Development and Nuclear History of the Teliospores of Urocystis colchici

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ABSTRACT

Development and nuclear history of the teliospores of *Urocytis colchici* were studied in infected onion seedlings by employing light- and electron microscope techniques. Teliospores developed from terminal cells of sporogenous hyphal branches which curved back upon themselves, and surrounding hyphal cells became appendage cells of the teliospores. Both appendage cells and central spores of young teliospores were dikaryotic initially, but central spore nuclei underwent karyogamy and became diploid before maturity. Appendage cells, initially binucleate, appeared to become mononucleate by disintegration of one of the nuclei. Nucleoli were evident in both haploid and diploid nuclei. Central spore walls differentiated into three distinct layers. Appendage cells contained organelles similar to those found in central spores, but contained fewer lipid bodies. Appendage cell walls had only two distinguishable layers, and were attached to the central spore wall by an amorphous matrix. Only appendage cells germinated when freshly collected teliospores were plated on artificial media.

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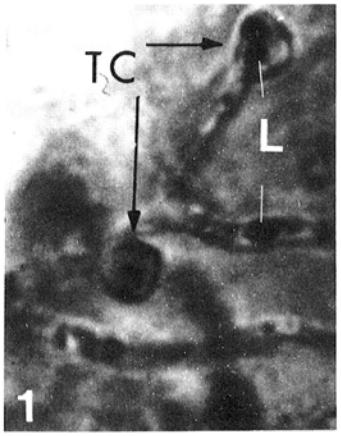
Additional key words: Ultrastructure of fungi, onion smut, fungal spore morphogenesis.

Infection of onion seedlings by the onion smut fungus, *Urocystis colchici* (Schlecht.) Rabenh. (= *U. cepulae* Frost) occurs by direct penetration of the cotyledons before emergence from the soil (1,4). The mycelium grows

