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Reproductive biology and evidence for water dispersal of teliospores in *Chrysomyxa weirii*, a microcyclic spruce needle rust

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Abstract: *Chrysomyxa weirii* (Uredinales) is the only autoecious, microcyclic species of *Chrysomyxa* occurring in North America. The telia form on second-year needles of spruce, causing premature needle loss. The morphology of the telia was studied in herbarium specimens from diverse locations, and the teliospore germination, nuclear condition, and reproductive biology of fresh collections were studied on microscope slides and on artificially and naturally infected host tissue using light and scanning electron microscopy. Basidiospore production was infrequent in mature sori, but teliospores dispersed readily in water and germinated to produce a two-celled basidium and two basidiospores. The two cells of the basidium could also separate to form two sporelike cells that could produce germ tubes, or the teliospore produced a long hyphalike promycelium. The type of germination was influenced by temperature. The ready dispersal of teliospores in water and their presence on the surface of current-year needles confirms that they function as diaspores. The distribution pattern of this rust and the elongated, smooth, thin-walled spores that are held rigidly together until wet suggest a water-dispersal mechanism. A cytological study showed that the vegetative hyphae are mostly monokaryotic. Dikaryotization and karyogamy occur in the cells at the base of the telium and result in teliospores with one large nucleus. During germination, the teliospore nucleus migrates into the basidium, where it divides once before a septum forms. A second nuclear division occurs in each cell during basidiospore formation. Both nuclei move into the basidiospore, and subsequently divide one or more times. The two-celled basidium, the fragmenting basidium and other unusual forms of germination, and teliospore dispersal have not been previously reported in the genus *Chrysomyxa*.

Key Words: basidium, cytology, microcyclic rust, *Picea*, splash dispersal, Uredinales

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INTRODUCTION

Among the North American species of the genus *Chrysomyxa*, *C. weirii* Jacks. is unique in being autoecious and microcyclic. The rust fungus forms reddish-orange, tonguelike telia in early spring on spruce needles of the previous year, and the infection spreads to young needles on newly opened buds before the old infected needles drop off. The natural host range for *C. weirii* includes *Picea engelmannii* Parry, *P. glauca* (Moench) Voss, *P. mariana* (Mill.) B.S.P., *P. rubens* Sarg., and *P. sitchensis* (Bong.) Carr. (Arthur 1962, Savile 1950, Ziller 1974). Although its occurrence is more sporadic than most other spruce needle rusts in boreal and subalpine forests (Peterson 1961), it has an extensive range that includes northern Oregon, Idaho, western Montana, Washington, South Dakota, British Columbia, Alberta, Saskatchewan, Manitoba, New Brunswick, Northwest Territories, Yukon Territory, as well as the southern Appalachian Mountains (Weir 1923, Boyce 1943, Peterson 1961, Ziller 1974). In recent years *C. weirii* has been introduced to blue spruce (*P. pungens* Engelm.) nurseries in the northeastern United States (Vermont, New Hampshire, Pennsylvania) (Pawuk 1971, Bergdahl and Smeltzer 1983, Merrill et al 1993), where serious outbreaks affecting up to 100% of new needles on the lower branches have been recorded (Bergdahl and Smeltzer 1983). There is also one report of *C. weirii* on *P. schrenkiana* Fischer & Meyer from south-central Asia (Kuprevich and Tranzschel 1957).

As in other members of the genus *Chrysomyxa*, the teliospores of *C. weirii* are thin-walled and catenulate, and they germinate without a period of dormancy. However, this single stage provides few systematic characters for comparison with other *Chrysomyxa* spe-

cies, most of which are heteroecious and macrocyclic, with their telia on various genera of Ericaceae. Therefore a study of the entire life cycle of *C. weirii* was undertaken in a search for characters that might elucidate its relationship with other members of the genus. In this study we describe the cytology and the unique form of teliospore germination in *C. weirii,* and discuss the possible implications of these features for its dispersal.

MATERIALS AND METHODS

Specimens of *C. weirii* were examined from the Mycological Herbarium of the Northern Forestry Centre (CFB), Edmonton, Alberta, and the Pacific Forestry Centre (DAVFP), Victoria, British Columbia, Canadian Forest Service; and the Arthur Herbarium (PUR), Purdue University, Lafayette, Indiana. The following collections were examined (host, location, collection number): *P. engelmannii,* Alberta, CFB 7967, British Columbia, CFB 458, CFB 7960, CFB 22109 (ex DAVFP), CFB 22111 (ex DAVFP 69-9-0775-01), Idaho, PUR 4906, Oregon, PUR 4907 (Type); *P. glauca,* Alberta, CFB 4903, CFB 5742, CFB 8089, CFB 20015, CFB 22077, CFB 22195, CFB 22196, CFB 22198, British Columbia, CFB 8498, Saskatchewan, CFB 20816, Yukon Territory, CFB 7495, CFB 8904; *P. mariana,* Manitoba, CFB 22175; *P. pungens,* New York, PUR 49269; *P. rubens,* West Virginia, PUR 44396; *P. sitchensis,* Oregon, CFB 8818. Three fresh collections, one from Kananaskis (CFB 22198) and two from Jasper (CFB 22195, CFB 22196), Alberta, were used to study the cytology and spore germination. Morphology was studied by light microscopy using squash mounts of telia and cross sections of spruce needles bearing sori mounted in lactophenol or lactophenol–cotton blue.

Teliospore germination.—Spruce needles bearing mature telia were attached with petroleum jelly to the lids of petri dishes containing moist filter paper. Glass slides were placed below the needles to capture basidiospores, and the dishes were sealed and kept in a refrigerator (4 C) or at room temperature (20 C) for up to 1 wk. Teliospores were also dispersed in droplets of water on glass slides and kept in a moist chamber. They were examined by light microscopy at regular intervals from 2 h to 3 d after dispersal. Because initial studies showed some variation in type of germination, slides were kept in a refrigerator in darkness or at room temperature either under fluorescent light or in darkness, and the results were compared.

