Use of Electron Microscopy to Characterize Teliospores of *Tilletia caries* and *T. controversa*

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ABSTRACT

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A specialized fixation procedure was used to distinguish morphological differences between teliospore walls of the wheat bunt fungi with transmission electron microscopy. The procedure consisted of hydration of spore sheaths, fixation in glutaraldehyde-acrolein, dehydration, critical-point drying, osmication, dehydration, critical-point drying, embedment in Spurr resin, and postsection staining with lead citrate. The sheaths of *Tilletia controversa* spores had a coarse, stippled appearance, whereas the sheaths of *T. caries* contained a fine network of parallel fibers. These characteristics of the wall were consistent and could be used to identify these species. Scanning electron microscopic procedures were also used for observing the outer layer of the spore wall, but they revealed inconsistent morphological differences that could not be used to identify these species.

Teliospores of two wheat bunt fungi, *Tilletia caries* (DC.) Tul. and *T. controversa* Kühn, are difficult to identify because their spore morphology is too similar (8). Teliospores of *T. foetida* (Wallr.) Liro, another common bunt fungus, do not have reticulations, so they are easily distinguished, and teliospores of *T. indica* (Mitra) Mund. are much larger than those of the other wheat bunt fungi. Hoffmann (9) stated that the criteria most frequently used to distinguish between teliospores of *T. caries* and *T. controversa* are the relatively wide and deep polygonal aerolae of the exospore and the presence of a hyaline sheath or capsule extending beyond the exospore of *T. controversa*. He stated that the extreme variability of teliospore characteristics of *T. controversa* and other similar-appearing species makes positive identification difficult or impossible and that there appears to be no way to determine with certainty whether a single teliospore is that of *T. controversa* or that of one of several other morphologically similar species.

Considering bunt spores associated with wheat shipments, the main need is to distinguish *T. caries* from *T. controversa*, primarily for marketing reasons (12). Trione (12) pointed out that physiological requirements and germination patterns differ for teliospores of these two species of *Tilletia*. If biochemical differences exist, it is likely that morphological differences may be detected. Therefore, the purpose of these investigations was to use specialized fixation procedures to identify teliospores of the wheat bunt fungi with transmission electron microscopy (TEM).

MATERIALS AND METHODS

In the specialized fixation procedures for TEM, spore samples were immersed in distilled water containing a small amount of wetting agent (Aerosol OT wetting agent or Shell Teepol detergent). Better results were obtained if the sheath or capsule was hydrated before fixation. Spores were filtered through glass wool to remove plant debris. Spores were pelleted with a clinical centrifuge at 950

