NAPPO
North American Plant Protection Organization
Organización Norteamericana de Protección a las Plantas

NAPPO Standards for Phytosanitary Measures (RSPM)

RSPM 21
A Harmonized Procedure for Morphologically Distinguishing Teliospores of Karnal Bunt from Ryegrass Bunt, Rice Smut and Similar Smuts

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Review

NAPPO Standards for Phytosanitary Measures are subject to periodic review and amendment. The next review date for this NAPPO Standard is 2019. This Standard was last reviewed in 2014. A review of any NAPPO Standard may be initiated at any time upon the request of a NAPPO member country.

Approval

This Standard was approved by the North American Plant Protection Organization (NAPPO) Executive Committee on October 17, 1999. The current revision was approved on October 20, 2014, and is effective from this date.

Approved and signed by:

Greg Wolff
Executive Committee Member
Canada

Rebecca A. Bech
Executive Committee Member
United States

Javier Trujillo Arriaga
Executive Committee Member
Mexico

Implementation

See the attached Implementation Plans for implementation dates in each NAPPO country.

Amendment Record

Amendments to this Standard will be dated and filed with the NAPPO Secretariat.

Distribution

This standard is distributed by the NAPPO Secretariat, to the Industry Advisory Group and Sustaining Associate Members, the International Plant Protection Convention (IPPC) Secretariat, and to other Regional Plant Protection Organizations (RPPOs).

Introduction

Scope

This standard describes the recommended procedures that NAPPO member countries should follow to morphologically distinguish teliospores of *Tilletia indica* (Karnal bunt), *Tilletia walkeri* (ryegrass bunt) and *Tilletia horrida* (rice smut) and several similar smuts.

References

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Definitions

Definitions of phytosanitary terms used in this standard can be found in NAPPO RSPM 5 and in ISPM 5.

Background

Karnal bunt (*Tilletia indica*), rice smut (*Tilletia horrida*) and ryegrass bunt *(Tilletia walkeri*) are morphologically similar. They can usually be distinguished on the basis of host preference whenever bunted seeds are found. However, if low levels of spores are found in seed washes, the probability of misidentification increases. In a seed wash situation, it cannot always be assumed that the main host and the source of the spores are identical. Therefore this standard provides guidance on morphologically distinguishing teliospores of *T. indica, T. horrida, T. walkeri* as well as teliospores of other *Tilletia* spp. that might be encountered as contaminants in seed washes.

Since the approval of this NAPPO standard, an international diagnostic protocol was approved for *Tilletia indica* (ISPM 27, Annex 04: 2014). The NAPPO Expert Group on Grains compared the two documents and found that the NAPPO standard covered some aspects not included in the international protocol and which were deemed sufficiently relevant so as to recommend keeping and updating the NAPPO standard. The NAPPO standard includes diagnostic details of *T. pulcherrima* and *T. barclayana*, species morphologically similar to *T. indica,* but not mentioned in ISPM 27, Annex 04.

Additionally, the international standard offered refinements to the morphological descriptions *of T. indica, T. horrida*, and *T. walkeri*. Accordingly, these refinements were included in the table found in section 3, Diagnostic Principals, of the NAPPO standard.

Outline of Requirements

This standard addresses the role of identifier and complexities of identification, sample preparation, diagnostic principles and diagnostic support as they relate to morphologically distinguishing teliospores of all bunts and smuts contained in this standard.

General Requirements

1. Role of Identifier and Complexities of Identification

The identifier must be an experienced mycologist who understands the complexity and subtleties of microscopic identifications. Since these fungi have confused experienced mycologists who have considerable expertise with the smut fungi, it should never be presumed that there is a simple formula to follow that will guarantee success. It must be recognized that an experienced mycologist is trained to note subtle differences and details and to focus on features that provide reliable characteristics of distinction. The identifier must know how to prepare a microscope slide.

The identifier must be capable of recognizing *Tilletia* spores in the *indica-horrida-walkeri* complex. Depending on the level of experience, there are a number of fungal spores and other artifacts that can be confounding and challenge the very best mycologists. However, this standard makes the assumption that the basic recognition of a *Tilletia* spore is within the competency of the identifier and that only appropriate spores are included in the diagnostic endeavour.

