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Tilletia walkeri, a new species on *Lolium multiflorum* and *L. perenne*

L. A. Castlebury[1]

USDA-ARS Systematic Botany and Mycology Lab, 10300 Baltimore Avenue, Beltsville, MD 20705-2350

L. M. Carris

Department of Plant Pathology, Washington State University, Pullman, WA 99164-6430

Abstract: *Tilletia walkeri* (Ustilaginales: Tilletiaceae) is described as a new species of partial bunt infecting *Lolium multiflorum,* annual ryegrass, and *L. perenne,* perennial ryegrass in the United States and Australia, respectively. The new species is characterized by large, tuberculate teliospores with the exospore ornamentation comprised of incompletely cerebriform ridges in surface view. Teliospores of *T. walkeri* are compared with those of *T. indica* and other similar species of *Tilletia,* and the issue of *Neovossia* versus *Tilletia* is discussed. A key is provided to smuts known to occur on species of *Lolium.*

Key Words: Karnal bunt, ryegrass, smut fungi, Tilletiaceae, Ustilaginales

INTRODUCTION

The first report of *Tilletia indica* Mitra, the Karnal bunt (KB) fungus, in Arizona durum wheat (*Triticum durum* Desf.) seed in March 1996 (Ykema et al 1996) initiated a national survey program to determine the extent of the disease in the USA. The presence of KB had serious implications for the $5 billion USA wheat export industry, as many countries importing wheat in 1996, including the USA, would not accept wheat from any country known to have *T. indica.* Teliospores of *T. horrida* Tak., the rice kernel smut fungus, are morphologically similar to those of *T. indica,* and the disease is widespread in the southern rice-growing regions of the USA (Whitney 1989). At the time *T. indica* was discovered in Arizona, a polymerase chain reaction (PCR)-based test was available to distinguish viable *T. indica* teliospores from *T. horrida* [as *T. barclayana* (Bref.) Sacc. and Syd. in Sacc.; Smith et al 1996].

During the national KB survey, *T. indica*-like teliospores were found in wheat seed washes from the southeastern USA, and from seed washes of pasture mixes containing wheat and ryegrass (*Lolium* spp.) from Oregon. The teliospores were on average smaller, paler in color, and had coarser ornamentation on the exospore than those of *T. indica.* The teliospores tested positive for *T. indica* using the PCR test (Bonde et al 1997), although bunted wheat seeds were not found in the southeastern USA or Oregon despite extensive searches. The source of the *T. indica*-like teliospores was unknown until Jan 1997 when bunted seeds of annual ryegrass (*L. multiflorum* L.) were recovered from commercial seedlots produced in Oregon's Willamette Valley (Bonde et al 1997). Bunted seeds were later found in annual ryegrass growing as weeds in wheat fields in the southeastern USA (Bonde et al 1997). In Feb 1997, one of us (LMC) found four specimen packets from Herb. DAR of a *Tilletia* species on *Lolium perenne* L., identified by Mr. John Walker from seed grown in 1967 and 1968 in Australia's Kangaroo Valley. The specimens were among the smut collection left by Prof. Rubben Durban after his retirement from the Department of Plant Pathology at Washington State University. The specimens had been sent to Durban for identification, and he had noted on one packet that the fungus appeared to be a new species, but the material in the specimens was inadequate to make a determination. The herbarium packets were later determined to be part of 22 specimens of the *L. perenne* smut deposited as *Tilletia* sp. at the Agricultural Research and Veterinary Centre, Orange, New South Wales, Australia. According to Michael Priest, curator of Herb. DAR (pers comm), 21 of the specimens in DAR are from the Kangaroo Valley, collected between 1967 and 1974, and each packet contains one or two bunted seeds. One collection of the *L. perenne* smut is a prepared slide of teliospores forwarded to Walker from New Zealand in 1976, but the precise date and location of the specimen is not known. Walker had noted on Australian specimen DAR 16719, collected Dec 28, 1967, that this was the first record of a *Tilletia* sp. on *Lolium* in New South Wales. The teliospores from Australian bunted perennial ryegrass seeds were similar to those recovered in the seed washes and bunted annual ryegrass seeds in the USA.

The DAR specimens demonstrate that this smut has been present on ryegrass for at least 30 yr. Sub-

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[1] Email: lisa@nt.ars-grin.gov

sequent surveys of commercial ryegrass from the Willamette Valley showed that the teliospores were present in at least 60% of the seedlots tested (Bonde et al 1997), and could be found in seedlots going back to 1989 (G. Milbraith, Oregon Department of Agriculture pers comm). The smut was also found in 1997 in annual ryegrass growing along a roadside ditch southwest of Portland in Yamhill County, Oregon (Carris unpubl). Molecular characterization of the ryegrass smut using RAPD, RFLP-ITS and ITS sequence analyses showed that it is distinct from *T. indica,* although it is more similar to this species than to the other tuberculate-spored *Tilletia* species tested, including *T. horrida* (Pimentel et al 1998; L. Levy, USDA-APHIS pers comm). The widespread nature of the ryegrass bunt, its relatively long association with ryegrass, and its morphological and molecular distinctions from *T. indica* provide support for describing it as a new species of *Tilletia.*

MATERIALS AND METHODS

Collections examined are listed in TABLE I. Teliospores were mounted in Shear's solution [50 mL 2% (w/v) potassium acetate, 20 mL glycerol, 30 mL 95% ethyl alcohol], warmed overnight at 45 C to evaporate excess mounting medium, and examined under bright field (BF) or differential interference contrast (DIC) microscopy at ×1000. Monochrome teliospore images were captured via a video camera mounted on the microscope and connected to a personal computer. Average diam (average of the longest and shortest lines through the center of the spore that join two points on the perimeter) of the total spore (including ornamentations) were automatically measured with ImagePro Plus 3.0 image analysis software (Media Cybernetics, Silver Spring, Maryland). Teliospore shape, color, and exospore ornamentation were also recorded. Thirty spores from each collection were examined. For scanning electron microscopy (SEM), dry teliospores were mounted on double-sided tape, coated with gold, and examined at ×1500, ×2000, and ×3500 with a JEOL JSM-T300 scanning electron microscope.

