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Morphological factors of the central whorl leaf associated with leaf surface wetness and resistance in sorghum to shoot fly, *Atherigona soccata**

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Summary

Earlier studies showed that leaf surface water on the central whorl leaf of sorghum seedlings is associated with resistance to shoot fly. In this study, the results of an experiment to determine if leaf surface wetness (LSW) originates from atmospheric condensation or from the plant are described. Morphological structures: trichomes, stomata, leaf cuticle and quantity of surface wax of the central whorl leaf were also examined for their role in LSW production. The results suggest that LSW of the central whorl leaf originates from the plant and is not due to condensation of atmospheric moisture. The presence of trichomes was indirectly associated with LSW and resistance to shoot fly but stomatal density was not associated with LSW production. The amount of wax extracted per 100 mg of fresh weight varied significantly between genotypes and seedling age. It was more in susceptible than in resistant genotypes; however, cuticular thickness was not associated with resistance. It is suggested that LSW could be the result of some form of cuticular movement of water to the leaf surface.

Key words: Sorghum, seedlings, shoot fly, central whorl leaf, leaf surface wetness, trichomes, stomata, epicuticular wax, cuticle, resistance, susceptibility

Introduction

Resistance to sorghum shoot fly, *Atherigona soccata* Rondani (Muscidae: Diptera) has been associated with the presence of trichomes on the leaf surface (Anon., 1978), the glossy leaf trait (Maiti & Bidinger, 1979) and moisture on the leaf (Raina, 1981). Nwanze, Reddy & Soman (1990) showed a highly significant correlation between the wetness of the central whorl leaf of young seedlings and shoot fly damage (deadhearts) and it was concluded that leaf surface wetness (LSW) is an important factor in susceptibility to shoot fly. In a more recent study we used low temperature scanning electron microscopy (LTSEM) to examine epicuticular wax structure and the nature of surface water on the leaves of shoot fly resistant and susceptible genotypes (Nwanze *et al.*, 1992*a*). While our results showed distinct

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differences between genotypes in wax morphology and leaf wettability, we had no indication if wax structure is related to the supply of LSW.

Several questions related to the role of physical and plant physiological factors in the production of water on the central whorl leaf remain unanswered. Here we report an initial investigation to determine the source of LSW production in a simple experiment. We also conducted microscopic examinations of the leaf cuticle, trichomes and stomata and measured epicuticular wax content to seek links between leaf morphological structures and LSW.

Materials and Methods

Plant material

Seedlings were obtained from small plot experiments (1 m × 1 m) grown at a spacing of 15 cm × 10 cm. Potted plants (grown in 10 cm diameter plastic pots) were also used for obtaining leaf samples. Five sorghum cultivars: two shoot fly resistant (IS 18551 and IS 1057), one moderately resistant (IS 1054) and two susceptible (IS 1046 and CSH 1) were used in various experiments. All plant material was irrigated to maintain adequate soil moisture for plant growth.

Moisture accumulation in the central whorl leaf

If moisture accumulates on leaf surfaces by condensation of atmospheric water vapour, it would be possible to detect the condensed water by an increase in seedling weight during the night. If, however, the surface moisture originates from the leaf, there would be no overall increase in weight as moisture accumulates on the leaf surface. We investigated this hypothesis using 10-day old seedlings of two cultivars, CSH 1 and IS 18551. Ten seedlings of each cultivar were excised directly above soil level and weighed immediately on a Mettler balance (model AE 160) and then arranged in an upright position on a wooden rack. Thereafter, they were weighed at two hourly intervals. To achieve accuracy in weighing, each plant was suspended on the balance from a hook attached to the weighing plate so that direct contact with the seedlings was minimal. This experiment was conducted from 1800 h to 0600 h on two dates in October 1989. During the same periods, we estimated LSW on intact seedlings of CSH 1 and IS 18551 by a visual score method (Nwanze *et al.*, 1990). We compared the change in weight of excised seedlings with LSW. The temperature of the central whorl leaf, air temperature and humidity were measured as described earlier by Nwanze *et al.* (1992*b*).

