Cytology and Histology

Teliospore Formation by *Ustilago scitaminea* in Sugarcane

Edward J. Trione

USDA-AR-SEA, and Department of Botany and Plant Pathology, Oregon State University, Corvallis 97331.

Cooperative investigations of Agricultural Research, Science and Education Administration, U. S. Department of Agriculture; the Hawaiian Sugar Planters' Association; and the Oregon Agricultural Experiment Station. Technical Paper 5215 of the Oregon Agricultural Experiment Station.

Appreciation is expressed to Linda Thatcher, Hawaiian Sugar Planters' Association, for assistance in staining the fungal preparations.

Mention of a trade name, proprietary product, or specific equipment does not constitute a guarantee or warranty by the U. S. Department of Agriculture and does not imply approval to the exclusion of other products that also may be suitable.

Accepted for publication 13 November 1979.

ABSTRACT

TRIONE, E. J. 1980. Teliospore formation by *Ustilago scitaminea* in sugarcane. Phytopathology 70:513-516.

The pathogen, which causes sugarcane smut, grew slowly in all sugarcane tissues other than the whiplike shoot, but in that modified apex the vegetative hyphae changed physiologically and cytologically into a reproductive phase that yielded large numbers of spores. The vegetative hyphae in the sori in the surface layers of the whip were mononucleate and irregular in shape and length. These hyphal cells aggregated, enlarged in size, and released their nuclei into a gelatinous matrix as their cell walls were hydrolyzed. Gradually cytoplasm and a cell wall formed around each nucleus. The spore initials were not connected directly to hyphae. The developing teliospores were held together in clusters by the gelatinous matrix. Mature teliospores also were formed in axenic culture on a medium containing aqueous extracts of sugarcane tissue.

Additional key words: binucleate cells, diploid cells, sorus.

The smut disease of sugarcane (*Saccharum officinarum* L.) caused by *Ustilago scitaminea* A. Syd. & P. Syd., is characterized by a long, whiplike sorus-bearing structure that develops from the apex of infected stalks (1,10). The hyphae of the sugarcane smut fungus develop systemically throughout the stalks but teliospores are formed only in the peripheral tissues of the whiplike structure (1,10) (Fig. 1). In many *Ustilago* spp., the mycelium partially fragments into segments before forming teliospores (3,6,15,16). Much attention has been focused on the nuclear status of teliospores, promycelia, and sporidia (3,6,7), but the nuclear changes that occur during teliospore formation have been described for only a few *Ustilago* spp. In *U. maydis,* binucleate

vegetative hyphae segmented to form diploid sporogenous cells (4). In *U. heufleri,* multinucleate hyphal cells break into binucleate segments that give rise to diploid spores (12). In *U. striiformis,* diploid hyphal cells break into short fragments that develop into diploid teliospores (9). The sporogenous hyphae of *U. avenae, U. bullata, U. cynodontis, U. hordei, U. nuda,* and *U. tritici* are composed of binucleate cells (8), the walls of which thicken and gelatinize after karyogamy.

The objectives of this study were to induce *Ustilago scitaminea* to produce teliospores in axenic culture and to describe the development of the teliospores in vivo and in vitro.

MATERIALS AND METHODS

Ustilago scitaminea-infected sugarcane stalks with terminal whips (Fig. 1) were collected from experimental field plots near the Experiment Station of the Hawaiian Sugar Planters' Association at Aiea, Hawaii. The cane sections were kept moist, and the developing sori in the peripheral 2-mm of the whips were carefully dissected, with the aid of a stereo microscope, within 1 hr after harvest. In addition to host cells, these samples contained vegetative and reproductive hyphae of *U. scitaminea* and all stages of developing teliospores. The cytological samples were teased apart, air dried on glass slides at 45 C, and processed through a nuclear-staining procedure similar to that described by Fernandez and Duran (5); fixed in anhydrous ethanol: acetic acid (3:1 for 30 to 60 min, washed in slowly running water for 4–16 hr, hydrolyzed in 6 N HCl at 25 C for 3–30 min, washed in water for 30 min, rinsed in 0.07 M KH_2PO_4 buffer (pH 6.5), stained in fresh Giemsa solution for 30 min, rinsed in water (containing 0.5 ml of Tween-20 per 100 ml), rinsed in acetone: xylene (19:1, v/v) for 10–30 sec, rinsed in acetone:xylene (14:6,v/v) for 10 sec, rinsed in acetone:xylene (6:14, v/v) for 10 sec, rinsed in two changes of xylene, and mounted in a xylene-soluble medium.

