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Bunts and Smuts Revisited: Has the Air Been Cleared?

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Introduction

In the current era when worldwide commerce of agricultural and food products is critical to the economic well being of many countries, great concern develops when movement of such commodities is interrupted by quarantines. In some cases, these quarantines involve plant pathogens. One example of this has involved the smut fungi; particularly dwarf bunt and Karnal bunt. Many countries have an embargo on receiving grain carrying teliospores or bunted kernels of Karnal bunt (Fig. 1), thus restricting movement of grain from areas where this pathogen is known to occur. This review is directed toward the current situation regarding these two pathogens of wheat.

Fig. 1. Karnal bunt infected wheat kernels showing black masses of teliospores (click image for larger view).

International Symposium

Since the 1996 electronic bulletin board that the American Phytopathological Society sponsored on Karnal bunt of wheat, there have been a number of developments that may be of interest to readers who are concerned with the issue of "new and emerging" diseases. First, in August 1997, an international symposium on the bunts and smuts of wheat was held in Raleigh, North Carolina with over 200 scientists, farmers, agribusiness people, and other interested parties in attendance. The proceedings of that symposium, *Bunts and Smuts of Wheat: An International Symposium*, are now available (3). Three major smuts were covered: Karnal bunt, dwarf bunt, and flag smut. Although no specific recommendations emerged from this meeting, there was a consensus that both flag smut and Karnal bunt are not diseases that cause major economic losses in and of themselves, but their major impact is on worldwide trade of the wheat commodity caused by quarantines. Dwarf bunt is known to cause some production losses, but they are restricted to very small geographic areas where winter wheat is produced with periods of long snow cover during the winter.

Smut Workshop

In June 1998, the 11th Biennial Workshop on the Smut Fungi was held in Moscow, Idaho where a number of papers and posters were presented. The abstracts for the workshop papers discussed below are provided as an appendix to this article. In July 2000, the 12th Biennial Workshop on Smuts will be held in Puerto Vallarta, Mexico.

Ryegrass Smut Look-Alike

In 1996, there was a major concern with the finding of Karnal bunt-like teliospores in ryegrass and in wheat where ryegrass had grown as a weed (Fig.

2). Initial tests suggested that the ryegrass isolate was nearly identical to the wheat Karnal bunt, and even pathogenicity tests showed that the ryegrass isolate could infect wheat when artificially inoculated. Now, based on work reported at the Moscow workshop, it appears that the ryegrass isolate is sufficiently different that it should be described as a new species of *Tilletia*, one that has wheat as an "experimental host" but which naturally does not move from ryegrass to wheat. This new species is *T. walkeri* (2).

