SMUT SPORE TRAPPING STUDIES CONDUCTED IN AUSTRALIAN EAST COAST PRODUCTION AREAS IN LATE 2007–2008

By

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Abstract

SUGARCANE SMUT has continued to spread to new regions during 2008 and more widely within already infested regions; the major spread mechanism is air-borne spores. In earlier 2007 studies, spore traps operating in un-infested districts were able to detect spores of the pathogen in the Mossman, Burdekin, Proserpine, Maryborough and New South Wales districts. In 2008, more extensive trapping was undertaken in some of these and other districts in order to determine the likely presence of the disease in un-infested areas. Spores were detected for the first time in the Mulgrave, Tableland, South Johnstone, Babinda and Tully areas, with disease symptoms sighted for the first time in Mulgrave, Tableland and Tully in the latter half of 2008. More extensive detections were made in the Innisfail and New South Wales regions and these have all confirmed the likely movement of smut to new mill areas. The finding of smut symptoms in crops in the Tableland, Mulgrave, Tully and Burdekin areas has confirmed the usefulness of the technique as an early warning tool. The disease is yet to be found in the Mossman, Babinda, Rocky Point and New South Wales mill areas, though it is highly likely that smut is present within these regions. Spore trapping is now planned for monitoring the intensity of atmospheric spore populations in more heavily infested regions as a means for assessing the progress, peak and decline of the Australian smut epidemic.

Introduction

Sugarcane smut occurs in almost all sugarcane-producing countries around the world and an epidemic has been progressing in the eastern Australian sugarcane industry since 2006. The disease is caused by the Basidiomycete pathogen *Ustilago scitaminea*. A characteristic smut symptom is the unique whip-like structure produced from the apex of infested shoots.

The whip-like structures produce spores which are released in abundance into the atmosphere to infest disease-free stools and crops in surrounding areas. Up to

10^8 spores per day are released from each whip over a three-month period (Simmonds, 1994). Being tiny (5–7 µm) and easily wind-borne, spores may blow over very long distances. Their presence in the atmosphere provides opportunity to detect the likely presence of the disease before symptoms are seen in crops.

Hoy *et al.* (1991) successfully used spore traps to detect sugarcane smut spores around Louisiana crops and showed how atmospheric populations varied with distance from infested crops. Magarey *et al.* (2008) reported how spore traps have been used for the detection of pathogens in other crops and environments and provided information on the success of spore traps for detecting *U. scitaminea* spores in the Australian sugarcane industry. Variations in atmospheric spore populations with time of day, distance from infested crops and with varying environmental conditions were reported.

In this paper, we report on spore trapping as an early-warning tool for smut in eastern Australian cropping areas, particularly those districts where either inadequate spore trapping had been undertaken previously, or no smut had been reported prior to 2008.

Methods

Spore traps

Magarey *et al.* (2008) describe the equipment used in spore trapping. Briefly, Burkard 'Sporewatch spore and pollen samplers' (spore traps) were used, consisting of an air pump (constant 10 L/min inflow), an orifice of slit design (14 mm × 2 mm), a round drum revolving at a constant speed, and a sticky tape coated with petroleum placed on the periphery of the drum.

The air pump draws air in through the orifice at right angles to the rotating drum. Spores entering the orifice are directed at the surface of the sticky tape and they embed as the air circulates past the drum.

Spore identification

Both light microscopy and molecular techniques were used to confirm the identity of *U. scitaminea* spores collected in spore traps. Other closely related fungal species were sourced and compared to *U. scitaminea* so that an accurate light microscope detection technique could be developed. In addition, a molecular test involving PCR was also developed; this provided greater certainty in assay results and allowed faster analysis of spore tapes (Magarey *et al.* 2008). The PCR test targets the ribosomal internal transcribed spacer (ITS). All DNA samples were initially screened with the universal ITS primers ITS1F and ITS4 (Gardes and Bruns, 1993; White *et al.*, 1990). This endogenous test is to assess the quantity and quality of DNA extracted from the tape. A negative result indicates the presence of PCR inhibitors such as dust or ash, or low DNA levels. The specific sugarcane smut test uses the primers ITS1F and Rev2 (5'-GATCCGCCAGCTCTTTCGTAAT-3'). The primer Rev2 was designed to be specific for sugarcane smut based on DNA sequences provided by Stoll *et al.* (2003).

District trapping

Following the development of the molecular assay, more intense spore trapping in uninfested districts/regions became feasible and the results provided an indication of the presence of smut.