Mycelium of *U. scitaminea* developed and teliospores were produced on an agar medium that contained the following components: minerals (13), L-asparagine, 3 g; citric acid, 2 g; sucrose, 30 g; thiamine-HCl, 5 mg; niacinamide, 1 mg; pyridoxine, 1 mg; riboflavin, 1 mg; pantothenic acid, 1 mg; D-biotin, 0.5 mg; folic acid, 0.5 mg; thioctic acid, 0.5 mg; sodium novobiocin, 50 mg; streptomycin sulfate, 100 mg; tetracycline, 20 mg; vancomycin, 20 mg; V-8 juice (soilds removed), 300 ml; aqueous extract from 50 g of stem tips of sugarcane in 200 ml of H_2O, and aqueous extract from 200 g of internodal tissue of sugarcane in 400 ml of H_2O, adjusted to pH 5.5 with ammonium hydroxide, then brought to 1 L with distilled water. Colonies were incubated in the dark at 30 and 25 C (12 hr each) for 2–6 wk.

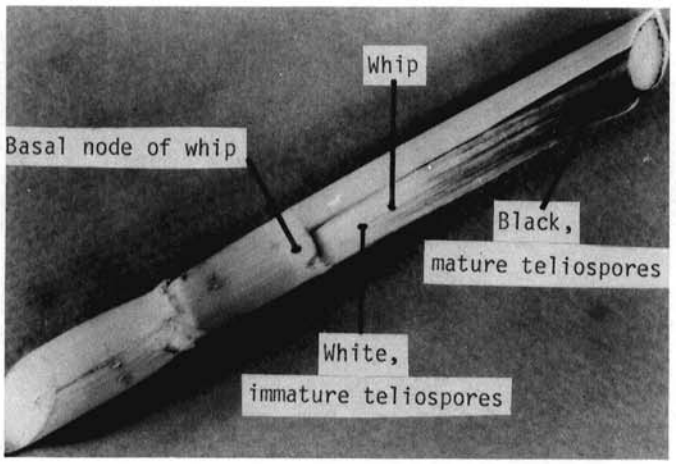

Fig. 1. *Ustilago scitaminea* in sugarcane. Detached basal section of whiplike sorus-bearing structure with the outer sugarcane leaves removed, showing the black teliospores in the peripheral layer. × 0.5.

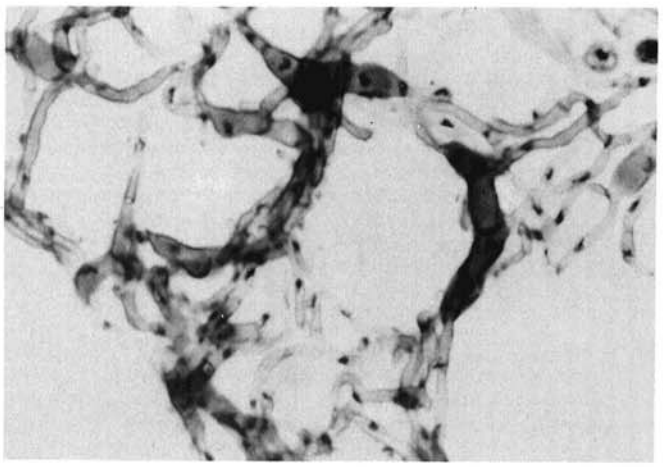

Fig. 2. Somatic, mononucleate, vegetative hyphal cells of *Ustilago scitaminea,* 1 hr after field harvest. Note aggregated and swollen cells. × 1000.

RESULTS

Vegetative hyphae in sori on the periphery of the whip tissues are mononucleate and less contorted and longer than the vegetative hyphae of *U. scitaminea* in other parts of the sugarcane plant. The hyphae within the sori were irregular in shape and length and were about 2 μm in diameter (Fig. 2). These somatic hyphae tended to develop into clumps or strands of cells before spore formation. Many of these hyphal cells swelled to 8 μm in diameter and were then easily dislodged from adjacent cells (Fig. 3).

