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Is rye bunt, *Tilletia secalis*, present in North America?

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Abstract: A volunteer rye plant (*Secale cereale*) infected by a reticulate spored species of *Tilletia* was collected in a wheat field in southeastern Idaho in 1993 (WSP 71279). The smut fungus was identified as *Tilletia contraversa*, the dwarf bunt pathogen of wheat, based on teliospore morphology and stunting of the host. Inoculation studies conducted in the greenhouse confirmed that the rye-infecting bunt was able to infect wheat. A phylogenetic analysis based on the internal transcribed spacer region rDNA, eukaryotic translation elongation factor 1 alpha, and the second largest subunit of RNA polymerase II demonstrated that the rye-infecting bunt was distinct from *T. contraversa* and the common bunt pathogens of wheat, *T. caries* and *T. laevis*. The rye-infecting bunt fits within the species concept of *T. secalis*, a pathogen of cultivated rye in Europe. The ability of *T. contraversa*, *T. caries* and *T. laevis* to infect rye and of *T. secalis* to infect wheat has resulted in confusion over the identity of bunts found infecting rye. This study is the first demonstration in North America of a bunt fungus infecting rye that is genetically distinct from the wheat bunt pathogens.

Key Words: Exobasidiomycetes, multilocus phylogenetic analysis, rye bunt, *Tilletia caries*, *Tilletia contraversa*, *Tilletia laevis*, *Tilletia secalis*, Tilletiales, Ustilaginomycotina, wheat bunt

Introduction: *Tilletia contraversa*, the dwarf bunt pathogen of wheat, *T. caries*, the common bunt pathogen of wheat, and *T. secalis* have reticulately ornamented teliospores and are known to infect cultivated rye (*Secale cereale*). *Tilletia caries* and *T. contraversa* occur in most cool and temperate wheat-growing regions of the world, whereas *T. secalis* is only reported from Europe and Eurasia (Vánky 1994). *Tilletia caries*, *T. contraversa* and *T. secalis* have similar teliospore morphology and overlap in host range (Durán and Fischer 1961; Vánky 1994). This has created confusion in the identification of reticulate-spored *Tilletia* spp. infecting rye, and uncertainty of species identity in published studies on these fungi. In his treatment of the species of *Tilletia* infecting rye, Fischer (1956) noted that the teliospores of *T. contraversa* and *T. secalis* have similar depth of ornamentation on the exospore (1.5-3.0 μm), whereas *T. caries* has relatively shallow ornamentation (0.5-1.5 μm). Fischer's description of *T. contraversa* also included rounded sori, teliospores encased in a hyaline, gelatinous sheath extending up to 4 μm beyond the exospore, and stunting of infected hosts. He described *Tilletia secalis* as having elongated sori, teliospores with little or no gelatinous sheath, and no stunting of infected plants. Fischer (1956) concluded that both *T. contraversa* and *T. secalis* were widespread on cultivated rye in Europe and Eurasia, but *T. secalis* did not occur in North America; reports of bunt on rye in the U.S. were *T. caries* or *T. contraversa*.

In a phylogenetic analysis of Tilletiales based on large subunit nuclear (nLSU) rDNA sequences, Castlebury et al. (2005) showed that *Tilletia* spp. infecting hosts in the grass subfamily Pooideae formed one, well-supported lineage comprising most of the reticulate-spored species that

germinate to form a small number of rapidly conjugating basidiospores. This lineage included *T. caries*, *T. contraversa* and *T. laevis*, but nLSU was insufficiently variable among the reticulate-spored taxa in the pooide clade to determine relationships within this group. Carris et al. (2007) recently used a multigene phylogenetic approach to compare the closely related species of *Tilletia* in the pooide-infecting lineage. An analysis of this lineage using the internal transcribed spacer region rDNA, eukaryotic translation elongation factor 1 alpha, and the second largest subunit of RNA polymerase II showed that collections of *T. contraversa* infecting hosts other than wheat were genetically distinct from *T. contraversa*, *T. caries* and *T. laevis* from wheat. Bunts from *Thinopyrum intermedium* and *Hordeum murinum* were recognized as *T. brevifaciens* and *T. trabutii*, respectively. A rye-infecting bunt from Idaho, considered to be *T. contraversa* based on host stunting and teliospore morphology, was also genetically distinct from the three wheat bunt species. The implications of this analysis and a reinterpretation of the species concept in *T. secalis* are presented.