Ryegrass seeds were soaked 1–2 d in tap water to render the palea and lemma transparent, and examined at ×20 for discrete darkened areas indicative of partial bunt. For germination, teliospores were soaked an additional 2 d in distilled water, surface-sterilized in 0.26% NaClO (5% commercial bleach) in a 1.5-mL Eppendorf tube for 50 s, pelleted by centrifugation at approximately 13 000 *g* in a benchtop microcentrifuge for 10 s, and rinsed once with sterile distilled water. Surface-sterilized teliospores were streaked on 2% water agar (WA) and incubated at room temperature (20–25 C). Primary sporidia from 10 teliospores per collection were counted, and 20 primary sporidia per teliospore were measured. Sporidia and mycelium of the ryegrass smut were fixed and stained with Giemsa-HCl following Durban (1980).

RESULTS

The highest level of infection among the *Lolium* seedlots examined in this study was TN 97-770, in which there were eight bunted seeds found in 40 g of seed (bunted seeds deposited as WSP 69698). Teliospores of the ryegrass smut started germinating in 2–5 d on WA at room temperature. Germination occurred by the formation of a promycelium with a terminal whorl of primary sporidia (FIGS. 2, 3). Primary sporidia germinated within 24 h by the formation of forcibly discharged allantoid secondary sporidia. The secondary sporidia germinated to produce secondary sporidia or mycelium and filiform secondary sporidia were also produced from the mycelium (FIGS. 4–10). No fusion was observed between primary or secondary sporidia on agar.

TAXONOMY

Tilletia walkeri L. A. Castlebury et L. M. Carris, sp. nov. FIGS. 1–16, 29–31

Sori in uno vel duobus ovariis per spicas, inconspicui; massa sporarum fusca; steriles cellulae globosae, subglobosae vel lacrimiformes, hyalinae vel pallidae fulvaeo, 13–26 μm diametro, cum crassis parietibus 3–5 μm; sporae globosae vel subglobosae, flavidae vel atro-badiae vel opacae, 23.7–44.4 μm diametro; episporium cum conicae vel acutae eminentiae 3–6 μm longae sunt.

Sori in the ovaries enclosed by the pericarp, inconspicuous, infecting only one or two per spike, ovaries usually only partially destroyed; infection mostly restricted to the palea side of the seed; spore mass dark brown; with no discernible odor. *Sterile cells* globose, subglobose to lacrimiform, hyaline to dark yellow-brown, 13–26 μm diam; walls smooth, laminated, 3–5 μm thick (FIG. 1). *Teliospores* globose to subglobose, pale yellow to dark reddish-brown, opaque to subopaque, 23.7–44.4 μm diam ($\bar{b}x$ = 34.0 μm); exospore ornamented with conical to truncate projections 3–6 μm high which from surface view appear coarsely and incompletely cerebriform to coralloid; with hyaline to yellowish brown sheath extending to tips of projections (FIGS. 11–16, 29–31). *Promycelium* multinucleate. *Primary sporidia* (36–)60–150(–230) per promycelium, formed in a terminal whorl, hyaline, filiform, curved, 38–75 × 1.3–1.8 μm (range of mean lengths and widths from a single teliospore = 44.3–67.8 × 1.3–1.7 μm); sporidia initially mononucleate, quickly undergoing nuclear divisions and forming inconspicuous septa between daughter nuclei, typically one septum per sporidium; fusions between sporidia lacking (FIGS. 2, 3). *Secondary sporidia* of two types: allantoid and filiform; allantoid type with truncate base, hyaline, 10.6–17.6 × 1.8–3.1 μm; filiform type, curved, hya-