Leaf trichomes and stomata

Leaf segments approximately 12 mm × 12 mm, from the central whorl leaves of 10 seedlings each of four age groups (5, 10, 14 and 21 days after emergence [DAE]) of each cultivar were examined for trichomes under a binocular microscope. For clearing leaf tissue, we used standard procedures as described by Maiti *et al.* (1980) with the following modifications. Each segment was initially placed in a glass vial (2 cm diameter) containing 20 ml of acetic acid and ethyl alcohol (2:1) for 24 h and was then transferred into 20 ml lactic acid (90%) for 24 h. For examination, each segment was mounted on a slide in a drop of lactic acid. Trichome and stomatal density were recorded in three randomly selected fields each measuring 0.16 mm^2. Trichome length and stomatal size were measured using an occular micrometer on 10 randomly selected trichomes and stomata respectively. Trichome angle was estimated visually to the nearest five degrees by comparison to a set of standard angles.

Leaf cuticle

The central whorl leaves of 10-day old seedlings of all five genotypes were excised and prepared for sectioning and staining using the method described by Johansen (1940). Leaf samples were initially fixed for a minimum of 48 h in FPA (40% formalin, propionic acid and 70% ethyl alcohol, 5:5:90) dehydrated in graded alcohol, then transferred into an absolute alcohol and tertiary butanol series and finally embedded in wax by infiltration for 24–72 h in pure paraffin wax. Sections (8–10 microns thick) of the wax embedded material were cut with a Cambridge rotary microtome, floated on water an de-waxed, stained in Sudan IV and mounted on microscope slides for examination. The thickness of the cuticle was measured using an ocular micrometer attached to the eye piece of a stereo microscope.

Extraction of surface waxes

The central leaves of four seedling ages (5, 10, 14 and 21 DAE) of each genotype were excised from the whorl, weighed on a mettler balance and then immediately immersed in distilled Analar chloroform for only 30 s in order to avoid the extraction of internal lipids and chlorophyll by a longer period of extraction. This technique results in the extraction of approximately 99% of the wax on the leaf surfaces (Gerald, David, David & Zander, 1970; Woodhead, Galeffi & Marini Bettolo, 1982). Using a flash evaporator, the resultant solvents which were held in round bottomed flasks, were evaporated under vacuum at temperatures below 40°C. The wax obtained was dried to a constant weight and expressed per 100 mg of fresh leaf weight.

Results

Soil water and seedling weight

There was a rapid decrease in the weight of excised seedlings during the first part of the night, followed by a transient increase between 0300 and 0600 (Fig. 1*a*). Dew was observed on the expanded leaves at the time of the 0500 h reading. Differences in the weight changes between the susceptible (CSH 1) and resistant (IS 18551) genotypes were not significant ($P = 0.05$).

In contrast with the change of weight, LSW on the central whorl leaf of intact seedlings increased continuously up to 0500 h, then there was a slight decrease (Fig. 1*b*). There were large differences in LSW between CSH 1 (scores > 2.7) and IS 18551 (scores < 1.5).

Leaf temperature decreased almost linearly from about 19°C at 2100 to 13–14°C between 0500 and 0600 h. There were no consistent differences in temperature between intact and excised seedlings, or between susceptible and resistant genotypes (Fig. 2*a*). The vapour pressure gradient between the leaf and the air (VPG) was calculated by taking the difference between the saturated vapour pressure at leaf temperature and the vapour pressure of the air. There were no significant differences ($P = 0.05$) between the VPG for intact and excised seedlings of CSH 1 and IS 18551 (Fig. 2*b*). The VPG was positive between 2100 and 0100 h, and negative between 0300 and 0600 h.

