Host-Parasite Relationships in Karnal Bunt of Wheat

Norma L. Cashion and E. S. Luttrell

Wheat Program, CIMMYT, Londres 40, Apartado Postal 6-641, 06600 México, D.F., and Department of Plant Pathology, University of Georgia, Athens 30602, respectively.

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ABSTRACT

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Tilletia indica, cause of Karnal bunt, is a local lesion pathogen that infects individual ovaries of wheat. Symptoms first appear at the soft-dough stage in the form of blackened areas surrounding the base of the grain and extending upward to varying degrees. In severe infections the entire grain is blackened and shriveled. This study by light and electron microscopy showed that the fungus is restricted to the pericarp, where it is entirely intercellular. Hyphae proliferate in the space formed by disintegration of the middle layers of parenchymatous cells of the pericarp during normal development of the grain and prevent fusion of the remaining outer and inner layers of the pericarp with one another and with the seed coat. The smut hyphae form a compact, hymenium-like layer over all surfaces of the pericarp tissue surrounding this cavity and give rise to short, septate sporogenous hyphal branches that produce teliospores singly from their terminal cells. Growth of the fungus ruptures the connection between the pericarp tissue surrounding the vascular bundle in the bottom of the adaxial groove in the pericarp and the nucellar projection along the length of the developing seed. The consequence is atrophy of the seed through disruption of the normal flow of nutrients from the pericarp. The endosperm is shrunken to varying degrees and cartilaginous in appearance. The embryo with attached endosperm may be easily dissected out and is germinable before or after removal from the grain. In the most severe infections, the grain is reduced to a black membranous sack of teliospores, and the shriveled embryo is dead.

Additional key word: *Neovossia indica*.

The history of Karnal bunt of wheat, caused by *Tilletia indica* Mitra [= *Neovossia indica* (Mitra) Mundkur]; its description in the Karnal District in 1931; its spread and development into a major disease in India between 1969 and 1979; its appearance in the Mayo and Yaqui valleys of Sonora, Mexico, in 1971; and the subsequent quarantine in 1981 against shipments of wheat, including breeders' seed, from Mexico into the United States has been reviewed in two recent papers (4,8). Karnal bunt, like the similar kernel smut of rice caused by *T. horrida* Tak. (2,9,10), is a local lesion disease affecting the ovaries. In both of these diseases the grain may be smutted to varying degrees, and in partially smutted grains the embryo usually is viable and capable of producing a healthy seedling. In their review of Karnal bunt, Joshi et al (4) refer to "the ramification of the fungus in the endosperm." Although no primary source was cited to support this statement, it may have been based on the conclusion of Munjal and Chatrath (7) that "the infection develops in the endosperm but the embryo is not attacked." In their discussion of rice kernel smut, Whitney and Fredericksen (10) stated that "...the fungus attacks...the endosperm while it is in the milk and dough stage."

The present study of host parasite relationships in Karnal bunt has demonstrated that the causal fungus does not invade the seed; its growth is limited to the pericarp where it intercepts nutrients from the vascular tissue and starves first the endosperm and then the embryo. These observations confirm the brief account of host relations given by Mitra (6) in his original description of the Karnal bunt fungus.

MATERIALS AND METHODS

Most of the material for light microscopy and all of the material for transmission electron microscopy was taken from a few inflorescences of the breeding lines S-5034 and S-5040 in disease nurseries at the CIMMYT spring wheat station at Ciudad Obregón, México, during the period 2–4 April 1984. Lines in the Karnal bunt screening nursery were inoculated in February by injecting sporidial suspensions from cultures of *T. indica* grown on PDA plates into the boot of plants approximately 2 days before emergence of the inflorescence. Grain sampled at 46–59 days after inoculation was in the soft to hard dough stage. Additional material was taken later from plants in the CIMMYT headquarters greenhouse at Mexico City at 7, 15, 21, and 28 days after inoculation by boot injection.

Transmission electron microscopy (TEM). Selected portions of wheat grains were cut in slices 1 mm thick in 0.1 M PO_4 buffer on dental wax under a dissecting microscope. The slices were transferred to vials of buffered 3% glutaraldehyde where they remained for 4–6 days during transit. During the washes in 0.1 M PO_4 buffer, the tissue slices were cut into smaller fragments, and selected pieces were postfixed in buffered 2% OsO_4 overnight, washed in water, and placed in aqueous uranyl acetate overnight. On day three the washed material was dehydrated in a graded acetone series and over the following 3 days was moved from acetone through a series of 30, 50, 75, and 100% Spurr's firm resin. Sections were stained on the grids with Reynold's lead citrate.

Light microscopy (LM). Whole or sliced grains were fixed in Formalin-propionic acid-alcohol (FPA), dehydrated in tertiary butyl alcohol, embedded in Paraplast, and sectioned at 8 μm. Sections were mordanted in 4% ferric ammonium sulfate 2 hr, stained in 0.5% hematoxylin 2 hr, differentiated for 15–20 min in a saturated aqueous solution of picric acid, and counterstained in 0.001% fast green in 95% alcohol 5 min. Drawings were made with a camera lucida on a dissecting microscope from whole grains fixed in FPA and immersed in 70% alcohol.

Scanning electron microscopy (SEM). Whole grains fixed in FPA were washed in 70% ethyl alcohol, dehydrated in an ethyl alcohol series to 100%, critical-point dried, and coated with gold-platinum. Some dried specimens were sliced with a razor blade before they were coated.

RESULTS

Symptoms. In the field at Ciudad Obregón, symptoms were well developed in grain approaching harvest 46 days after inoculation. In the greenhouse symptoms became evident in grain in the soft dough stage 28 days after inoculation. The mildest common

symptom was black point, a narrow, blackened band surrounding the base of the grain and extending higher up the groove on the adaxial face (Figs. 1–3). Variations in symptoms resulted from varying degrees of extension of the blackening, with the greatest extension always in the adaxial groove (Figs. 4 and 5), until the entire grain was blackened. External blackening resulted from development of masses of dark brown teliospores beneath the hyaline outer layers of the pericarp (Figs. 6–12 and 18–22). In the most severe infections, the grain was reduced to a fragile, shriveled, membranous sack filled with a black mass of teliospores (Figs. 13 and 14).

Normal grain. In normal florets, shortly after pollination the ovule consisted of an embryo sac embedded in a nucellar tissue surrounded by integuments. The ovary wall consisted of an outer layer of epidermal cells enclosing 12–19 layers of parenchyma cells bounded on the inside by a poorly defined inner epidermis. As the ovule developed, the embryo and endosperm absorbed the nucellus except for a narrow ridge of tissue, the nucellar projection, extending vertically along the adaxial face of the ovule. The ovule was surrounded by crushed remnants of the integuments of the ovule and an outer cuticularized layer except for a longitudinal gap filled by the pigment trace through which the nucellar projection made contact with the pericarp tissue in the bottom of the adaxial groove. The cuticularized layer, naturally colorless, stained a deep yellow in hematoxylin and clearly outlined the ovule. The cuticularized layer was further emphasized by the naturally brown layer of crushed nucellar tissue beneath it (Figs. 23 and 24). The periphery of the endosperm was marked by the thick-walled, cuboidal cells of the aleurone layer (Fig. 23). By the soft dough stage the pericarp was divided into two layers through disintegration of the intervening parenchyma: 1) an outer hyaline layer composed of the epidermis or of the epidermis and hypodermis, and 2) an inner layer composed of tube cells representing remnants of the original inner epidermis, cross cells running at right angles to the axis of the grain like an ordered series of segmented belts encircling the seed, and often the innermost pericarp parenchyma cells (Fig. 23). The cross cells and contiguous parenchyma cells were filled with chloroplasts and were responsible for the green color of the immature grain. Structure of the pericarp varied with its location. Larger amounts of parenchymatous tissue persisted in the bottom of the adaxial groove (Fig. 24) and in the base of the grain where the various layers were less well marked. The two separable layers of the pericarp (Fig. 23) could be easily stripped in turn from the grain. The tube cells, which were rarely distinguishable in cross sections (Fig. 23), appeared as a loose reticulum of tubular elements over the inner surface of the stripped layer of cross cells. By the hard dough stage the two layers of the pericarp were fused with one another and with the cuticularized layer of the seed. The chlorophyll in the cross cells disintegrated, and the mature grain was brown.

