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Cytology of Teliospore Germination and Basidiospore Formation in *Uromyces appendiculatus* var. *appendiculatus*[1]

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Summary

The cytology of teliospore germination and basidiospore formation in *Uromyces appendiculatus* var. *appendiculatus* was characterized with light and fluorescence microscopy. Meiosis of the diploid nucleus occurred in the metabasidium. The four haploid daughter nuclei migrated into the basidiospore initials where they underwent a post meiotic mitosis. Each basidiospore was delimited from the meatabasidium by a septum at the apex of the sterigma. Seventy-five percent of mature basidiospores were binucleate, 24.5% uninucleate, and 0.5% trinucleate. Mature, released basidiospores measured $\sim 16 \times 9$ µm, were smooth-surfaced, and reniform to ovate-elliptical in shape.

Keywords: Basidiospore; Cytology; Meiosis; Teliospore germination; *Uromyces appendiculatus*.

1. Introduction

The earliest illustrated descriptions of teliospore germination and basidiospore formation in the rusts date back to the classic studies of DE BARY (1863) and TULASNE (1854). Subsequently many studies have been conducted on the sequence of nuclear division in the basidium and post meiotic development of basidiospores (*e.g.*, ALLEN 1933, PAVGI 1975, ANIKSTER *et al.* 1980). These studies have revealed the extremely variable pattern of meiotic development in the rusts.

The purpose of the present study was to provide, for the first time in *Uromyces appendiculatus* var. *appendiculatus*, qualitative and quantitative cytological information on the processes of teliospore germination and basidiospore formation.

2. Materials and Methods

The isolate of *Uromyces appendiculatus* (Pers.) Unger var. *appendiculatus*[2] used in this study was collected in the field as urediniospores from infected leaves of *Phaseolus vulgaris* L. The collection was made in the Black Forest (Lahr Valley) in August, 1978. Teliospores were produced, stored, and activated to germinate according to methods described earlier (GOLD 1983, GOLD and MENDGEN 1983).

For the cytological observations teliospores were evenly brushed onto a hydrophilic cellulose nitrate filter (Sartorius, 8 µm pores) on purified 2% glass-distilled water agar (Merck) in plastic Petri dishes and incubated at 18 °C under 1000 lux (16 hours daily photoperiod).

Germinating teliospores at various stages of development were carefully scraped from the filter paper surface and suspended in a single drop of 0.05% Tween 20 in distilled water on a clean glass slide. A cover slip was gently set in place and the living spores were examined and photographed with Nomarski differential interference contrast optics with a Zeiss Planachromate 40/0.65 or 100/1.25 objective. The measurements of teliospores, metabasidia, and basidiospores were made from calibrated light microscopic negatives. Fresh, unfixed spores were photographed in the focal plane that gave

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[2] Synonyms: *Uromyces phaseoli* (Pers.) Wint. and *Uromyces phaseoli* (Pers.) Wint. var. *typica* Arth. See BOEREMA and VERHOEVEN (1979) and CUMMINS (1978) for an accurate nomenclatural account for these and related species.

maximum breadth. One hundred of each spore type and 30 metabasidia were measured.

To study nuclear movement and behaviour during and after meiosis, germinating teliospores were fixed in 2% glutaraldehyde in 0.05 M sodium phosphate, pH 7,2 for 1 hour at 20 °C and then stained with the fluorochrome DAPI (4,6-diamidino-2-phenylindol ~ 2 HCl, Serva) as described by HOOLEY *et al.* (1982). Following fixation the spores were washed in buffer, suspended in the staining solution (= 5 µg/ml DAPI made up in buffer) and observed immediately with a Standard Zeiss microscope equipped with interference contrast and incident light fluorescence. Observations were made using a 365 nm excitation filter and a 420 nm barrier filter. All micrographs were taken with a 35 mm camera on Agfaortho 25 Professional or Ilford Pan F and developed in Emofin (Tetenal-Photowerk).