Leaf trichomes

Trichomes were present only in the resistant genotypes IS 18551, IS 1057 and IS 1054 (Table 1). In susceptible CSH 1 and IS 1046, instead of trichomes, very sparse, blunt and short glandular cells were located in deep portions of the upper and lower leaf surfaces. Trichomes in resistant genotypes were usually thorn-like and were oriented towards the

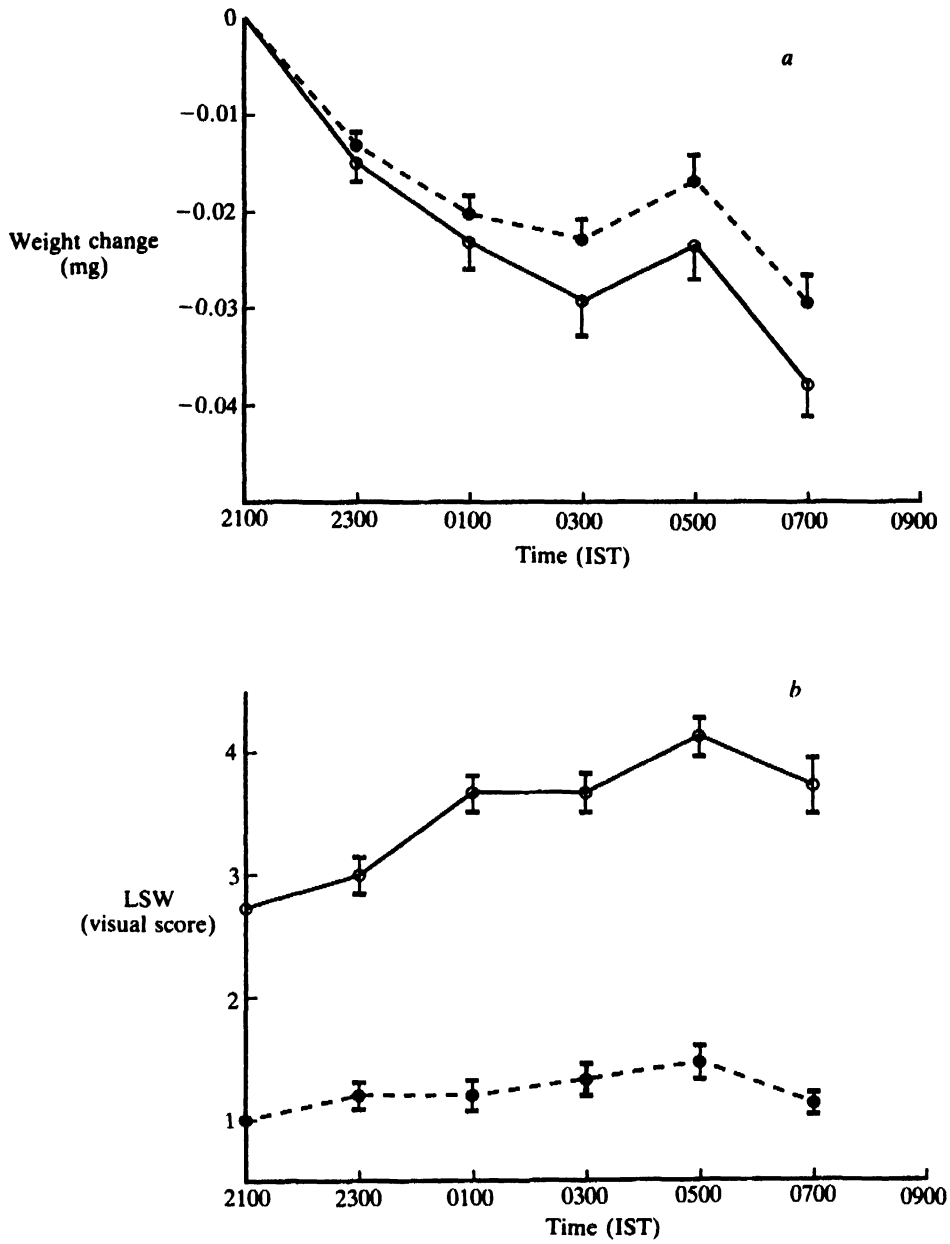

Fig. 1. Changes with time of (*a*) weight of excised seedlings and (*b*) LSW on the central whorl leaf of intact 10-day seedlings. (CSH 1 —○—, IS 18551 - - -●- - -).

base of the leaf at an acute angle of about 30–70° on upper and lower leaf surfaces. Trichome lengths ranged from 37.5 μm in IS 18551 to 17.5 μm in IS 1054.

