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Splash dispersal in *Ramulispora sorghi* Olive and Lefebreve, the casual organism of sooty stripe of sorghum

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Abstract. The conidia of *Ramulispora sorghi* were produced in a mucilaginous mass and dispersed through splash-off and wash-off mechanisms during rain, while the sclerotia of *Ramulispora sorghi* were produced freely and attached superficially to the host leaf surfaces, dispersed through air over short distances and deposited on the sampling surfaces at the rate of 22 sclerotia/cm^2/day.

Greater number of conidia were monitored by splash traps during the kharif crop period as compared with that of rabi. The peak conidial dispersal was noticed during the 10-leaf stage flag leaf growth stages of sorghum crop. Both incident water drops (splash-off) and flowing water drops (wash-off) liberated the conidia from the sporulating lesions. Peak liberation of conidia occurred with the water drops from 3–7 and most conidia were removed from the sporulating lesion within 60 s indicating the possible dispersal of the pathogen even during short traces of rain.

Keywords. *Ramulispora sorghi*; sooty stripe of sorghum; splash dispersal; epidemiology; aerobiology.

1. Introduction

It is now well established that splash-off and wash-off during rain are important modes of spore dispersal in several plant pathogens (Gregory *et al* 1959; Gregory 1973; Rajasab and Ramalingam 1985). In an investigation concerning the dispersal of sorghum pathogens, studies were undertaken on *Ramulispora sorghi* inciting sooty stripe disease of sorghum, the results of which are reported.

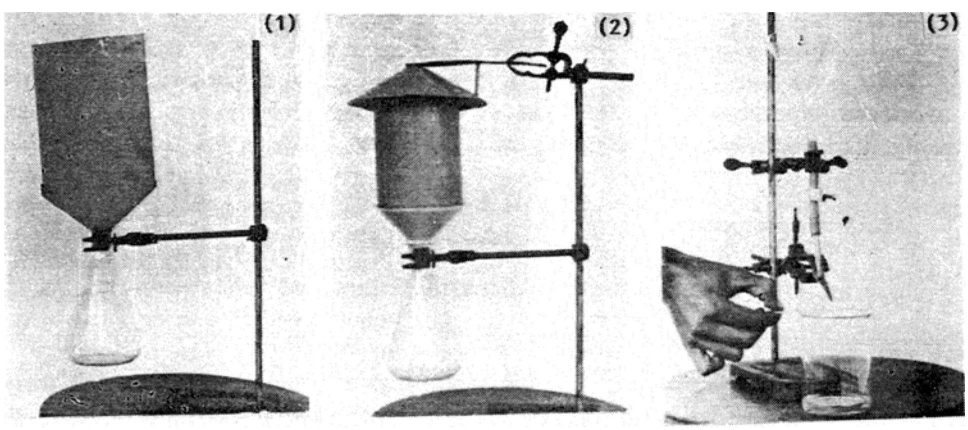
2. Materials and methods

Four crops of sorghum, two each during the kharif and rabi seasons during the years from 1977 to 1979 were raised in an experimental field measuring half an acre. Kharif crops were raised during the period from May to August and rabi from October to January each year. The seeds of a local sorghum variety, 'bilijola', were consistently used in all sowings and in associated experiments. While sowing, the apparently healthy sorghum seeds were mixed with powdered infected leaves of sorghum containing sclerotia and sporodochia of *R. sorghi*. The disease incidence was recorded at 5-day intervals throughout the crop period by adapting a random quadrat method. At each sampling time 30 quadrats (m^2) were randomly placed in the field and all the plants falling in the quadrat area were examined to record the percentage of plants bearing sooty stripe symptoms. The percentage of leaf area infected was estimated using Feak's scales.

Bidirectional (figure 1) and multidirectional splash traps (figure 2) were used to collect splash-dispersed spores under field conditions. The traps were placed between the rows at ground level or at a chosen height, at least 50 cm away from the leaves. About 2 ml of 50% formaldehyde solution was added to each collection bottle before commencing sampling to inhibit conidial germination. Rain samples were collected after 24 h during rainy days. The volume of water collected in each day's sample was measured after adding a drop of Tween-20 (wetting agent) and the water was centrifuged at 3000 rpm. Three-fourth's of the supernatant was discarded, microscopic examinations of the supernatant having always showed the absence of conidia. The centrifugation was continued until the solution was reduced to 10 ml. The conidial concentrations in the final solution (10 ml) was estimated using a haemocytometer following the method described by Tuite (1969).