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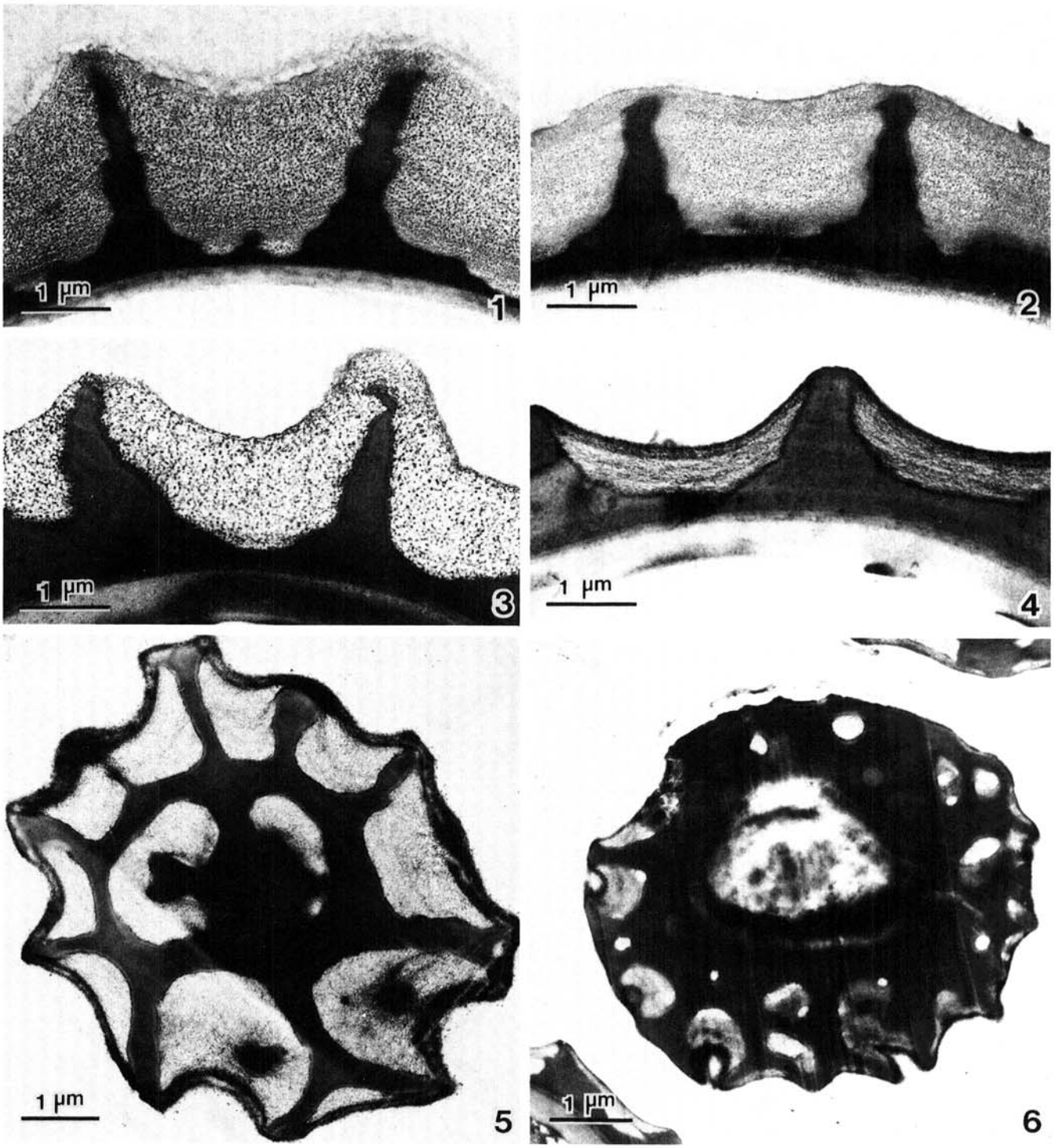
rpm, then fixed with 3% glutaraldehyde 3% acrolein in 0.1 M sodium cacodylate buffer (pH 7.2–7.4) as described by Hess (3). After fixation for 2 hr or longer, spores were washed in water:buffer (1:1) and dehydrated in an ethanol series followed by three changes in absolute acetone (3).

The spores were placed on Whatman No. 50 filter paper, which was folded and secured with C-clamps made of 30-gauge aluminum. After two changes in absolute acetone (treated with $CaSO_4$ to remove water), spores were critical-point dried with liquid CO_2. After critical-point drying, aluminum clamps were removed to prevent excessive blackening during postfixation with 2% OsO_4 buffered with sodium cacodylate buffer (pH 7.2–7.4) for 2 hr in an ice bath. After the OsO_4 treatment, aluminum clamps were replaced to prevent unfolding of the filter paper packets. The spores were then dehydrated with an ethanol series followed by three changes in absolute acetone (3). After two changes in absolute acetone, the spores were again critical-point dried with liquid CO_2 followed by dehydration with an ethanol series and three changes in absolute acetone. The spores were then embedded in Spurr resin (11) and were sectioned and stained with lead citrate (10).



Figs. 1–6. Thin sections of portions of *Tilletia* teliospores: **(1)** *T. controversa* teliospore wall fixed with standard glutaraldehyde-acrolein in sodium cacodylate buffer (×15,000). **(2)** *T. caries* teliospore wall fixed with standard glutaraldehyde-acrolein in sodium cacodylate buffer (×15,000). **(3)** *T. controversa* teliospore wall fixed with specialized procedures (×15,000). **(4)** *T. caries* teliospore wall fixed with specialized procedures (×15,000). **(5)** Tangential section of a *T. controversa* teliospore fixed with specialized procedures (×8,000). **(6)** Tangential section of a *T. caries* teliospore fixed with specialized procedures (×8,000).