Materials and Methods: Collections of *Tilletia* spp. used in this study are listed in Table 1. Host names and authorities are listed as in the US Department of Agriculture PLANTS Database (<http://plants.usda.gov>). Teliospores were mounted in Shear's mounting medium [50 mL 2% (w/v) potassium acetate, 20 mL glycerol, 30 mL 95% ethyl alcohol], examined and measured using differential interference contrast (DIC) microscopy at X1000. Teliospore color, diam (including exospore), thickness of exospore, number of meshes per spore diam, and sterile cell shape, color and wall thickness were recorded (Table 2). For germination, teliospores were

surface-sterilized in 0.26% NaClO (5% commercial bleach) in a 1.5 mL Eppendorf tube for 50s, pelleted by centrifugation at approximately 13000 *g* in a benchtop microcentrifuge for 10s and rinsed twice with sterile distilled water. Surface-sterilized teliospores were streaked on 1.5% water agar (WA) and incubated at 5C, 15C and room temperature (20–25C). Teliospore germination was examined directly on the surface of the agar using the 40X objective of a compound microscope to determine number of basidiospores per basidium. Basidiospores were mounted in water and measured (Table 2). Germinating teliospores and basidiospores were transferred to potato sucrose agar (PSA) or M-19 agar (Trione 1964) to generate colonies for DNA extraction (see below). All digital images were captured using a Nikon CoolPix 4300.

Sequence analysis: Sequences for the isolates used in this study were generated by Carris et al. (2007) and obtained from GenBank. Alignments were edited with GeneDoc 2.6.03 (<http://www.psc.edu/biomed/genedoc/>) or Jalview version 2.3 (Clamp et al. 2004). The three genes were aligned individually, concatenated into a single alignment and a combined three-gene analysis was performed for all available taxa. The EF1A gene was not available for *T. laguri*.

Trees were inferred by maximum parsimony (MP) using the heuristic search option with the random addition sequence (1000 replications) and the branch swapping (tree bisection-reconnection) option of PAUP* 4.0b10 (Swofford 2002). For MP analyses, a limit of 10 trees per random addition sequence was enforced with a MAXTREE limit of 10 000. All aligned positions were included. All characters were unordered and given equal weight during the analysis. Gaps were treated as missing data and trees were midpoint rooted. Relative support for branches was estimated with 1000 bootstrap (BS) replications (Felsenstein 1985) with MULTREES off and 10 random sequence additions per bootstrap replicate for the MP bootstrap analysis.

Results: Herbarium WSP (Department of Plant Pathology, Washington State University) had 39 specimens of *T. secalis* on *Secale cereale* and 6 specimens of *T. contraversa* on *S. cereale*. Collection data are available on-line at <http://www.herbaria.wsu.edu/web/default.aspx>. Representative specimens examined as part of this study are listed in Table 1. The morphological and germination characters of *T. contraversa* and *T. secalis* collections analyzed in this study are summarized in Table 2, and illustrated in Figures 1–17.