Fig. 1-7. Development of *Urocystis colchici* teliospores. **1)** Terminal cells arising from sporgenous branches (× 4,100). TC = terminal cell; L = lipid body. Osmium stained. **2)** Terminal cells of sporogenous branches in the "hooked-over" configuration with the earliest stage represented by "a" and later stages "b" and "c" (× 4,100). Osmium stained. **3)** The binucleate central spore which developed from the terminal cell (× 23,100). N = nucleus; M = mitochondria; SW = spore wall; MX = matrix material. **4)** The central spore lying in the center of a coil composed of sporogenous branch cells which became the appendage cell primordia (× 4,100). CS = central spore; ACP = appendage cell primordia. Osmium stained. **5)** Appendage cell primordia (ACP) surrounding the central spore (CS) walls (× 9,000). **6)** Binucleate young spore showing nuclei (N) (× 7,600). Feulgen stained. **7)** Nuclei (N) with their nucleoli (NU) in a pre-fusion position with lipids (L) in the background (× 6,000). Hematoxylin stained.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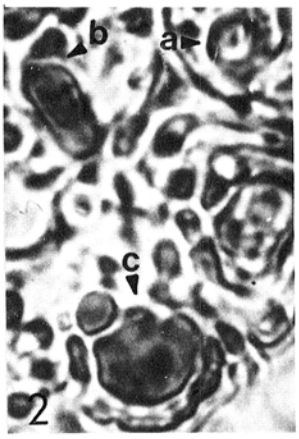
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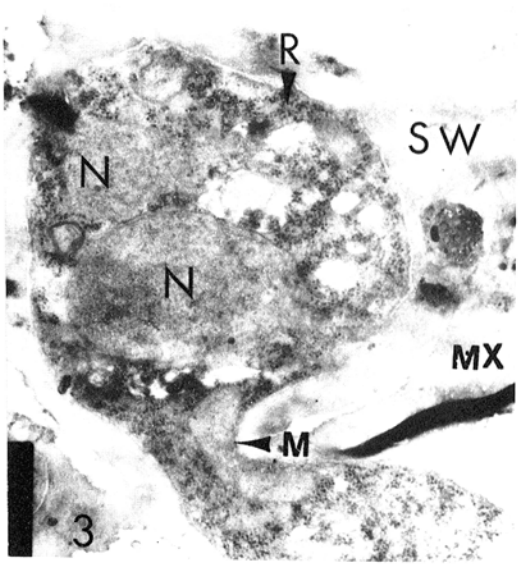
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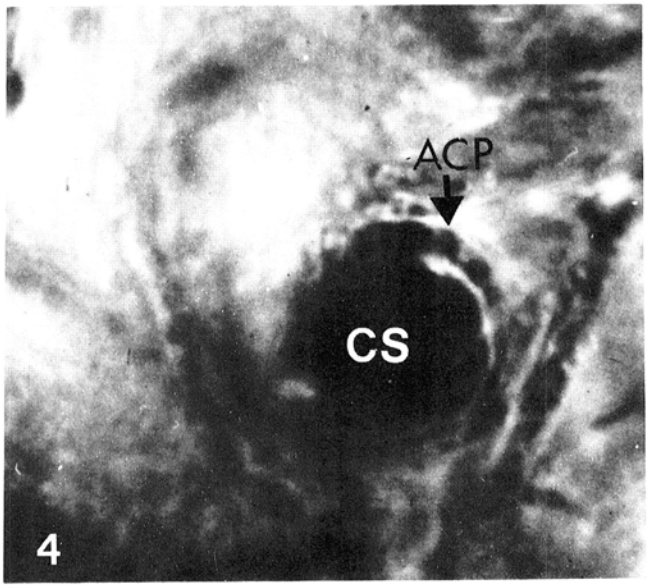
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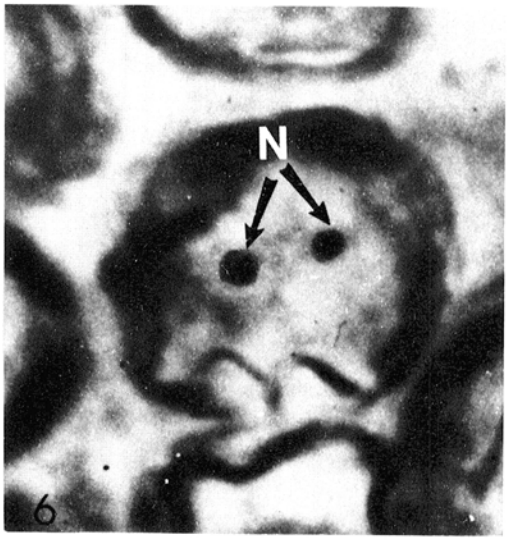
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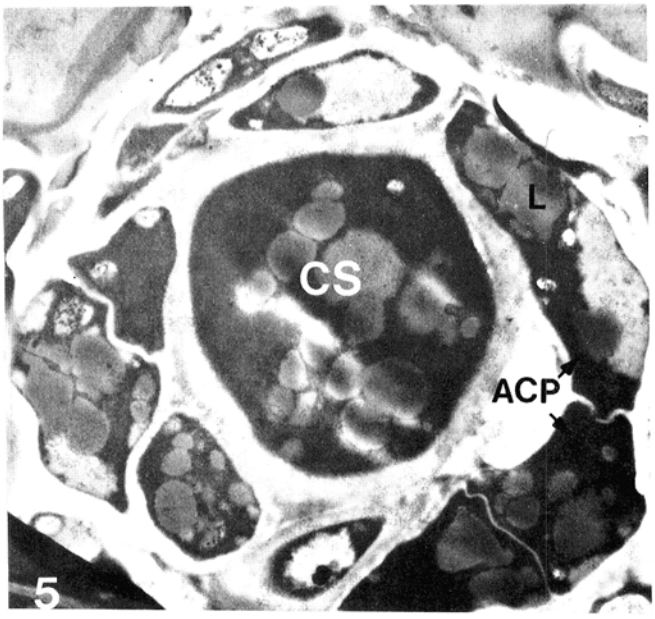
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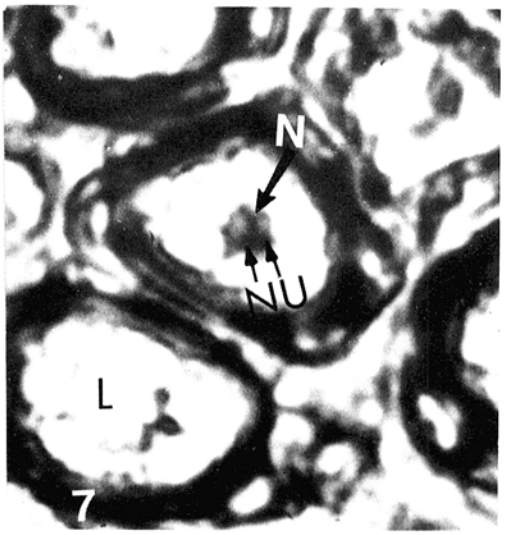
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intercellularly without formation of haustoria, and becomes binucleate prior to sporogenesis (2). The mature teliospore is composed of a single, dark-pigmented, thick-walled, central spore to which is attached a variable number of thinner-walled appendage cells that have also been called nurse cells, sterile cells, accessory cells, or pseudospores (1,2,12,13).