TABLE I. *Tilletia* collections examined

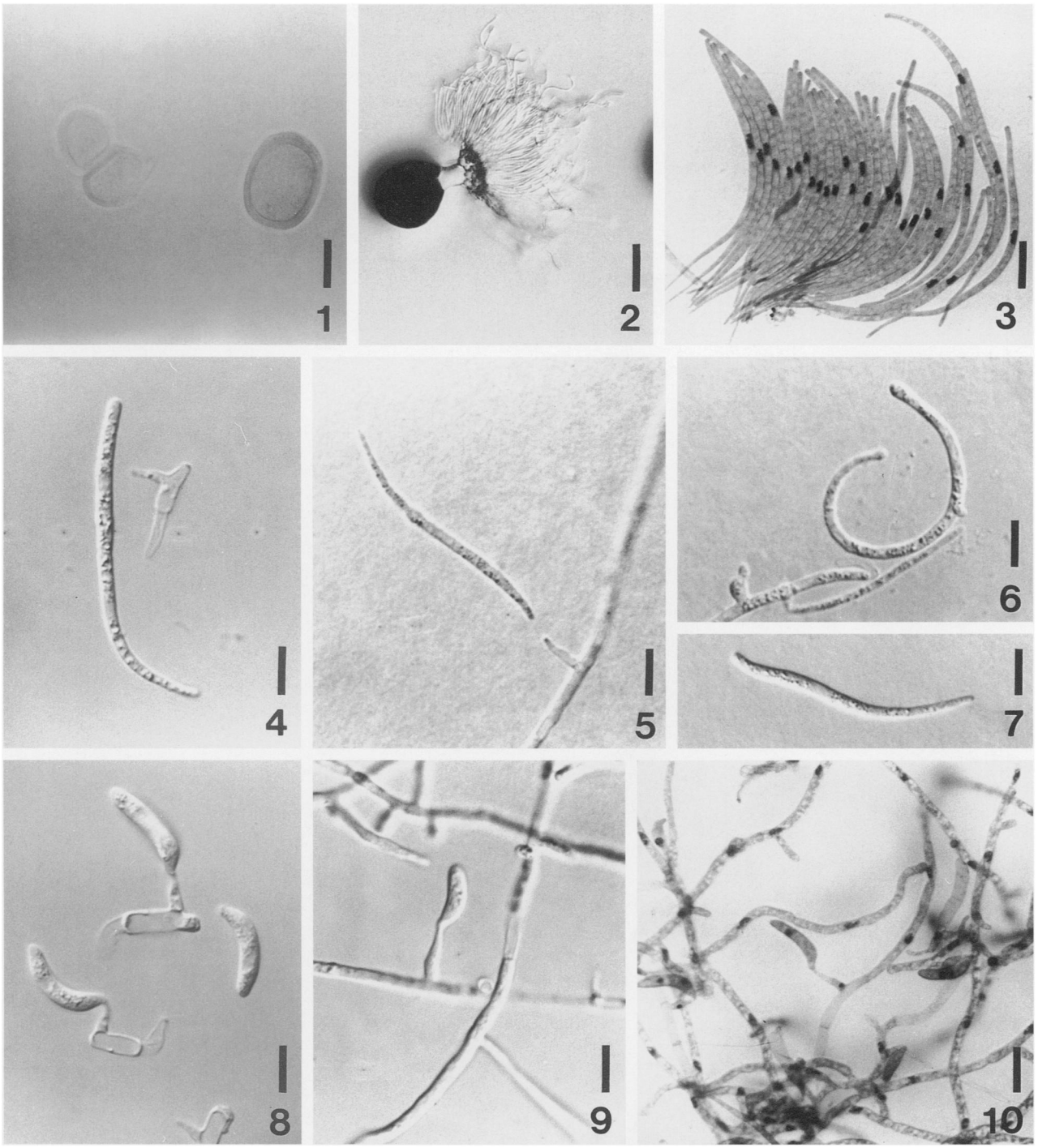
Specimen	Location	Host	Date collected
T. eragrostidis			
WSP 60857	Australia (QLD)	*Eragrostis japonica*	8 Jul 1954
WSP 63954	USA (MS)	*E. glomerata*	10 Sep 1904
WSP 34668	USA (MS)	*E. glomerata*	8 Sep 1904
BPI 172896	USA (MS)	*E. glomerata*	8 Sep 1904
T. horrida			
BPI 173389	Burma	*Oryza sativa*	29 Nov 1912
BPI 173388	China	*O. sativa*	15 Sep 1926
BPI 174228	India (Punjab)	*O. sativa*	17 Oct 1954
BPI 174224	Senegal	*O.* sp.	20 Dec 1967
BPI 174227	Pakistan	*O. sativa*	Nov 1952
BPI 174229	Philippine Islands	*O. sativa*	31 Mar 1964
BPI 802756	USA (AR)	*O. sativa*	27 Jun 1993
BPI 173394	USA (TX)	*O. sativa*	1936
BPI 174226	USA (TX)	*O. sativa*	1940
T. indica			
BPI 174231	India	*Triticum* sp.	5 Apr 1948
BPI 174230	India	*Triticum* sp.	Dec 1942
BPI 174233	India (Karnal)	*T. aestivum*	Apr 1954
BPI 195164	India (Karnal)	*T. vulgare*	Mar 1937
BPI 174235	India	*T. vulgare*	23 Dec 1943
BPI 174236	India	*T. vulgare*	20 Apr 1942
BPI 174232	India	*Triticum* sp.	10 Aug 1948
BPI 174234	Afghanistan	*T. aestivum*	31 Jan 1955
BPI 749197	USA (AZ)	*T. durum*	4 Mar 1996
BPI 032241	Mexico	*T. aestivum*	8 Jul 1981
BPI 032255	Mexico	*T. aestivum*	Mar 1982
BPI 196268	Mexico	*T. aestivum*	24 Jul 1985
BPI 032227	Mexico	*T. aestivum*	19 Apr 1982
BPI 196271	Mexico	*T. aestivum*	13 May 1985
BPI 749272	Mexico	*T. aestivum*	29 Jun 1986
T. inolens			
BPI 195159	Australia (Victoria)	*Deyeuxia forsteri*	Nov 1894
Tilletia sp.			
DAR 16719	Australia (NSW)	*Lolium perenne*	28 Dec 1967
DAR 16722	Australia (NSW)	*L. perenne*	24 Jan 1968
DAR 16745	Australia (NSW)	*L. perenne*	16 Feb 1968
DAR 16774	Australia (NSW)	*L. perenne*	2 Mar 1968
BPI 744421	USA (OR)	*L. multiflorum*	1996
WSP 69699	USA (OR)	*L. multiflorum*	1996
WSP 69698	USA (TN)	*Lolium* sp.	1997
WSP 69697	USA (TN)	*Lolium* sp.	1997
BPI 744575	USA (TN)	*Lolium* sp.	Feb 1997

line, 26–57.2 × 1.8–2.6 μm; allantoid sporidia forcibly discharged, produced asymmetrically from sporogenous cells formed on hyphae, primary or secondary sporidia; sporogenous cells hyaline, subulate, 4.5–7.0 μm high, 1.3–2.2 μm wide at base, 0.9 μm wide at apex (FIGS. 4–10).

Etymology. Honoring Mr. John Walker, an eminent Australian mycologist.

HOLOTYPE. USA. OREGON: Benton County, on *Lolium multiflorum* seeds, 1997, L.M. Carris (BPI 744421). ISOTYPE (WSP 69700).

Additional specimens examined. AUSTRALIA. NEW SOUTH WALES: Kangaroo Valley, *Lolium perenne,* 11 Mar 1968, det. J. Walker (DAR 16774); Kangaroo Valley, *Lolium perenne,* 16 Feb 1968, det. J. Walker (DAR 16745); Kangaroo Valley, *Lolium perenne,* 28 Dec 1967, det. J. Walker (DAR 16722); Kangaroo Valley, *Lolium perenne,* 24 Jan 1968, det.

