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Resistance in sorghum to shoot fly *Atherigona soccata*: Evidence for the source of leaf surface wetness

By S SIVARAMAKRISHNAN, P SOMAN*, K F NWANZE[1], Y V R REDDY and D R BUTLER

International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, Andhra Pradesh 502 324, India
**Farm Division, M/s Valli Textile Mill, Venkateswarapuram, Sattur 626 203, Tamil Nadu, India*

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Summary

Leaf surface wetness (LSW) of the central whorl leaf of sorghum seedlings has been associated with susceptibility to shoot fly. Previous physical and physiological evidence suggested that LSW originates from the plant. This was confirmed by radioactive labelling methods using tritium and carbon-14. Tritiated water applied to the soil of potted seedlings was translocated to the surface of the whorl leaf. There were significant differences in the amount of tritiated water collected from susceptible (CSH 5) and resistant (IS 18551) genotypes. Studies with carbon-14 labelling of sorghum seedlings indicated the presence of (small amounts of) solutes in the surface water that may affect larval movement and survival.

Key words: Shoot fly, sorghum, leaf surface wetness, radiolabelling

Introduction

Deadheart damage to sorghum (*Sorghum bicolor* Moench) seedlings due to shoot fly, *Atherigona soccata* Rondani (Diptera: Muscidae), has been associated with the amount of surface wetness on the central whorl leaf, (Nwanze, Reddy & Soman, 1990). There is more leaf surface wetness (LSW) in shoot fly susceptible sorghum genotypes than resistant ones and physical and physiological evidence suggests that LSW is exuded from the plant (Nwanze *et al.*, 1992; Soman *et al.*, 1994). If this is so, it should be possible to trace the movement of water from the soil through the plant and on to the leaf surface. To do this tritiated water was applied to the soil, and the level of radioactivity in LSW was measured for resistant and susceptible sorghum genotypes. Carbon-14 labelling was used to verify the presence of solutes in LSW.

Materials and Methods

Plant material

Sorghum genotypes CSH 5 (shoot fly susceptible) and IS 18551 (shoot fly resistant) were grown in an Alfisol and peat mixture (4:1) with 4–5 seedlings in each pot (12.5 cm diameter).

[1]Corresponding author

Tritium labelling

Labelling of the plants with tritium was done with a modification of the procedure described by Krishnamurthy, Gogate, Sarma & Soman (1979) and L'Annunziata (1979). Pots containing 2-wk old seedlings were watered to field capacity and 1–2 ml tritiated water (^3H_2O, 10–20 μCi) was applied to the soil centrally with a hypodermic needle at a depth of about 5 cm. No tritiated water was added to control plants.

After 18 h, water droplets on the surface of the whorl leaf were absorbed onto 3 cm × 1 cm strips of Whatman No. 3 mm filter paper. Excised leaves were stored at 0°C for further analysis. Five samples, each from two seedlings were taken for the measurement. Two methods were used for analysis:

(a) *Wet filter paper method*: The filter paper strips were quickly placed in vials and 5 ml scintillation fluid (Insta-Gel, Packard Inc., USA) was added. The vials were placed in a Liquid Scintillation Counter (Beckman, USA) to obtain the disintegrations per minute (dpm), using a set of tritium standards for the quench correction. This method gave the radioactivity in LSW, which may include both water and other solutes.

(b) *Dry filter paper method*: The procedure was similar to (a) except that the vials were put in an oven at 80°C for 2–3 h to evaporate the water before adding the scintillation fluid. This method determined any radioactivity, remaining on the filter paper (in non-volatile solutes) after evaporation of water.

The amount of radioactivity in the stored leaf tissue was determined using ≈50 mg leaf from each sample. Each sample was cut into small pieces, spread on a porcelain weigh boat and combusted in a stream of oxygen in a Biological Oxidizer (Model 300, R J Harvey Inc., USA). Tritiated water was trapped in glass vials containing 15 ml scintillation fluid and the level of radioactivity recorded as described above.

Carbon-14 labelling

^{14}C-labelling of plants was carried out as described by Kumarasinghe (1990) and Mahalakshmi, Sivaramakrishnan & Bidinger (1993) with modifications. Air-tight glass syringes with teflon plungers were filled with $^{14}CO_2$ generated from ^{14}C-sodium bicarbonate ($NaH^{14}CO_3$) and HCl. Three pots each containing sorghum seedlings of genotypes CSH 5 and IS 18551 in a plexiglass chamber were exposed to $^{14}CO_2$ for 20 min on two occasions separated by 24 h, by injecting 5 ml gas (about 50 μCi) into the chamber. LSW was sampled by absorbing on filter paper 42 h after the second $^{14}CO_2$ application or by washing whorl leaves from two plants five times with 2 ml of distilled water into 20 ml glass vials. Radioactivity in the wash was measured by the addition of 10 ml of scintillation fluid using a set of carbon-14 standards for the quench correction. This method ensured fairly complete removal of the solutes from the leaf surface. Five sets of measurements were carried out with two plants in each sample. Control plants were not exposed to $^{14}CO_2$.