Cytological studies.—The fluorescent dye 4′,6-diamidino-2-phenylindole (DAPI) was used to study the nuclear condition of the various stages in the life cycle of *C. weirii*: hyphae in cross sections of infected needles, squash mounts of the base of telia, ungerminated teliospores, germinating teliospores (at 2, 3, 4.5, and 22 h after dispersal of teliospores in water), and basidiospores. DAPI (0.2 μg/mL) was dissolved in McIlvaine's buffer (0.1 M citric acid:0.2 M dibasic sodium phosphate, 1:6.7) (pH 7.0) (Meixner and Bresinsky 1988). The fresh stain was applied to the fungal material on glass slides, which were viewed immediately under epifluorescent illumination using a Zeiss Axiophot Photomicroscope. In some cases slides of germinating teliospores were dried on a slide warmer (55 C) and stained later (Imazu et al 1989). To verify the results with DAPI, portions of spruce needles bearing telia were also stained with hematoxylin (Sass 1958). Tissue was first fixed in formalin–acetic acid–ethanol for 3 d, then rinsed in 50% ethanol, dehydrated in a *n*-butanol series, and embedded in paraffin (Sass 1958). Sections (10 μm thick) were made using a rotary microtome and mounted on glass slides with Haupt's adhesive (Gurr 1965). Slides were dewaxed in xylene, rehydrated in an ethanol series, placed in 4% iron alum mordant for 4 h, rinsed in distilled water, and stained in Heidenhain's hematoxylin for 4 h, then destained in diluted iron alum as necessary (Sass 1958). Slides were dehydrated in an ethanol series then placed in xylene before coverslips were permanently mounted with Permount (Fisher Scientific Co.).

Microscopy of inoculated spruce.—To study spore germination on host tissue, newly opened vegetative buds of 3-yr-old greenhouse-grown *P. glauca* were inoculated in two ways. Needles bearing mature sori were attached to the lids of petri dishes above excised shoots in a moist chamber, as above. In other cases, sori were wetted with droplets of distilled water, then telia were rubbed along the young needles, and the shoots were kept in a moist chamber either at room temperature or in a refrigerator. At regular intervals from 5 to 30 h after inoculation, thin segments of the upper surface (epidermis and part of underlying mesophyll) of individual needles were removed with a razor blade, mounted in lactophenol–cotton blue, and examined by light microscopy for spore germination on the host surface. In fresh collections of infected spruce, young current-year needles adjacent to sporulating needles were examined for evidence of natural spore deposition and germination. Host epidermis was removed and examined microscopically in the same manner as for artificially inoculated needles. For scanning electron microscopy (SEM), young current-year spruce shoots, both artificially and naturally inoculated as above, were vapor-fixed in OsO_4: shoots were enclosed in sealed petri dishes containing 3 or 4 drops of 2% OsO_4 in phosphate buffer, pH 7, for 24 h. They were then either air-dried or frozen in liquid nitrogen and freeze-dried, coated with gold using a sputter coater, and examined with an Hitachi S-510 scanning electron microscope operated at 15 kV.

Light microscope images were made by digitizing black and white photographic negatives made from Kodak TMAX 100 or 400 film. SEM images were captured using Quartz PCI software, version 4.0. Image contrast was adjusted and all halftone plates were composed using Adobe Photoshop 5.0.

RESULTS

Morphology.—Telia of *C. weirii* occur on well-defined chlorotic bands on second-year needles; they are about 0.3–2 mm long, amphigenous, and seldom confluent. Within the needle tissue, the telium is

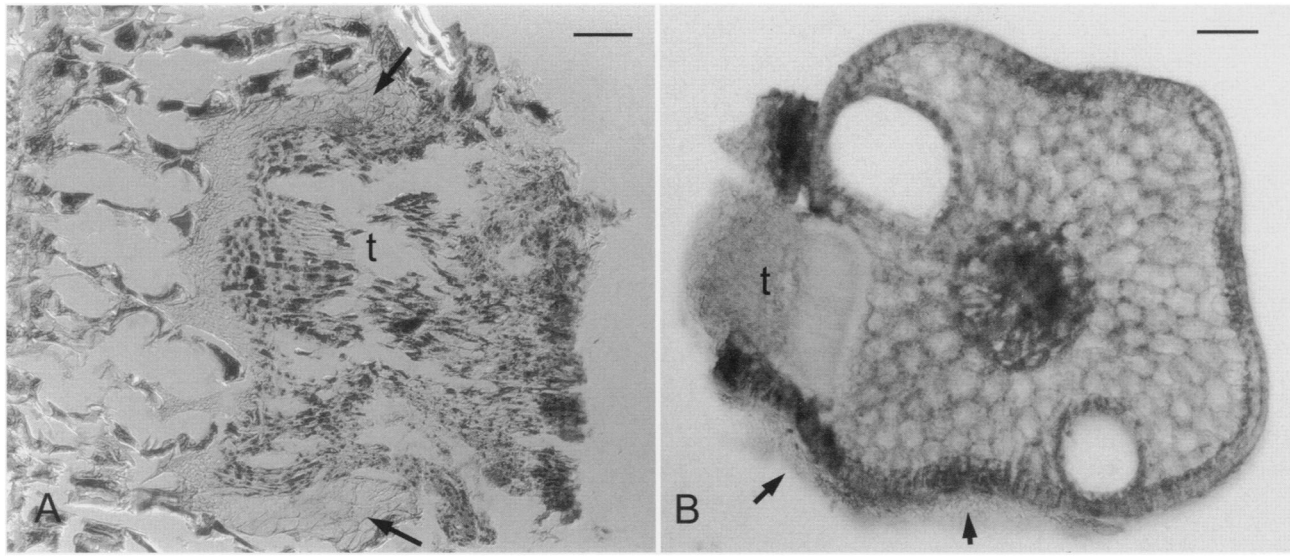
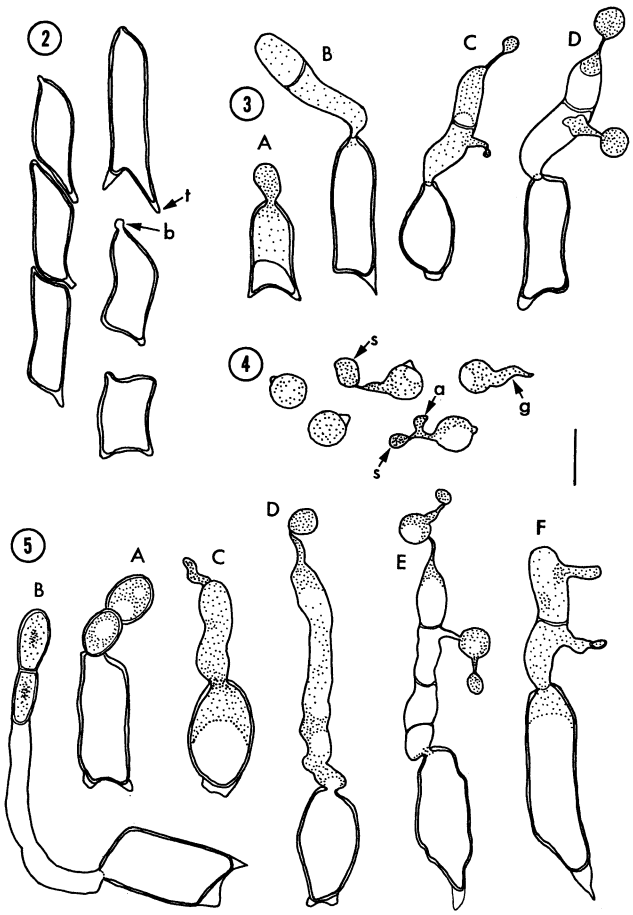