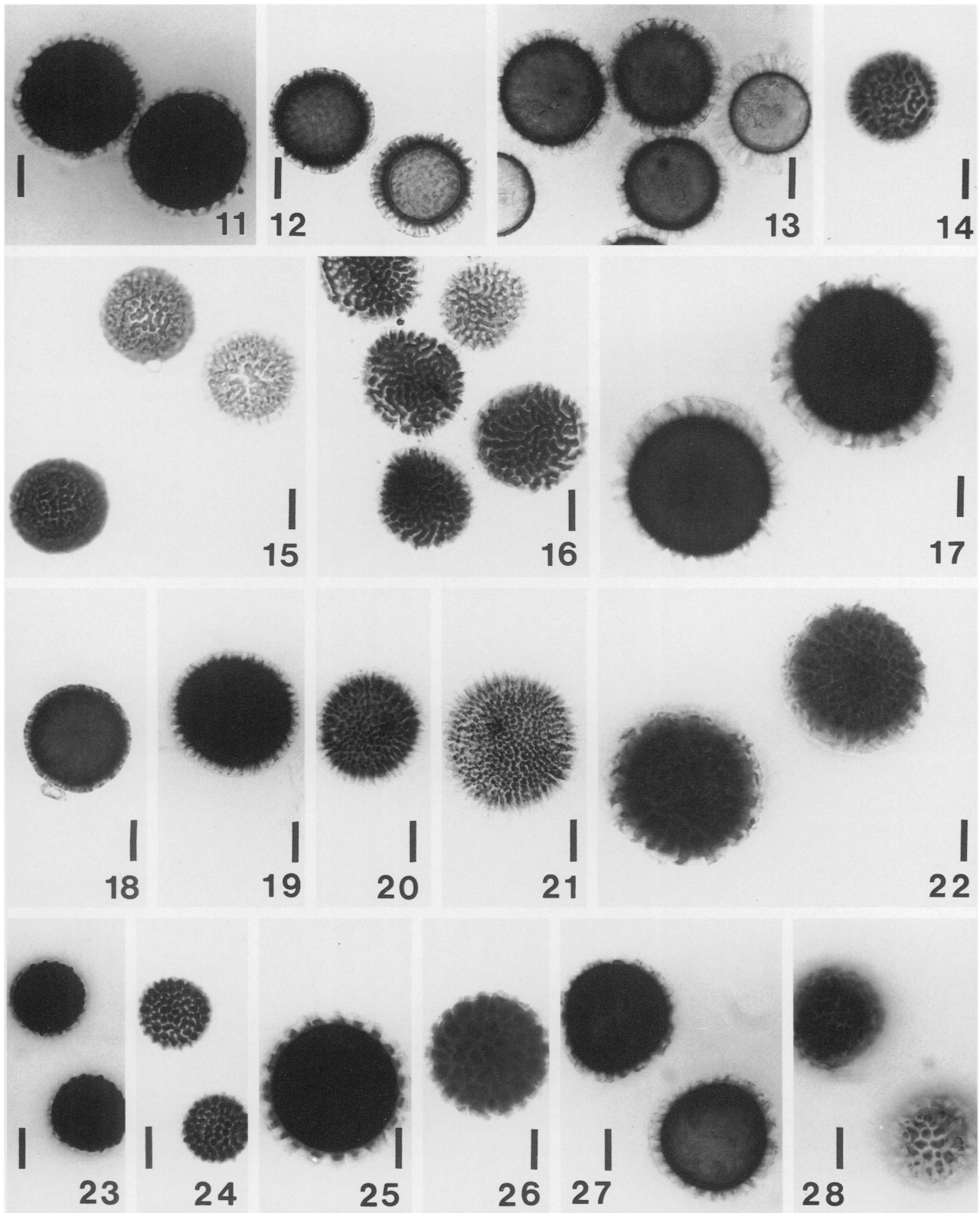


FIGS. 1–10. *Tilletia walkeri*. 1. Sterile cells. 2. Germinated teliospore with primary sporidia (BF). 3. Giemsa-HCl stain of primary sporidia (BF). 4–7. Filiform secondary sporidia [DIC (4, 6, 7) and BF Giemsa-HCl stain (5)]. 8, 9. Allantoid secondary sporidia (DIC). 10. Giemsa-HCl stain of allantoid secondary sporidia (BF). Scale bars: 1 = 12 μm; 2 = 20 μm; 3 = 8 μm; 4–10 = 6.7 μm.