Teliospores of *T. contraversa* and rye bunt, WSP 71279, required low temperature (5C) for germination, and no germination occurred at 15C, in contrast to teliospores of *T. brevifaciens* and *T. trabutii*, which germinated at both 5C and 15C (Table 2). Considerable overlap existed in size, color and ornamentation of teliospores, and size of sterile cells among the four taxa. Teliospores of some *T. secalis* collections, e.g., WSP 14455 from Russia (Figs. 3, 4) had a yellowish tint to the exospore, whereas other collections, such as WSP 11814 from Russia (Figs. 5, 6), the exospore color was variable. In the rye bunts, WSP 71279 from Idaho (Figs. 1, 2) and WSP 65551 from New York (Figs. 11, 12), the exospore was hyaline. In *T. brevifaciens*, the sterile cells had somewhat thinner walls, and there were fewer primary basidiospores per basidium than in *T. contraversa*, *T. trabutii* and WSP 71279 (Table 2). The teliospores in the rye bunt, WSP 71279, germinated to form 8 – 19 basidiospores; conjugation occurred either mid-spore or at the base of basidiospores (Fig. 17). Basidiospores that did not conjugate appeared to lyse (Fig. 17). Sori of selected specimens of *T. secalis* and *T. contraversa* on rye are shown in Figs. 13 – 16. The sori in WSP 71279 were rounded and conspicuous (Fig. 13), whereas those in *T. secalis* specimens from Russia (WSP 11814 and 14455), were somewhat more elongated, retaining the shape of the caryopsis (Figs. 14, 16). In *T. contraversa* from rye (WSP 63890; Fig. 15), the sori were also elongated. The limited amount of

material available in other specimens of *T. secalis* and *T. contraversa* from rye in Herbarium WSP was insufficient to determine shape of sori. The combined sequence alignment consisted of EF1A (719 bp), ITS (647 bp), and RPB2 (554 bp) sequences for 24 isolates of *Tilletia* spp. infecting grass hosts in the Pooideae for a total of 1920 characters. Of these, 76 were parsimony-informative, 1833 were constant and 11 were variable but not parsimony-informative. MP phylogenetic analysis of the combined alignment resulted in 37 equally parsimonious trees (length= 115) differing in the arrangement of the interior branches (trees not shown). Species-level clades were present in all trees. Fig. 18 shows one randomly chosen MP tree generated for the combined alignment, with MP BS values above the branches. Only BS values 70 % or greater are shown. Collection WSP 71279 did not fall within any species clades formed by this analysis but was most closely related to *T. trabutii* (BS=81%). *Tilletia trabutii*, *T. brevifaciens*, *T. bromi*, and *T. vankyi* were all supported as species at bootstrap levels of 98 % or greater. A clade containing the dwarf and common bunt isolates was supported at 80%, but clades correlating with *T. caries*, *T. contraversa* or *T. laevis* were not resolved.

Discussion: In his treatment of *Tilletia* spp. on rye, Fischer (1956) wrote “it is difficult, if not impossible, to know for sure what organism the binomial *Tilletia secalis* refers to.” The ability of the rye bunt fungus to infect wheat, and the dwarf and common bunt species to infect rye, has resulted in considerable uncertainty regarding the identity of *T. secalis*. Jackson (1920) reported that an 1892 specimen in the Underwood herbarium at the New York Botanic Garden labeled “Ustilago on Rye” from Syracuse, NY, consisting of a single “somewhat abnormal head” agreed morphologically with *T. secalis*. This would represent the first report of *T. secalis* in North America. Fischer (1956) cited Jackson’s (1920) report of *T. secalis* from New York; he examined this specimen and retained a portion as WSP 65551 (Figs 11, 12), but in his opinion, this

specimen did not represent *T. secalis*. Fischer (1956) distinguished *T. secalis* from the reticulate-spored wheat bunts, *T. caries* and *T. contraversa*, by elongated, fragile sori, somewhat larger diameter teliospores with narrower areolae and little or no gelatinous sheath, somewhat larger sterile cells, and little or no dwarfing of the infected host. In contrast, Niemann (1956) dismissed host stunting as a character that could be used to distinguish dwarf bunt from rye bunt, and cited a study (Niemann 1954) in which a bunt fungus from unstunted rye plants caused considerable (50%) stunting in artificially inoculated rye. He pointed out that factors including temperature and host cultivar could influence the extent of stunting in infected hosts, and stunting is seen in several other grass hosts infected by *Tilletia* spp., including “*Agropyron*” (which may be a reference to *Elymus repens*), *Hordeum murinum* and *Agrostis*.