FIG. 1. Cross sections of spruce needles infected with *Chrysomyxa weirii*. A. Telium (*t*) and large thin-walled fungal cells (arrows) adjacent to sorus (from paraffin-embedded material stained with hematoxylin). Bar = 50 μm. B. Telium (*t*) and teliospore mass (arrows) crusted on needle surface. Bar = 100 μm.

bounded by a layer of small pseudo-parenchymatous cells at the base, and several layers of much larger, thin-walled cells at the sides (FIG. 1A). The teliospores are produced in narrow chains from the basal cells and are extruded between flaps of the host epidermis to form a waxy, reddish-orange, tonguelike mass. The mature spores separate readily, and crusts of spore masses are common on the needle surface around the sori (FIG. 1B). The teliospores are extremely variable in shape, from irregular to ellipsoidal, cylindrical, or rhomboidal with ends obtuse, attenuate, or truncate (FIG. 2); the width covers a narrow range of 5–9 μm, but the length is variable, 14–36(-42) μm. The wall is thin (0.4–0.8 μm) and colorless. The proximal ends of the spores have one or two hyaline tails that are either pointed or truncated and about 1.5–3.5 μm long. On the distal end of mature spores there is a narrow extension of the cytoplasm within the wall, suggesting a pore, and at this point a globose, knoblike process often extends beyond the end of the mature spore (FIG. 2). The knob expands to become the basidium during teliospore germination. Neither the tails nor the basidial knobs were included in teliospore measurements. Small globose, subglobose, or irregularly shaped spores about 5–8 × 5–7 μm were often seen among the teliospores in squash mounts from the sorus or from the spore crusts taken from the needle surface. These seldom had an apiculus. Empty teliospores, sometimes with a basidium forming from one end, were also occasionally present in these mounts. Although some basidia had one septum, sterigmata were not seen, and basidiospores were infrequent.

Teliospore germination and basidiospore formation.—Attempts to capture basidiospores beneath mature telia in a moist chamber were unsuccessful. However, when telia were touched to a droplet of water on a glass slide the spore mass disintegrated and teliospores dispersed readily into the water and germinated. When dispersed in water, the spores remained suspended or sank to the bottom. The most common type of germination is shown in FIG. 3A–D. Shortly after dispersal of teliospores in water (2 h at room temperature), the knoblike processes on the spores expanded into elongated promycelia (basidia) into which the spore contents moved. By 3 h, a single septum had formed across many basidia, and one sterigma (3–4 μm long) had grown from each basidial cell. As the basidiospore formed at the end of each sterigma, the cytoplasm moved from the basidial cell into the basidiospore. The distal cell of the basidium usually formed a sterigma and basidiospore earlier than the proximal cell. Basidiospores were globose to subglobose (5–6 × 5–6 μm) with a small apiculus; they often germinated on the slides, sometimes while still attached to the sterigma, to produce a short sterigma and secondary spore or a short germ tube (FIG. 4). In many cases, basidia became septate but failed to develop further to produce sterigmata or basidiospores even after 24 h (FIG. 5A). In one sample of teliospores on a glass slide for 3 d, many of the cells of the basidia had become rounded and the distal cell had produced a short germ tube, sometimes with a swollen end.

The mode of germination varied with the conditions under which the slides were incubated. At 4 C



FIGS. 2–5. Spore morphology and germination of *C. weirii* on glass slides. 2. Chain of teliospores as formed in sorus, and some variations in spore shape. *b,* knob that will expand to become the basidium; *t,* tail-like extension of hyaline wall. 3. Most common mode of teliospore germination. A. Basidium expands from knoblike process on end of teliospore by movement of cytoplasm from teliospore. B. Septum forms in fully formed basidium. C. A sterigma forms on each basidial cell. D. Cytoplasm moves out of basidial cells into two expanding basidiospores. 4. Ungerminated basidiospores, and basidiospores germinating to produce secondary spore (*s*), short germ tube (*g*), or appressorium-like swelling (*a*). 5. Other types of teliospore germination, more common at room temperature than at 4 C. A. Basidium fragments into two sporelike cells. B. Hyphalike promycelium with two distal sporelike cells. C. Germ tube on nonseptate promycelium. D. Hyphalike promycelium with single sterigma and basidiospore. E. Three-septate promycelium with the two distal cells producing basidiospores. F. Septate basidium producing germ tubes. Bar = 10 μm.