The walls of the swollen, segmented, sporogenous cells were hydrolyzed and the resulting nuclei appeared to be suspended in a matrix composed of a mixture of host and fungal elements (Fig. 4). The organelles for cellular syntheses and function remained associated with the fungal nuclei, however, and the nuclei were soon surrounded by a clearly defined cytoplasm, and the young spores appeared as distinct protoplasts. These spore initials were not connected directly to hyphal strands, but were held in a viscous matrix surrounded by the parent somatic hyphae. As the spores

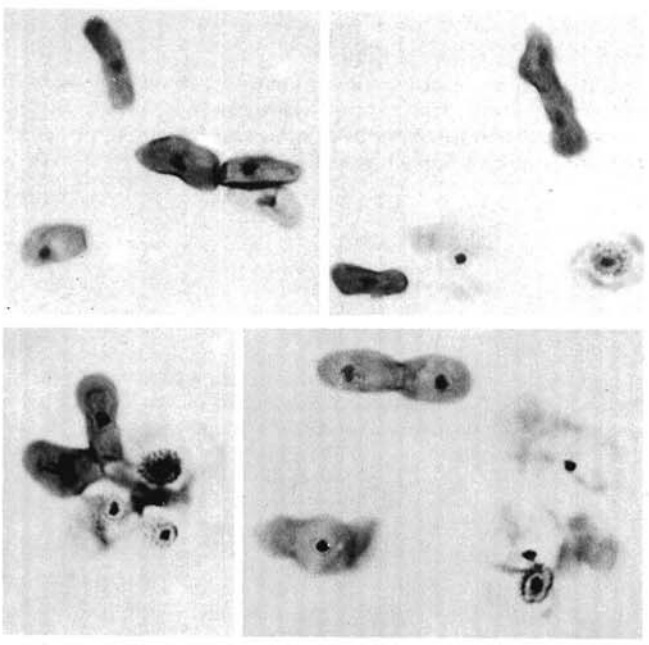

Fig. 3. Irregular, enlarged, mononucleate, sporogenous cells of *Ustilago scitaminea,* various stages of cell wall autolysis and several stages of teliospore formation. × 1000.

matured, their outer walls formed, thickened, and became pigmented and a clear capsule formed around each spore (Fig. 5). The gelatinous matrix that held the spore mass together did not dry out and release the spores until the outer peridium of the whip had dried and fragmented.

At the base of the whip, host cells continued to grow, increasing the diameter and length of the whip. Vegetative, mononucleate hyphae were sparse in the central pith tissues of the whip, and their growth appeared to be slow, in contrast to the vigorous hyphal developmental that occurred in the peripheral region of the whip. Nonsporogenous hyphae continued to grow and extend the infection into newly developed areas of the peripheral whip tissue. Irregular sites of abundant spore formation were found in the peripheral 1-mm of whip tissue. These pockets of sporulation enlarged and coalesced during the development so that eventually the entire surface of the whip was covered with sporulating loci. The most mature spores were in the centers of the sporulation pockets. The youngest nonpigmented teliospores formed at the base of the whip; the older brownish-black, mature spores were higher on the whip. Teliospores collected in the black area, 20 cm above the base of the whip, usually germinated in about 6 hr, whereas spores found in the white basal whip tissue were immature and did not germinate. There was a gradation in the degree of maturity between those zones (Fig. 1).

Smut-infected tissue was dissected from the basal pith tissue of freshly cut whips. The samples contained only host cells and vegetative hyphal cells. These samples were placed on the agar medium described in Materials and Methods. Mature teliospores were formed in these axenic cultures grown at 30 C (12 hr) and 25 C (12 hr). The formation of teliospores in pure culture differed from the development of teliospores in the sori on the sugarcane whip tissue. In vivo, the lysed sporogenous cells, the unlysed vegetative hyphae, and the peripheral cells of the whip tissue probably all contributed components to the developing teliospores in the sori, for the teliospore initials had no direct hyphal attachments (Fig. 4); ie, spore initials in the sori were suspended in a mixed cytoplasmic milieu. In contrast, teliospores formed in axenic culture were attached terminally to mononucleate, bulbous, beadlike hyphal cells. During the formation of teliospores in culture there was no apparent hydrolysis of hyphal walls, and the developing teliospores were formed on mycelial tissue 2–5 mm above the agar surface. Hence, metabolites required for spore development were transported through the connecting hyphae. Also, the teliospores formed in pure culture were not surrounded by a gelatinous matrix that held them together. Teliospores formed in culture germinated normally and sporidia were produced.