Infected grain. In infected grain, sporogenous hyphae of the fungus lined all surfaces of the cavity produced in the pericarp by dissolution of the middle parenchyma and prevented normal fusion of the remaining inner and outer layers (Figs. 18 and 20–22). Teliospores in various stages of maturity filled the resulting cavity (Figs. 6–11 and 18–22). The production and enormous expansion of the teliospores during their development resulted in a mechanical splitting of the tissue at the bottom of the adaxial groove (Fig. 25) that separated the nucellar projection of the ovule from the pericarp (Fig. 26) and the major vascular supply of the grain (Fig. 27). The base of the seed was surrounded by teliospores and was almost completely isolated from the ovary wall (Fig. 28). Sheets of tissue composed of the cross cells or of the cross cells and an inner layer of pericarp parenchyma were stripped from the seed and covered on both surfaces by the sporulating fungus (Figs. 26 and 29). In completely blackened grains the shrunken seed was embedded in the mass of teliospores and was entirely isolated from the pericarp (Figs. 13, 14, 30, and 31). The reduction in development of the seed resulting from its separation from the vascular tissue (Figs. 7–11, 20, and 30) was apparent first in the endosperm, which was markedly shriveled before the embryo showed signs of abortion. Even in the severest infections in which the embryo was still evident, the fungus never penetrated the cuticularized layer surrounding the seed (Figs. 14 and 30). There was no invasion of either endosperm or embryo. However, in some of these severely infected grains no remnant of the seed could be found. In less severely infected grains the endosperm was shrunken to correspondingly lesser extents (Figs. 6–12 and 20–22). The size of the grain was little altered (Figs. 1–5) or was reduced (Fig. 13) by infection, and space within the epidermis of the grain for the expanding mass of teliospores was produced by corresponding shrinkage in the seed.

No infections were demonstrated in sections of ovaries and immature grains fixed before the appearance of external symptoms made identification of diseased grains possible.

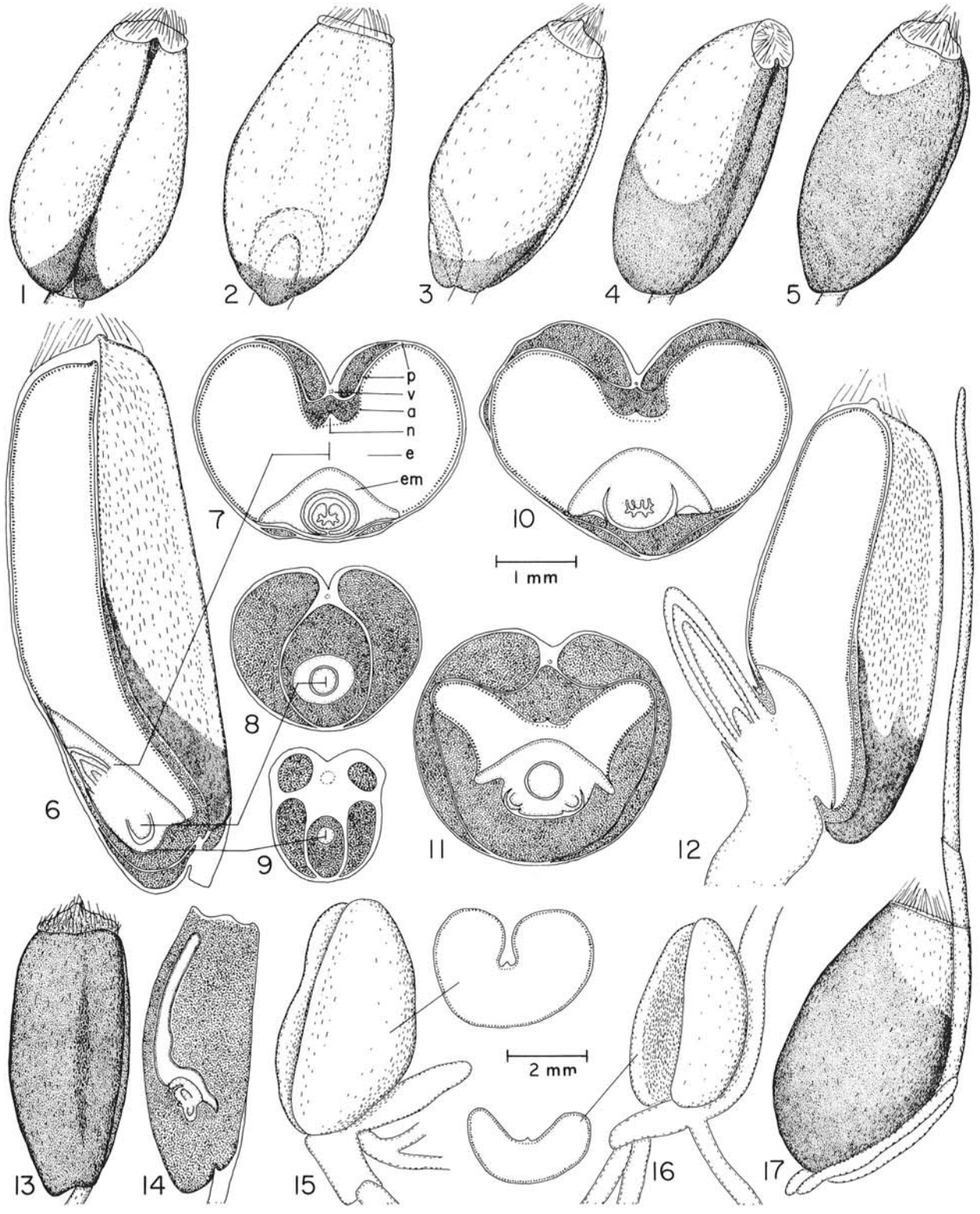
Mycelium and sporulation. Within the pericarp the fungus mycelium was entirely intercellular (Figs. 32 and 36–39). At the margins of the infected areas the mycelium formed wefts between the outer and inner layers of the pericarp (Fig. 32), which were pushed apart by further development of mycelium and teliospores (Fig. 18). A hymenium-like layer of mycelium producing teliospores on short, septate sporogenous hyphae covered all surfaces of the pericarp surrounding the central cavity (Figs. 19 and 33–37). When a detached sheet of inner pericarp covered on both surfaces by the fungus hymenium was examined in sections, it resembled the gill of an agaric (Fig. 29). In the base of the grain these sheets of tissue were thicker and even more like agaric gills in section (Fig. 28). The teliospore-producing hymenium remained thin as it produced successive teliospores, and many of the hyphal cells became empty (Fig. 37). Although maturing teliospores sometimes remained attached to the sporogenous hyphae (Fig. 35), most teliospores were detached from the hymenium at an early stage and completed their development after abscission (Fig. 26).