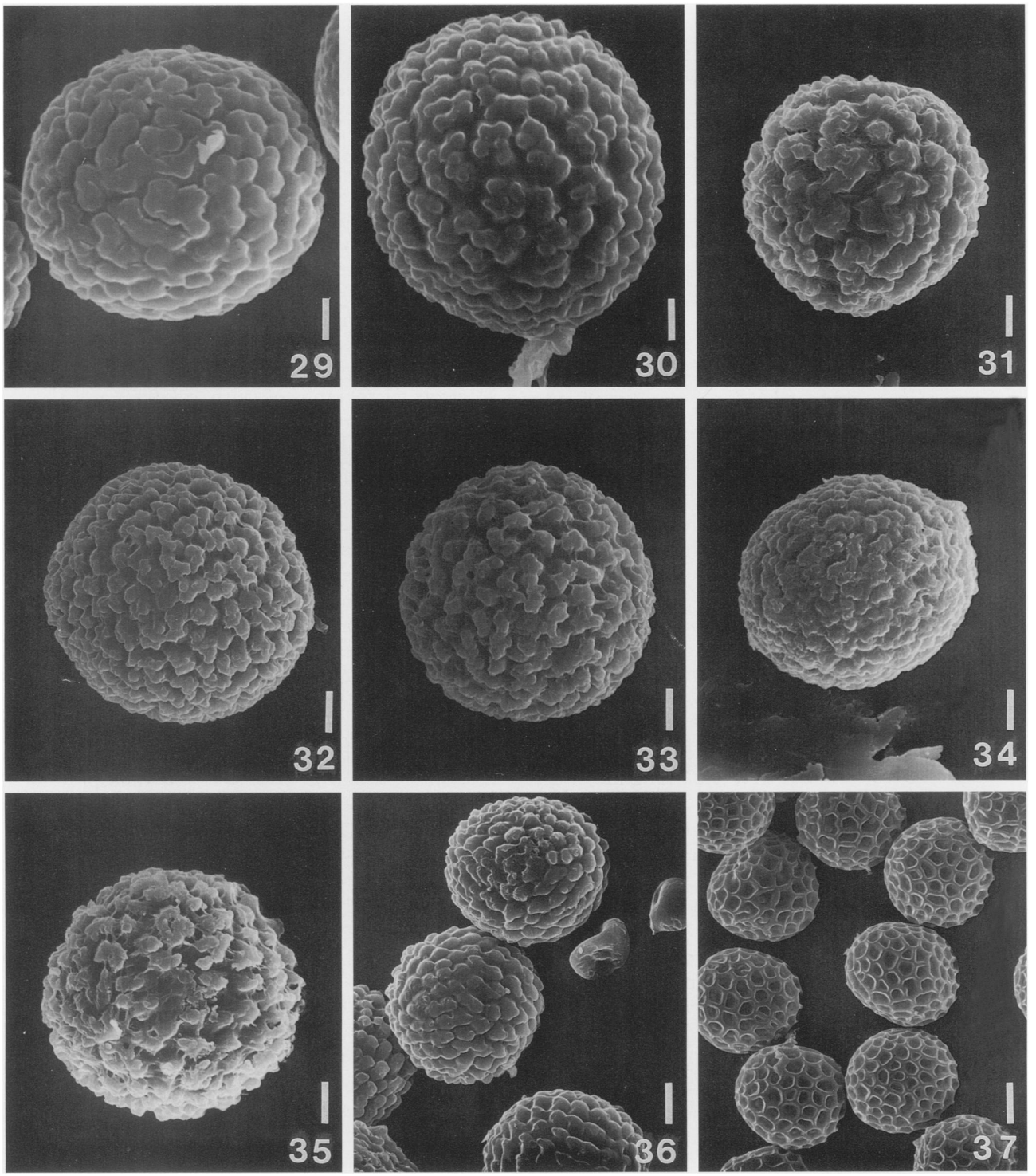
J. Walker (DAR 16719). USA. OREGON: Lane County, 1996 (Tetraploid seedlot WSP 69699); Yamhill County, 2 Jul 1997, coll. & det. L. M. Carris; TENNESSEE: *Lolium* sp., Feb 1997, coll. G. L. Peterson, det. L. A. Castlebury (BPI 744575); 97-770 (WSP 69698); 97-774 (WSP 69697).

DISCUSSION

Tilletia walkeri is similar to *T. indica* and *T. horrida* in causing a partial bunt of the host, the large number of nonfusing primary sporidia, and overlapping



FIGS. 11–28. Teliospores (BF). 11–16. *Tilletia walkeri*. 17–22. *Tilletia indica*. 23, 24. *Tilletia horrida*. 25, 26. *Tilletia eragrostidis*. 27, 28. *Tilletia inolens*. Scale bars: 12 μm.



FIGS. 29–37. Teliospores (SEM). 29–31. *Tilletia walkeri*. 32–34. *Tilletia indica*. 35. *Tilletia eragrostidis*. 36. *Tilletia horrida*. 37. *Tilletia lolii*. Scale bars: 29, 30 = 3 μm; 31–37 = 5 μm.

teliospore morphology. The teliospores of *T. walkeri* are distinguished from *T. indica* and similar *Tilletia* spp. by their coarser ornamentation, which in surface view gives the appearance of wide, incompletely cerebriform ridges or thick clumps (FIGS. 14–16, 29–31). Teliospore color in *T. walkeri* ranges from pale yellow to dark reddish brown, with a hyaline to yellowish brown sheath enveloping the exospore and range in size from 23.7–44.4 μm ($\bar{b}x$ = 34.0 μm, FIG. 38). Teliospores of *T. indica* are more variable in size, color,

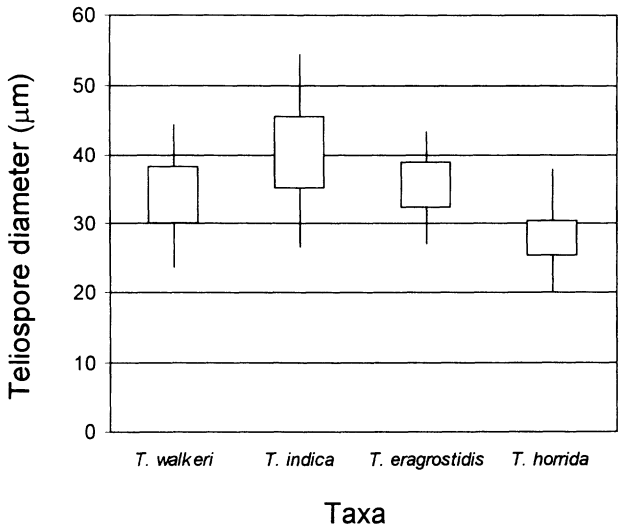


FIG. 38. Mean teliospore diameters. The box indicates the mean ± SD and the lines above and below each box indicate the range of measurements.

and ornamentation, ranging in size from 26.6–54.5(–63.8) µm ($\bar{b}x$ = 40.2 µm) among the collections examined (FIG. 38). These measurements are slightly larger than those reported by Durban and Fischer (1961) and Durban (1987). One factor that might explain this slight discrepancy is that the teliospores in this study were measured after warming at 45 C overnight. These teliospores tend to swell once placed in the mounting medium and it is not clear how long teliospores were in the mounting medium before measurement in previous studies.