Niemann’s (1954, 1956) species concepts for the wheat, rye and *Hordeum*-infecting bunts differed from those of Fischer (1956), in part because he recognized a higher degree of host specificity than did Fischer. Niemann (1956) considered *Tilletia secalis* as a distinct species, but one that was morphologically intermediate between *T. caries* and *T. contraversa*. According to Niemann (1956), the yellowish tinge to the gelatinous sheath surrounding the teliospores of *T. secalis* could generally be used to distinguish this species from the wheat dwarf bunt.

The 1993 specimen of *Tilletia* sp. from an infected rye plant growing as a volunteer in a wheat field in southeastern Idaho (WSP 71279) was initially identified as *T. contraversa* for several reasons. First, the teliospores were more similar to those of *T. contraversa* than *T. caries*, the only other reticulate-spored bunt known to infect rye in North America. Secondly, the infected plant was collected in a wheat-growing area with conditions favorable for dwarf bunt, and common bunt at that time had been largely eliminated from Pacific Northwest wheatfields by the widespread use of

fungicide seed treatments containing carboxin or pentachloronitrobenzene (Goates, 1996). Subsequent inoculation studies conducted with this collection by P. M. Gray (unpublished) using a modification of the hypodermic inoculation method of Fernandez and Durán (1978) demonstrated the rye bunt fungus was able to infect 'Red Bob', a spring wheat cultivar widely used for these types of studies due to its susceptibility to all known races of common and dwarf bunt. Only one of twenty-five (4%) wheat plants injected with germinating teliospores was infected in this study. Hypodermic inoculation at the coleoptile stage is used in the Carris lab to increase common and dwarf bunt collections due to the efficiency of this method relative to other inoculation methods. For example, when *T. contraversa* WSP 71413 (used in Carris et al 2007) was inoculated into 25 seedlings of cv. Red Bob, 16 (66%) of the plants were infected. Pimentel et al (2000) used coleoptile inoculation to characterize interspecific hybrids of *T. bromi*, *T. contraversa* and *T. laevis*. In that study, 58 of 175 (33%) of cv. Red Bob inoculated at the coleoptile stage with *T. contraversa* x *T. laevis* were infected, whereas only 0.8 - 8% of seedlings inoculated with *T. contraversa* x *T. bromi* were infected. Wheat is not a known host for *T. bromi*, and the low level of infection with *T. contraversa* x *T. bromi* was used as indication of reduced fitness of this interspecific hybrid. Hypodermic inoculation can be an effective method for infecting wheat with *Tilletia* spp. However, hypodermic inoculation cannot be used to screen for resistance in wheat lines, as even resistant varieties are susceptible using this method (Fernandez and Durán 1978; Goates 1996).

Niemann (1957) provided examples of inoculation studies with bunt fungi from wheat and other grasses in which a relatively high level of infection occurred in the original host, but in transfer to other host plants only low levels of infection typically occurred—only one or a few plants, as was the case with our inoculations of wheat with the rye bunt.

Accurate identification of *Tilletia* spp. infecting rye and wheat based only on morphological features is problematic. Durán and Fischer (1961) described the teliospores of *T. contraversa* as 19-24 µm diam., with areolae 1.5-3 µm high and embedded in a hyaline, gelatinoid sheath 1.5-5.5 µm thick. They did not describe a sheath for teliospores of *T. caries*, and described a hyaline, gelatinoid sheath that extends "little if any beyond the reticulations" for *T. secalis*. The exospore of most reticulate-spored species of *Tilletia* is enveloped in a gelatinous sheath to some extent, and the thickness of the sheath can be variable among spores within a species. For example, Holton and Kendrick (1956) unambiguously stated that "virtually every characteristic used in the separation of dwarf bunt (*T. contraversa*) from common bunt (*T. caries*) is one of degree only." They documented variability in these species in teliospore ornamentation, presence and degree of thickness of a gelatinous sheath, and degree of host stunting and tillering. Hess and Trione (1986) showed that the thickness of the gelatinous sheath varied considerably among *T. contraversa* samples, with some teliospores showing no sheath, and others possessing a prominent sheath. For that reason, the protocol developed by the USDA to identify *T. contraversa* spores for export certification of wheat does not utilize the gelatinous sheath, but instead uses exospore (reticulation) depth as the diagnostic character (Mathre 1996).