Germination of grain. Partially infected grains and even some that were completely blackened, were capable of germination (Fig. 12). Some germinated in the heads in the field (Fig. 17). The coleoptile emerged between the tips of the lemma and palea; the roots were coiled within the floret. The endosperm and embryo could be dissected from healthy grains (Fig. 15) only with difficulty but were easily freed from diseased grains (Fig. 16). In severely infected grains they could be washed free by shaking the grains in a flask of water. The endosperm in healthy grains was white and opaque (Fig. 15). In infected grains it was shrunken, brownish, semitranslucent, and cartilaginous (Fig. 16). Such embryos germinated on moist filter paper either before (Fig. 12) or after (Fig. 16) they were removed from the grain.

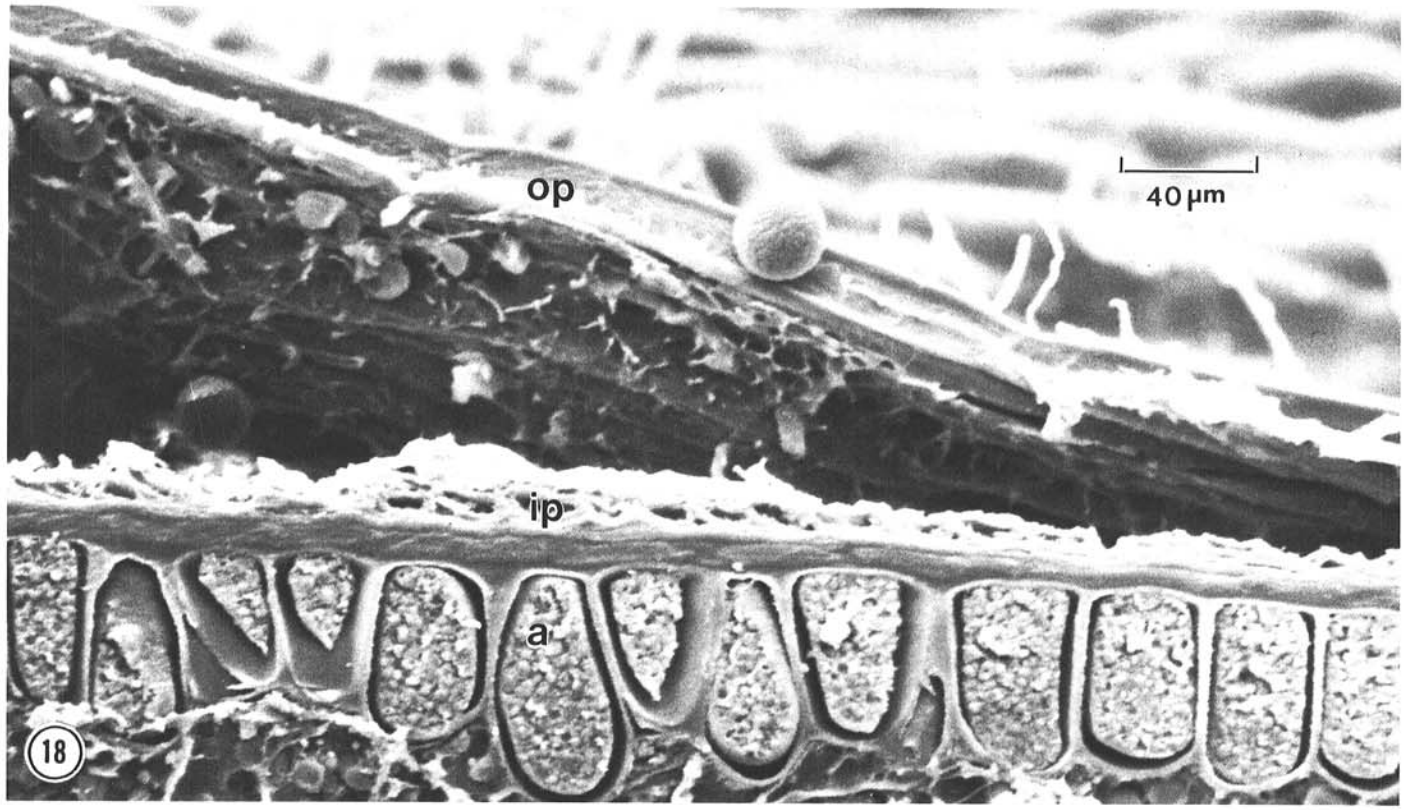
DISCUSSION

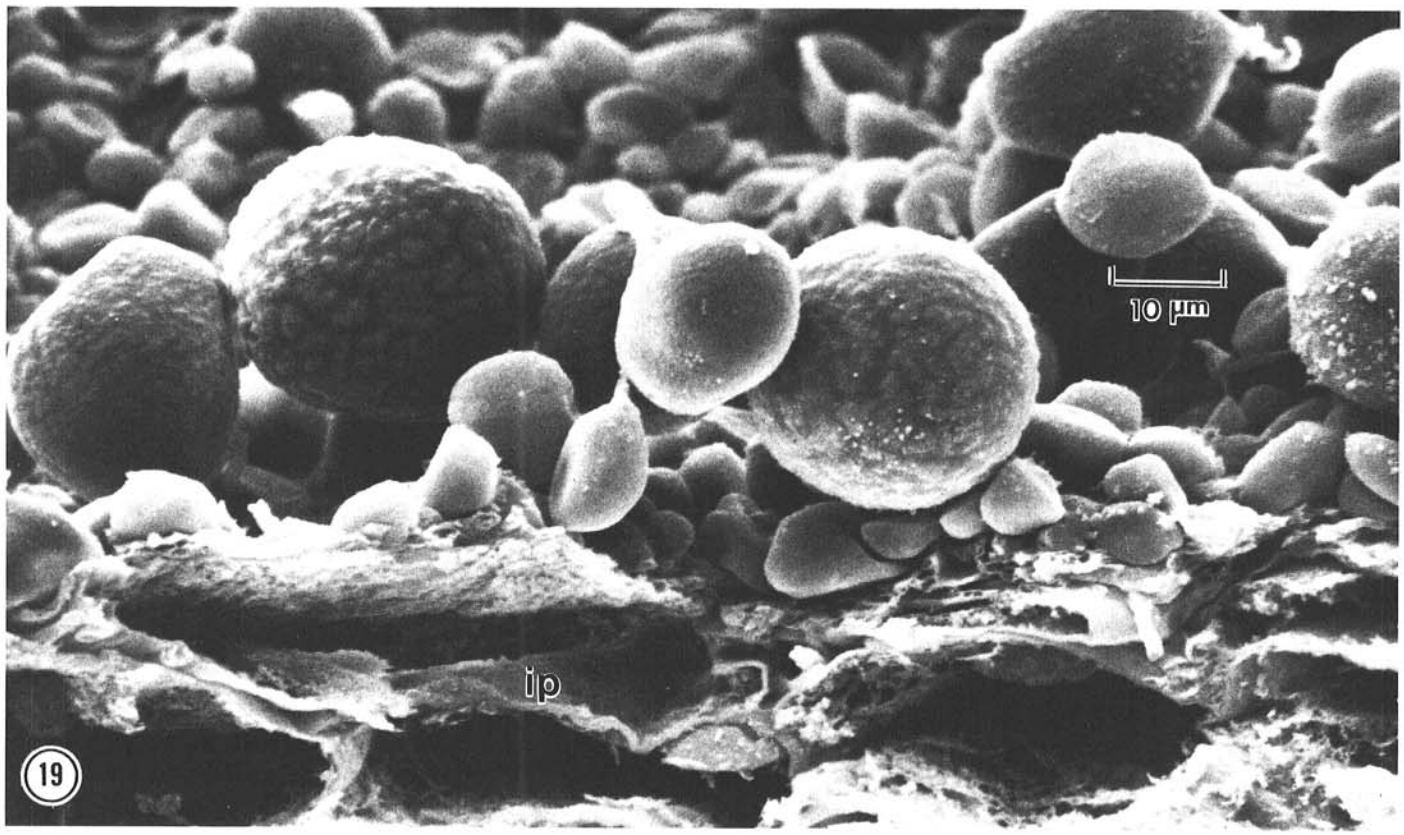
In light of our observations, it is evident that Mitra (6) in a brief note in 1931 presenting *T. indica* as a new species correctly described the relationship of host and parasite in three sentences and illustrated it with a series of excellent photomicrographs. His description reads, "This smut infects only partially the kernels which are not swollen. The embryo tissue is not destroyed by the smut and in a large number of cases only the embryo portion [end of the grain] is affected. In some cases the infection spreads to the tissues along the groove, but the endosperm material lying along the smooth side of the grain is uninfected." The micrographs in his Figures 1–4 (Plate XV) are now readily interpretable as corresponding to the area of detached pericarp immediately surrounding the bottom of the adaxial groove in our Figure 21. Mitra's micrographs demonstrate the fungus hymenium lining the cavities in the pericarp, as in our Figure 28, but do not show any of the adjacent tissues of the grain. Unfortunately, he did not describe his micrographs, and his text was ignored by later workers who wrote at greater length.