Trichomes were consistently more numerous on the upper than on the lower surface and density varied significantly ($P < 0.01$) with seedling age, increasing with growth stage to a maximum in 10-day old seedlings (5th leaf stage)(Table 1). The lowest trichome density (4.7 mm^{-2}) was recorded on the lower surface of 5-day old seedlings (3rd leaf stage) of IS 1054 while the highest (38.9 mm^{-2}), was on the upper surface of 10-day old seedlings of IS 18551.

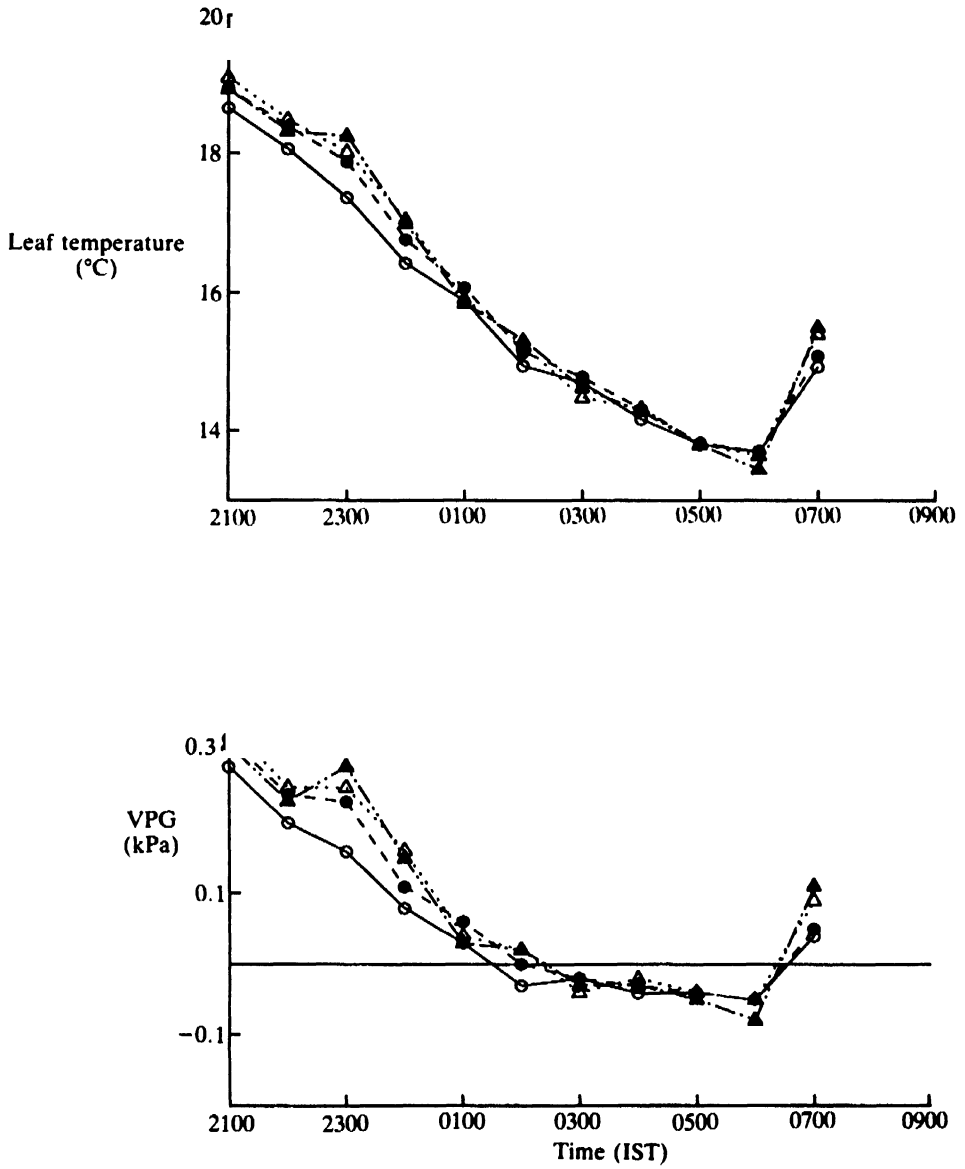

Fig. 2. Changes in (*a*) leaf temperature and (*b*) vapour pressure gradient (VPG) with time in intact CSH 1 (——○——); excised CSH 1 (·····△·····); intact IS 18551 (---●---); and excised IS 15881 (-·-·-▲-·-·-) 10-day old seedlings.

Leaf stomata

Differences in stomatal density between resistant and susceptible cultivars were not significant but the differences between seedling ages were significant. The highest stomatal densities were recorded in 10-day old seedlings in all cultivars and they were more abundant on the upper (mean of 116 mm^{-2}) than on the lower surface (mean of 94 mm^{-2})(Table 2). Stomata were arranged along parallel rows mainly over leaf veins and did not vary in shape between leaf surfaces nor between cultivars. Variation in stomatal size was independent of resistance. The largest stomata were observed in CSH 1, with a size of 35.5 $\mu m \times 24$ μm on the upper surface and 34 $\mu m \times 23$ μm on the lower surface. The smallest size (24.4 $\mu m \times 15$ μm) was recorded on the upper surface of IS 1054. Change in stomatal size with seedling age was negligible.