A few previous cytological investigations of *U. colchici* were accomplished with the light microscope. The limitations of light microscopy and the size, morphology, and pigmentation of the spores led to some confusion among earlier investigators regarding the cytology of this fungus. Opinions concerning sporogenesis, nucleus size, condition of the spore nucleus, and spore germination in culture differed (1,2,12,13).

The objectives of this investigation were: (i) to study the histology of the teliospores of *U. colchici* from sporogenesis through germination; (ii) to study the nuclear history of the spore; and (iii) to establish the genesis of the appendage cells and to discover, if possible, their function.

MATERIALS AND METHODS.—Onion seeds were sown in flats of steamed soil infested with field-collected teliospores. Sections from 12- to 16-day-old infected seedlings were cut in a drop of cold 0.2 M sodium cacodylate buffer (pH 7.2). Sections containing sori were transferred to shell vials containing modified chromic acid-acetic acid-formalin (CRAF) solution (6), 3% glutaraldehyde - 3% acrolein (5), or formalin-acetic acid-alcohol (FAA) (7) as fixatives. Modified CRAF was prepared according to Jensen (6) except that the formalin was replaced by an equivalent amount of glutaraldehyde. The tissues prepared for examination by light microscopy remained in the fixative for 4 to 16 hours under vacuum. Following a 2-hour rinse in water, the tissues were dehydrated in the same vials using an ethanol-tertiary butyl alcohol series with eventual embedding in Bioloid wax. Serial sections 4- to-6-μm thick were affixed to slides with Haupt's adhesive (7) and stained with Mayer's hematoxylin (7) or Feulgen stain (3). Deoxyribonuclease and ribonuclease tests were used to verify that the materials stained were nuclear (6). The presence of lipid-containing bodies in the mycelia and spores were confirmed by reaction with osmium tetroxide, and by the Sudan test (6).

Photomicrographs were made using Kodak Ektachrome film in a Nikon Microflex microscope fitted with an AFM photomicrographic attachment, or in a Zeiss microscope equipped with phase-contrast and Nomarski interference-contrast optics.

Sections 2 mm in diameter were prepared for electron microscopy by fixing fresh tissue in 3% glutaraldehyde - 3% acrolein in 0.1 M sodium cacodylate buffer (pH 7.2) for 1 hour, or in cacodylate-buffered 3% $KMnO_4$ for 15 minutes (5). The glutaraldehyde-acrolein-fixed tissues were transferred through several changes of the same buffer followed by post-fixation in 1% osmium tetroxide in cacodylate buffer for 1 hour. Both sets of tissues were then washed in buffer, dehydrated in an ethanol and propylene oxide series, and embedded in ERL epoxy resin (10). Sections were cut with a diamond knife, poststained in a saturated ethanol solution of uranyl acetate for 30 minutes, washed in water, and stained for 7 minutes in a saturated lead citrate solution. Observations and photographs were made with a Philips 300 electron microscope.

Teliospores for germination studies were collected according to the method of Lacy (9) and plated on agar for germination. Spores were removed from agar plates after 4 days, fixed in glutaraldehyde-acrolein, post-fixed in osmium tetroxide, and treated as above.