Although smut fungi generally cause necrosis of host tissue as a space-making process during sporulation (5), they are basically growth-altering parasites. The growth alterations commonly result in hypertrophy, but atrophy may occur also, as in suppression of pistils in normally pistillate flowers of Caryophyllaceae infected with anther smut caused by *Ustilago violacea* (Pers.) Fckl. (1). In

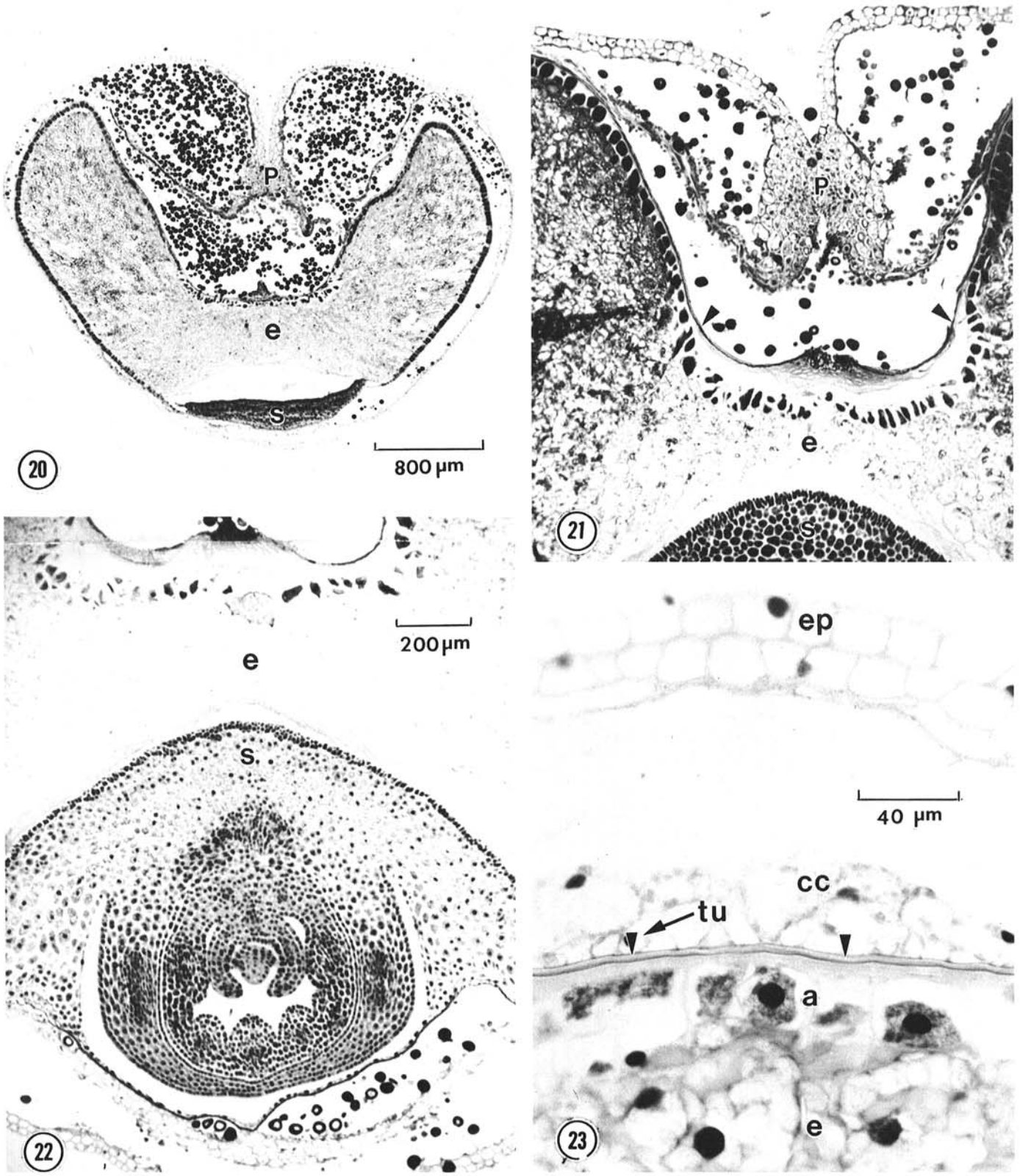


Figs. 1–17. Semidiagramatic drawings of wheat grains infected with *Tilletia indica* (1–5 and 13–17 to scale in 16; 6–12 to scale in 10). **1–3,** Adaxial, abaxial, and lateral views of grain with symptoms of black point. **4 and 5,** Lateral views of more severely infected grains. **6,** Longitudinal-section through adaxial groove showing at right external surface of groove and at left in section embryo and endosperm separated from pericarp at base by mass of teliospores. **7–9,** Cross sections at progressively lower levels showing seed separated from vascular bundle in pericarp at bottom of adaxial groove. (p = outer and inner layers of split pericarp, v = vascular bundle, a = seed coat and aleurone layer, n = nucellar projection of seed with pigment trace at apex in gap in seed coat, e = endosperm, em = embryo) **10 and 11,** Cross sections of more severely infected grains at level of coleoptile and at level of coleorhiza. **12,** Longitudinal section of germinated partially bunted grain. **13 and 14,** Lateral surface view and longitudinal section of severely infected grain with shrunken endosperm and dead embryo. **15,** Surface view of embryo and endosperm from germinated healthy grain and cross section of endosperm. **16,** Same from infected grain. **17,** Infected grain germinated in the head in the field with roots coiled by pressure of the enclosing glumes.