As undertaken previously (Magarey *et al.*, 2008), spore traps were placed close to crops of susceptible varieties. Up to nine traps were operated concurrently in order to

better define the presence of *U. scitaminea* spores in an area. Trapping periods were generally 18–48 hours. At each sampling site, starting and end points were marked on spore trap tapes to aid identification of samples; samples were separated by 1/12 turn (approximately 30 mm) to avoid overlap. A summary sheet was also completed which contained details of weather conditions, run times and GPS co-ordinates. Tapes from spore traps were removed and dispatched to BSES Indooroopilly for assay.

Details of the trapping undertaken in late 2007 and 2008 (northern Queensland, Sunshine Coast, Rocky Point and New South Wales regions) are outlined in Table 1. Intense trapping occurred in individual regions at different times of the year. Trapping was undertaken at many sites in northern Queensland in March 2008. One of the difficulties with spore trapping is predicting weather conditions. Prior to the March sampling, fine weather was predicted. This soon changed and sites in the Innisfail, Babinda, Mulgrave and Mossman areas were trapped under typical northern wet season conditions – heavy rainfall arising from monsoonal influences.

Most trapping in northern Queensland in July 2008 was under much more favourable weather conditions, with harvest operations in full swing.

Table 1—The timing of spore trapping undertaken in each region of the east coast Australian sugarcane industry from late 2007 to October 2008. Numbers refer to the number of sites where spore trapping occurred during that period.

	Year	2007		2008				
District	Local area	November	December	January	March	April	July	October
Northern	Mossman				7		49	
	Tableland				7		9	
	Mulgrave				8		6	
	Babinda				2		9	
	Innisfail				13		18	
	Tully				7		7	
	Kennedy		4	4			4	
Southern	Sunshine Coast	9						
	Rocky Point	8						
New South Wales	Condong		7		6	12		2
	Broadwater		7		3	10		
	Harwood		7			10		

Results

The results from the trapping undertaken in each district are detailed below.

Northern

March sampling: In the March 2008 round of spore trapping, only one positive spore trap site was identified and this was in the Innisfail area. Despite positive spore trap sites being identified in Mossman in mid-2007 (Magarey *et al*., 2007), no positive sites were detected in March 2008.

July sampling: A number of positive spore trap sites were identified during July 2008 (Table 2). Data for percentage positive sites should be treated with caution due to low sample numbers in some locations. However, it is obvious that sugarcane smut spores were present across the whole northern region.

Table 2—Details of the percentage of sites where sugarcane smut spores were located in July 2008 spore trapping undertaken in northern Queensland mill areas. (Tot. = total number of trap sites; + = number of positive smut spore trap sites; % = percentage of trap sites where spores were detected).

Location	Tully			Innisfail			Babinda			Tableland			Mulgrave			Mossman		
	Tot.	+	%	Tot.	+	%	Tot.	+	%	Tot.	+	%	Tot.	+	%	Tot.	+	%
# sites	4*	1	25	18	2	11	9	1	11	9	2	22	6	2	33	49	5	10

* Only four sites are shown here as three could not be analysed because of dust contamination

Due to adverse weather conditions, the Mossman sampling was undertaken in two batches. The first was under showery conditions and 3% of 30 sites were positive for smut. The second batch was sampled under clear conditions when crop harvesting was occurring; 21% of 19 samples were positive under these conditions.

A map of the Tully Mill area illustrates the spatial distribution of spore trap sites in July 2008 and the first finding of smut symptoms in sugarcane crops in the Murray district of the Tully Mill area in October 2008 (Figure 1). Three trap sites were contaminated by dust arising from haul-out machinery operating close by the traps (Murray, Lower Tully and El Arish). This was unfortunate as the Murray spore trap site was in the midst of what were later to be identified as smut-infested crops. This illustrates the potential for other influences to affect spore trap outcomes.