The fewer the spores present, the more difficult the identification. It should be possible to make a good identification on the basis of 10 clearly seen spores. In practice, the diagnostician often will have access to only one or several spores.

The identifier must also understand the complexities that help form an opinion on the identification of an organism. In addition to morphological data, information on the presumed host and geographic origin is also very important. However, it cannot be automatically assumed that the product the spore is found on is also the host of origin. Spores in a grain lot may be derived from weeds or other contaminants or may be introduced as cross contaminants from another lot during shipping or processing. A comparison of the geographical origin of the shipment with the known geographical distribution range of each fungus may be very helpful in narrowing down the diagnostic options if the information on sample origin is reliable.

2. Sample Preparation

Preparing the sample is a simple procedure, but like all aspects of science, it has its subtleties in producing a clear observation of the object in question. A seed wash, most likely the size selective sieve method developed by Peterson et al. 2000 or some other process must be utilized to produce a microscope slide on which a spore or several spores are clearly visible and not obstructed by debris.

3. Diagnostic Principles

Some "guiding diagnostic principles" have been established to aid the identification process and distinguish these fungi from one another. These are:

3.1 Characteristic teliospores in excess of 36 microns in diameter are most likely *T. indica*.

3.2. Characteristic mature (colored) teliospores less than 22 microns in diameter are

probably neither *T. indica* nor *T. walkeri*. They could be *T. horrida* or another related grass pathogen.

3.3. Characteristic teliospores associated with wheat from an area known to be infected with *T. indica* should be presumed to be *T. indica* unless there is clear evidence to the contrary.

3.4. Characteristic teliospores from locations where ryegrass is produced or known to intermingle with wheat production and which tend to be 28.8-34.8 microns in diameter, which are translucent brown and very spherical in shape, which have blunt spines in median focus and which appear to form blunt ridges on the exospore and which have visible gaps between the spiny ridges can safely be called *T. walkeri*, even when the primary commodity is wheat. The presence of ryegrass seed as a contaminant in a sample will further increase the possibility that *T. walkeri* may be present.

3.5. Teliospores less than 36 microns in diameter with distinctly curved spines have a high probability of being *Tilletia horrida*.

3.6. Teliospores that are opaque black, which do not transmit light, are probably either *T. indica* or *T. horrida. T. walkeri* is never opaque black. Teliospores of all three species can be translucent and appear brown.

Pertinent diagnostic details of similar species are summarized in the Table below:

Diagnostic Feature	***T. indica****	***T. horrida****	***T. walkeri****	***T. pulcherrima***	***T. barclayana***
Diameter size in um	22-64	14-36,<25 if mature	28-35	17-30	18-35
Color	Pale orange brown to dark Reddish brown, Mature spores black to opaque	Light to dark brown	Pale yellow to dark reddish brown	dark reddish brown	Reddish brown to subopaque
Shape	globose to subglobose	globose to subglobose	globose	globose	globose to subglobose
Ornamentation	Teliospore spines 1.4–5(up to 7) 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	Teliospore spines 1.5–4 µm.	Teliospore spines 3–6 µm. Coarse +/– cerebriform. Wide incompletely cerebriform ridges in surface view. In median view, profile is irregular with gaps between spines.	Teliospore spines blunt, 1-1.8 µm long, encased in a hyaline sheath.	Teliospore spines 1.5-5 µm long Warts dense, truncate.

	with curved tips.				
Primary host	*Triticum* spp.	*Oryza* spp	*Lolium perenne* and *Lolium multiflorum*	*Panicum*spp.	*Pennisetum* spp.

*Figures here are taken from ISPM 27, Annex 4 (2014) DP 4: *Tilletia indica* Mitra.

4. Evaluation of Information

Using the information from the previous items, the identifier and regulatory officials should be able to identity a spore or a group of spores. It should satisfy a combination of an appropriate morphological match of features with a direct or indirect association of a suitable host and come from an area where Karnal bunt is present or is likely to occur as determined on the basis of pest risk analysis. Whenever a deviation from this combination occurs, further investigation, trace-backs, sampling, analysis and verification of information may be necessary to confirm the identification.