Table 1. *Mean trichome densities on the central whorl leaf of sorghum seedlings at different ages*

Seedling age/ leaf surface	Number of trichomes/mm² IS 18551 Resistant	IS 1057 Resistant	IS 1054 Moderately resistant	IS 1046 Susceptible	CSH 1 Susceptible	Mean
5 DAE[2]						
upper	12.9	11.6	10.7	0.0	0.0	6.9
lower	8.5	5.3	4.7	0.0	0.0	3.8
10 DAE						
upper	38.9	17.7	20.2	0.0	0.0	18.2
lower	33.3	19.7	19.2	0.0	0.0	14.4
14 DAE						
upper	37.7	15.4	16.4	0.0	0.0	13.8
lower	33.0	12.9	8.3	0.0	0.0	10.8
21 DAE						
upper	28.7	13.1	10.2	0.0	0.0	10.3
lower	18.5	11.6	7.2	0.0	0.0	7.4
Mean	26.4	13.4	12.0	0.0	0.0	

SE for comparison:

	Upper surface	Lower surface
Age	± 0.97	± 1.15
Genotype	± 0.98	± 2.12
Genotype × age	± 2.13	± 3.08

[1] = Mean of 30 observations
[2] DAE = Days after emergence

Thickness of the leaf cuticle

Results of light micrographs showed differences between genotypes in the thickness of the cuticular membrane. However, these differences were not statistically significant ($P = 0.05$) and did not appear to be associated with resistance. In resistant IS 18551, the cuticle was well defined and measured 2 μm, whereas in resistant IS 1057 it was only 0.78 μm thick. In susceptible genotypes it varied from 1.18 μm in IS 1046 to 0.80 μm in CSH 1.

Wax content

Shoot fly susceptible genotypes contained more surface wax than resistant ones. In 10-day old seedlings, the wax content ranged from 2.31 mg/100 mg of fresh leaf weight in CSH 1 to 0.9 mg/100 mg in IS 18551 (Table 3). In all genotypes, the greatest values occurred at 10 DAE and the smallest values at 21 DAE. Practically no significant differences were observed between genotypes at 21 DAE (Table 3).