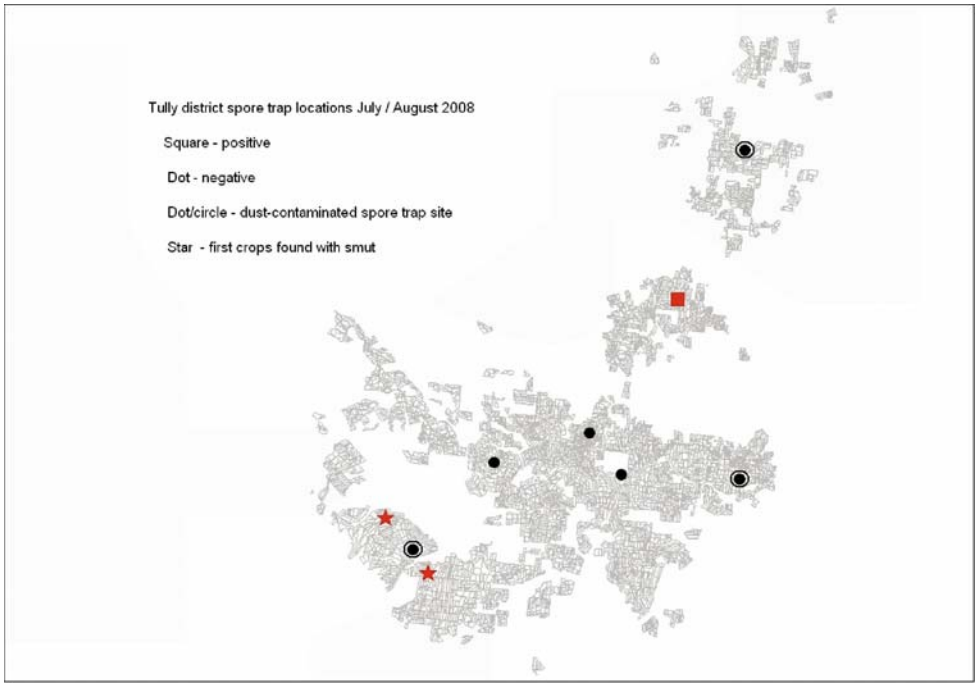

Fig. 1—Crop outlines from the Tully Mill area showing the locations of the July 2008 spore trapping sites (square = positive smut spore site, circle = negative site, circle with outer ring = dust-contaminated trapping site) and the crops where smut symptoms were first detected in October 2008 (star).

Sunshine Coast

No positive spore trap sites were identified in November 2007 near Sunshine Coast sugarcane crops.

Rocky Point

As for the Sunshine Coast, no positive spore trap sites were identified in the Rocky Point Mill area in November 2007.

New South Wales

Spore trapping was undertaken at various times during the 2007–2008 period. A larger number of sites were sampled in the Condong area and several of these (for instance October 2008) were specifically to follow up on earlier positive spore trap detections. Results for the 2007–2008 samplings are outlined in Table 3.

Table 3—Details of the percentage of trapping sites where sugarcane smut spores were located in 2007–2008 spore trapping undertaken in New South Wales sugarcane production areas. (Total = total number of trap sites; + = number of positive smut spore trap sites; % = percentage of trap sites where spores were detected).

Location	Condong			Broadwater			Harwood		
	Total	+	%	Total	+	%	Total	+	%
December 2007	7	2	29	7	0	0	7	0	0
March 2008	6	1	17	3	0	0			
April 2008	12	1	8	10	0	0	10	0	0
October 2008	2	2	100						

Spore trap detections and first identified crop symptoms

Table 4 provides information from 2007 and 2008 spore trapping activities relating to when smut spores were first detected in specific districts / regions and when crop symptoms were first found. These indicate how much warning cane farmers in these districts had of a possible disease incursion, as provided by spore trapping.

Table 4—Information on spore trap smut spore detections in selected districts and the date when smut crop symptoms were first seen illustrates the length of warning provided by spore trapping to local cane farmers with regard to the likely presence of smut in their area.

District	Spores detected	Crop symptoms identified
Mossman	July 2007	December 2008
Tableland	July 2008	September 2008
Mulgrave	July 2008	September 2008
Burdekin	April 2007	October 2008
Proserpine	July 2007	December 2007
Maryborough	March 2007	January 2008

Discussion

A definitive outcome of the work reported in this paper is the identification of sugarcane smut spores (via spore trapping) in many districts where the disease had not been observed prior to 2008. Smut is obviously spreading to new areas within Queensland and New South Wales, just as expected from our knowledge of smut epidemiology (Magarey *et al*., 2009). There were several areas where positive spore assays were accompanied by the observation of smut symptoms in crops for the first time in 2008. These include the Tableland, Mulgrave and Tully. The time delay associated with the molecular smut assays meant that on several occasions, the positive spore trap assay results were received just after smut was observed in the field (Tully), around the same time as whips were located in crops (Tableland) or prior to crop symptom identification (Mulgrave). The positive assays provided additional information on the likely distribution of the disease in some of the affected districts.

In the Tully mill area, smut was observed in the drier Murray district in the susceptible Q174$^{(\dagger)}$. This variety is known from other work to be highly susceptible and one of the varieties in which smut is often first seen (Magarey *et al*., 2009). The first disease symptoms were seen in October 2008 close to a July 2008 spore trap site. Two farms were infested, one on the north-western side of the trap site and another on the eastern side. Unfortunately, in-field haul-out traffic adjacent to the trap site caused high levels of dust contamination on the collection tape and no conclusion could be drawn from molecular assays. However, positive spore trap results from the wetter Feluga area, on the northern outskirts of Tully, provide little doubt that the disease is more widespread that in just the Murray district. Cane farmers should keep implementing a proactive smut management strategy in all parts of the Tully area.