DISCUSSION

The pathogen appeared to grow slowly in all tissues other than the whip, but in the surface layers of the whip the vegetative hyphae were physiologically and cytologically transformed into a reproductive phase of the fungus that yielded tremendous numbers of teliospores. *Ustilago scitaminea* has one of the highest sporulation capacities ever recorded (11), as one whip was estimated to have produced 10^{11} spores (14). Surrounding the sori on the whip was a fragile peridium, derived from epidermal host tissue, which dried and ruptured as the sorus matured.

Ustilago spp. can form teliospores by several different procedures. The general features of the differentiation of teliospores are similar; ie, vegetative hyphae dissociate into individual or small clumps of sporogenous hyphae, which then develop into teliospores. There are species differences, however, within this general plan of development. As indicated in Table 1, a variety of nuclear patterns have been reported for the cells of *Ustilago* spp. undergoing reproductive changes. In addition to these patterns of teliospore development in specific host tissues, 12 *Ustilago* spp. have been reported to form teliospores in pure culture (6). The in vitro development of teliospores appears to be much less complicated than the in vivo pattern of differentiation, at least in *U. scitaminea.*

Caten and Day (2) stated that naturally occurring somatic diploids are rare in fungal plant pathogens. In several *Ustilago* spp., including *U. scitaminea,* the somatic hyphal cells that give rise to the sporogenous hyphae are mononucleate and presumably diploid, for they give rise to diploid teliospores. The morphology and cytology of the vegetative phases of this polymorphic fungus, *U. scitaminea,* in the host and in vitro culture will be discussed in a separate paper.

Host factors must strongly influence the transition from vegetative to reproductive growth of many *Ustilago* spp. Although many of these pathogens grow systemically throughout their host plants, sporulation occurs only in specific tissues, often the

TABLE 1. Nuclear configurations reported in three cell types involved in teliospore development in several *Ustilago* spp.

	Nuclear configuration in:			
Ustilago species	Vegetative hyphae[a]	Sporogenous hyphae[a]	Teliospores	References
U. striiformis	diploid	diploid	diploid	9
U. maydis	binucleate	diploid	diploid	4
U. crameri	binucleate	binucleate	diploid	15
Six *Ustilago* spp.	binucleate	binucleate	diploid	8
U. heufleri	multinucleate	binucleate	diploid	12
U. scitaminea	diploid	diploid	diploid	[b]

[a] In host tissue.
[b] This manuscript.

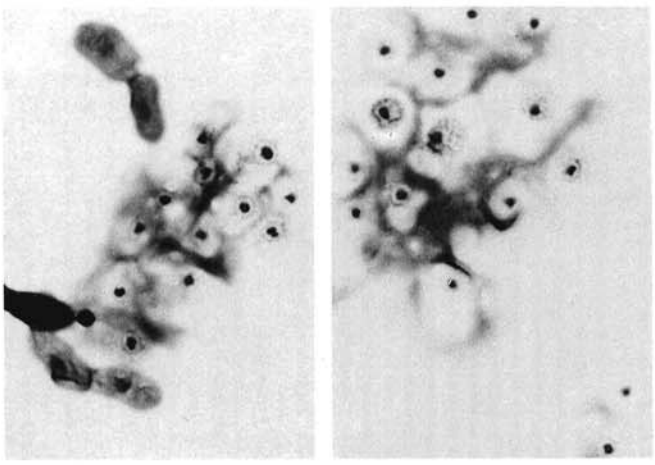

Fig. 4. Early stages of development of mononucleate teliospores of *Ustilago scitaminea* in a viscous matrix. × 1000.

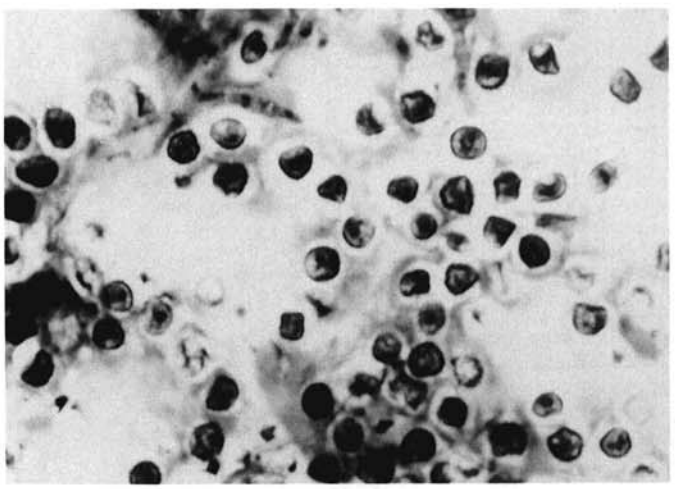

Fig. 5. Late stages of teliospore development in *Ustilago scitaminea,* showing a thick, pigmented, outer wall and a clear zone around each spore. × 1000.