the two-celled basidium with basidiospores was produced almost exclusively. At room temperature, about one-half of the teliospores formed two-celled basidia and basidiospores (FIG. 3), whether in light or darkness. The following types of germination were also observed, more commonly at room temperature: (i) a narrow, greatly elongated, septate or aseptate hyphalike promycelium, with or without a swollen sporelike end, developed from one end of the teliospore (FIG. 5B); (ii) the basidium was elongate, remained aseptate and formed a narrow germ tube from the distal end (FIG. 5C) or a single, unusually long, tapered sterigma (FIG. 5D); (iii) the basidium consisted of a single small cell that formed a more typical short sterigma; (iv) a single sterigma with a basidiospore formed directly from the end of the teliospore; (v) two or more septa formed in the basidium, but the basal cells were empty and only the two distal cells contained cytoplasm and developed sterigmata and basidiospores (FIG. 5E); (vi) typical basidia formed, but the sterigmata were thick, rounded and hyphalike and without basidiospores (FIG. 5F). Basidia never formed more than two basidiospores.

Teliospore germination on spruce needles.—As with glass slides, basidiospore deposition did not occur on spruce shoots when telia were suspended above young needles in a moist chamber. In freshly collected spruce samples, teliospores were abundant on the surface of immature current-year needles adjacent to second-year needles infected with *C. weirii.* Various stages of spore germination were seen by both light and scanning electron microscopy. A mucilaginous material that appeared to hold the spores to the host surface could be seen by SEM (FIG. 6A). Although teliospores were more often clumped together in artificially inoculated samples, the characteristics of teliospore germination on artificially inoculated spruce needles were similar to those on naturally infected material. Teliospore germination occurred more slowly on host tissue than in water.

Two main forms of teliospore germination were observed on host tissue. (i) Where many teliospores were clumped together on the host surface, basidiospore formation was rare and basidia often stood out from the host surface and divided to form two cells separated by a constriction (FIG. 6A, B). As seen by light microscopy, 20.5 h after inoculation, the cells of the basidia had become round and separated into two sporelike cells. Small rounded cells that may have resulted from fragmentation of basidia were sometimes also seen on the needle surface by SEM; they were smooth-walled, larger than basidiospores, and had a depressed center, perhaps resulting from shrinkage during preparation (FIG. 6B, lower cell). (ii) Elongated basidia formed along the host surface and produced two sterigmata and basidiospores (FIG. 6C, D). Basidiospores were often seen on the needle surface. These spores were globose with a tiny apiculus and a rough surface and were the same size as basidiospores produced on glass slides. Infrequently,

