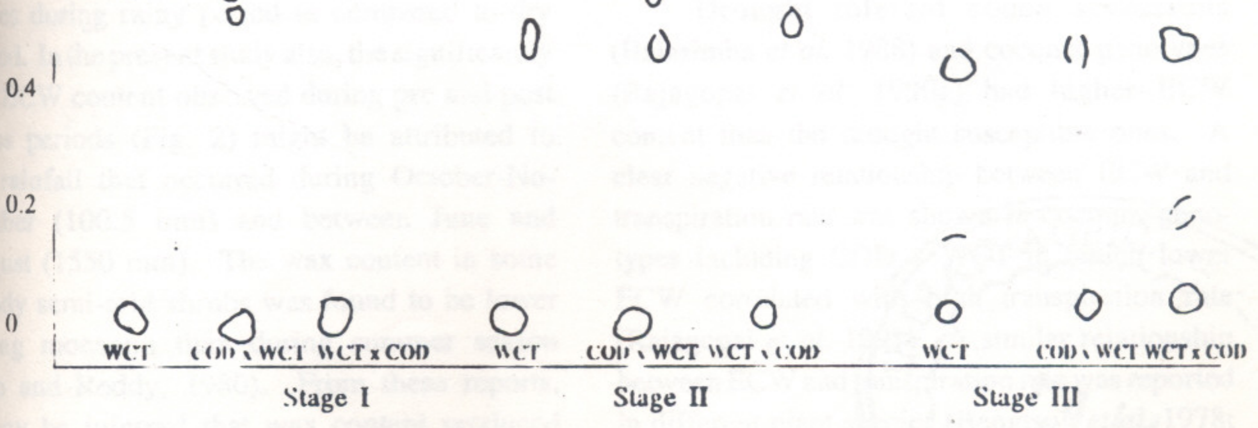

Fig. 4. Thin layer chromatography of ECW from the 6th leaf of three coconut genotypes

depleted soil moisture availability (Kurup *et al.* 1993). Coconut genotypes responded to the moisture stress to different degrees in terms of leaf water potential and membrane integrity (Kurup *et al.* 1993). The data on ECW serves as further evidence on the response of genotypes to changes in weather parameters (Fig. 2 to 4).

All the three genotypes showed a peak in ECW content during stress period, coinciding with high light intensity, temperature and low relative humidity. These weather factors have been shown to enhance wax accumulation in different plant species (Daland, 1964, Wilkinson and Kasperbauer, 1980). In *Brassica oleracea* maximum deposits of ECW was reported during high temperature and low relative humidity conditions (Baker, 1974). Giese (1975) reported that barley plants grown at high temperature and light showed higher ECW (46 μg. cm^{-2}) than those grown in dark (6 μg. cm^{-2}). In addition to the weather factors, the soil moisture deficit contributes to the increase in ECW as observed in the present study and supported by other reports (Ebercon *et al.* 1977; Bengtson *et al.* 1978; Weete *et al.* 1978; Bhat *et al.* 1991).

According to Mayeux and Jordan (1987) rainfall removes wax content, which was supported by 15 to 35 per cent decrease in ECW of *Isocoma* leaves during rainy period as compared to dry period. In the present study also, the significantly low ECW content observed during pre and post stress periods (Fig. 2) might be attributed to the rainfall that occurred during October-November (100.5 mm) and between June and August (1550 mm). The wax content in some woody semi-arid shrubs was found to be lower during monsoon than during summer season (Rao and Reddy, 1980). From these reports, it may be inferred that wax content produced as protective layer during dry periods is lost once the stress is relieved in plants due to rainfall.

The increase in ECW content during stress period could be related to prevalence of dry weather with high light intensity, high temperature and low relative humidity (Fig. 1). In tobacco, leaves grown under long photoperiods had more ECW (Wilkinson and Kasperbauer, 1980). Baker *et al.* (1975) found a 58 per cent decrease in ECW of lemon during July and October when compared to the content during drought period. The variations in ECW content between first and sixth leaf in three genotypes is in agreement with such variations in other plant species (Tulloch, 1973; Bass 1982, Bukovac *et al.* 1979; Mayeux and Jordan, 1987; Baker and Greyson, 1988).

The formation of wax layer on the leaf surface is an adaptive mechanism to withstand water deficit under field condition in plant species (Hall and Jones, 1961; Baker, 1974).

As an important component in reducing transpirational loss of water, deposition of wax on the leaf surface was correlated with drought resistance in sorghum (Ebercon *et al.* 1977), Oat (Bengtson *et al.* 1978), *Eragrostis* (Hull *et al.* 1978), and other plant species (Baker and Procopiou, 1980).

Drought tolerant cocoa accessions (Balasimha *et al.* 1988) and coconut genotypes (Rajagopal *et al.* 1990a) had higher ECW content than the drought susceptible ones. A clear negative relationship between ECW and transpiration rate was shown in coconut genotypes including COD & WCT in which lower ECW correlated with high transpiration rate (Rajagopal *et al.* 1992). A similar relationship between ECW and transpiration rate was reported in different plant species (Bengtson *et al.* 1978;

O'Toole *et al.* 1979; Mohammed *et al.* 1986).

There was also a qualitative difference in the components of ECW among the genotypes during the three periods (Fig. 3 and 4). The major components of ECW are esters of higher fatty acids and fatty alcohols. In general, the number of components of ECW was more in both the leaf positions of all the three genotypes during the stress period than either of the other two stages. The major components namely hydrocarbons (Rf. 0.94) and esters (0.86) were detected in all the three genotypes during the stress period than either of the other two stages. The major components namely hydrocarbons (Rf. 0.94) and esters (0.86) were detected in all the three genotypes with varying intensities during different stages. The alcohols (Rf 0.49) could be identified in the three genotypes only during the stress period, while fatty acids(Rf 0.31) only during the post-stress period. The esters were separated into two bands in case of WCT and COD x WCT, whereas in WCT x COD only one band of ester was observed during stress period. Similarly during post-stress period acids were observed in WCT and WCT x COD, while it was absent in COD x WCT. In Oat, primary alcohols increased during drought (Bengtson *et al.* 1978), while in other species aldehyde components and fatty acids increased during summer season (Rao and Reddy, 1980). A decrease in soil water potential increased the proportion of alkanes and esters in apple (Darnell and Ferreed, 1983). A new class of wax components such as β-diketones, secondary alcohols and aldehydes were detected in monsoon season in some semi-arid shrubs (Rao and Reddy, 1980), similar to the observations on coconut genotypes during post-stress period. Ramadas *et al.* (1979) reported that the alcohols and aldehydes act as suppressors of cuticular transpiration, while fatty acids promote transpiration. In *Isocoma* leaves, cuticular transpiration was associated with waxes that contained high concentrations of fatty acids and alcohols, while low rates of cuticular transpiration were associated with high aldehyde content (Wilkinson and Mayeux, 1987).

Thus, the present investigation revealed the role of weather variables on the changes in both the content and composition of leaf surface wax in coconut genotypes, although the precise mechanism of these changes are not clear. While extensive work has been done in annual crops on the ECW, the present paper highlights the relevance of initiating such studies in a tropical perennial plantation crop like coconut. The efficacy of ECW estimation in screening coconut genotypes for drought tolerance has been reported recently (Rajagopal *et al.* 1990a).

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