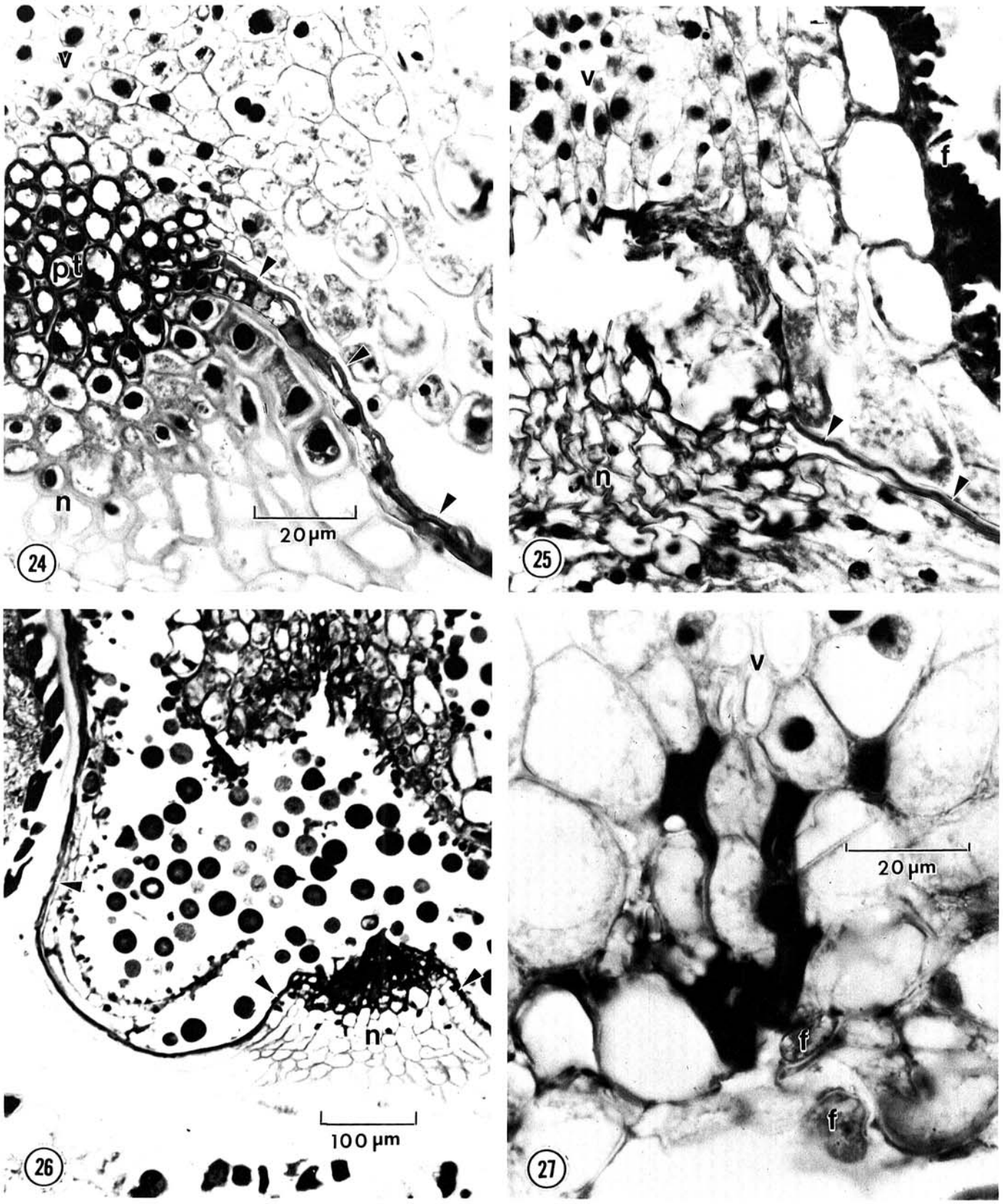




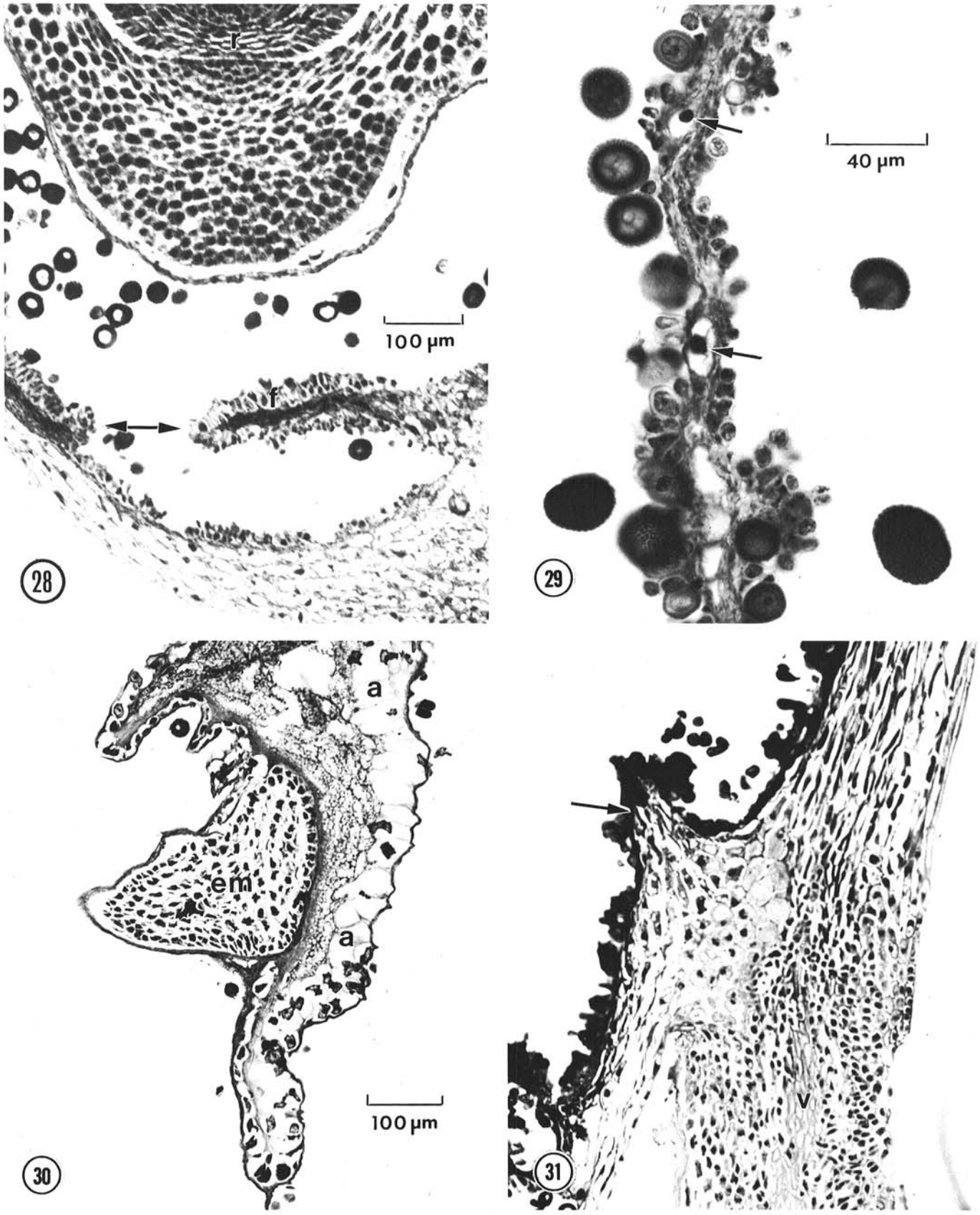
Figs. 18 and 19. Scanning electron micrographs of wheat grains infected with *Tilletia indica*. **18,** Cavity formed by raised outer layer of pericarp (op) consisting only of epidermis, widening at left toward base of grain; floor of cavity lined by inner layer of pericarp (ip) composed of innermost parenchyma cells and cross cells resting on cuticularized layer of seed coat that covers the cuboidal aleurone cells (a) of the endosperm; teliospores mostly washed out of cavity. **19,** Teliospores in various stages of development in hymenium over surface of inner layer of pericarp (ip).