inflorescence. This localization of sporulation suggests that substances from specific tissues may be required for reproductive development of these smut fungi. During the vegetative growth of these fungi in the host, often there is no outwardly visible sign of the disease. However, as reproductive development of the pathogen begins, black spores of the smut pathogen are produced and the morphology of the host often is drastically altered; eg, by the formation of sugarcane whips, corn smut galls, dwarfing of culms, excessive tillering, and smut-induced phyllody, hermaphroditism, heterostyly, and dehiscence (6). Thus, not only do the hosts influence the reproductive development of the *Ustilago* spp. pathogens, but the pathogens often influence the reproduction of the hosts. Bioactive substances may be involved in these morphological developments.

The hydrolytic enzymes that degrade the cell walls of the sporogenous hyphae apparently were present in the peripheral tissue at the base of the whips. These enzymes must be very specific, for they do not hydrolyze the vegetative hyphae or the peridium. These hydrolytic enzymes probably include glucanases, chitinases, and proteinases that may be useful in studying fungal cell-wall biochemistry.

LITERATURE CITED

1. BOCK, K. R. 1964. Studies on sugar-cane smut (*Ustilago scitaminea*) in Kenya. Trans. Br. Mycol. Soc. 47:403-417.
2. CATEN, C. E. and A. W. DAY. 1977. Diploidy in plant pathogenic fungi. Annu. Rev. Phytopathol. 15:295-318.
3. CHRISTENSEN, J. J. 1963. Corn smut caused by *Ustilago maydis*. Phytopathological Monograph No. 2, The American Phytopathological Society, St. Paul, MN. 41 pp.
4. EHRLICH, H. G. 1958. Nuclear behavior in mycelium of a solopathogenic line and in a cross of two haploid lines of *Ustilago maydis* (DC.) Cda. Mycologia 50:622-627.
5. FERNANDEZ, J. A., and R. DURAN. 1975. Sporulation by *Ustilago enneapogonis* in axenic culture. Mycologia 67:1229-1233.
6. FISCHER, G. W., and C. S. HOLTON. 1957. Biology and control of the smut fungi. Ronald Press, New York. 622 pp.
7. HOLTON, C. S., J. A. HOFFMAN, and R. DURAN. 1968. Variation in the smut fungi. Annu. Rev. Phytopathol. 6:213-242.
8. LANGDON, R. F. N., and R. A. FULLTERON. 1975. Sorus ontogeny and sporogenesis in some smut fungi. Aust. J. Bot. 2:915-930.
9. LEACH, J. C., and M. A. RYAN. 1946. The cytology of *Ustilago striiformis* forma *Poae pratensis* in artificial culture. Phytopathology 36:876-886.
10. LEE-LOVICK, G. 1978. Smut of sugarcane—*Ustilago scitaminea*. Rev. Plant Pathol. 57:181-188.
11. ROBINSON, R. A. 1976. Plant Pathosystems. Springer-Verlag, Berlin, Heidelberg, New York. 184 pp.
12. SARTORIS, G. B. 1924. Studies in the life-history and physiology of certain smuts. Am. J. Bot. 11:617-647.
13. TRIONE, E. J. 1964. Isolation and in vitro culture of the wheat bunt fungi, *Tilletia caries* and *T. controversa*. Phytopathology 54:592-596.
14. WALLER, J. M. 1969. Sugarcane smut (*Ustilago scitaminea*) in Kenya. I. Epidemiology. Trans. Br. Mycol. Soc. 52:139-151.
15. WANG, C. S. 1943. Studies on cytology of *Ustilago crameri*. Phytopathology 33:1122-1133.
16. WANG, D. T. 1934. Contributation à l'étude des Ustilaginées (Cytologie du parasite et pathologie de la cellule Hôte). Botaniste 26:540-670.