Similar observations were made for the Tableland where smut symptoms were first seen in crops on the western side of Mareeba. A positive spore trap site was identified on the eastern side of the Mareeba township, again suggesting that the disease is more widespread than field observations suggest. The generally favourable environment for smut in the Mareeba region also suggests that smut spread will be rapid.

In the Mulgrave mill area, positive spore trap sites were widely divergent; detections were made in both the northern and southern parts of the area. The disease has been initially seen in northern crops only. Trapping suggests that smut will soon be distributed right across the Mulgrave mill area. New reports of smut-infested farms are being received and the disease is likely to rapidly escalate (Magarey *et al*., 2009).

There are other mill areas where smut spores have been detected widely across the area. The most prominent example is the Mossman mill area, where positive spore trap detections were made in early-mid 2007 (Magarey *et al*., 2007) and also in 2008. It was only in late 2008 (December) that disease symptoms were first seen in the field. Consistent trapping of smut spores across the Mossman area suggests that many crops are likely to be infested. The length of time between the first spore detection (mid-2007) and ‘now’ suggests there has been significant opportunity for smut to increase in severity in affected crop(s). As a result, we predict disease severity is likely to be high in some newly identified smut-infested crops. Spores have also been detected in the Babinda mill area in northern Queensland. It can only be a matter of time before crop symptoms are seen in this mill area also.

In southern districts, no spores were detected in either the Sunshine Coast or Rocky Point production areas. Being somewhat isolated, and also with cooler temperatures than in other Queensland cane production areas, the disease may take longer to infest crops. Given the relatively long time period since the last spore trapping activity (November 2007), further spore trapping could be beneficial in these areas.

Spore traps have been operating in New South Wales production areas on a consistent basis since mid-2007. Positive trap sites were confirmed in the Condong mill area only; no positive sites have been detected in either Broadwater or Harwood. As with the Mossman area, it seems highly likely that smut has infested Condong crops too. On more than one occasion spores were detected close to specific farms and continued inspections in susceptible crops close-by seems an appropriate response. The potential significance of sugarcane smut to New South Wales crops remains to be seen, given that the lower temperatures influencing the area may decrease disease severity.

The operation of spore trapping technology has proved valuable in assisting the sugarcane industry to identify likely areas where the disease may be present – especially given the results reported in this paper and previously (Magarey *et al*., 2008; Table 4). The recent sugarcane smut finding in the Burdekin has finally confirmed the conclusions drawn from extensive trapping undertaken there in early-2007. If local cane farmers took note of the implications of the early spore trap results, a speedy implementation of disease management plans could have been undertaken, particularly the transition to more resistant varieties. Identifying the urgency for transition is a key outcome from an early detection tool, and farmers in Mossman, Burdekin, Proserpine and Maryborough all had some warning of a likely incursion. Farmers in the Babinda and Condong mill areas are in the same position as Burdekin farmers were earlier this year—smut spores have been locally detected, but no disease symptoms have been seen in crops. We recommend these farmers continue to take steps to ensure sufficient resistance is present in crops on their farms, to withstand the inevitable local smut epidemic. Only time will tell of the timeliness and scale of adoption of this strategy by farmers in each of the production areas.

Weather conditions are likely to have influenced trapping results. Heavy rainfall experienced in March 2008 no doubt affected atmospheric smut spore densities; there were few positive assays even from areas where smut spores had been detected previously. On the other hand, in July 2008, a number of positive detections were made. There could be several contributing factors, including: (i) harvesting operations, with the use of extractors and intense agitation of shoots leading to additional wind movement of spores from whips, and higher spore densities in the surrounding atmosphere; (ii) drier conditions – Hoy *et al.* (1991) reported on the increased spore densities surrounding infested crops associated with lower relative humidity. These factors should be considered when planning the timing of spore trapping operations.

A recently suggested strategy has been to use spore traps as a means of quantifying the atmospheric spore concentrations present in heavily infested cropping regions (such as the Herbert, Mackay and Bundaberg-Childers areas). This would assist the local industry to identify when the peak of the smut epidemic occurs and to relate observed levels of disease in varieties of intermediate resistance with progress of the epidemic. This will provide information on how these varieties are able to withstand the developing infection pressure and whether they are able to fulfil an important role as alternative high-yielding varieties for

cane farmers as they transition from susceptible to resistant crops. The success of this technique will rest on whether quantitative PCR technology can be developed to quantify spore levels on spore trap tapes. If so, then several very useful outcomes may arise from spore-trap technology in the Australian sugarcane industry.

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