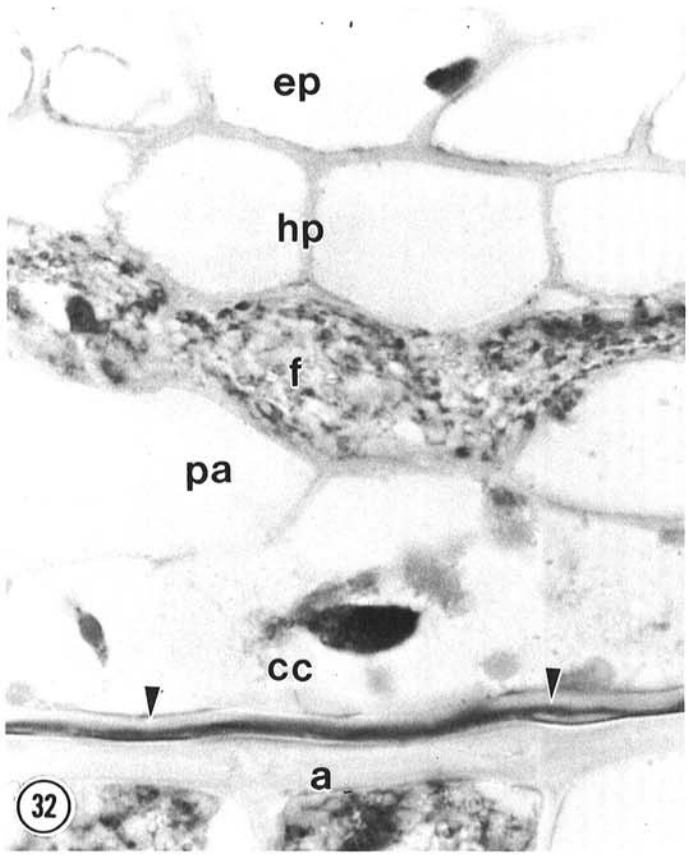
Figs. 20–23. Light micrographs of cross sections of wheat grains infected with *Tilletia indica* (20–22) and healthy grain (23) (21 and 22 to same scale). **20,** Section of entire grain near upper tip of scutellum (s) with teliospores occupying space between pericarp (p) and shrunked endosperm (e). **21,** Adaxial side of grain showing teliospores in spaces between outer and inner layers of pericarp (p) and between inner pericarp and seed (arrowheads). **22,** Abaxial side of grain showing well-developed embryo (s = scutellum) with shrunken endosperm (e) above and split layers of pericarp below. **23,** Abaxial side of grain showing top to bottom: outer layer of pericarp composed of epidermis (ep) and hypodermis, cavity created by normal dissolution of middle layer of pericarp, inner layer of pericarp composed of cross cells (cc) and tube cells (tu), cuticularized outer layer of seed (arrowheads), cuboidal cells of aeurone layer (a), and cells of endosperm (e) filled with starch grains.

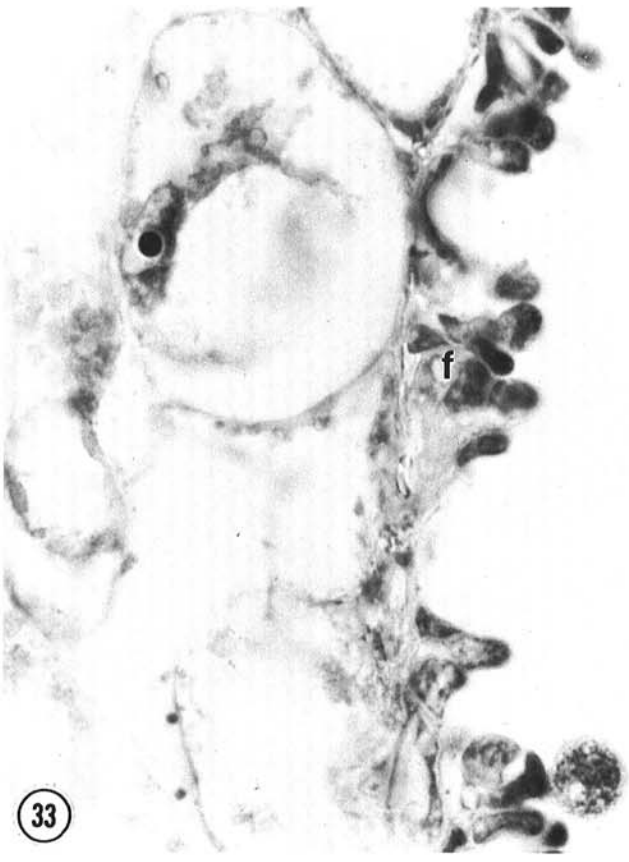


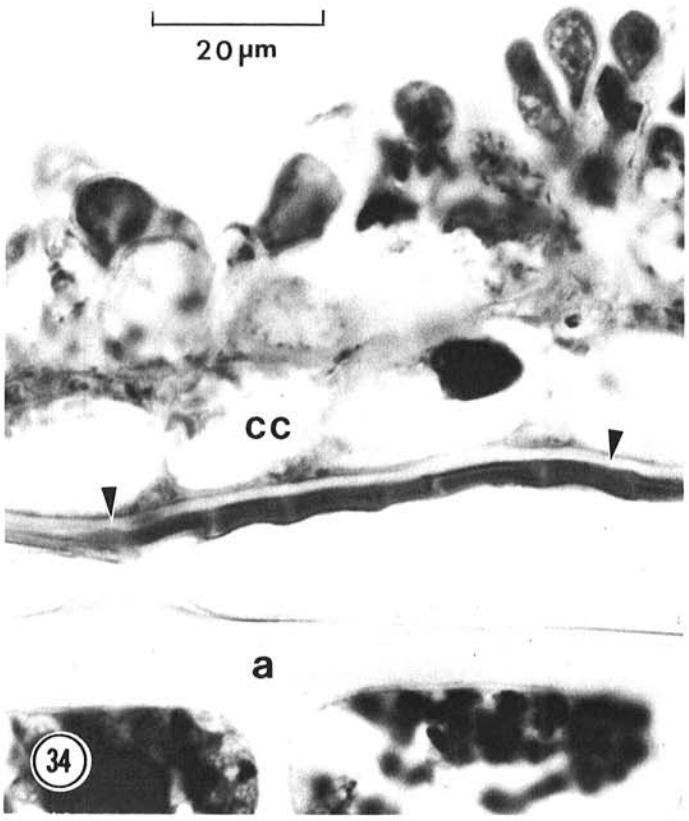
Figs. 24–27. Cross sections of wheat grains, healthy (24) and infected with *Tilletia indica* (25–27) (25 and 26 to same scale). **24,** Area of healthy grain near adaxial groove showing part of nucellar projection (n) bounded on right by cuticularized layer (arrowheads) of seed coat and connected through pigment trace (pt) in gap in seed coat with pericarp parenchyma surrounding vascular bundle (v) at upper left. **25,** Comparable area of infected grain showing pericarp surrounding vascular bundle (v) splitting away from nucellar projection (n) of seed; fungus hymenium (f) lining pericarp cavity at upper right. **26,** Pericarp tissue at bottom of adaxial groove split away from nucellar projection (n) of seed (arrowheads mark cuticularized layer). **27,** Vessels (v) in vascular bundle of pericarp with fungus hyphal cells (f) on lower surface, which has split away from nucellar projection.