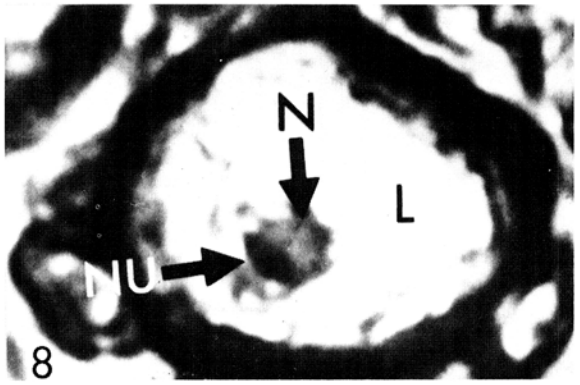
RESULTS.—*Sporogenesis.*—Numerous longitudinal and transverse sections of young developing sori in onion cotyledons were observed by light- and electron microscopy. *U. colchici* did not produce haustoria or invade vascular bundles of the onion cotyledon. Various stages of sorus and spore development were observed and mature spores were clearly evident in sori in the more advanced states. The host mesophyll cells were pushed apart as the sori developed.

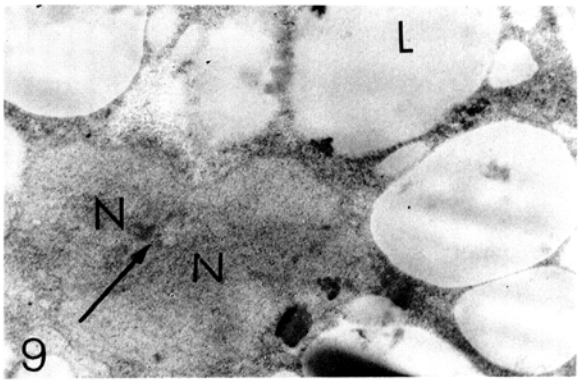
During sporogenesis, lateral branches arose from the mycelia which grew parallel with the vascular tissues of the host. As the lateral branches continued growth, terminal cells enlarged into bulbous-shaped spore initials 3.4 $\pm$ 0.5 μm in diameter (Fig. 1). The sporogenous branches curved inwardly upon themselves so that subterminal cells were wrapped around the enlarged spore initials. This hooking over of the terminal branches was called a crozier by Anderson (1). Light microscopy sections showed spiraling growth of the sporogenous branches, and a rapid increase in the size of terminal cells and numbers of lipid bodies (Fig. 2). The binucleate terminal cells also contained nuclei slightly larger than 1.0 μm, mitochondria, rough endoplasmic reticulum, and a few vacuoles. Spore initials (terminal cells) developed into binucleate central spores whose walls appeared to be covered with a gelatinous matrix (Fig. 3). Curving of sporogenous branches resulted in coils with central spores lying in the center surrounded by the other hyphal cells of sporogenous branches. The outer hyphal cells became

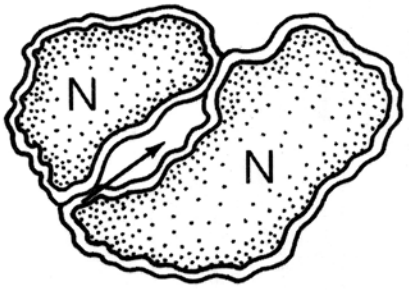
Fig. 8-14. Histology of *Urocystis colchici* teliospores. **8)** Nuclei (N) containing nucleoli (NU) tightly appressed along their margins prior to fusion (karyogamy). (× 8,300). Hematoxylin stained. **9)** Pre-karyogamy in the juvenile spore indicated by the position of the nuclei (N) which are surrounded by lipid bodies (L). The cytoplasm (arrow) will be squeezed out upon completion of fusion (× 19,100). Figure **9a** is a diagrammatic representation of Fig. 9. **10)** The diploid nucleus (N) of the central spore. Lipid bodies (L) have encroached around the nucleus. The bi- and mononucleate condition of the appendage cells (ACN-arrows) were evident at this stage of development (×5,000). Hematoxylin stained. **11)** Diploid nucleus (N) of a central spore with a nucleolus (NU) present (× 10,500). L = lipid body; M = mitochondria; ACN = appendage cell nucleus. **12)** $KMnO_4$ - fixed material indicates that lipid bodies (L) are bounded by a half unit membrane (× 9,000). N = nucleus; ACN = appendage cell nucleus. **13)** Globular lipid bodies (L) which filled most of the cytoplasmic area of mature spores (× 2,200). Sudan stained. **14)** Mature teliospore walls with the central spore wall composed of the inner layer (il), middle layer (ml), and outer layer (ol). The appendage spore walls were composed of an outer layer (a) and an inner layer (b), and were cemented to the central spore by the matrix (mx). (× 17,500).

appendage-cell primordia (Fig. 4, 5) which adhered to central spores as they expanded.