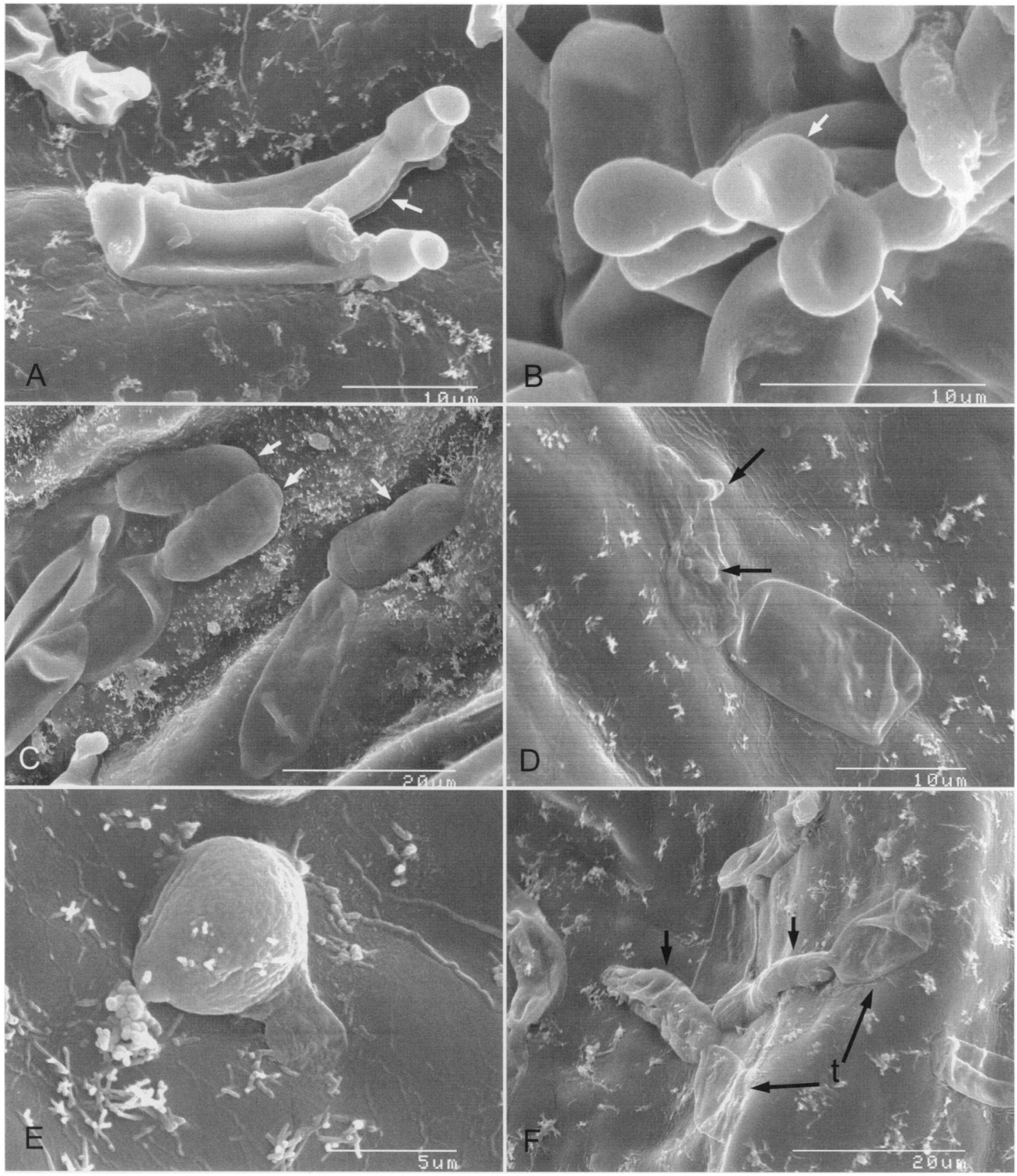


FIG. 6. SEM micrographs of *C. weirii* on spruce needles of the current year. A. Teliospores germinating to produce two basidial cells. Note mucilaginous material (arrow) at contact between spore and host. B. Disarticulating basidial cells (arrows) at a later stage of development than in A. C. Teliospores with elongate basidia (arrows). D. Collapsed teliospore and basidium that has already released its basidiospores. Arrows indicate sterigmata. E. Basidiospore with short germ tube that appears to be directly penetrating the needle surface. F. Teliospores (*t*) with long hyphalike promycelia (short arrows). A–C are on artificially inoculated needles; D–F are on naturally infected needles.

basidiospores produced a germ tube or a secondary spore. Rarely, direct host penetration by a short germ tube was observed (FIG. 6E). An appressorium was observed on only one germ tube. Some of the other types of teliospore germination seen on glass slides were also observed on host tissues (FIG. 6F).

Cytology.—The nuclear condition of hyphae in spruce needle tissue was difficult to interpret, because nuclei were often diffuse or greatly elongated. The DAPI stain sometimes showed two small nuclei side by side across the width of a hypha, but with the greater detail revealed by hematoxylin staining these were confirmed to be two parts of one dumbbell-shaped nucleus joined by a narrow isthmus. Interpretation was further complicated by the presence of many small scattered nuclear fragments in cells rather than distinct nuclei (FIG. 7A). Of the cells in which distinct nuclei couid be discerned, about 75% were monokaryotic (FIG. 7A) and 25% dikaryotic. Below the base of the telium, globose pseudo-parenchymatous cells were dikaryotic or monokaryotic. The thin-walled cells bounding the sorus at the sides contained one small nucleus, usually at the periphery. In the sorus, about 30–50% of the basal cells in the chains of teliospores were dikaryotic; the rest were monokaryotic (FIG. 7B). Teliospores of *C. weirii* contained one large diffuse nucleus that occupied up to one-quarter of the spore volume (FIG. 7C). When a teliospore germinated (FIG. 7D–I), the single nucleus migrated into the forming basidium (FIG. 7D), where it divided near the proximal end. The nuclei then moved apart (FIG. 7E) and a septum formed across the basidium (FIG. 7F). As the sterigma and basidiospore began to form, the nucleus in each basidial cell divided again, and both nuclei moved, one at a time, into the basidiospore along with the cytoplasm (FIG. 7G, H). Mature basidiospores most commonly contained four nuclei (FIG. 7I), but up to six tiny nuclei or nuclear fragments were sometimes present.

Where the basidium fragmented into two rounded cells, these cells contained one or two nuclei, but after long incubation (3 d), many small nuclear fragments were present, as in mature basidiospores. Long hyphalike promycelia generally contained one large nucleus, or one nucleus in each of two cells, if septate. The nuclear condition during other variations of germination was not observed.

DISCUSSION

Spore dispersal.—In *C. weirii* the teliospores function as diaspores. This is supported by their ready separation and dispersal in water, and their presence on field-collected current-year needles adjacent to year-old infected needles. Although the ready separation of the teliospores has previously been noted (Jackson 1917), the significance of this feature to the epidemiology of the fungus has not been recognized. We propose that dissemination of *C. weirii* occurs mainly through water dispersal of the teliospores rather than by wind dispersal of basidiospores produced in the sorus, as previously assumed (Weir 1923, Ziller 1974). A water-dispersal mechanism is consistent with both the teliospore morphology of this fungus and its distribution pattern. The teliospores are wettable, and they have a smooth surface, thin hyaline wall, and elongate shape, common features of splash-dispersed fungi (Fitt and McCartney 1986, Fitt et al 1989). In contrast, wind-dispersed spores, such as the aeciospores of heteroecious *Chrysomyxa* species, are borne dry, are nonwettable, have an ornamented surface, and are generally more rounded (Fitt and McCartney 1986). When dry, the spore masses of *C. weirii* form rigid crusts on the needle surface and in the sorus, but when water is present these masses dissolve, suggesting that an adhesive substance holds them together and prevents their removal by wind (Gregory et al 1959). Such a substance would also protect the spores from desiccation during dry weather (Fitt et al 1989). A few drops of moisture from mist, fog, or drizzle may be enough to float the spores from the sorus to the needle surface. Subsequent droplets from rain or dripping from branches above would further disperse the spores to susceptible young needles.

Chrysomyxa weirii is mainly confined to the lower branches of mature trees, and individual trees become infected year after year while neighboring trees remain free of infection (Weir 1923). The sporadic distribution of this rust is consistent with the fact that dispersal by water occurs over much smaller distances than by wind (Fitt et al 1989). During this study, we observed *C. weirii* in four locations in the Rocky Mountains of western Alberta. In all cases, the rust occurred on isolated trees or groups of trees in damp places near rivers or streams, where, in addition to rain, water-splash and heavy mist might provide enough moisture for dissemination and germination of the teliospores. Bergdahl and Smeltzer (1983) noted the correlation of infection with a period of rainfall in a blue spruce nursery. This dispersal mechanism should be an important consideration in the spacing of trees and in the method of irrigation chosen for spruce orchards where infection with *C. weirii* is prevalent.