3. Results

During rehydration on agar the teliospores swelled slightly to their normal turgid dimensions, but thereafter no further swelling occurred. The average size of turgid, mature teliospores was 31 × 24 µm, ranging 27.1–35.5 µm long and 21.6–26.8 µm wide. Fusion of the dikaryotic nuclei occurred immediately after the formation of teliospores in the telium and the diploid nucleus was characterized by its large volume, central position in the spore and its single nucleolus. Mature, dikaryotic teliospores were very rarely observed. Following rehydration the first visible sign of germination was the accumulation of cytoplasmic vacuoles (Fig. 1). Parallel to the time of maximum vacuolation (~ 4 days after rehydration), teliospores germinated apically to produce a metabasidium (Fig. 2). After a portion of the cytoplasm had passed into the expanding metabasidium, the diploid nucleus followed, becoming elongated as it moved through the germ pore region. The nucleus occupied a central position in the metabasidium and underwent the first meiotic division (Figs. 3, 15, and 16). Thereafter, 2 septa were laid down in the metabasidium- one at the middle point (Fig. 4) which separated the 2 daughter nuclei and one at the base (Fig. 5). The second division followed immediately giving rise to 4 haploid daughter nuclei (Figs. 5 and 17) separated into 4 cells by 3 septa (Fig. 7). A fifth cell at the base of metabasidium contained little cytoplasm and remained anucleate (Figs. 5 and 8). The metabasidium attained an average length of ~ 80 µm; however, extremely long metabasidia up to 280 µm were also occassionally observed.

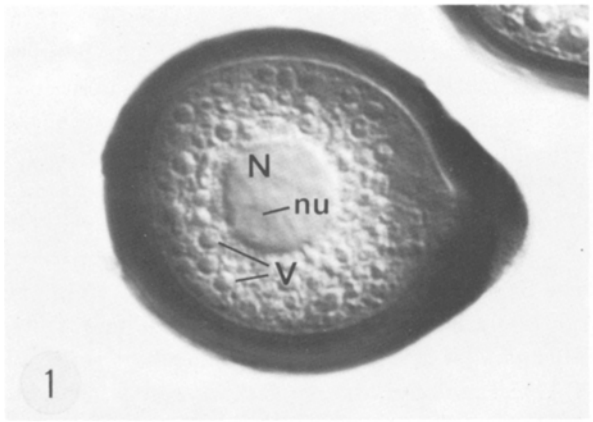
Each cell of the metabasidium gave rise to a sterigma which initially appeared as a small bump-like protuberance of the cell wall (Fig. 6). These bumps then elongated narrowly to form the characteristic conical sterigmata with bluntly rounded tips (Figs. 7 and 8). The apex of the sterigma developed a spherical swelling which enlarged to produce a single basidiospore (Fig. 8). During development of sterigmata and basidiospores the metabasidial cells slowly began to vacuolate. The nucleus and most of the protoplasm in the metabasidial cell migrated into the expanding basidiospore initial (Figs. 9 and 18). Frequently, a portion of the protoplasm remained behind and later degenerated in the sterigma or in the vacuolated cell there below (Figs. 10 and 12). Soon after its entrance into the basidiospore the nucleus typically divided mitotically-producing a binucleate, mature basidiospore (Figs. 11 and 19). The basidiospore was separated from the sterigma by a septum which also simultaneously formed the apiculus (Fig. 11). Based on fluorescence microscopic observations of 420 mature basidiospores it was found that 24.5% were uninucleate, 75.0% binucleate and 0.5% trinucleate.