Two haploid central spore nuclei, each measuring about 1.25 μm in diameter, were initially present in the







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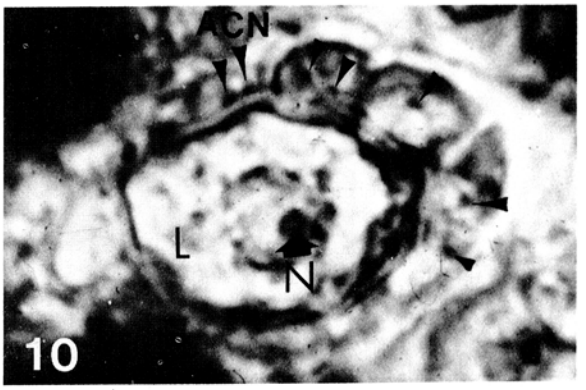

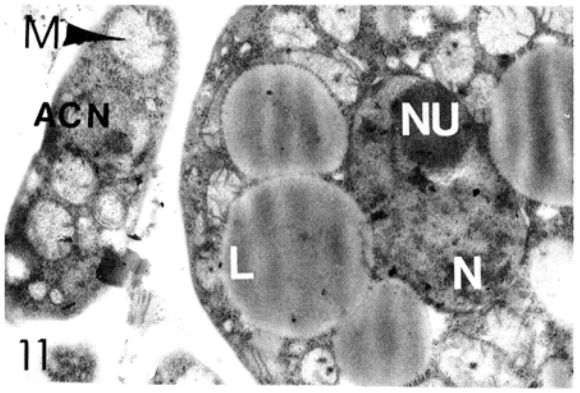

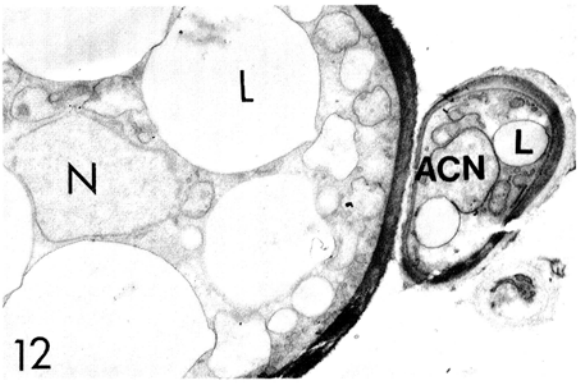

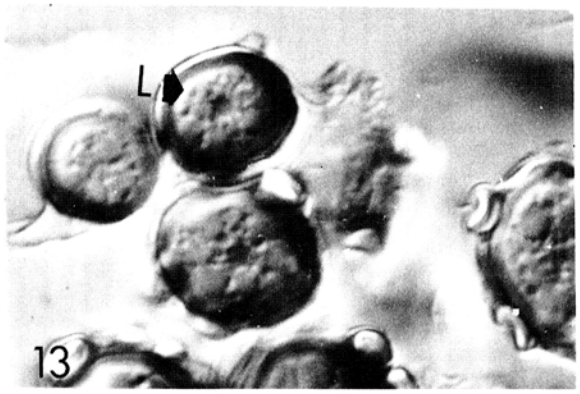

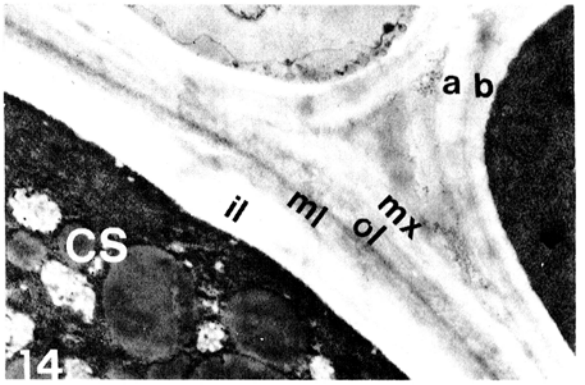

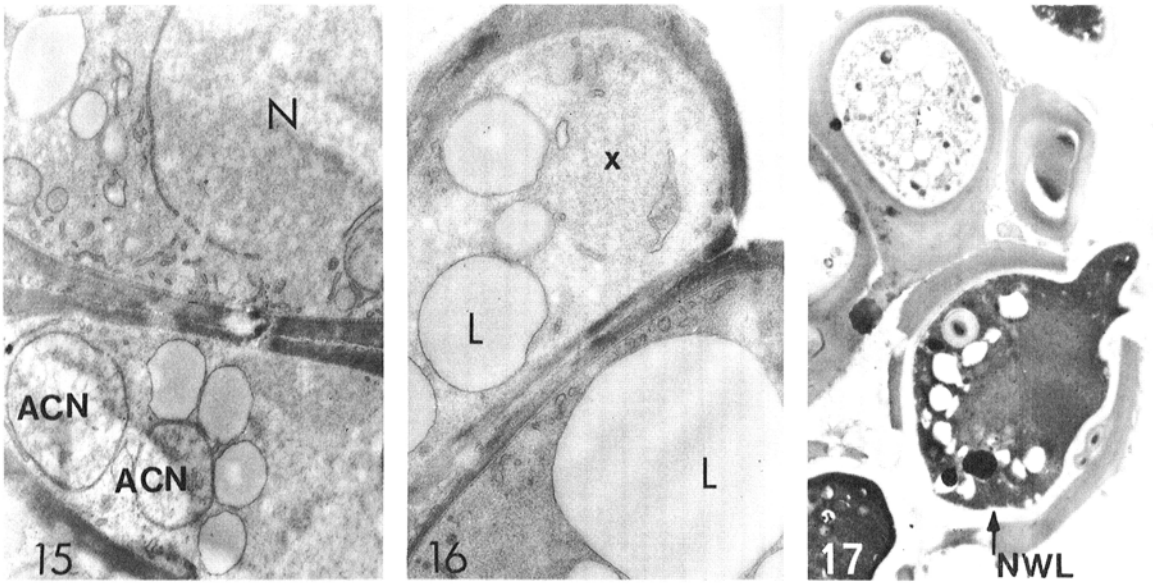

Fig. 15-17. Histology of *Urocystis colchici* teliospores. **15)** Binucleate appendage cell fixed in $KMnO_4$ (× 16,000). ACN = appendage cell nucleus; N = nucleus. **16)** Area (x) which may represent a disintegrating nucleus in the appendage cell (× 22,400). L = lipid bodies. **17)** (Lower) A germinated appendage cell located near the agar surface which has synthesized a new cell wall layer (NWL). (Upper) Some appendage cell cytoplasms appeared highly disorganized suggesting cell degeneration (× 7,700).

cytoplasm of young spores (Fig. 6). In slightly older spores, nuclei eventually contacted each other in the center of the spores (Fig. 7), became appressed to each other (Fig. 8, 9, 9a), and fused together (Fig. 10, 11). After karyogamy in the juvenile spore, the diploid nuclei, measuring about 2.0 μm in diameter, remained centrally located. Lipid bodies increased in size and numbers (Fig. 10, 11), and filled the free cytoplasmic area. These lipid bodies (Fig. 1, 7, 9, 12) appeared to be bound by what may be a half-unit membrane (8,15) (Fig. 9, 11, 12, 16). Thus electron microscopy confirmed observations made with light microscopy that much of the cytoplasmic area was filled with globular lipid bodies (Fig. 13) which appear white in the preparations treated with nuclear stains (Fig. 6, 7, 8, 10).

Spore wall layers >0.3 μm thick became evident during the juvenile stage. When spores reached maturity, walls of central spores were composed of three distinct layers covered with a matrix to which the appendage cells were attached (Fig. 14).