The wash-off of the conidia by flowing water drops under laboratory conditions was studied by a simple device (figure 3). Water drops of 3 mm diameter were generated from a funnel at predetermined intervals and allowed to flow on a sporulating lesion incited by *R. sorghi*, mounted on inclined aluminium foil (45°). Each or a selected flowing drop was collected on a clean microscope slide and mounted using 22 × 22 mm sized cover glass after adding a trace of cottonblue with lactophenol. With the help of a microscope, 5 scans were taken across the mounted area under a scanning width of 500 μm and the data were computed for the estimation of total number of conidia in each drop.

The design of the assembly used to study the splash-off of the conidia by impacting water drops is given in figure 4. The assembly consisted of a water-dropping device, a glass tube, a splash-collecting unit and the infected leaf piece which was mounted on a glass plate. Numbered water drops of 2–3 mm diameter were made to fall from a height of 3·5 m at 10 s intervals and to bombard the sporulating lesion mounted on a thick glass plate. A square tin box (15 × 15 cm) open at both



Figures 1–3. 1. Bidirectional spore trap used to study the splash-off of the conidia of *R. sorghi* under field conditions. **2.** Multidirectional spore trap used to study the splash-off of the conidia of *R. sorghi* under field conditions. **3.** Wash-off assembly used to study the wash-off of the conidia of *R. sorghi* by flowing water drops under controlled conditions in the laboratory.

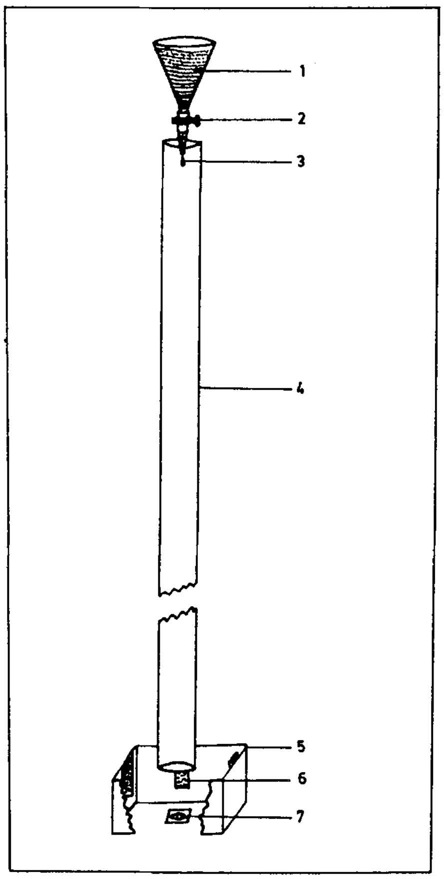


Figure 4. Assembly used for the study of splash-off of the conidia of *R. sorghi* from the infection spots by impacting water drops. (1), Water reservoir; (2), pinch cock; (3), water drop (3 mm diameter); (4), glass tube; (5), splash collecting unit; (6), microscope slide; (7), infected leaf bit mounted on a glass plate.

ends and containing 4 microscope slides at 4 equal angles was used to catch the splash-off droplets resulting from each incident drop. Before the commencement of the experiment the microscope slides were coated uniformly with fast green dissolved in absolute alcohol. The splash drops impacting on stained glass slides formed circular haloes. The conidia carried in them took the fast green colour readily which made their identification easy.

The splash droplets (0·5 mm diameter) thrown out by each, or each selected, incident (impacting) drop were observed in the microscope and the number of conidia carried by each splash droplet arising out of each incident drop was estimated.

To know the possibility of air dispersal in the conidia and sclerotia of *R. sorghi* if any, the 7 day volumetric Burkard spore trap (Hirst 1952), and the vertical cylinder spore trap (Ramlingam 1968) were established in the centre of the sorghum field containing sooty stripe infections and operated through out the crop period. In addition vaseline-coated sticky glass slides were exposed on the ground surface on non-rainy days and rotorod samplers (Perkins 1957) were operated on selected days. The sampling traces of Burkard spore trap, vertical cylinders, rotorod samplers and gravity slides were examined under the microscope to determine the presence of conidia and sclerotia of *R. sorghi.*

3. Results

3.1 *Sclerotial deposition*

Sticky microscope slides coated with vaseline were exposed at ground level in the severely infected sorghum field for 24 h on days with no rainfall and scanned for sclerotial deposition by using a graticuled occular micrometer. An average of 22 sclerotia per cm^2 were found to be deposited over a slide surface in a period of 24 h. Dry weather with strong winds gave higher deposition of sclerotia.

