

NAPPO Standards for Phytosanitary Measures (RSPM)

**RSPM# 21
A Harmonized Procedure for Morphologically Distinguishing Teliospores of
Karnal Bunt, Ryegrass Bunt and Rice Bunt**

The Secretariat of the North American Plant Protection Organization
59 Camelot Drive, Nepean, Ontario, Canada
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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 October, 2004. A review of any NAPPO Standard may be initiated at any time upon the request of a NAPPO member country.

Endorsement

This standard was approved by the North American Plant Protection Organization (NAPPO) Executive Committee on October 17, 1999.

Approved by:

Executive Committee Member
CANADA

Executive Committee Member
UNITED STATES

Executive Committee Member
MEXICO

Amendment Record

Amendments to this Standard will be given a consecutive number, dated and filed with the NAPPO Secretariat.

Distribution

This Standard is distributed by the Secretariat of the NAPPO within NAPPO, including Sustaining Associate Members and Industry Advisory Groups, to the FAO/IPC Secretariat, to the ICPP, and to the Administrative Heads of the Regional Plant Protection Organizations (RPOs). Copies are available upon request to the NAPPO Secretariat and are available on the NAPPO web page: www.nappo.org.

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 bunt).

REFERENCES

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OUTLINE OF REQUIREMENTS

Karnal bunt (*Tilletia indica*), rice bunt (*Tilletia horrida*) and ryegrass bunt (*Tilletia walkeri*) are morphologically similar. They can usually be distinguished on the basis of host preference whenever banded seed 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. In this standard, the issue of how to distinguish these three fungi is addressed.

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 endeavor.

The fewer the spores present, the more difficult the diagnosis becomes. It should be possible to make a good diagnosis on the basis of 10 clearly seen spores. In the real world, the diagnostician is often confronted by a small number or even few as a single spore.

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 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 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

In essence preparing the sample is a simple procedure, but like all aspects of science, has its subtleties in order to produce a clear observation of the object in question. A seed wash, most likely the size selective sieve method developed by Gary Peterson 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 are most likely *T. indica*.
- 3.2 Characteristic mature (colored) teliospores less than 22 microns 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 size, 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 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 each species are summarized in the Table below:

Diagnostic Features	<i>T. indica</i>	<i>T. horrida</i>	<i>T. walkeri</i>
Size (microns)	22-61	17-36	26-44
Color	brown to opaque black	brown to opaque black	light to dark brown, never opaque black
Shape	globose to subglobose	globose to subglobose	very spherical
Spines	dense, 1.5-7.0 microns long; form a verrucose surface ornamentation	may appear curved; tips are often large blunt dots	often blunt in median view; forms ridges on the spore surface, sometimes similar to those of <i>T. indica</i>
Main Host	wheat	rice	ryegrass

4. Evaluation of Information

Using the information from the previous items, the identifier and regulatory officials should be able to come to an opinion about the identity of 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 known to occur 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 resolve the identification.

All identifications should gravitate to the highest level of risk that results from the available information. If the identity of a 35 micron teliospore is in question and it can be associated with wheat, for regulatory purposes, it should tentatively considered to be *T. indica* until more information becomes available or another method is utilized to make a more definitive 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).

Dr. Lori Carris, Washington State Univ., Pullman, WA - carris@mail.wsu.edu
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