This report of dispersal of rust teliospores by water appears to be unique. Dispersal of urediniospores by water, however, has been studied in several rust fungi

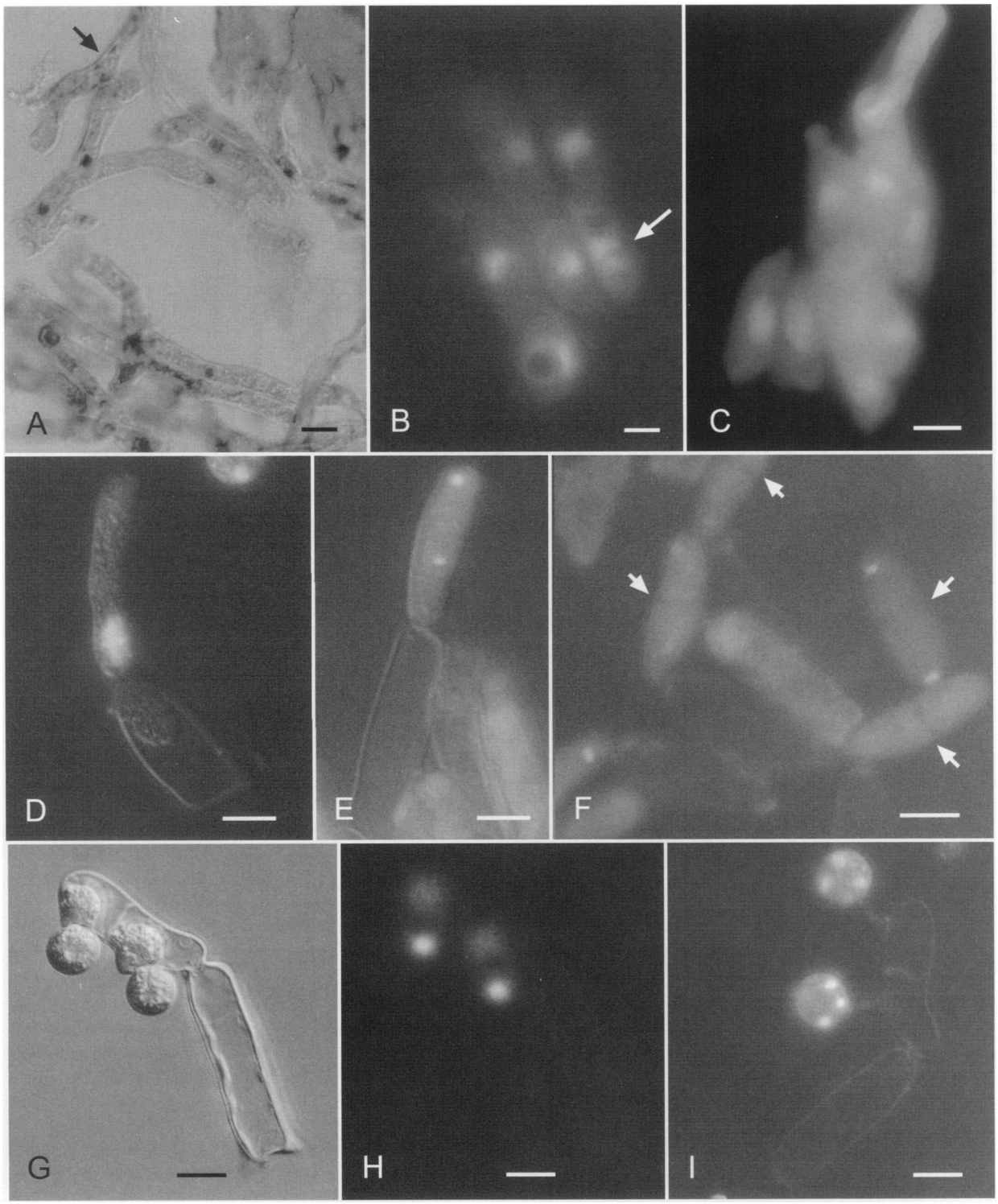


FIG. 7. Nuclear cycle of *C. weirii*. A. Monokaryotic hyphae in cross section of infected spruce needle, stained with hematoxylin. Arrow indicates a hyphal cell with many small nuclear fragments. B. Monokaryotic and dikaryotic (arrow) cells at base of teliospore chains. C. Mass of monokaryotic teliospores. D. Germinating teliospore. Nucleus has migrated from teliospore to basidium. E. Nucleus has divided, and the two nuclei have moved apart. F. Septa (arrows) have formed in several basidia. G. Germinated teliospore and basidium, photographed using differential interference contrast optics. Cytoplasm is moving into the forming basidiospores. H. Same as G, but under epifluorescent lighting to show DAPI-stained nuclei. I. Fully formed basidiospores containing four nuclei (fourth nucleus in each spore is slightly out of focal plane). B–F, H, I, stained with DAPI. Bars: A, B, D–I = 5 μm; C = 7 μm.

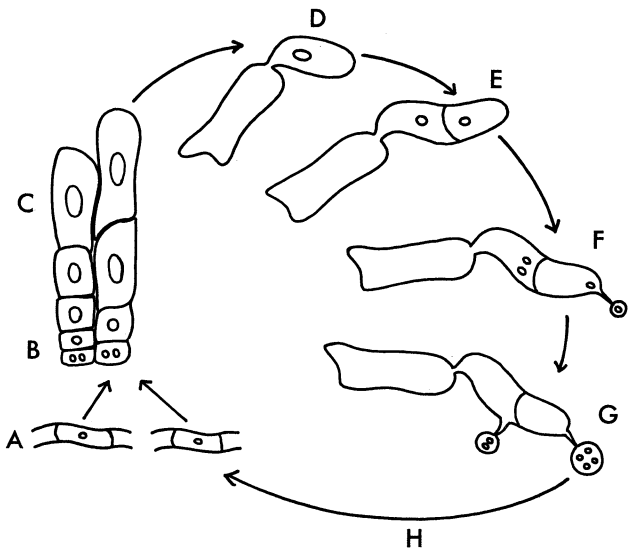


FIG. 8. Schematic drawing showing the major nuclear events in the life cycle of *C. weirii*. A. Monokaryotic hyphae in spruce needle tissue. B. Binucleate cells at base of sorus after dikaryotization. C. Teliospores in sorus, each containing one large diploid nucleus after karyogamy. D. Germinating teliospore; nucleus has migrated into basidium. E. Diploid nucleus has divided, either by mitosis or the first meiotic division, and a septum has formed across the basidium. F. Nuclei in basidial cells divide again and move into the forming basidiospores. G. Nuclei in basidiospores divide again either by the second meiotic division, or by mitosis to form tetranucleate basidiospores. H. Basidiospores germinate and infect new host needles. The mechanism by which multinucleate basidiospores result in uninucleate hyphae in the host was not observed.