Excised leaves were dried in an oven at 80°C for 48–72 h and about 10 mg of the dried sample was combusted in a Biological Oxidizer (R J Harvey, USA). $^{14}CO_2$ from the sample was trapped in vials containing carbon-14 scintillation fluid (R J Harvey, USA). The amount of radioactivity was determined as before. This gave the total incorporation of ^{14}C in the leaf tissue.

Results and Discussion

Filter paper blots of LSW (wet filter paper method) from CSH 5 showed the presence of significantly more ($P < 0.001$) radioactivity than the untreated controls (Table 1), indicating that tritium applied to the soil was translocated by the plants to the leaf surface. IS 18551

Table 1. *Detection of tritium in leaf surface wetness and leaf tissue from shootfly resistant and susceptible genotypes of sorghum*

Treatment	dpm in LSW[1]		dpm/50 mg wet leaf[1]	
	CSH 5	IS 18551	CSH 5	IS 18551
Wet Filter Paper	3812 ± 217	169 ± 24	4207 ± 469	4186 ± 429
Dry Filter Paper	97 ± 4.3	83 ± 1.1	3081 ± 322	2250 ± 355
Control	109 ± 2.18	107 ± 1.4	132 ± 4.85	193 ± 23.1

[1]Mean ± SE of 10 observations

showed very small amounts of radioactivity in wet filter paper blots, significantly less ($P < 0.001$) than CSH 5, confirming more LSW in the susceptible genotype (mean mass of LSW = 8.25 mg/whorl leaf) than in the resistant one (mean mass of LSW = 0.1 mg/whorl leaf).

Larger amounts of radioactivity in LSW from the susceptible genotype could have resulted from more tritiated water within the leaf caused by differential uptake of water by the two genotypes. However, analysis of the whorl leaf tissue showed that the radioactivity (dpm/50 mg wet leaf) was similar in both genotypes (Table 1). These results confirmed that, although the ability of resistant and susceptible genotypes to move water from the soil into the leaf did not differ, the mechanism for transfer of water to the leaf surface in susceptible genotypes is reduced in resistant ones (Soman *et al.*, 1994).

In the plant system, tritium from water may be incorporated in metabolites and exuded in solution. In the dry filter paper method, any radioactivity would come from non-volatile solutes with which the label is exchanged. Our results showed that all the radioactivity was lost on drying the filter paper (Table 1), indicating that all the radioactivity was present in water and any solute in the LSW did not contain detectable levels of tritium.

More detailed information on solutes is provided by carbon-14 labelling. The data in Table 2 show that there was significantly more ($P < 0.01$) ^{14}C radioactivity in LSW in both susceptible and resistant seedlings than in the control. However, there was significantly more ($P < 0.01$) ^{14}C in LSW of CSH 5 than IS 18551. Furthermore, there were similar amounts of radioactivity in the leaf tissue of both genotypes (Table 2), indicating similar incorporation of labelled carbon.

The results of the tritium and carbon-14 experiments confirm that LSW is an exudate from the central whorl leaf and that it contains solutes. The low recovery of ^{14}C-radioactivity indicates that these solutes are present in small quantities. Furthermore, because there is much less LSW in IS 18551 than in CSH 5 (Table 1), and the quantity of solute is more similar (Table 2), it can be inferred that the solute concentration is greater in IS 18551 than in CSH 5. A higher solute concentration in LSW of IS 18551 may partly explain the differences in larval behaviour and survival between resistant and susceptible genotypes as reported earlier (Nwanze *et al.*, 1990). It is possible that the lower solute concentration in

Table 2. *Recovery of carbon-14 in LSW and leaf tissue from shoot fly resistant and susceptible sorghum*

Treatment	dpm in LSW[1]		dpm/10 mg dry leaf[1]	
	CSH 5	IS 18551	CSH 5	IS 18551
Wet Filter Paper	306 ± 33	98 ± 11	11531 ± 1407	15923 ± 1776
Wash	360 ± 46	159 ± 24	17839 ± 1491	15572 ± 2613
Control	79 ± 6.2	66 ± 1.4	190 ± 19.0	161 ± 6.0

[1]Mean ± SE of 10 observations

LSW of CSH 5 (than IS 18551) provides a more favourable substrate for larval movement and survival. Further research is needed to determine the nature and composition of the solute component of LSW and how this affects larval physiological functions.

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