Teliospores of *T. indica* are densely ornamented, with sharply pointed to truncate spines which in surface view appear as either individual spines (densely echinulate), or as closely spaced, narrow ridges (finely cerebriform) (FIGS. 17–22, 32–34). The teliospores may have an apiculus or a short mycelial fragment, more common on immature spores but occasionally present on mature teliospores. The apiculus is a remnant of the sporogenous hypha from which the spore was produced. Spore color in *T. indica* ranges from pale orange to dark opaque reddish brown with a bright yellow to yellowish brown halo. Most collections of *T. indica* also contain some teliospores that are nearly black and opaque. This feature was not found in field collections of *T. walkeri*. Khanna and Payak (1968) reported that the exospore ornamentations of *T. indica* were truncate with flattened to occasionally curved tips, which at times became torn or forked. This type of ornamentation has also been observed in *T. walkeri*. Because the characters of color and size of the teliospores intergrade between these two species, these cannot be considered reliable characters for distinguishing these fungi based on the examination of only a few teliospores. Additionally, when the teliospores are examined with SEM, the sheath present on the teliospores may partially mask the exospore preventing a reliable comparison of the exospore of the two species. Matsumoto and Bell (1989) used a chemical treatment to remove the sheath on teliospores of *T. horrida* (as *Neovossia barclayana*) and showed SEM comparing the teliospores with and without the sheath. The exospore on the teliospore with the intact sheath was bluntly echinulate (similar to the illustrations in this paper), whereas the teliospore without the sheath was densely echinulate with sharply pointed spines. Unfortunately the authors did not describe the method they used to remove the exospore. The scanning electron micrographs of Durban (1987) and Vbanky (1994) illustrate *Tilletia* teliospores with intact sheaths.

A direct comparison of teliospore germination in *T. indica* and *T. walkeri* was not possible because of the limitations in working with living material of *T. indica* due to its quarantined status. However, based on published reports there appear to be several differences in germination between the two species. In a study examining the physical and chemical factors affecting teliospore germination in *T. indica,* Krishna and Singh (1982a) reported that the maximum germination occurred at 20–25 C in teliospores soaked 7 d in tap water. Teliospores of *T. indica* germinate in 5–7 d under optimal conditions (Durban 1972, Holton 1949, Smilanick et al 1985). Teliospores of *T. walkeri* began germinating in 2–3 d in the Tennessee collections, and in 5 d in the Oregon collections. Teliospores of *T. indica* germinate to produce 26–171 primary sporidia (Holton 1949), similar to *T. walkeri.* Peterson et al (1984) reported the range of the mean length and width of the primary sporidia in *T. indica* as 64.4–78.8 × 1.6–1.8 µm based on a comparison of four collections from India and three collections from Mexico. The authors noted that there were no significant differences for these parameters among the collections examined. If these dimensions are representative of the species, then the primary sporidia of *T. indica,* on average, are somewhat longer than those of *T. walkeri.* The significance of differences in primary sporidia is difficult to assess because there are no studies directly comparing the size of primary sporidia between closely related species. A more comprehensive comparison of primary and secondary sporidia in *T. indica, T. walkeri,* and other species of *Tilletia* is needed to demonstrate whether sporidial morphology is a reliable character in the taxonomy of this group.

Prior to the discovery of *T. walkeri, T. horrida* was considered the species most likely to be mistaken for

T. indica in the national KB survey. Teliospores of *Tilletia horrida* (FIGS. 23, 24, 36) range from 20.3–37.9(–41.4) μm ($\bar{b}x$ = 27.8 μm) in total spore maximum diam with curved sharply pointed to truncate spines that in surface view appear as polygonal scales. The teliospores of *T. horrida* are distinctly smaller than those of *T. indica* and *T. walkeri* (FIG. 38). Other species of *Tilletia* with tuberculate teliospores in the size range of *T. indica* and *T. walkeri* include *T. inolens* McAlp. on *Deyeuxia forsteri* Kunth. from Australia and *T. eragrostidis* Clint. & Ricker on *Eragrostis glomerata* (Walter) L. Dewey from the southern USA (Durban and Fischer 1961). Walker had indicated on DAR 16719 that the ryegrass smut was most similar to *T. inolens*. Only a few teliospores of *T. inolens* (FIGS. 25, 26) were available for study (TABLE I) and no viable collections of *T. inolens* and *T. eragrostidis* were available for any of the molecular studies cited. However, Durban and Fischer (1961) state that the spores of *T. inolens* are 31–41 μm diam with coarse warts. Teliospores of *T. eragrostidis* (FIGS. 27, 28, 35) range from 27.0–43.3 μm diam ($\bar{b}x$ = 35.6) μm (FIG. 38). In surface view, the exospore ornamentation of both species appears much more coarse and blunt than that of *T. walkeri*. In addition, these two species apparently do not have the cerebriform appearance that teliospores of *T. walkeri* typically exhibit.