(Rajasab and Rajendran 1983, Savary and Janeau 1986, Zadoks 1988).

Spore germination and nuclear cycle.—It is apparent that *C. weirii* has a number of germination strategies, depending on the environmental conditions. Most teliospores produced basidiospores at 4 C, a temperature at which these delicate spores would be unlikely to be harmed by desiccation. At room temperature, however, teliospores were much more likely to form fragmenting or hyphalike promycelia. Observations of naturally infected needles confirmed that these variations also occur under natural conditions. The slightly thicker walls of the basidial cells may render these propagules more resistant than basidiospores to the harmful effects of ultraviolet light or desiccation.

It also appears that most basidiospore production does not occur in the telium. For seven other *Chrysomyxa* species from western Canada, teliospore germination and production of basidiospores has been readily obtained by enclosing infected plant material in a moist chamber in the same manner as described herein (P. E. Crane unpubl). The absence of basidiospore deposition by this method with *C. weirii* and the ready germination of teliospores when dispersed in water suggests that basidiospore formation in *C. weirii* occurs primarily after teliospore dispersal and that free water is required for teliospore germination. Although basidiospores were occasionally seen within the mass of teliospores from a sorus, the globose or irregular spores most often observed did not have an apiculus and were slightly larger than basidiospores. They were identical with teliospores in pigmentation and morphology except for their size. They may have been unusually small teliospores or basidial cells resulting from fragmentation of the basidia.

The nuclear cycle in macrocyclic rust fungi is remarkably uniform; however, considerable variation in this pattern has been documented in microcyclic rusts (Jackson 1931, Petersen 1974, Hiratsuka and Sato 1982). Most rust fungi produce four-celled basidia, but in our studies of *C. weirii* the basidia were consistently two-celled except for a few cases where they were one-celled or four-celled, but in the latter case only two cells were functional. A photograph of *C. weirii* by Bergdahl and Smeltzer (1983) also appears to show a two-celled basidium. Weir (1923) claims to have observed four-celled basidia in *C. weirii* from which a single basidiospore was produced. It is possible that Weir misinterpreted the knoblike immature basidium as a basidiospore. The two-celled basidium has not previously been reported in the genus *Chrysomyxa*. However, a two-celled basidium may not in itself be unique within a genus (Oberwinkler 1982), because species with two-celled basidia are thought to be derived from simplification of forms with typical four-celled basidia. For example, *Kunkelia nitens* (Schw.) Arth. on *Rubus* spp. lacks spermogonia and develops a two-celled basidium, but is likely derived from *Gymnoconia peckiana* (Howe) Trott., which has four-celled basidia and spermogonia (Dodge 1924, Cummins and Hiratsuka 1983). The two-celled basidium has been reported in several other microcyclic rusts, for example, *Uromyces aloes* (Cke.) Magn. (Thirumalachar 1946), *Frommea obtusa* (Strauss) Arth. (Cunningham 1966), and *Puccinia rutainsulara* D. E. Gardner (Gardner 1994). Some of the variations in *C. weirii* teliospore germination that we observed, such as the disarticulating basidial cells and hyphalike promycelia have also been reported in other rusts, either as aberrant forms under conditions of excessive moisture or low oxygen (Thirumalachar 1946, Petersen 1974, Cunningham 1966) or as the usual mode of germination (Pady 1935, Gardner 1988).

Based on the cytological study of *C. weirii,* the nuclear cycle is most likely as follows (FIG. 8). The nu-

clei in the vegetative hyphae are haploid; dikaryotization occurs at the base of the sorus, followed by nuclear fusion. The single nucleus in the teliospore is much larger than in other parts of the life cycle, suggesting that it is diploid and the product of karyogamy. During germination, the teliospore nucleus migrates into the basidium, where it divides. Two interpretations of the events in the basidium and basidiospores are possible, as follows. (i) The first division in the basidium is meiotic, and the second meiotic division is delayed until after septum formation in the basidium. The two products of the second division in each of the basidial cells both enter the forming basidiospore, where they subsequently divide mitotically to produce a tetranucleate basidiospore. Delay of the second meiotic division until after septum formation in the basidium has been reported in other rust fungi (Dodge 1924, Jackson 1935, Petersen 1974, Kohno et al 1977). (ii) The first division in the basidium is mitotic, then a septum forms. The first meiotic division then occurs in each basidial cell, producing two nuclei per cell. These both migrate into the basidiospore, where they undergo the second meiotic division. In either case, tetranucleate basidiospores would result, the most common condition observed. Accounts of more than one nucleus in basidiospores are not unusual (e.g., Allen 1933, Berkson and Britton 1969, Petersen 1974, Kohno et al 1977, Gardner 1987, 1988, 1994). The advantage to the fungus of having all products of meiosis in the same basidiospore is unknown, but also occurs in the microcyclic *Uromyces alyxiae* Arth. (Gardner 1987). Studies of the nuclear condition of germinating spores on the host surface (not observed in this study) would clarify the mechanism for transition from the tetranucleate basidiospores to the uninucleate mycelium in the host.

The presence of thin-walled bounding cells at the sides of the telia of *C. weirii* requires explanation, since a telial peridium is not characteristic of *Chrysomyxa* species. These thin-walled monokaryotic cells may not be a true peridium, but are likely remnants of a mass of fungal tissue in the host before telium initiation. They appear to be much like the undifferentiated cells that form a "protoaecium," as defined by Buller (1950), or like the "dermal layer" surrounding the telia of *Puccinia rutainsulara* (Gardner 1994). These cells contain a single small nucleus and were probably part of the monokaryotic mycelium present before initiation of teliospore formation by dikaryotization and karyogamy. They are unlike the modified spores produced in a catenulate manner that form a peridium around the aecia of macrocyclic *Chrysomyxa* species.