The morphological features of the two collections of rye bunt fungi from the U.S. (WSP 71279 from Idaho and WSP 65551 from New York) fit within the broad species concept of *T. secalis*. Our data demonstrate that the Idaho rye bunt is genetically distinct from the common and dwarf wheat bunt species, although it has limited ability to infect wheat under artificial conditions. Confirmation of the identification of WSP 71279 requires comparison with European collections of *T. secalis*. We concur with Niemann's (1954, 1956) contention that teliospore morphology and host stunting cannot be used to distinguish *T.*

contraversa from *T. secalis*, just as they are insufficient for distinguishing *T. contraversa* and *T. caries*.

Although Fischer (1956) rejected the Underwood specimen (cited by Jackson 1920) as *T. secalis*, it may well represent the first report of this species in North America. The lack of additional records of *T. secalis* in North America may be due to misidentification of this species as *T. contraversa* or *T. caries*, and more recently, by the effective control of bunt fungi by fungicide seed treatments. Results from this study demonstrated that *T. secalis* has the ability to infect both wheat and rye, but infection in wheat occurs under extraordinary conditions. *Tilletia secalis* has the ability in some instances to cause dwarfing in infected host plants, but whether this is dependent upon certain pathogen by host genotype interactions or environmental conditions requires additional study.

Acknowledgements: Historically, high levels of wheat bunt infection in the Pacific Northwest gave this region the title “wheat smut center of the world”. In the 1950s, intense research efforts on wheat bunts resulted in the accumulation of a massive body of literature on smut fungi at Washington State University, then known as the State College of Washington. George W. Fischer, one of the leading smut authorities of this era, donated a large collection of bound reprints and English translations of non-English language articles to the Department of Plant Pathology reprint collection, now housed in the Fischer Agricultural Library at Washington State University. The reprint collection has proved invaluable in piecing together the details of work on *T. contraversa* and *T. secalis*, most notably by providing access to English translations of work by Niemann and other German smut workers. Professor Jack D. Rogers, a long-time proponent of the libraries at WSU, deserves much of the credit for keeping the collections in the Fischer Agricultural Library intact. It is therefore fitting that this article be part of the Festschrift honoring

his forty years of service to Washington State University.

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Table 1. Collections used in study.