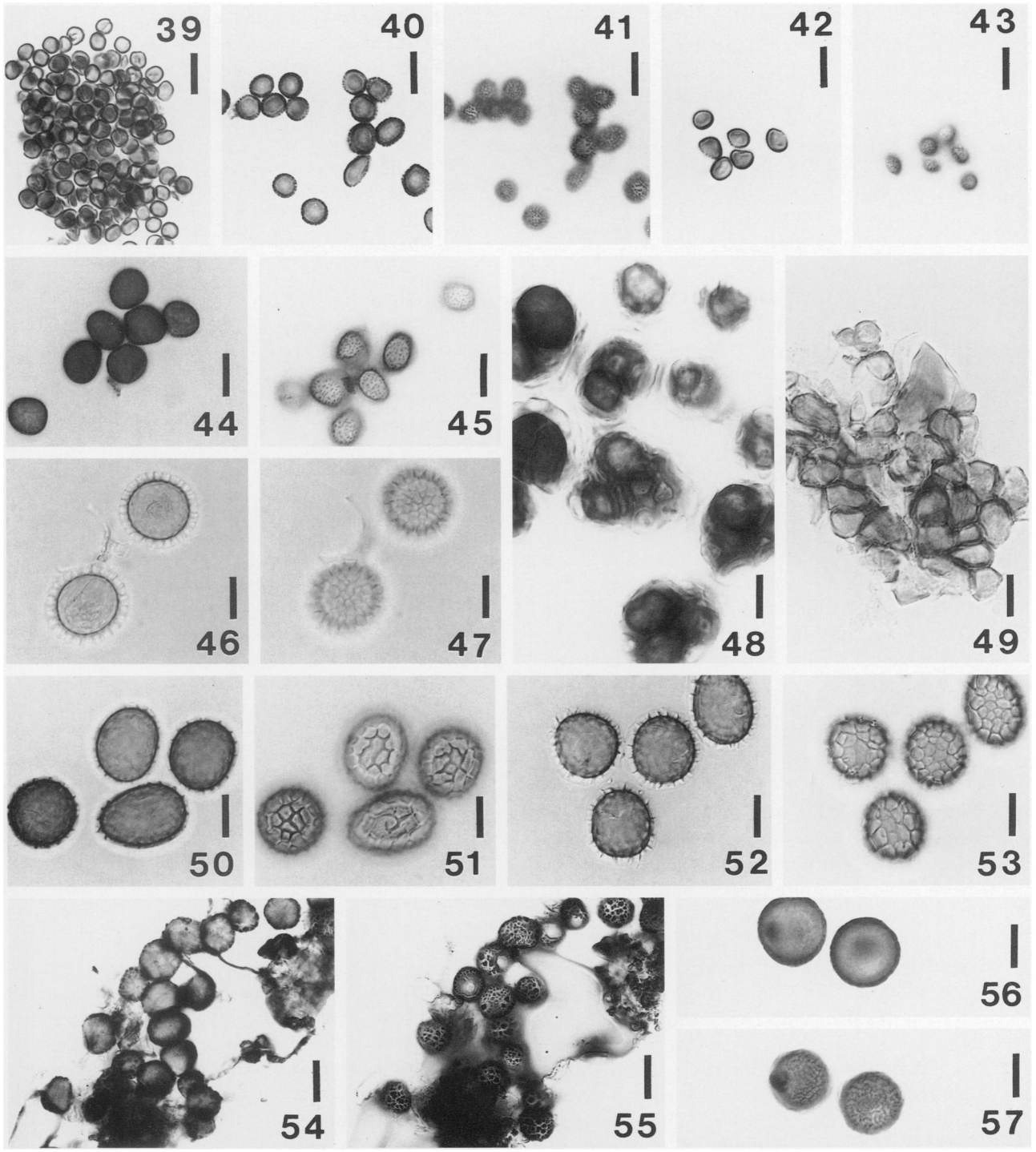
In an experimental host range study utilizing a boot injection inoculation technique, Royer and Rytter (1988) showed that *T. indica* was able to infect 20 species of grasses in *Aegilops, Bromus, Lolium, Oryzopsis* and *Triticum*. However, *T. indica* is only known to infect *Triticum* species and triticale (× *Triticosecale*) under natural conditions (Fuentes-Davila 1996). Other smuts known to infect *Lolium* include *Ustilago hypodytes* (Schlechtend.) Fr., *U. bullata* Berk., *U. lolii* P. Magnus, *U. striiformis* (Westend.) Niessl, *Urocystis bolivari* Bub. & Gonz. Frag., and *Entyloma dactylidis* (Pass.) Cif. (FIGS. 39–45, 48, 49) (Farr et al 1989, Vánky 1994, Zundel 1953). The only species of *Tilletia* reported to infect *Lolium* spp. are *T. controversa* Kühn, *T. laevis* Kühn, *T. lolii* Auersw. and *T. tritici* (Bjerk.) Wint. (Farr et al 1989, Vánky 1994). Vánky (1994) lists *T. laevis* Kühn as an experimental host (artificially inoculated). *Tilletia lolii, T. tritici,* and *T. controversa* (FIGS. 46, 47, 50–53) have reticulate teliospores, while the spores of *T. laevis* are smooth. Zundel (1953) also lists *Lolium* spp. as hosts for *Ur. agropyri* (G. Preuss) J. Schröt. (Hungary) and *Ur. occulta* (Wallr.) Rabenh. ex Fuckel (Hungary and Australia). Two specimens identified as *Ur. occulta* on *Lolium perenne* in the U. S. National Fungus Collection (BPI) could not be distinguished from *Ur. bolivari*. Neither Vánky (1994) nor Lindeberg (1959) list *Lolium* as a host for *Ur. agropyri* or *Ur. occulta* and Vánky (1994) lists *Lolium* as a questionable host for *U. tritici* (Pers.) Rostr. Due to the uncertainty of the identifications of hosts and fungi in these cases, *Ur. agropyri, Ur. occulta,* and *U. tritici* will not be discussed further.

Two other fungi with spores superficially resembling smut teliospores were frequently found in the seed washes from the national KB survey. Conidia of *Epicoccum purpurascens* Ehrenb. (FIGS. 53, 54), a common hyphomycete, and resting spores of *Neozygites parvispora* (MacLeod & Carl) Remaudière & S.Keller (FIGS. 55, 56), an obligate entomophthoralean pathogen of thrips, were found in both wheat and ryegrass seed samples. *Neozygites parvispora* was previously known only from Europe (Keller 1991). Carris and Humber (1998) expanded the known distribution of *N. parvispora* to include New Zealand, and Arizona, Oregon and Tennessee in the USA based on the results of the survey.

Both *T. walkeri* and *N. parvispora* are examples of fungi which have become relatively widespread in North America but were not reported until the national KB survey was initiated. The spores of both species are cryptic in ryegrass seed, and can only be detected in seed washes or by soaking the seed overnight in water to render the palea and lemma transparent. The low level of infection of *T. walkeri* in ryegrass may also account for its delayed discovery.

The known geographic distribution of *T. walkeri* includes the southeastern USA, the Willamette Valley in Oregon, and the Kangaroo Valley in Australia. The actual distribution of this species is probably greater, as indicated by the DAR specimen purportedly from New Zealand. In the two regions in the USA where *T. walkeri* is known to occur, infected *L. multiflorum* is growing in or around wheat fields, although no infection has been detected in wheat in either the Southeast or Oregon after extensive searches. The Kangaroo Valley is a coastal valley approximately 100 km southwest of Sydney in New South Wales, Australia. The Kangaroo Valley is separated from the inland wheat-producing areas of New South Wales by hills and forests, but it is a major producer of ryegrass seed which is distributed to other areas of Australia. The ryegrass smut has not been reported outside the Kangaroo Valley (Gordon Murray pers comm), nor are there any known reports of Karnal bunt in wheat in Australia.

