How the misidentification of a pathogen can cause an emergency response – a real life case study of an Australian grain export incident

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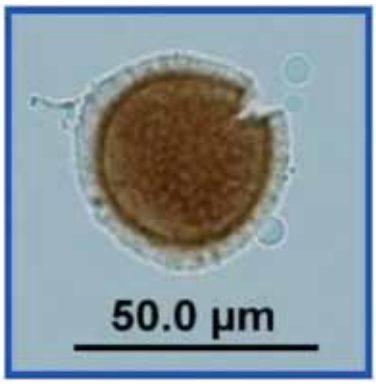
In February 2004, a shipment of Australian wheat was rejected by an importing country that alleged the grain contained ustilospores of *Tilletia indica*. This allegation caused all wheat trading and shipments from Australia to be stopped. At this time, Australian wheat was worth A$4 billion and provided 14% of the world's export demand[1]. How did this misidentification occur? And why is correct identification of pathogens so important when trading with other countries?

The disease Karnal bunt of wheat is caused by the smut fungus *Tilletia indica.* It was first identified in Karnal, India in 1931[2]. The pathogen replaces part of the seed with a black powdery mass containing millions of spores and produces a strong unpleasant odour like rotten fish. This is due to the production of trimethylamine, which makes the grain unpalatable. It causes a very small reduction in yield. Because of the reduction in quality, the marketability of the grain is reduced. *T. indica* is not widely distributed throughout the world, and as a result it is subject to very strict quarantine regulations to prevent its introduction into Australia and other countries not known to have the disease.

In March 2004, Australian wheat harvested from the 2003 growing season that was still in storage awaiting export or in the process of being exported was surveyed. The 300 samples were processed by a task force of plant pathologists using the National diagnostic protocol[3] which was based on the EU protocol[4] that had been developed previously. The results from these samples found that there was a range of smuts and bunts present in the grain samples including *Tilletia caries* (common bunt), *T. laevis* (common bunt) and *Urocystis agropyri* (flag smut) which are known to infect wheat and two smuts, *T. walkeri* and *T. ehrhartae,* that do not infect wheat[2]. These two smut fungi infect Lolium spp. and *Ehrharta calycina* (perennial veldt grass) respectively. Both of these ustilospores are similar enough to *T. indica* for misidentification to occur when there are very few ustilospores present in a sample and were the reason for the rejection of the Australian grain shipment.

Table 1 provides descriptions of the *Tilletia* species that can be detected in Australian wheat and individual ustilospores of the *Tilletia* species are shown in Figure 1. *Tilletia walkeri* is morphologically similar to *T. indica* due to its overlapping size, its dark reddish brown colour and spines that are conical to truncate in side profile. However, the ornamentation on the surface of *T. walker* is coarsely cerebriform rather than finely cerebriform, and *T. indica* is generally much larger, having a mean spore size of 41 μm compared to that of *T. walker of* 30 μm. *Tilletia ehrhartae* is much smaller than these being only 25 μm and has very coarse polygonal scales. Pascoe *et al.*[2] provide essential criteria to differentiate *T. walker* and *T. ehrhartae* from *T. indica* and other *Tilletia* species. However, the ability of someone being able to differentiate ustilospores morphologically is highly dependent upon their skill and training as a taxonomist in plant pathology, and is made easier if there are a large number of ustilospores present in the sample being tested.

How do we prevent a similar situation from occurring? The Cooperative Research Centre for National Plant Biosecurity (CRC NPB) supported further work to improve the identification methods currently used by many countries. The key outcome of



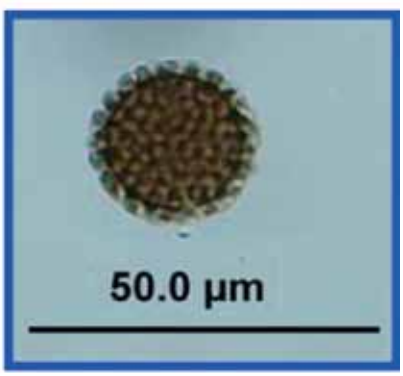


Figure 1. *Tilletia indica* *Tilletia walker* *Tilletia ehrhartae*

Table 1. Morphological description of *Tilletia* species detected in Australian wheat.