As appendage cells matured, they became rounded at the end, flat on the side attached to the central spore, and torulose. This resulted in oval-shaped appendage cells which were nearly hyaline, 3.0 − 4.0 μm in length and 1.5 − 3.0 μm in width, and contained one or more small lipid bodies (Fig. 5, 11, 12, 16). Mature appendage cell walls were composed of two layers. There was no evidence of cytoplasmic connections through the wall layers of central spores and appendage cells (Fig. 14).

Numerous observations of serial sections of appendage cells by light and electron microscopy indicated that these cells had either one or two nuclei (Fig. 10, 11, 12, 15). In glutaraldehyde-acrolein fixed material, younger appendage cells initially had two nuclei, but usually only one at maturity. Due to electron-dense cytoplasm, however, it was difficult to determine the fate of the second nucleus. Therefore material fixed in $KMnO_4$ was examined for indications of variation in number of nuclei. In many sections areas were found that suggested disintegrating nuclei (Fig. 16). These areas were the same diameter (1.0 μm) as appendage cell nuclei.

Organelles indicated to be nuclei by the Feulgen reaction became Feulgen-negative after treatment with deoxyribonuclease but not after treatment with ribonuclease.

Germination.—It was impossible to determine with the light microscope if germ tubes arose from central spores or appendage cells of teliospores. Teliospores originally 17 μm in diameter increased to 22.5 μm prior to germ tube emergence. A swollen hyaline appendage cell was always proximal to the germ tube. This swollen cell probably represented what Anderson described as a vesicle (1). As observed by Tachibana (11), detached appendage cells germinated. Sporidia were not produced by *U. colchici* in culture.

Electron microscopy of serial sections of teliospores germinated on agar media revealed that the central diploid spores failed to germinate, but that one or (rarely) two appendage cells located near the agar surface germinated. Some other appendage cells in the same vicinity appeared to be cytologically capable of germination, as indicated by formation of a new wall layer within the existing cell wall, an electron-dense cytoplasm, and lipids (Fig. 17). Most other appendage cells had highly disorganized cytoplasms which suggested cell degeneration. Repeated attempts to locate a nucleus within the central spore associated with a germinated appendage cell failed.

DISCUSSION.—Although this study supported some of the conclusions of Anderson (1), there was no evidence

for speculations of other investigators that central spores and appendage cells originated from different sporogenous hyphae (13,14), or that a sporogenous hyphal cell in a "favorable" position became the central spore, while the contents of appendage cells served as "food" for the central spore (2). Appendage cells were cytologically similar to central spores, except that appendage cells had smaller amounts of stored lipids, a two-layered spore wall, and a variable number of haploid nuclei. The cytological evidence indicated that nuclear disintegration of one of the two haploid nuclei could account for the variation in the nuclear condition of appendage cells, and also explain the differences between the reports of Anderson (1) and Blizzard (2) on nuclear number.

Previous investigators of spore germination (1,2,11,12,13,14) proposed that the germ tube originated from the central spore between appendage cells. Only appendage cells germinated in culture under the conditions described in this study. A single teliospore has 20 to 40 appendage cells and only one, or rarely two, of these germinated. These cells were always located near the agar surface and contained one nucleus at maturity. At no time was there any cytological evidence that karyogamy or meiosis occurred in appendage cells or germ tubes. The majority of appendage cells on a germinating teliospore appeared to be degenerating as indicated by their disorganized cytoplasm. In another study (12), 62% of appendage cells detached from central spores produced cultures. Therefore, the position of the appendage cell on the teliospore relative to the nutrient source and oxygen supply may play a role in determining which cells germinate on intact teliospores.

Tachibana and Duran (12) concluded that *U. colchici* was heterothallic, and that teliospores contained only one of two possible mating types (+ and −). They assumed that meiosis occurred in the central spore with subsequent disintegration of three of the resulting haploid nuclei, leaving one haploid nucleus of one mating type. They overlooked the possibility that appendage cells could germinate and provide mycelia of one or the other mating types.

Experiments on the cytology of overwintered teliospores germinating on onion seedlings may reveal the roles that appendage cells and central spores play in infection in nature.

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