The generic placement of *T. indica, T. horrida* and several other species of *Tilletia* characterized by large numbers of nonfusing primary sporidia has been controversial. The question of *Neovossia* Körn. versus *Tilletia* has not been adequately resolved. According to Tullis and Johnson (1952), the distinguishing characters of *Neovossia* include the formation of two types of secondary sporidia, numerous primary spo-



FIGS. 39–57. *Lolium* fungi (BF). 39. *Ustilago hypodytes*. 40, 41. *Ustilago bullata*. 42, 43. *Ustilago lolii*. 44, 45. *Ustilago striiformis*. 46, 47. *Tilletia lolii*. 48. *Urocystis bolivari*. 49. *Entyloma dactylidis*. 50, 51. *Tilletia tritici*. 52, 53. *Tilletia controversa*. 54, 55. *Epicoccum purpurascens*. 56, 57. *Neozygites parvispora*. Scale bars: 39–53 = 12 μm.; 54, 55 = 17 μm; 56, 57 = 12 μm.

ridia (20–60+) that do not fuse, the presence of a hyaline sheath around the teliospores, and the remnants of the sporogenous hyphae that remain as a conspicuous appendage on the mature teliospores. A gelatinous sheath is frequently present on mature teliospores of *Tilletia* spp., but the sporogenous hyphae are not evident as an appendage. As described by these authors, *Tilletia* species produce only one type of secondary sporidium, fewer primary sporidia (usually 12), and the primary sporidia fuse. Goates and

Hoffman (1979, 1986) noted that the formation of two types of secondary sporidia is typical in *Tilletia* spp. Goates (1996) and Ingold (1987) clearly illustrate the formation of both allantoid and filiform secondary sporidia in *T. tritici,* the type species of *Tilletia.* Tullis and Johnson's (1952) assertion of only one type of secondary sporidium in *Tilletia* is erroneous, but unfortunately has been used by other authors (Singh and Pavgi 1972, Whitney 1989) to support the placement of *T. horrida* and *T. indica* in *Neovossia.*

Differences in number and fusion of primary sporidia were also used by various authors to support the placement of *T. horrida* and *T. indica* in *Neovossia* (Joshi et al 1983, Khanna and Payak 1968, Krishna and Singh 1982b, Singh and Pavgi 1972, Singh et al 1979, Whitney 1989). However, Vȃnky (1994) used a somewhat different set of characters to distinguish species of *Tilletia* and *Neovossia.* In addition to the large number of primary sporidia that germinate without conjugation, Vȃnky considered localized infection, conspicuous appendages on the teliospores, absence of sterile cells, and absence of trimethylamine smell as important characteristics of *Neovossia* (type = *N. moliniae* on *Molinia caerulea* (L.) Moench, also known from *Phragmites australis* (Cav.) Trin. ex Steudel in Europe, Asia, and N. America). The last two characters are particularly relevant to this discussion, because *T. indica, T. horrida* and *T. walkeri* produce sterile cells between the teliospores (Durȃn and Fischer 1961), and *T. indica* has a strong trimethylamine odor (Fuentes-Davila 1996, Mitra 1935). No trimethylamine odor has been detected in *T. horrida* or *T. walkeri.* Durȃn and Fischer (1961) rejected the disposition of either *T. indica* or *T. horrida* in *Neovossia,* noting that the presence of "occasional, fragmentary appendages on immature spores was not sufficiently diagnostic" of *Neovossia,* and that several other *Tilletia* species also produce short apiculi on teliospores. Durȃn (1987) also showed that 15 Mexican species of *Tilletia* produced primary sporidia that did not fuse on artificial agar, and noted several species other than *T. indica* that produce large numbers of primary sporidia. The only character used to support the placement of *T. barclayana, T. horrida* and *T. indica* in *Neovossia* that has not been adequately demonstrated in other *Tilletia* species is floret infection. Vȃnky (1994) considered systemic infection a characteristic of the genus *Tilletia.* He also noted the need for a critical revision of the genus *Neovossia.* In consideration of the questions surrounding the distinction between the two genera, we concur with Durȃn (1987) and recommend the use of *Tilletia* for *T. barclayana, T. horrida, T. indica* and *T. walkeri* until a more thorough investigation nomically significant differences between the two genera.

KEY TO SMUTS KNOWN TO NATURALLY OCCUR ON *LOLIUM* SPP.

1. Spores single 2
1. Spores in balls or densely packed in groups 9
   2. Spore diam typically less than 15 μm 3
   2. Spore diam typically greater than 15 μm 6
3. Spores apparently smooth, typically < 7 μm . . . . . *Ustilago hypodytes*
3. Spores ornamented 4
   4. Spores sparsely ornamented 5
   4. Spores densely ornamented, typically >7 μm . . . . . *U. bullata*
5. Spores typically 5–9 . . . . . *U. lolii*
5. Spores typically 8–15 μm . . . . . *U. striiformis*
   6. Spores reticulate 7
   6. Spores tuberculate to subcerebriform . . . . . *Tilletia walkeri* sp. nov.
7. Spore walls shallowly reticulate (typically < 1.5 μm) . . . . . *T. tritici*
7. Spore walls deeply reticulate (typically > 1.5 μm) . . 8
   8. Spores with inconspicuous sheath, yellowish to pale yellowish-brown.. . . . . *T. lolii*
   8. Spores with gelatinous sheath, yellowish to reddish-brown . . . . . *T. controversa*
9. Spores surrounded by sterile cells . . . *Urocystis bolivari*
9. Spores typically densely packed in groups . . . . . *Entyloma dactylidis*

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