Differences in surface morphology between *T. caries* and *T. controversa* teliospores were also investigated with scanning electron microscopy (SEM). Carbon, gold, and platinum shadowing techniques and stereomicroscopy were used to investigate untreated spores and spores that were fixed in 3% glutaraldehyde 3% acrolein in 0.1 M sodium cacodylate buffer (pH 7.2–7.4) followed by dehydration in acetone. Spores were critical-point dried before shadowing, and various voltage settings from 1 to 15 kV were used.

RESULTS AND DISCUSSION

In TEM studies with standard glutaraldehyde-acrolein fixation procedures, the two outer wall layers of *T. caries* and *T. controversa* teliospores appeared similar (Figs. 1 and 2). However, it was possible to distinguish between *T. controversa* (Fig. 3) and *T. caries* teliospores (Fig. 4) when the specialized procedures described in this paper were used. These procedures help maximize differences in the nature of the sheath and polygonal aerolae or reticulated wall layers. The sheaths of *T. controversa* spores had a coarse, speckled or stippled appearance (Fig. 3), whereas those of *T. caries* commonly were composed of a fine network of parallel fibers (Fig. 4). The physical or chemical bases for these ultrastructural differences are not known. However, the morphological differences in the exospore and sheath of these two species of *Tilletia* are not evident if any of the steps of the specialized procedures are omitted. Tangential sections often were valuable in characterizing the species for they quickly indicated the larger, deeper aerolae of *T. controversa* (Fig. 5) in contrast to the smaller, shallower aerolae of *T. caries* (Fig. 6).

Previous attempts were made to process dormant *Tilletia* teliospores for TEM studies (1,4–6,8). Because of the presence of four distinct spore wall layers (8), resins and fixatives do not penetrate adequately until after spores germinate. Gardner et al (2) used special ultracryomicrotomy procedures to overcome these limitations. Hess and Gardner (7) reported that a lamellar material was present in the spore wall layer, which they also called the partition layer (1). They suggested that this spore wall layer may account for the resistance of this layer to fixatives and resins. To characterize *T. caries* and *T. controversa* teliospores, as described herein, it was not necessary to fix and preserve the partition layer and the inner wall layer.

To determine the capability of our TEM procedure for distinguishing *T. caries* from *T. controversa* teliospores, coded samples were obtained from known collections. Twenty-seven samples of *T. caries*, 20 samples of *T. controversa*, and three samples containing mixtures of these two species were all correctly identified on the basis of the morphological appearance of exospores and sheaths. Also, samples were mixed thoroughly before sectioning to increase the opportunity to observe representative spores.

Spore wall characteristics varied significantly within samples, even within one bunt sorus. In some collections of *T. controversa*, spores within a sample varied from little or no sheath to a full sheath, and in other collections, inclusions were present in the sheath material. However, the appearance of characteristic aerolae was a constant feature for both species. Teliospores of *T. caries* were more consistent in appearance from collection to collection. Although the spores of most collections were easy to identify, some samples had individual ambiguous spores. By examining additional spores in the same sample, it was possible to identify the sample. Because morphological differences can be demonstrated between teliospores of *T. caries* and *T. controversa*, it is likely that further investigations with chemical procedures may make it possible to more precisely characterize species of *Tilletia* teliospores. However, for routine examination of wheat samples, the specialized TEM procedures described in this paper can be used to accurately evaluate relative percentages of *T. caries* and *T. controversa* teliospores in wheat.

With both coated and uncoated *Tilletia* teliospores, examined with SEM at regular voltage settings of 1–15 kV, it appeared that the electrons penetrated the porous outer sheath of spores and reflected from the electron impermeable exospore layer. When the teliospores were coated with carbon, gold, or platinum and voltage settings of 1–5 kV were used, the outer surface of the sheath could be photographed. With these techniques, the larger, deeper aerolae of *T. controversa* teliospores were sometimes easy to distinguish from the smaller, shallower aerolae of *T. caries*. Unfortunately, with SEM, the exospore and sheath characteristics were not consistent enough to use as a primary factor for identifying these two species of wheat bunt fungi.

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