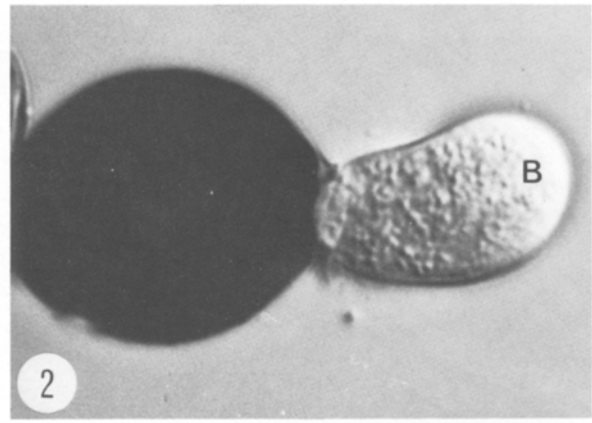
Mature basidiospores were reniform to ovate-elliptical, smooth-surfaced, hyaline, and each possessed a prominent apiculus (Figs. 10 and 11). They measured ~ 16 × 9 µm, ranging 10.7–20.7 µm long and 5.8–11.4 µm wide. The development of sterigmata and basidiospores commonly proceded in a basipetal succession, however, the dispersal of basidiospores sometimes followed a less orderly pattern.

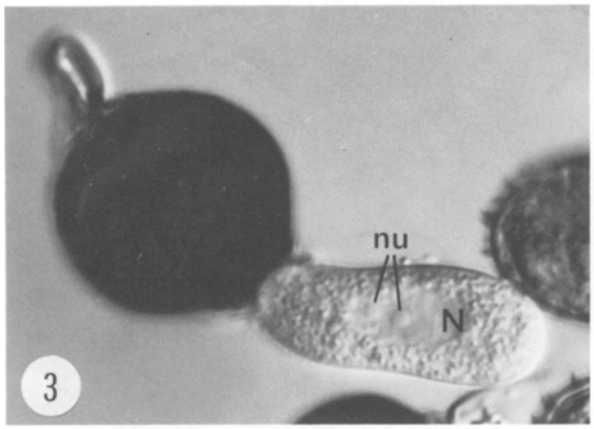
Occasionally, abnormal teliospore germination was observed. The most common cytological aberrations were (1) direct teliospore germination from the spore itself or from a single metabasidial cell (Fig. 13), (2) sudden death of a portion or the whole metabasidium, (3) basidiospore germination *in situ*, and secondary basidiospore production (Fig. 14).

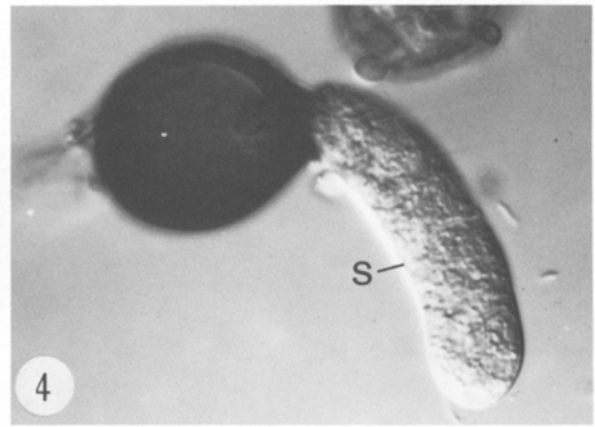
4. Discussion

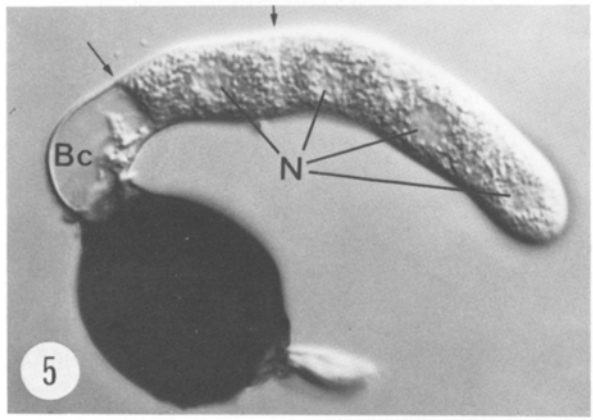
Migration of the diploid nucleus from the teliospore into the metabasidium occurs early (*i.e.*, when the metabasidium is about half the length of the spore and still elongating, (MIMS 1981, PAVGI 1975) or late (*i.e.*, when the metabasidium is as long or longer than the spore and nearly mature, O'DONNELL and MCLAUGHLIN 1981). In *U. appendiculatus* var. *appendiculatus* nuclear migration occurred early, soon after emergence of the metabasidium. Light microscopy of living structures showed that a single nucleolus (rarely two) was present in the diploid nucleus from karyogamy until migration into the metabasidium (~ prophase I of meiosis). Thereafter, the nucleolus dispersed, but reappeared in the haploid daughter nuclei during interphase II. These observations concur with reports for other rusts (see O'DONNELL and MCLAUGHLIN 1981).