3.2 *Conidial dispersal*

Examination of the exposed traces of various spore traps used to monitor airborne conidia of *R. sorghi* if any failed to reveal the presence of conidia, indicating the absence of air dispersal of the conidia of the pathogen. However, the rain samples collected by the two splash traps employed in the study revealed the presence of conidia, indicating the operation of splash-off and wash-off dispersal mechanisms.

3.3 *Field experiments*

The data on the seasonal incidence of sooty stripe of sorghum, the incidence of *R. sorghi* conidia in each day's rain samples and the rainfall patterns during the two crop seasons are presented in figures 5 and 6. No conidia were encountered

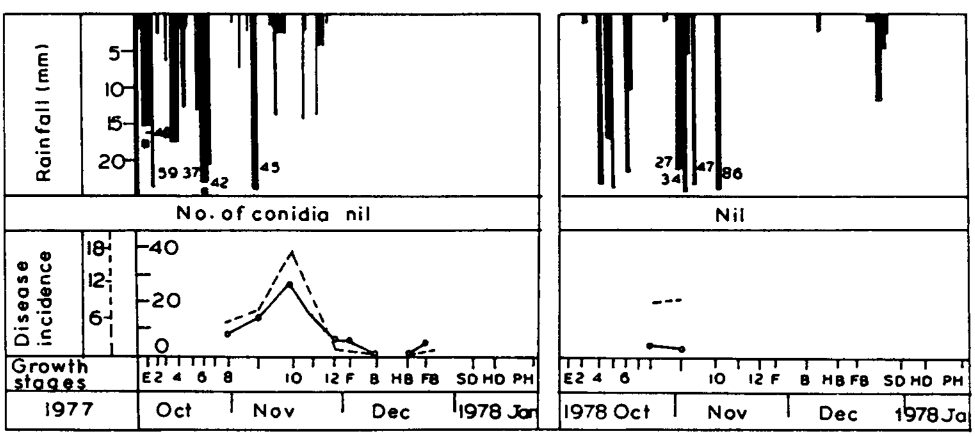


Figure 5. Incidence of sooty stripe of sorghum in two rabi crop seasons during 1977 and 1978 related to crop growth stages, rain fall and splash borne conidial catches of *R. sorghi.* (——), Percentage of plants infected; (----), percentage of leaf area infected. The numbers on histograms indicate the actual rainfall (mm).

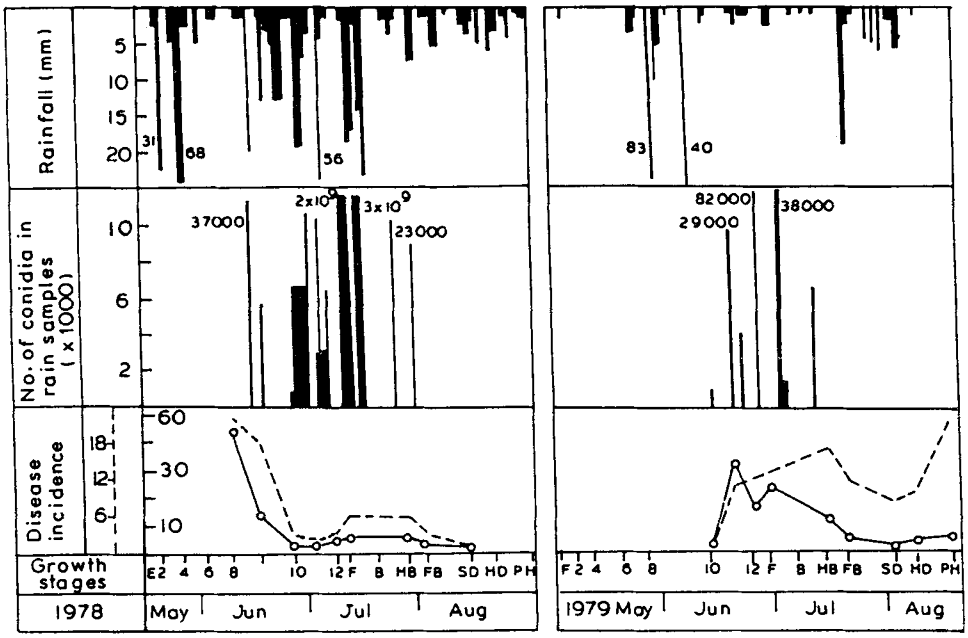


Figure 6. Incidence of sooty stripe of sorghum in two kharif crop seasons during 1978 and 1979 related to crop growth stages, rain fall and splash borne conidial catches of *R. sorghi*. (———), Percentage of plants infected; (----), percentage of leaf area infected. The numbers on histograms indicate the actual rainfall (mm)/the number of conidia.

during the rabi crop period although the disease incidence was considerable at 9 and 10-leaf growth stages of the crop. However, a good number of conidia were encountered in splash traps during Kharif crop period from the 9-leaf stage to flowering growth stages of the crop. Their number varied from a few to 3×10^9/day. No correlation was seen between the number of conidia monitored in the splash traps and the progress of the disease in the field, indicating that the splash traps are efficient only for qualitative estimation of splash-borne conidia in rain water but not for quantitative estimations.