Discussion

Any change in the dry mass of excised seedlings during the period under consideration would be negligible compared with changes in their water content. It is reasonable to assume therefore that changes in seedling weight give a measure of water lost or gained. Between

Table 2. *Mean stomatal densities on the central whorl leaf of sorghum seedlings at different ages*

Seedling age/ leaf surface	Number of stomata/mm² IS 18551 Resistant	IS 1057 Resistant	IS 1054 Moderately resistant	IS 1046 Susceptible	CSH 1 Susceptible	Mean[1]
5 DAE[2]						
upper	99.1	94.3	84.5	80.4	95.5	90.7
lower	79.9	59.1	83.5	56.6	70.4	69.0
10 DAE						
upper	113.2	135.8	103.5	122.0	113.2	117.5
lower	82.4	105.7	98.0	101.8	109.4	99.4
14 DAE						
upper	109.4	103.1	98.1	110.7	104.5	105.1
lower	98.5	70.4	85.5	90.6	84.3	85.8
21 DAE						
upper	90.6	97.0	91.4	101.9	80.1	92.2
lower	73.3	65.4	89.3	80.5	85.5	78.8
Mean	93.3	91.3	91.7	93.0	92.8	

SE for comparison:

	Upper surface	Lower surface
Age	± 2.52	± 2.38
Genotype	± 3.57	± 3.61
Genotype × age	± 6.04	± 5.86

[1] = Mean of 30 observations
[2] DAE = Days after emergence

Table 3. *Wax content of the central whorl leaf of sorghum seedlings at different ages*

Seedling age (DAE)[1]	Wax content (mg/100 mg fresh weight) IS 18551 Resistant	IS 1057 Resistant	IS 1054 Moderately resistant	IS1046 Susceptible	CSH 1 Susceptible	Mean
5	0.7	0.9	1.1	1.4	1.6	1.1
10	0.9	1.0	1.3	1.6	2.31	1.4
14	0.3	0.5	0.4	0.5	0.6	0.4
21	0.3	0.3	0.4	0.5	0.4	0.4
Mean	0.53	0.67	0.8	1.1	1.23	

SE for comparison:

Age	± 0.12
Genotype	± 0.08
Genotype × age	± 0.24

[1] = Mean of 5 determinations
[2] DAE = Days after emergence

2100 and 0100 h, when a positive VPG provided the potential for evaporation, water was lost from the seedlings. Between 0300 and 0600 h, however, the VPG was negative causing water vapour from the air to condense on the plant to form dew. Dew was seen on the leaves at 0500 h, and its presence was detected by an increase in seedling weight. A similar pattern was observed for both susceptible and resistant genotypes.

Surface wetness of the central whorl leaf of intact seedlings showed an entirely different pattern to the changes in water content of the excised seedlings. Between 2100 and 0100 h, when water was being lost by evaporation (see Fig. 1*a*), LSW was increasing. Leaf temperature and VPG were similar for intact and excised seedlings, so the potential for evaporation would be similar. We must conclude therefore that leaf surface wetness originated from the plant and, between 2100 and 0100 h, the rate of supply of water exceeded the rate of evaporation. Between 0300 and 0700 h the pattern of changes in LSW was similar to the pattern of weight change of excised seedlings. There was a marked difference in the amount of LSW on CSH 1 and IS 18551, although the leaf temperatures of these two genotypes were similar, so the difference in LSW was not caused by the microclimate. It is probable that differences between cultivars are related to differences in the genetic potential to supply LSW.

It has been observed at other times of the year that LSW commonly decreases from about 0200 h (Nwanze *et al.*, 1992*b*). This happens on nights when there is no dew and the VPG remains positive throughout the night. It appears therefore that the rate of supply of LSW usually decreases several hours before sunrise, but we do not know the reason for this. In the examples shown in Fig. 1, it is possible that the rate of supply of LSW from the plant decreased after 0200 h, but condensation from the atmosphere between 0300 and 0500 h caused a continued increase in observed LSW.