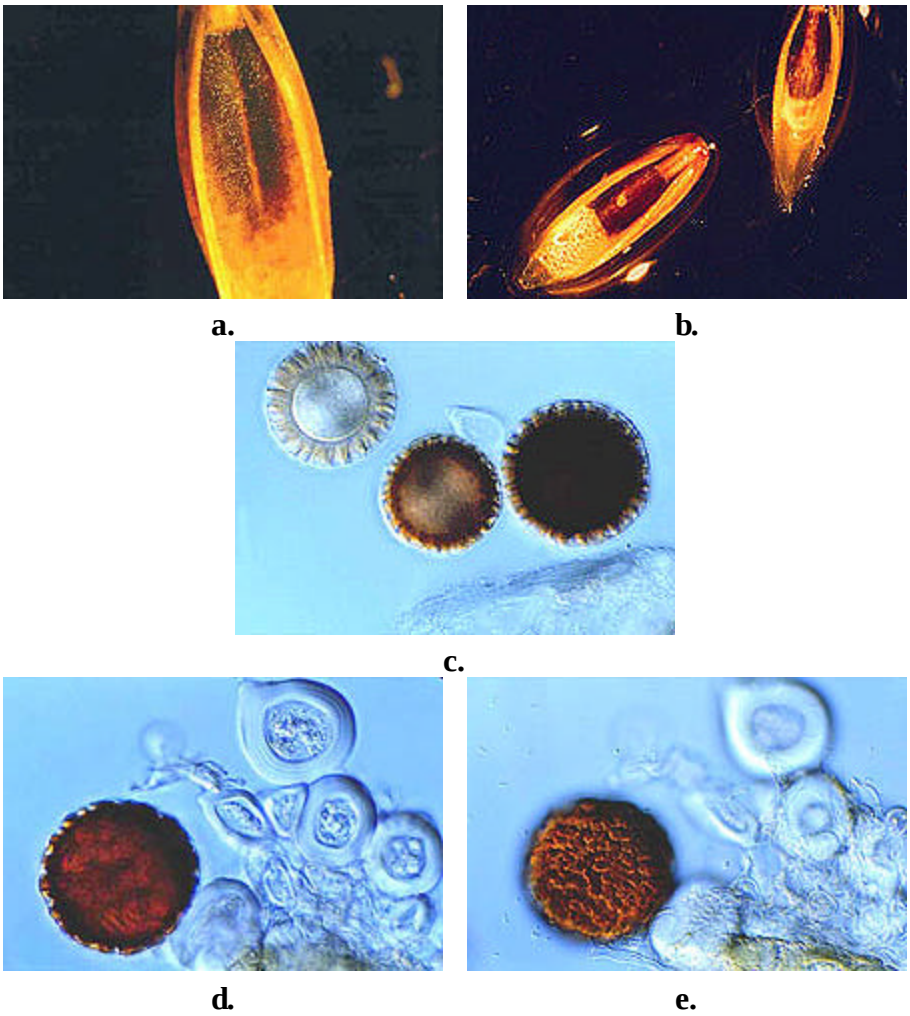

Fig. 2. Ryegrass seed infected with *Tilletia walkeri* that is the karnal bunt "look-alike" along with teliospores of *T. walkeri* (click each image for larger view).

Some of the Highlights of the Moscow Workshop

L. M. Carris reported on the first record of the ryegrass smut, which looks like *Tilletia indica*, and its known distribution (see Appendix). Herbarium specimens of ryegrass collected in 1967-68 in the Kangaroo Valley of Australia were infected. Levels of infection were less than 1% with most consisting of just a few bunted seeds. This smut also occurs in low levels in annual ryegrass in Oregon in both commercial fields and as weeds in wheat fields.

L. A. Castlebury and L. M. Carris (2) studied the morphology of teliospores of the ryegrass isolate of *Tilletia* using teliospores from Tennessee, Oregon, and Australia. Based on teliospore color, size, and ornamentations, they were able to distinguish this isolate from *Tilletia indica* from wheat thus supporting the description of the ryegrass isolate as a new species, *T. walkeri*.

Barry Cunfer and L. A. Castlebury surveyed 190 fields of wheat in Georgia, Alabama, and Tennessee in 1997 for the presence of annual ryegrass seeds and suspected karnal bunt (1). Teliospores from four samples were similar to those of karnal bunt. Annual ryegrass is a common weed in southeastern USA wheat fields and was associated with all teliospore positive samples in APHIS surveys.

L. Levy and others studied the *Tilletia* species from ryegrass and compared it to *Tilletia indica* from wheat (see Appendix). Using molecular techniques, they

found two restriction enzyme sites in the ITS-1 region that were present in the ryegrass isolates, but were absent in the wheat isolate. Sequence analysis showed them to be unique to the ryegrass isolate.

G. Pimentel and L. M. Carris compared the genetic relationships of *Tilletia indica* and related species, especially the ryegrass isolates (see Appendix). "Based on PCR-RFLP and RAPD analyses, the ryegrass smut is most similar to *T. indica*, but the low level of similarity (ca., 25%) in the RAPD analysis does not support conspecificity of both taxa".

Evidently, the feeling is that although the ryegrass isolates of *Tilletia* can infect wheat if inoculated, wheat is just an "experimental" host and not a "natural" host. Therefore, this isolate deserves to be described as a new species of *Tilletia*, *T. walkeri*. This would certainly help explain why there has been no karnal bunt of wheat found in Oregon, even in wheat fields in close proximity to ryegrass fields naturally infected with the karnal bunt-like organism.

Karnal Bunt Locations

New findings of Karnal bunt of wheat occurred in 1997 in central Texas, but no other areas of the United States covered by the second year of extensive surveys of the wheat crop by APHIS showed any evidence of the disease. Surveys of the 1997-98 and the 1998-99 crops of wheat were conducted with no new occurrences of Karnal bunt being reported. Previously reported findings of Karnal bunt in New Mexico and eastern Texas are now known to be false (Gary Peterson, USDA, *personal communication*).

Update on Dwarf Bunt and the Embargo by the People's Republic of China

In 1999, the USDA presented a Pest Risk Assessment for dwarf bunt and the People's Republic of China. This document indicated that the chances of dwarf bunt establishing in the winter wheat crop in China were extremely small, based on an analysis of the weather conditions for the winter wheat growing areas, and the number of teliospores that might be introduced into the country on imported grain. Based on this and other information, the Chinese in March 2000 purchased a "test" shipment of wheat from the Pacific Northwest with the caveat that it not contain more than a specified number of teliospores per kilogram of grain.