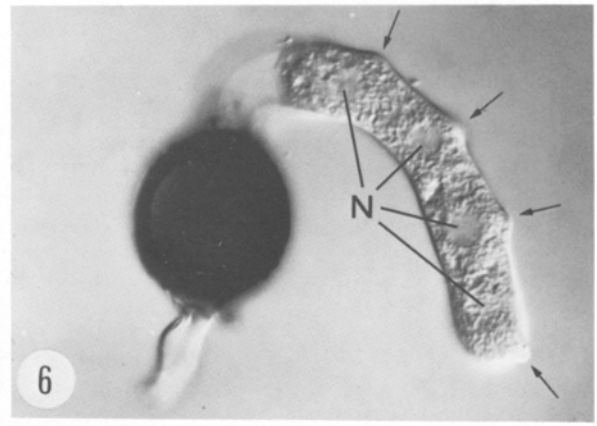


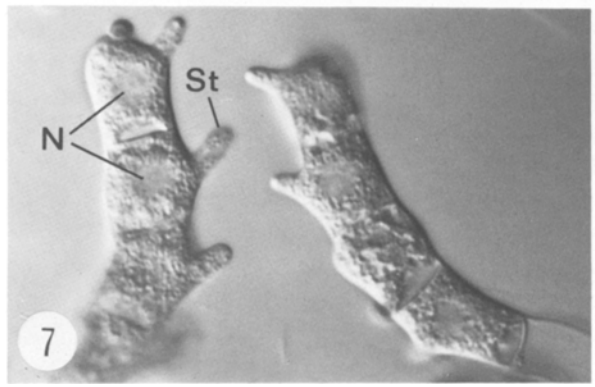


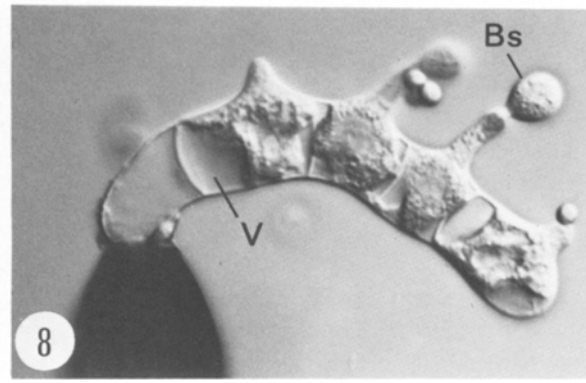




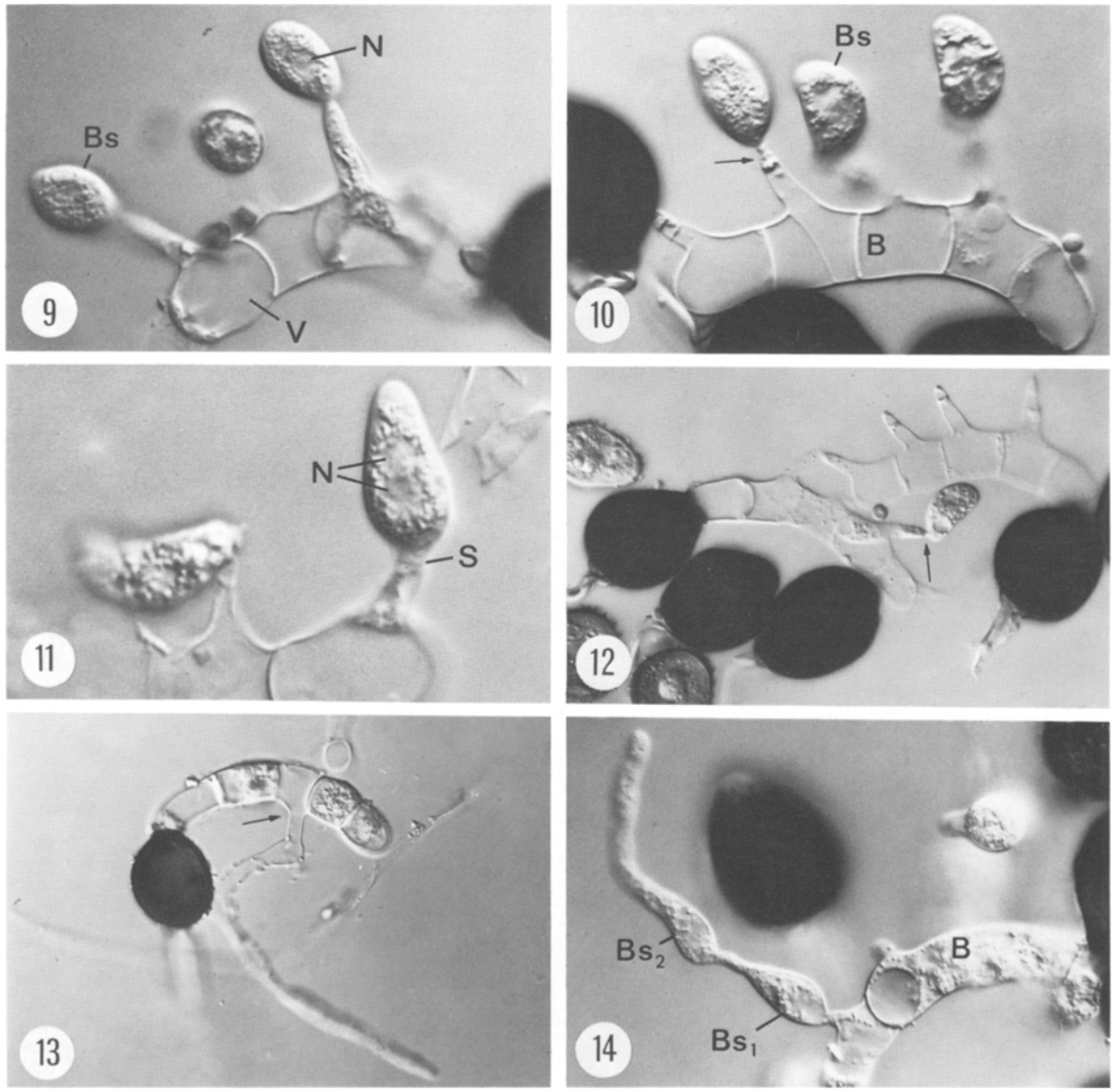






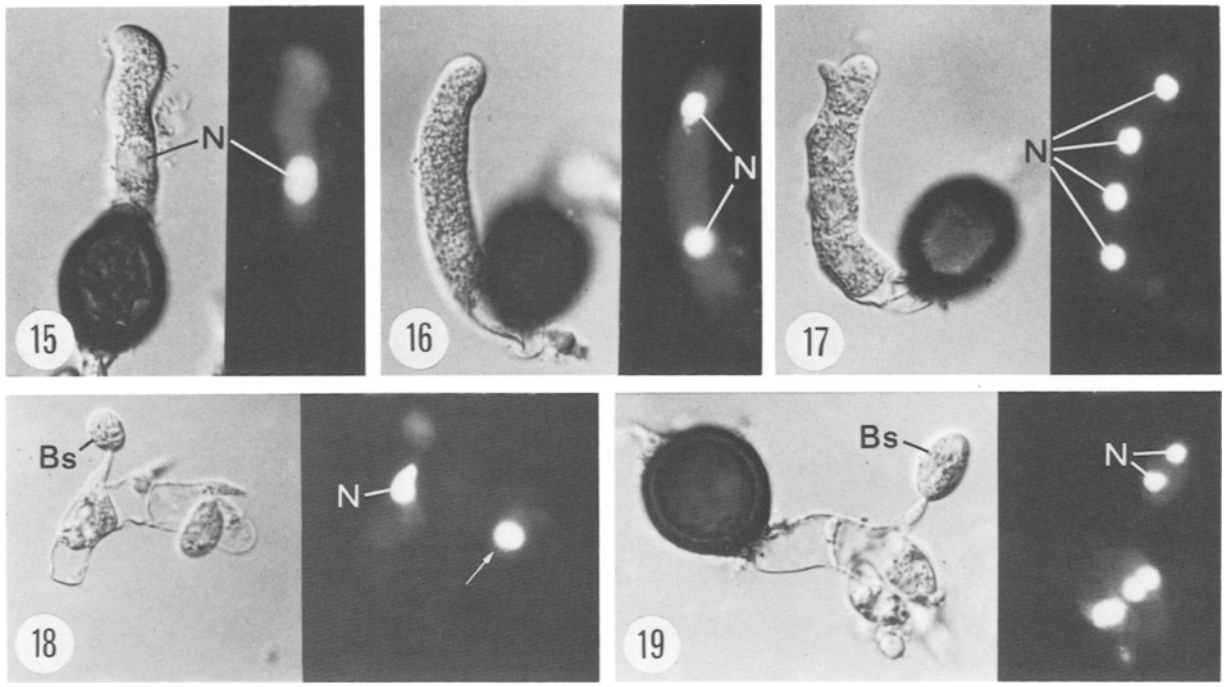


Figs. 1–8



List of Abbrevations: *B* metabasidium; *Bc* basal cell; *Bs* basidiospore; *N* fungal nucleus; *nu* fungal nucleolus; *S* septum; *St* sterigma; *V* fungal vacuole.

Figs. 1–14. LM (intereference-contrast optics) of teliospore germination and basidiospore formation from living, unstained material of *Uromyces appendiculatus* var. *appendiculatus:* Fig. 1. Ungerminated teliospore; note prominent vacuoles (*V*) and nucleolus (*nu*). × 1,790. Fig. 2. Early stage of metabasidium (*B*) emergence. The diploid nucleus and much cytoplasm (not visible in