Taxon	Collection/Culture numbers	Year	Host	Origin	Collector/Source
<i>Tilletia brevifaciens</i> G. W. Fisch.	HUV 20.802 (CBS 121948)	2004	<i>Thinopyrum intermedium</i> (Host) Barkworth & D. R. Dewey x <i>E. repens</i> (L.) Gould	Poland	K. Vánky
<i>T. brevifaciens</i>	WSP 68945 (Vanky 412)	1999	<i>Thinopyrum intermedium</i> (Host) Barkworth & D. R. Dewey	Austria	K. Vánky
<i>T. bromi</i> (Brockm.) Brockm.	WSP 71271 (LMC 148)	1992	<i>Bromus tectorum</i> L.	US (WA)	L. Carris
<i>T. bromi</i>	WSP 71272 (LMC 167)	1992	<i>Bromus arvensis</i> L.	US (WY)	L. Carris
<i>T. bromi</i>	WSP 71273 (LMC 75)	1991	<i>Bromus hordeaceus</i> L. ssp. <i>hordeaceus</i>	US (WY)	L. Carris
<i>T. caries</i> (DC.) Tul.	WSP 71304 (DAR 73302)	1997-1999	<i>Triticum aestivum</i> L.	Australia	G. Murray
<i>T. caries</i>	WSP 71303 (LMC J-19; CBS 121951)	1995	<i>Triticum aestivum</i>	Sweden	L. Johnsson
<i>T. contraversa</i> Kühn	WSP 71280 (LMC 282; CBS 121952)	1994	<i>Triticum aestivum</i>	US (OR)	L. Carris
<i>T. contraversa</i>	WSP 71301 (LMC 94)	1991	<i>Bromus marginatus</i> Nees	US (ID)	L. Carris
<i>T. contraversa</i>	WSP 69062 (Vanky 528)	1984	<i>Triticum aestivum</i>	Germany	K. Vánky
<i>T. contraversa</i>	WSP 63890	1953	<i>Secale cereale</i> L.	Turkey	Gassner
<i>T. contraversa</i>	WSP 63887	1954	<i>Secale cereale</i>	Austria	Niemann
<i>T. contraversa</i>	WSP 63892	1930	<i>Secale cereale</i>	Switzerland	Volkart
<i>T. laevis</i> Kühn	WSP 71278 (LMC 178; CBS 121949)	1971	<i>Triticum aestivum</i>	US	L. Carris
<i>T. laevis</i>	WSP 71300 (Vanky 766; CBS 121950)	1988	<i>Triticum aestivum</i>	Iran	K. Vánky
<i>T. laevis</i>	WSP 71302 (LMC 98-194)	1998	<i>Triticum aestivum</i>	Australia	G. Murray
<i>T. laguri</i> G. M. Zhang, G. X. Lin & J. R. Deng	HUV 16.352	1992	<i>Lagurus ovatus</i> L.	Italy	K. Vánky
<i>T. lolii</i> Auers. ex Rab.	WSP 71298 (Vanky 767)	1990	<i>Lolium rigidum</i> Gaudin	Iran	K. Vánky
<i>Tilletia</i> sp.	WSP 71279 (LMC 255)	1993	<i>Secale cereale</i>	US (ID)	J. Sittón & J. Waldther unknown
<i>T. secalis</i> (Cda.) Koern.	WSP 64744	1955	<i>Secale cereale</i>	Turkey	
<i>T. secalis</i>	WSP 64743	1955	<i>Secale cereale</i>	Switzerland	H. Aebi
<i>T. secalis</i>	WSP 65551	1892	<i>Secale cereale</i>	US (NY)	L. M. Underwood
<i>T. secalis</i>	WSP 64730	1910	<i>Secale cereale</i>	Czech Republic	F. Bubak
<i>T. secalis</i>	WSP 14455	1910	<i>Secale cereale</i>	Russia	A. Bondarzew
<i>T. secalis</i>	WSP 11814	1923	<i>Secale cereale</i>	Russia	S. Burov
<i>T. trabutii</i> Jacz.	WSP 71299 (Vanky 764; CBS 122324)	1990	<i>Hordeum murinum</i> L. ssp. <i>glaucum</i> (Steud.) Tzvel	Iran	K. Vánky
<i>T. trabutii</i>	VPRI 32106	2005	<i>Hordeum murinum</i> L. ssp. <i>leporinum</i> (Link) Arcang.	Australia	I. Pascoe
<i>T. vankyi</i> Carris & Castl.	WSP 71266 (V21-713; CBS 121953)	1997	<i>Lolium perenne</i> L.	Australia	L. Carris
<i>T. vankyi</i>	culture	2003	<i>Festuca rubra</i> L. ssp. <i>fallax</i> (Thuill.) Nyman	US (Chinese Intercept)	G. Huang
<i>T. vankyi</i>	culture	2002	<i>Lolium perenne</i>	Australia (Chinese Intercept)	G. Huang
<i>T. vankyi</i>	WSP 71270 (FF1-1)	2005	<i>Festuca rubra</i> L. ssp. <i>fallax</i> (Thuill.) Nyman	US (OR)	L. Carris
<i>T. vankyi</i>	culture FF7/8	2005	<i>Festuca rubra</i> L. ssp. <i>fallax</i>	US (OR)	L. Carris

^a HUV, Herbarium Ustilaginales Vánky, Tübingen, Germany; CBS, Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands; DAR, Orange Agricultural Institute, Orange, NSW, Australia; Vánky, *Ustilaginales Exsiccata* by K. Vánky; WSP, Washington State Mycological Herbarium, Pullman, WA; LMC, Lori M. Carris, Department of Plant Pathology, Washington State University, personal collection. Sequences of EF1A, ITS and RPB2 gene regions were deposited in GenBank as accessions EU257524- EU257620

Table 2. Host, morphology and germination of reticulate-spored *Tilletia* species.