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Further Reading

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Appendix
Selected Abstracts from the 11th Biennial Workshop on the

Smut Fungi, Moscow, Idaho

The first record of the ryegrass smut and known distribution. L. M. Carris. *Department of Plant Pathology, P.O. Box 646430, Washington State University, Pullman, WA 99164-6430 USA.*

The discovery of *Tilletia indica*-like teliospores in mixed grass seed mixtures from Oregon in late 1996 generated a flurry of activity directed at identifying the source of the spores. Annual ryegrass *(Lolium multiflorum)* was confirmed as the source of the teliospores when the first partially bunted seeds were found in a commercial seedlot in January, 1997. In February, four specimen packets from Herbarium DAR (Australia) of an undescribed *Tilletia* sp. infecting perennial ryegrass *(Lolium perenne)* were found among the smut collections housed in the Department of Plant Pathology at Washington State University. Examination of teliospores in the four packets showed that they were similar to those from the smut found in Oregon annual ryegrass. The Australian specimens had been forwarded to Rubén Durán for identification by John Walker. The specimens consisted of partially bunted ryegrass seed grown in 1967 and 1968 in Australia's Kangaroo Valley. According to Prof. Duran's notation on one packet, he believed the specimen represented a new species of *Tilletia* if the host was indeed *Lolium*. The Australian specimens were later determined to be part of a larger collection of 22 specimens from perennial ryegrass collected between 1967 and 1974 in Australia. According to Mr. Walker, the ryegrass smut has been found only in the ryegrass-growing regions of the Kangaroo Valley and neighboring Nowra districts of New South Wales, Australia. The level of smutted ryegrass seed detected by the Australian Seed Testing Laboratory was less than 1%, with most samples consisting of only a few bunted seeds. The ryegrass smut has not been found in the field, despite intensive search by Mr. Walker and other researchers in the late 1960's to mid-1970's. In addition to Australian specimens, the collection in Herb. DAR contains one packet with spores from a bunted perennial ryegrass seed found in New Zealand in the 1974-1975 harvest. Based on correspondence between Mr. Walker and Dawn Matthews, the scientist at the Seed Testing Station in Palmerston North, New Zealand, the *Tilletia* sp. on ryegrass was found once, and it could not be identified as a known species by mycologists at the Commonwealth Mycological Institute, Kew. The ryegrass bunt occurs in low levels in annual ryegrass *(Lolium multiflorum)* in commercial ryegrass fields in Oregon, and on annual ryegrass growing as weeds in wheat fields in the southeastern U.S. Teliospore counts from 16 commercial fields sampled in the Willamette Valley in 1997 ranged from 0 to 162 spores/25 g of seed. There was no apparent correlation between the level of spore counts and whether the fields had been burned or plowed. The ryegrass bunt occurs as far north as Yamhill County, southwest of Portland, Oregon, based on a collection made in 1997 from annual ryegrass growing as weeds along a roadside ditch.

A Survey of the Southeastern U.S. for Smut Fungi Associated with Ryegrass in Wheat Fields and Their Relation to Karnal Bunt. Barry M. Cunfer and Lisa A. Castlebury. *Department of Plant Pathology, University of Georgia, Griffin Campus, Griffin, GA 30223-1797 and USDA-ARS SBML, Room 304, Bldg. 011A, Beltsville, MD 20705-2350 USA.*

Between April and June, 1997 annual ryegrass *(Lolium multiflorum)* seed were collected from 190 fields of wheat *(Triticum aestivum)* in 47 counties in Georgia and from 26 fields in 17 counties in Alabama and south central Tennessee where suspected Karnal bunt teliospores were found in USDA-APHIS surveys of wheat seed in 1996. About 100 g of ryegrass seed were collected from each field. A 5 g subsample was washed in deionized water containing Tween 20 overnight on a reciprocal shaker. Seed washings were centrifuged and a portion of the pellet was inspected at 200X for teliospores of smut fungi. Spore numbers ranged from 1-50 teliospores in an 1100 square mm area examined on a glass slide. No smutted florets were found in the remnant seed of samples in which teliospores were found. Fields with teliospores generally matched the locations where teliospores were found in APHIS wheat seed surveys in 1996 and 1997. Teliospores were recovered only from completely ripe seed. One sample had spores tentatively identified as *Neozygites parvispora*, an obligate parasite of

thrips. These spores also have been recently identified in ryegrass seedlots from Oregon examined for smut fungi by other investigators. Two samples had teliospores tentatively identified as those of *Ustilago serpens*, a common leaf smut of grasses. Teliospores from four samples (three from Georgia and one from Tennessee) were confirmed to be those typical of the *Tilletia* sp. found on *Lolium* species in other areas of the U.S. and originally thought to be Karnal bunt. This smut is morphologically similar to *Tilletia indica*. The teliospores are 28-39 µm in diameter and range from light to dark reddish brown. They have coarse, widely spaced cerebriform ridges on the surface. The ryegrass smut is the probable source of teliospores found on wheat seed from the southeastern states in APHIS surveys in 1996 that were initially identified as those of *Tilletia indica*. Annual ryegrass is a common weed in southeastern wheat fields and was associated with all teliospore positive samples in APHIS surveys. No bunted wheat caused by *Tilletia indica* or teliospores of the fungus have been found in the southeastern U.S. The survey of ryegrass will be repeated in 1998.