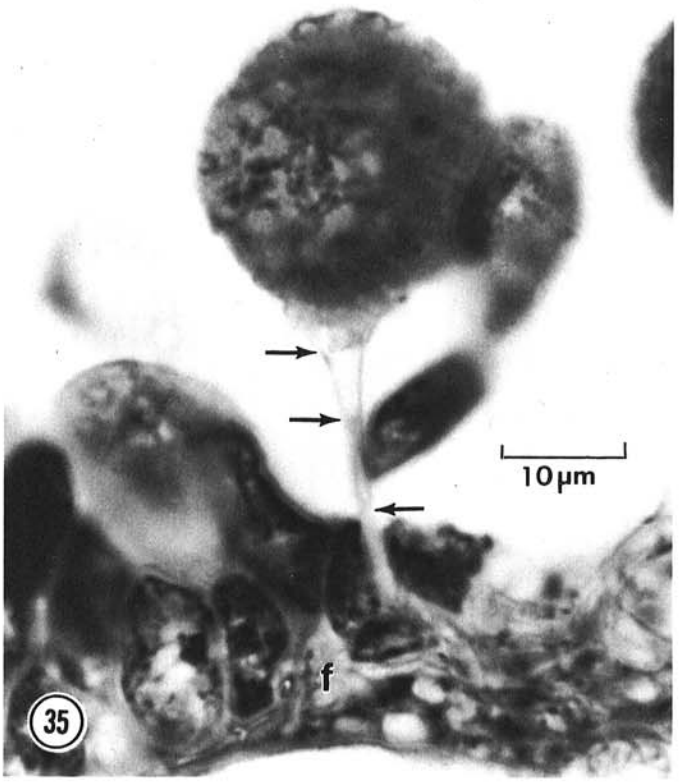


Figs. 28–31. Longitudinal sections of wheat grains infected with *Tilletia indica* (30 and 31 to same scale). **28,** Base of grain on abaxial side showing tip of coleorhiza and root tip (r) of apparently normal embryo surrounded by teliospores and detached strip of inner pericarp covered over both surfaces by fungus hymenium (f) resembling gill of an agaric. **29,** Hymenium covering both surfaces of detached strip of cross cells, two with nuclei (arrows) still visible. **30 and 31,** From grain reduced to membranous sack of teliospores (washed out in processing). **30,** Aborted embryo (em) and shrunken endosperm with empty aleurone cells (a). **31,** Base of ovary wall showing vascular bundle (v) in rachilla and funicular projection (arrow) into cavity lined with remanants of fungus hymenium.