micrograph) are still within the teliospore. × 1,600. Fig. 3. Migration of the slightly elongated diploid nucleus (*N*) and bulk of cytoplasm into the metabasidium. Note the two nucleoli (*nu*) in nucleoplasm. × 1,210. Fig. 4. One-septate (*S*) metabasidium with two uninucleate haploid cells following meiosis I (nuclei not in plane of focus). × 1,110. Fig. 5. Four-septate metabasidium (two septa in plane of focus at arrows) containing four daughter nuclei (*N*). The basal cell (*Bc*) is essentially empty. × 1,010. Fig. 6. Initiation of sterigma formation (arrows). × 850. Fig. 7. Elongation of cytoplasm-filled sterigmata (*St*). × 1,020. Fig. 8. Metabasidium with developing basidiospores (*Bs*). Basidiospore formation occurs in conjunction with vacuolation of metabasidial cell. × 950. Fig. 9. Migration of haploid nucleus and cytoplasm into expanding basidiospores (*Bs*). Note highly vacuolated metabasidial cells and single nucleus (*N*) in the newly formed basidiospore. × 960. Fig. 10. The metabasidium (*B*) becomes highly vacuolated after basidiospore (*Bs*) formation is completed. A small amount of cytoplasm remains behind in the sterigma (arrow). × 830. Fig. 11. Basidiospore development commonly terminates with septum formation (*S*) and mitosis of the haploid nucleus. The basidiospore is binucleate. × 1,290. Fig. 12. Basidiospore release is almost completed; one spore is still attached to the sterigma (arrow). × 720. Fig. 13. Direct germination of metabasidial cell (arrow). × 480. Fig. 14. Secondary basidiospore production *in situ*. × 760