Differentiation of *Tilletia India* from the Undescribed *Tilletia* species on Ryegrass by ITS Sequence-Differences. L. Levy[1], R. J. Meyer[1], L. Carris[2], G. Petersen[3], A. T. Tschanz[1].

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[2]Department of Plant Pathology, P.O. Box 646430, Washington State University, Pullman, WA 99164-6430 USA;
[3]USDA-ARS-FDWSRU, 1301 Ditto Avenue, Fort Detrick, Frederick, MD 21702 USA

During the National Karnal Bunt Survey conducted by APHIS in 1996-97, spores that were morphologically similar to those of *Tilletia indica* were found in ryegrass seed samples. These spores gave the same results as *T. indica* with the PCR-based diagnostics test that the Agency used to distinguish *T. indica* from the morphologically similar *Tilletia horrida*. Morphological, epidemiological and distributional information indicated that the spores in the ryegrass samples were not those of *T. indica*, but rather those of an undescribed species of *Tilletia* that infects ryegrass, referred to here as the ryegrass bunt fungus. Because of the morphological similarity of the spores of *T. indica* and the ryegrass bunt fungus, a molecular test was needed that would distinguish the two species. During the survey, we observed a variation in the length of the PCR products from the Internal Transcribed Spacer (ITS) 2 region of the nuclear ribosomal DNA (rDNA). This result, combined with the fact that the ITS regions of the rDNA have often been useful in species delineation and molecular systematics, led to a study of this region for a possible diagnostic method that would distinguish these two species. Initially the ITS1 and ITS2 from several isolates of *T. indica* and the ryegrass bunt fungus were PCR amplified and sequenced. The sequences could be fully aligned with >98% sequence similarity between the two species in both the ITS1 and ITS2 regions. Despite this similarity, a computer analysis of the sequences revealed two restriction enzyme sites in the ITS1 that were present in the ryegrass bunt fungus but were absent in *T. indica*. Sequence analysis of additional isolates of these species and six other *Tilletia* species suggested that these ITS1 restriction enzyme sites were unique to the ryegrass bunt fungus. One of the restriction enzymes, *Sca* I, was commercially available. Digestion of the ITS1 amplification product from the ryegrass bunt fungus with *Sca* 1 produces two bands, one 155 bp and the other 146 bp. The ITSl product from all five ryegrass bunt fungus sources cut with *Sca* I. However, *Sca* I did not cut the ITS1 product from eleven other species of *Tilletia*, including 26 sources of *T. indica* and 21 sources of all the remaining species. The results indicate that an amplification of the ITS1 region followed by digestion with *Sca* I would be a simple and robust molecular method for distinguishing *T. indica* from the ryegrass bunt fungus.

Genetic relationships among isolates of *Tilletia indica* and allied species based on RAPD and PCR-RFLP markers. G. Pimentel and L. M. Carris. *Department of Plant Pathology, Washington State University, PO BOX 646430, Pullman, WA 99164-6430 USA.*

When *Tilletia indica* was discovered for the first time in the USA in March of 1996, it became a major threat to the export wheat industry. Later in 1996, *T. indica*-like teliospores were discovered in seed washes from seedlots of annual ryegrass from Oregon's Willamette Valley. *Tilletia indica*-specific primers amplified DNA from the teliospores collected from the Oregon ryegrass suggesting that they were from *T. indica*. Morphological analysis demonstrated that the spores from the ryegrass smut and *T. indica* were similar but not identical. The objective of this research was to study the genetic relationships among *T. indica* and other floret-infecting smuts with similar teliospore morphology, including the ryegrass smut and *T. barclayana*. Three hundred and ten RAPD markers and 105 restriction fragments from the ITS region were used in the analysis. Based on PCR-RFLP and RAPD analyses, the ryegrass smut is most similar to *T. indica*, but the low level of similarity (ca., 25%) in the RAPD analysis does not support conspecificity of both taxa. Both analyses suggested that among the *T. barclayana* isolates studied two distinct taxa were present (ca., 10% similarity). One group corresponded to the isolates from rice, and the other to isolates infecting wild species of *Panicum and Paspalum*. Hence, *T. horrida* and *T. barclayana* are proposed for the rice and *Panicum and Paspalum*-infecting smuts, respectively. *Tilletia boutelouae and T. rugispora* were supported as distinct species in both analyses.