5. Diagnostic Support

NAPPO countries have access to the following individuals who can provide diagnostic advice or will direct inquires with their country to appropriate individuals. These include but are not limited to the following scientists (in alphabetical order):

Stephan C. Briere, M.Sc., Canadian Food Inspection Agency, Ottawa, Canada - Stephan. Briere@inspection.gc.ca
Dr. Lori Carris, Washington State Univ., Pullman, WA - carris@mail.wsu.edu
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Dr. Guillermo Fuentes-Davila, INIFAP-CIRNO, Mexico - guillermofuentes_davila@hotmail.com
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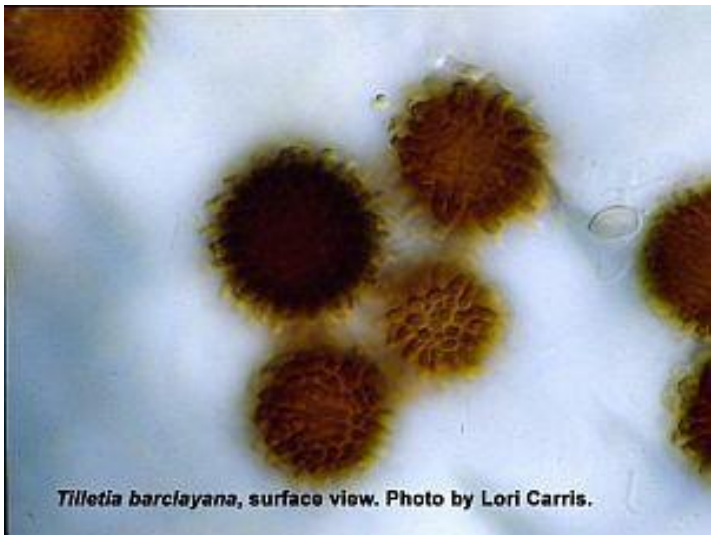
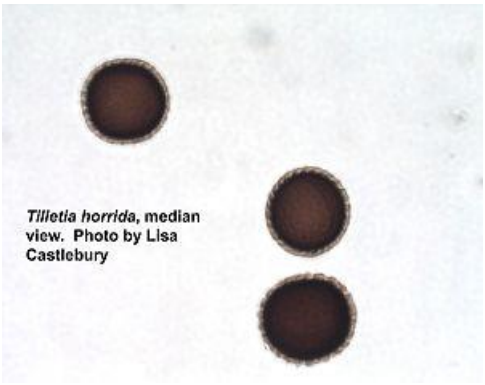
Appendix 1

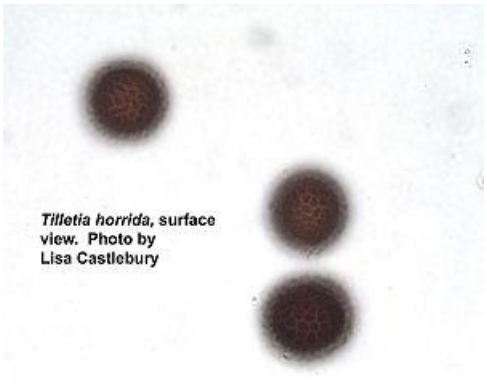
Pictures of teliospores of Karnal bunt, ryegrass bunt, rice smut and similar smuts

barclayana



horrida





indica

Tilletia indica, surface view.
Photo by Lisa Castlebury

Tilletia indica, median view.
Photo by Lisa Castlebury.

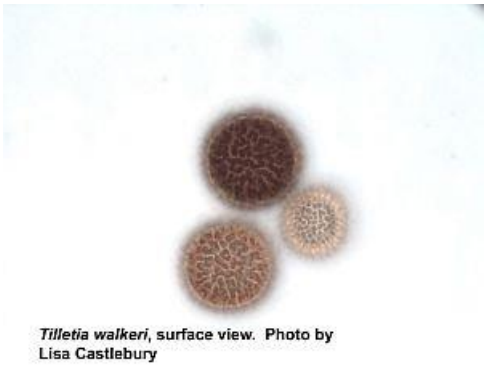
pulcherrima

Tilletia pulcherrima, median view.
Photo by Lori Carris

Tilletia pulcherrima, surface view.
Photo by Lori Carris.

walkeri

Tilletia walkeri, median view.
Photo by Lisa Castlebury.



Tilletia walkeri, surface view. Photo by
Lisa Castlebury

Tilletia indica

Tilletia barclayana