Analysis of means across genotypes and seedling ages indicated that although differences were not statistically significant between genotypes for most parameters (except for trichome density and stomata on the lower leaf surface), the interactions between genotype × seedling age for each parameter were highly significant. Thus the leaf characteristics studied varied significantly across different stages of seedling development. This finding indicates an indirect relationship between leaf morphological factors and seedling susceptibility and therefore insect behaviour, since the susceptibility of sorghum to shoot fly is associated with seedling age and is highest in 10-day old seedlings, a stage at which trichome density and wax content are also greatest.

The presence of trichomes on the abaxial and adaxial leaf surfaces of fully expanded leaves has been associated with reduced frequency of both egg laying and deadhearts (Maiti *et al.*, 1980). Glandular cells in shoot-fly susceptible genotypes have also been reported by Maiti (1986) and, although the role of these cells is not known, Maiti (1986) suggested that they may be linked with the secretion of plant volatiles.

In our study, in which we used unexpanded leaves of the central whorl, although we found that resistant cultivars (with less deadhearts) had more trichomes and little LSW (< 1.5) while susceptible cultivars (with more deadhearts) had no trichomes and LSW was more (> 2.7), there was no evidence that LSW production was dependent on trichome density. In pearl millet, trichome-less cultivars were shown to accumulate more dew and to stay wet longer than trichomed ones (Burton *et al.*, 1977). Raina (1981) suggested that a similar situation in sorghum would facilitate the movement of freshly hatched larvae to the base of the central shoot. On the other hand, trichomed cultivars would tend to dry faster, making the downward journey of the larvae more difficult.

Since stomata are closed at night the weight loss of excised seedlings would be an indicator of cuticular transpiration. At the same time, we observed LSW to be highest at night and our results did not show any direct relationship between stomatal density and LSW.

Surface waxes are usually deposited on young leaves and especially during or shortly after the period of leaf expansion and are known to change with plant age (Schieferstein & Loomis, 1956; Hallam, 1970). In the present study, we observed an increase in the amount of wax up to 10 DAE, followed by a decrease. Blaker & Greyson (1988) working with maize, reported a large increase in the amount of wax per unit weight from 10 to 20 days followed by a rapid decline until 40 days. Atkin & Hamilton (1982) also reported similar changes in sorghum leaves.

Cuticular thickness varied irrespective of resistance or susceptibility. Resistant IS 18551 and susceptible IS 1046 had thicker cuticular layers than susceptible CSH 1 and resistant IS 1057. We would expect the thickness of the cuticle to influence cuticular transpiration (Schieferstein & Loomis, 1956) but this may not necessarily relate to the accumulation of water on the leaf surface. In a separate study (Nwanze *et al.*, 1992*a*), we found that susceptible genotypes were characterised by dense wax crystals and that water droplets showed spreading at the edges, indicating a tendency to wet easily. In resistant genotypes with an amorphous wax layer and sparse wax crystals, no water droplets were observed. Differences in LSW between genotypes indicate some form of cuticular movement of water to the leaf surface which may be affected by the nature of the cuticle (but not its thickness) or by the potential to supply water (i.e. a difference in plant water relations).

Our findings indicate possible linkages in some areas between the morphology of the central leaf whorl and resistance through modification of shoot fly behaviour. The glandular cells in susceptible genotypes may well be associated with volatiles which affect adult shoot fly orientation behaviour and ovipositional preferences, but direct evidence for this is not available. The presence of dense trichomes in other genotypes may retard larval movement and result in reduced susceptibility, but the functional importance of leaf surface wax is less obvious. Similarly, no clear association exists between resistance and leaf stomata and cuticular thickness.

With specific reference to LSW, the results of this study and our earlier studies (Nwanze *et al.*, 1990, 1992*a*,*b*) confirm and/or suggest that: (1) LSW is associated with resistance to shoot fly, (2) LSW originates from the plant and is not the result of atmospheric condensation, (3) the presence of trichomes in resistant genotypes may be associated with the retention of LSW and not its production, (4) LSW is not related to stomatal activity but probably results from cuticular movement of water, and (5) LSW is not related to cuticular thickness nor the amount of surface wax on the central whorl leaf. It, however, remains to be shown what plant physiological process is involved in the supply of LSW to the leaf surface.

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