Taxon	Host(s)	color	Teliospore morphology			Sterile Cells		Teliospore germination		Primary basidiospores		
			diam (µm)	exospore depth (µm)	meshes/spore diam	diam (µm)	wall (µm)	temp.	days	shape	#/basidium	size (µm)
<i>T. brevifaciens</i>	<i>Thinopyrum intermedium</i>	pale to medium yellowish-brown	17--21	1.8	4--6	7--14	0.5--1	5--15C	> 21	filiform	6--8	81--88 x 2.5
<i>T. contraversa</i>	<i>Triticum</i> spp.	pale to medium reddish brown	15--27	1.8--3.5	5--7	7--22	1--1.3	5C	>21	filiform	14--30	57--84 x 2--2.5
<i>T. contraversa</i>	<i>Secale cereale</i>	pale to medium reddish brown	17--23	1.3--2.2	5--8	9--20	1--2	NA	NA	NA	NA	NA
<i>T. trabutii</i>	<i>Hordeum murinum</i>	pale to medium reddish-brown	20--23	1.3--1.8	4--8	10--16	1--1.8	5--15C	>14	filiform	12--15	66--75 x 2.5
<i>Tilletia</i> sp. WSP 71279	<i>Secale cereale</i>	medium reddish brown	17--24	1.3--1.8	5--8	10--17	1--1.8	5C	>40	filiform	8--19	57--67 x 2--2.5

Figs. 1–6 *Tilletia* spp. on *Secale cereale*. (1, 2) WSP 71279 (Idaho, 1993); (3, 4) *Tilletia secalis* WSP 14455 (Russia, 1910); (5, 6) *Tilletia secalis* WSP 11814 (Russia, 1923). (1, 3, 5) Teliospores and sterile cells, plane view showing exospore and enveloping sheath. (2, 4, 6) Teliospores and sterile cells, surface view showing reticulate ornamentation. Bar = 10 μ m.

Figs. 7 – 12. *Tilletia* spp. on *Secale cereale*. (7, 8) *T. contraversa*, WSP 63887 (Austria, 1954); (9, 10) *T. contraversa*, WSP 63890 (Turkey, 1953); (11, 12) *Tilletia* sp. WSP 65551 (New York, 1892). (7, 9, 11) Teliospores, plane view showing exospore and enveloping sheath. (8, 10, 12) Teliospores, surface view showing reticulate ornamentation. Bar = 10 µm.

Figs. 13 -- 17. *Tilletia* spp. on *Secale cereale*. (13) *Tilletia* sp. WSP 71279 (Idaho, 1993), sori. (14) *T. secalis* WSP 11814 (Russia, 1923), sori. (15) *T. contraversa* WSP 63890 (Turkey, 1953). (16) *T. secalis* WSP 14455 (Russia, 1910). (17) *Tilletia* sp. WSP 71279 (Idaho, 1993), germinating teliospore with conjugated basidiospores ("H-bodies"). Bar = 2.5 mm for Figs. 13 – 16, and 10 μ m for Fig. 17 .

Fig. 18. One of 37 equally parsimonious trees with score of 115 resulting from analysis of 1920 bp from the EF1A, ITS and RPB2 genes for selected reticulate-spored species of *Tilletia*. Consistency Index = 0.800, retention index = 0.934, rescaled consistency index = 0.747, homoplasy index = 0.200. Numbers above the branches indicate MP bootstrap support proportions from 1000 pseudoreplicates with 10 random additions of taxa per pseudoreplicate.