Species	Spore size range (µm)	Spore size mean (µm)	Spore colour	Spore shape	Spore sheath	Spore spines	Host
T. indicaa	28–54	35–41	Brown to dark reddish-brown, opaque	Globose to sub globose	Present	1.4–5 µm In surface view densely echinulate or as closely spaced, narrow ridges (finely cerebriform). In median view, smoother more complete outline due to spines being densely arranged occasionally with curved tips	Triticum sp.
T. walkerib	28–35	30–31	Pale yellow to dark reddish-brown (never black/opaque)	Globose	Present. Extending to tips of projections, hyaline to yellowish brown	3–6 µm coarse +/– cerebriform. Wide incompletely cerebriform ridges in surface view In median view, profile is irregular with gaps between spines	Lolium perenne and L. multiflorum.
T. ehrhartaed	17–25	23–25	Very dark olivaceous brown when mature. Can be opaque due to melanisation of the scales.	Globose to sub globose	Present. Extending to the apex of the spines or slightly beyond	1–2.5 µm cylindrical or slightly tapered spines In surface view, rarely cerebriform. Larger, acute polygonal scales In median view, broadly truncated to slightly rounded at apex	Ehrharta calycina,

[a]Authors' data. [b]Based on: Castlebury 1998; Milbrath *et al* 1998; Castlebury & Carris 1999; Cunfer & Castlebury 1999. [c]As T. barclayana: Durán & Fischer 1961; CMI Description no. 75 (1965); Durán 1987; Castlebury & Carris 1999; or as *T. horrida*: Khanna & Payak 1968; Aggarwal *et al* 1990; Castlebury 1998. [d]Pascoe *et al* 2005.

this work was the development of a new method to extract DNA from a single ustilospore and perform multiplex real-time PCR on this DNA sample[5]. In the past, molecular methods relied upon the ability to germinate and grow the ustilospores detected in a sample, to enable enough DNA to be extracted and identified using various molecular methods. This is very difficult when the number of ustilospores is less than 10 in a sample, and also because the spores have an average germination rate of 50%. The 5-plex fluorescent PCR methods developed[5] allow for the rapid identification of common *Tilletia* species detected in grain samples.

The National Diagnostic Protocol for *Tilletia indica* has been updated based on this new method and is currently undergoing a review process before being endorsed by The Australian Plant Health Committee. This new updated protocol will facilitate the rapid identification of *Tilletia* species that may be found in Australian grain. At the same time the International Plant Protection Committee has been working on an international protocol that will provide a methodology for all countries to use, to reduce the number of mistaken identifications. This incident highlights the importance of protocols being available when trying to identify pathogens of international economic and quarantine significance, and emphasises the need for plant pathologists to maintain their traditional skills and abilities in fungal taxonomy.

Acknowledgement

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References

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Biography

Ms Dominie Wright is a plant pathologist with the Department of Agriculture and Food, Western Australia. She has a broad background in plant pathology, having worked in both the broadacre and vegetable sectors as a diagnostician for fungal and bacterial pathogens in Queensland and Western Australia. Dominie has a strong interest in biosecurity, having developed a national diagnostic protocol for *Tilletia indica,* and is currently preparing the international protocol for *T. indica* for the International Plant Protection Committee. She has also developed with colleagues from NSW two national contingency plans for *T. indica* and *Tilletia contraversa.* Dominie enjoys being able to combine methodologies from different disciplines; for example, the use of forensic tape to detect fungal spores on clothing and has a very strong interest in using web-based technology to increase skills and abilities of diagnosticians in Australia.