3.4 *Wash-off experiments*

When a drop of water was placed on the surface of leaf near sporodochia of *R. sorghi*, the mucilaginous mass of the sporulating lesion suddenly melted into water. The first drop of water that was allowed to flow over an infected spot carried only a little mass of mucilage and no conidia. The second drop carried a few conidia with it and drops 4–10 carried with them the largest number of conidia (up to 7,000). The number of conidia carried in the sixth water drop and onwards decreased to reach minimum (less than 500) at the seventy first drop, the last one to be tested (figure 7A).

3.5 *Splash-off experiments*

Upon bombarding the infection spot, the incident water drops split into many

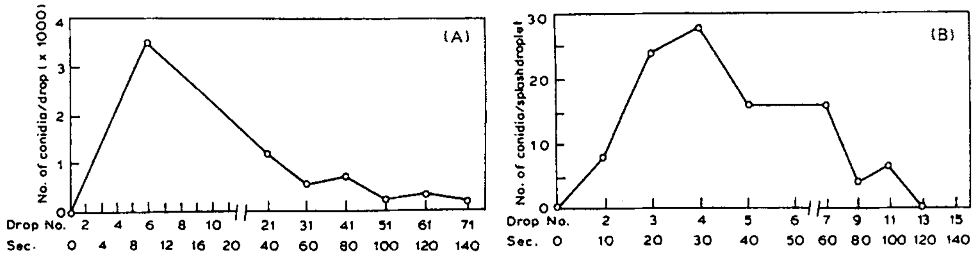


Figure 7. Liberation of *R. sorghi* conidia from sporodochia by flowing (A) and splashing (B) water drops. The intervals between water drops were 2 and 10 s respectively.

splash droplets and some were trapped on microscope slides exposed in the splash-collecting unit of the splash-off assembly. They formed roughly circular haloes on the fast green coated slides. The number of the conidia released into splash droplets varied from drop to drop (figure 7B). The highest number of conidia were observed in the splash droplets arising out of impacting water drops from 3–7 (up to 28,000) and the conidial liberation fell thereafter to a figure less than 100 in the thirteenth drop, the last one to be tested.

4. Discussion

Practically no information is available on the dispersal of inoculum of *R. sorghi*. The present study has revealed that the sclerotia are liberated from lesions by mechanical disturbances are dispersed through the air, become deposited on the soil, and function as perpetuating propagules. Further work is necessary to understand the airborne dispersal of sclerotia of *R. sorghi*.

The conidia of *R. sorghi* were not encountered in the spore trap traces used to monitor airborne particles, but they were caught in splash traps. The field observations revealed the close relation between the appearance of the sooty stripe symptoms and the rainfall patterns. Further, the results of the experiments involving splash-off and wash-off techniques have conclusively established that the conidia of *R. sorghi* are splash-borne, 'wet type' and require the energy of flowing water or splashing water drops for their liberation and dispersal. A similar mechanism of splash dispersal has been reported for *Collectotrichum graminicola* (Rajasab and Ramalingam 1985) *C. coffeanum* (Waller 1972), *Hemileia vastatrix* (Rajasab and Rajendran 1983) and *Fusarium moniliforme* (Ooka and Kommendahl 1977).

No correlation was observed between the number of conidia monitored in the spore trap and the progress of disease in the field. This is probably because of the inefficiency of splash traps in determining the quantitative concentrations of splash-borne conidia and also because of the possibility of counting *Gloeocercospora sorghi* conidia along with those of *R. sorghi*, although the latter species differs markedly from the former in having usually branched conidia.

An analysis of experimental data obtained in the present study reveals that the dispersal of conidia in *R. sorghi* is related to the amount of water flowing over the infection spot rather than the time interval between the incident water drops. This suggests the capacity of spore dispersal in the pathogen even during small showers of rain. The possibility of splash-borne conidia becoming air borne during small showers of rain is indicated by Gregory (1973). This phenomenon may not operate during heavy showers of rain when the falling rain drops scrub down the airborne conidia. In such conditions the phenomenon of transport of conidia by flowing water has an important bearing on the epidemiology of sooty stripe of sorghum.

Acknowledgements

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