Figs. 15–19. LM and FM of metabasidia and basidiospore development from fixed, fluoro-stained material (all magnifications, × 620): Fig. 15. Migration of diploid nucleus (*N*) into metabasidium. Fig. 16. Meiosis I. Fig. 17. Meiosis II. Fig. 18. Migration of haploid nucleus (*N*) into basidiospore (*Bs*). The released basidiospore seen near the apex of the detached metabasidium still contains a single nucleus (arrow). Fig. 19. Post-meiotic mitosis of the haploid nucleus occurs in the basidiospore (*Bs*). Note the progressively smaller size of nuclei from Figs. 15→19

The development of basidiospores in basipetal succession appears to be characteristic of the rusts (ALLEN 1933, O'DONNELL and MCLAUGHLIN 1981, PAVGI 1975). Basidiospore production in *U. appendiculatus* var. *appendiculatus* also showed a strong tendency for basipetal development, however, the release of basidiospores did not consistently follow the same pattern. The basidial cells commonly undergo extensive vacuolation during the initiation and development of basidiospores (*e.g.*, ALLEN 1933, MIMS 1981, O'DONNELL and MCLAUGHLIN 1981, PAVGI 1975). The simplest explanation of the mechanism causing nuclear migration into the basidium and later into the basidiospore initials is expansion of vacuoles in teliospore and basidial cells, respectively (BULLER 1933, cited by MADELIN 1981). MADELIN (1981) recently examined this hypothesis and concluded it can only be valid under the assumption that the cytoplasm can absorb water from the external environment to replace that which it lost to the vacuoles.

The normal nuclear complement of basidiospores in the rusts has been generally viewed to be uninucleate (BULLER 1950, LITTLEFIELD and HEATH 1979). Accordingly, binucleate and quadrinucleate basidiospores have been treated as abnormalities and/or exceptions to the rule. Recently, PETERSON (1974) pointed out the numerous variations that occur in nuclear behaviour and development of the basidium and basidiospores in the *Uredinales*. However, in agreement with other authors (ALLEN 1933, KOHNO *et al.* 1975 b, 1977), he concluded that the binucleate condition appears to be common in rusts. A thorough examination of the literature has shown, indeed, that binucleate basidiospores have been frequently reported (BAUER 1983, KAPOORIA 1968, KULKARNI 1963, METZLER 1982, MIMS 1981, SANWAL 1953, ALLEN 1933, ANIKSTER *et al.* 1980, MENDGEN 1983, MIMS 1981, PETERSON 1974). In those studies the binucleate condition most typically arose as a result of post-meiotic mitosis or due to the formation of simplified basidia (*e.g.*, two- or three-celled, yielding two binucleate or two uninucleate and one binucleate spore, respectively). Quadrinucleate basidiospores have been also reported by several authors (KAPOORIA 1968, KOHNO *et al.* 1975 a, b, MIMS 1981). In *U. appendiculatus* var. *appendiculatus* ~ 75% of the cast basidiospores were binucleate. The post-meiotic mitosis typically occurred after migration of the haploid nucleus into the basidiospore initial; binucleate metabasidial cells were very rarely observed (< 0.1%).

In this study teliospore germination and development of basidiospores showed a very regular sequence of events with little tendency for abnormalities (*cf.*, PAVGI). GROTH and MOGEN (1978) have shown that basidiospores of *U. appendiculatus* var. *appendiculatus* may germinate *in situ* when teliospores are incubated on water agar.

Several authors have described the formation of a "water droplet" prior to discharge of rust basidiospores (BULLER 1924, cited in PRINCE 1943, DIETEL 1912 b, PRINCE 1943). However, only PRINCE recognized septum formation proximal to the basidiospore in the sterigma and discussed its importance in the release process. Since then, septum formation has been reported in three other studies for *C. ribicola* (BEGA and SCOTT 1966, LITTLEFIELD and HEATH 1979) and for *G. clavipes* (MIMS 1981). Basidiospores formation in *U. appendiculatus* var. *appendiculatus* closely resembled PRINCE's description for *Gymnosporangium nidus avis*, with the exception or droplet formation.

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