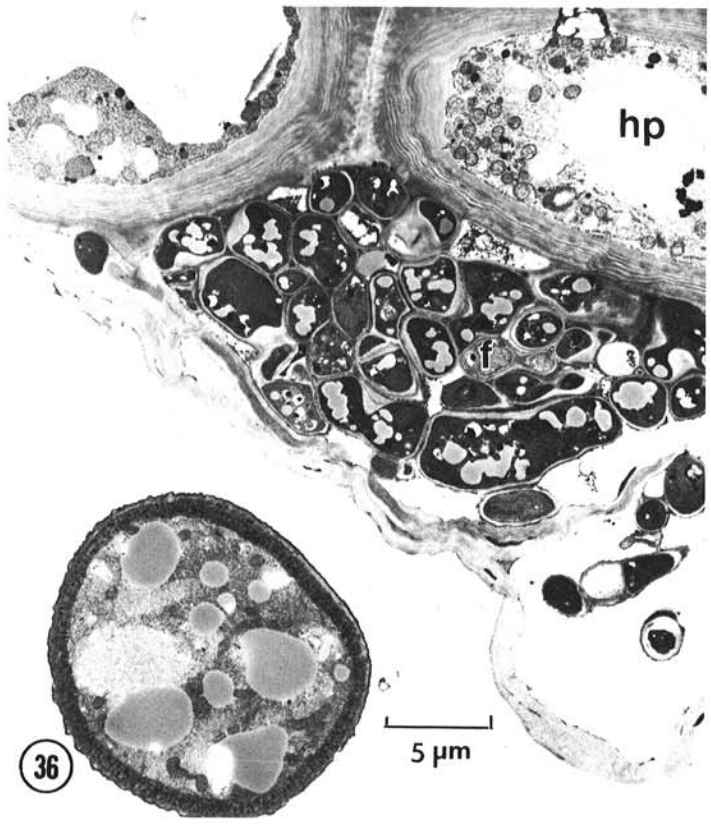


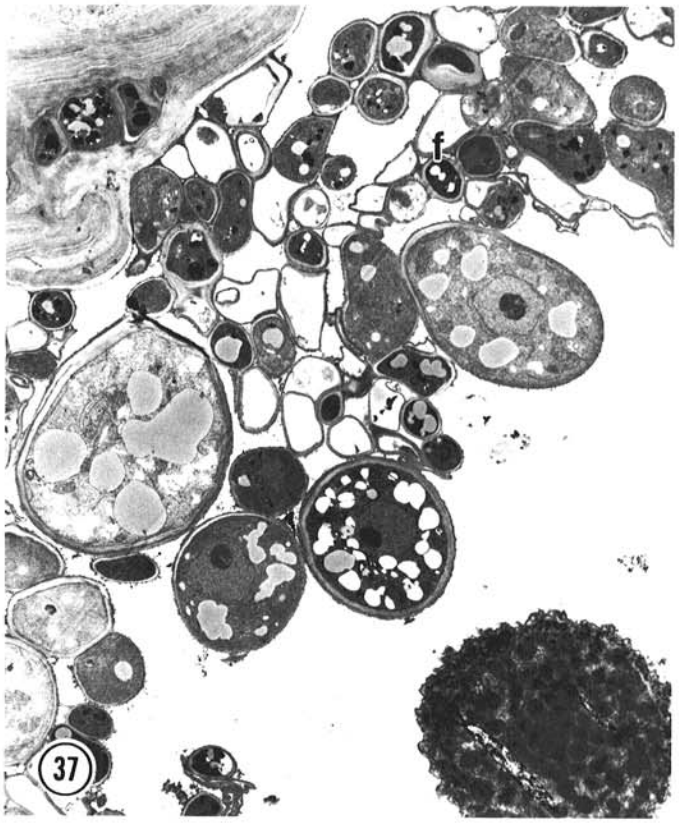


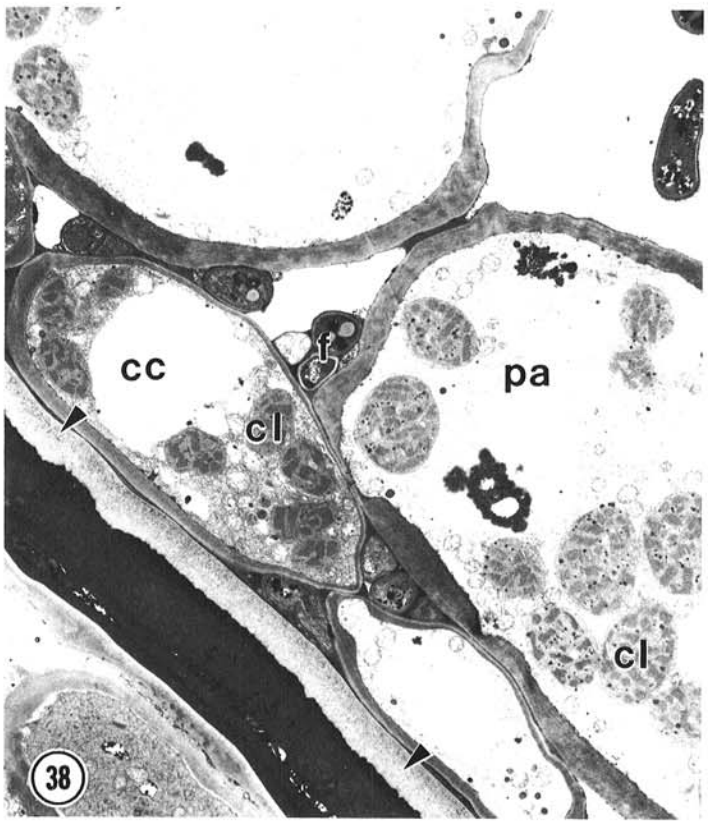


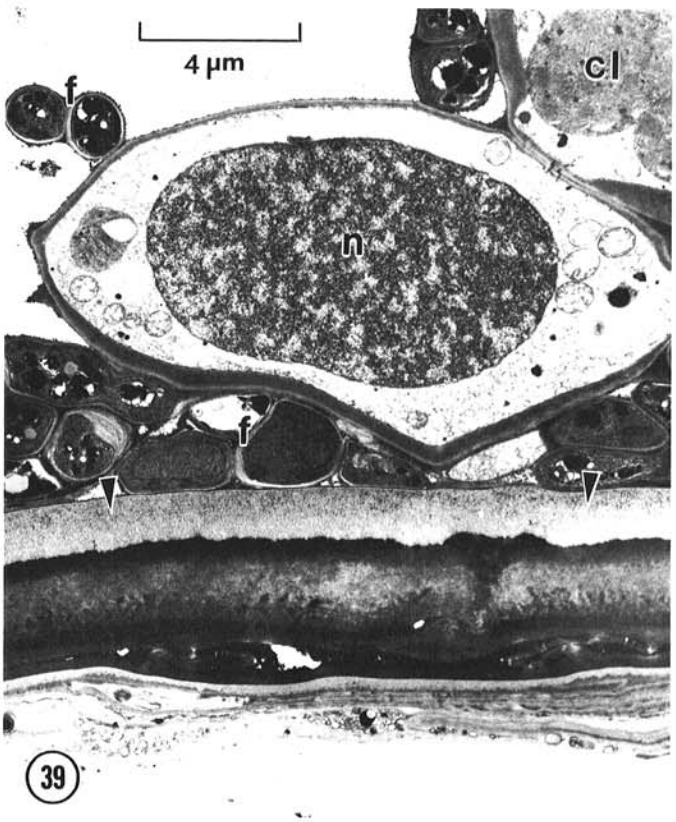


Figs. 32–35. Sections of wheat grains infected with *Tilletia indica* (32–34 to same scale). **32,** Pocket of fungus hyphae (f) in middle of pericarp between epidermis (ep) and hypodermis (hp) above and inner layer of parenchyma (pa) and cross cells (cc) below resting on cuticularized layer (arrowheads) and aleurone (a) of seed. **33,** Hymenium (f) covering inner surface of pericarp parenchyma lining adaxial groove. **34,** Hymenium covering cross cells (cc), one with nucleus, resting on cuticularized layer (arrowheads) bounding crushed integuments and aleurone (a) of seed. **35,** Teliospore at tip of empty, septate (arrows) sporogenous hypha projecting from hymenium (f).









Figs. 36–39. Transmission electron micrographs of wheat grains infected with Karnal bunt (36–38 to same scale). **36,** Mass of fungus hyphae between hypodermis (hp) and disintegrating peripheral layers of parenchyma beneath epidermis of pericarp. **37,** Hymenium of sporogenous hyphae (f) and developing teliospores lining cavity beneath outer layer of pericarp. **38,** Fungus hyphae (f) between innermost pericarp parenchyma cells (pa) and cross cells (cc) both with intact chloroplasts (cl) resting on cuticularized layer (arrowheads) of seed coat. **39,** Fungus hyphae (f) between innermost parenchyma cells and cross cells (with large nucleus, n) of pericarp above cuticularized layer (arrow heads) and crushed integuments of seed coat.

anther smut, atrophy of pistils is merely one of a complex of growth changes. In Karnal bunt, atrophy of the endosperm and embryo is the only obvious host reaction to infection. The fungus intercepts nutrients that normally flow from the vascular bundle in the pericarp to the endosperm, and the endosperm deteriorates. Atrophy also serves the space-making function usually performed by necrosis. The fungus proliferates in the space created by normal disintegration of parenchymatous tissue in the middle layers of the pericarp, and shrinkage of the endosperm provides space for the development of masses of teliospores.

Karnal bunt has some remarkable parallels in physiogenic diseases represented by the shrunken endosperm mutants of barley (3). In four of these genetic diseases, shrinkage of the endosperm results from disruption of movement of sugars from the sieve tubes of the vascular bundle across the chalaza (corresponding to the pigment strand in wheat) and through the nucellar projection into the endosperm. As in Karnal bunt, "except for the reduced size and crenulated outline, the endosperm [is] not otherwise abnormal or disrupted" (3). Also as in Karnal bunt, shrinkage of the endosperm results in loosening and separation of layers of the pericarp. In the shrunken-endosperm mutants the immediate cause of the interruption of nutrient flow into the endosperm is necrosis of the cells of the chalaza and the nucellar projection resulting from failure of normal compartmentation of tannins in the central vacuoles of chalazal cells. Suggested underlying mechanisms (3) were synthesis of abnormal quantities of tannins, aberrations in synthesis of tannin precursors, or leakage from the small vesicles in which the tannins were originally contained. The mechanism of pathogenesis in Karnal bunt, on the face of it at least, is more straightforward. Atrophy of the endosperm, and less often of the embryo also, results from mechanical disruption of host tissue in the pathway of nutrient transport from the vascular bundle in the pericarp to the developing ovule.

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