Relationship of C. weirii *to other members of* Chrysomyxa.—The results of this study raise questions about the relationship of *C. weirii* to other members of the genus *Chrysomyxa*. Teliospores that function as diaspores, the two-celled basidium, and the other unusual forms of teliospore germination have not been previously reported in the genus. In many rust genera, it is possible to correlate microcyclic species with a macrocyclic species of similar morphology, and from which the short-cycled form is presumed to have arisen (Jackson 1931). The morphology of *C. weirii* and the new information on its biology presented here do not suggest correlation with known heteroecious species of *Chrysomyxa*. Similarly, the dispersal of the teliospores before germination is different from other members of the genus. Although the telia of *C. weirii* superficially resemble the telia of other species of *Chrysomyxa,* further studies are needed to determine whether placement in this genus is appropriate.

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LITERATURE CITED

Allen RF. 1933. A cytological study of the teliospores, promycelia, and sporidia in *Puccinia malvacearum*. Phytopathology 23:572–586.

Arthur JC. 1962. Manual of the rusts in United States and Canada. New York: Hafner Publishing Co. p 32–33.

Bergdahl DR, Smeltzer DLK. 1983. *Chrysomyxa weirii* on *Picea pungens* in Vermont. Pl Dis 67:918.

Berkson BM, Britton MP. 1969. Cytological studies on the teliospore and teliospore germination in *Puccinia lobata*. Mycologia 61:981–986.

Boyce JS. 1943. Host relationship and distribution of conifer rusts in the United States and Canada. Trans Conn Acad Arts Sci 35:329–482.

Buller AHR. 1950. Researches on fungi. Vol. VII. The sexual process in the Uredinales. Toronto: University of Toronto Press. 458 p.

Cummins GB, Hiratsuka Y. 1983. Illustrated genera of rust fungi. Rev ed. St. Paul, Minnesota: The American Phytopathological Society. 152 p.

Cunningham JL. 1966. Germination of teliospores of the rust fungus *Frommea obtusa*. Mycologia 58:494–496.

Dodge BO. 1924. Uninucleated aeciospores in *Caeoma nitens* and associated phenomena. J Agric Res 28:1045–1058.

Fitt BDL, McCartney HA. 1986. Spore dispersal in splash droplets. In: Ayres PG, Boddy L, eds. Water, fungi, and

plants. Cambridge: Cambridge University Press. p 87–104.

———, ———, Walklate PJ. 1989. The role of rain in dispersal of pathogen inoculum. Annu Rev Phytopathol 27:241–270.

Gardner DE. 1987. Teliospore germination of *Uromyces alyxiae,* an endemic Hawaiian rust. Mycologia 79:914–917.

———.1988. Teliospore germination of *Puccinia vitata,* an endemic Hawaiian rust. Mycologia 80:590–593.

———. 1994. Teliospore germination and nuclear behavior of *Puccinia rutainsulara,* a microcyclic Hawaiian rust. Mycologia 86:486–493.

Gregory PH, Guthrie EJ, Bunce ME. 1959. Experiments on splash dispersal of fungus spores. J Gen Microbiol 20: 328–354.

Gurr E. 1965. The rational use of dyes in biology and general staining methods. London: Leonard Hill. 422 p.

Hiratsuka Y, Sato S. 1982. Morphology and taxonomy of rust fungi. In: Scott KJ, Chakravorty AK, eds. The rust fungi. London: Academic Press. p 1–36.

Imazu M, Kakishima M, Kaneko S. 1989. *Endocronartium sahoanum,* a new stem rust fungus on *Pinus pumila* in Japan. Trans Mycol Soc Japan 30:301–310.

Jackson HS. 1917. Two new forest tree rusts from the northwest. Phytopathology 7:352–355.

———. 1931. Present evolutionary tendencies and the origin of life cycles in the Uredinales. Mem Torrey Bot Club 18(1):1–108.

———. 1935. The nuclear cycle in *Herpobasidium filicinum* with a discussion of the significance of homothallism in Basidiomycetes. Mycologia 27:553–573.

Kohno M, Nishimura T, Noda M, Ishizaki H, Kunoh H. 1977. Cytological studies on rust fungi (VII) the nuclear behavior of *Gymnosporangium asiaticum* Miyabe et Yamada during the stages from teliospore germination through sporidium germination. Trans Mycol Soc Japan 18:211–219.

Kuprevich VF, Tranzschel W. 1957. Cryptogamic plants of the USSR. Vol. IV. Fungi (1), rust fungi, family Melampsoraceae. Akad Nauk SSSR Bot Inst Komarova. (Translated from Russian by Israel Program for Scientific Translations, Jerusalem, 1970). Jerusalem: Kieter Press. 518 p.

Meixner B, Bresinsky A. 1988. Cytofluorometric determination of relative DNA content in nuclei of Coniophoraceae (Boletales) using DAPI. Trans Br Mycol Soc 90:175–180.

Merrill W, Wenner NG, Peplinski JD. 1993. New host distribution records from Pennsylvania conifers. Pl Dis 77: 430–432 (Abstr).

Oberwinkler F. 1982. The significance of the morphology of the basidium in the phylogeny of Basidiomycetes. In: Wells K, Wells EK, eds. Basidium and basidiocarp. Evolution, cytology, function, and development. New York: Springer-Verlag. p 9–35.

Pady SM. 1935. The role of intracellular mycelium in systemic infections of *Rubus* with the orange-rust. Mycologia 27:618–637.

Pawuk WH. 1971. *Chrysomyxa weirii* found on blue spruce (*Picea pungens*) in New Hampshire. Phytopathology 61: 131 (Abstr).

Petersen RH. 1974. The rust fungus life cycle. Bot Rev 40: 453–513.

Peterson RS. 1961. Notes on western rust fungi. I. *Chrysomyxa.* Mycologia 53:427–431.

Rajasab AH, Rajendran C. 1983. Dispersal of *Hemileia vastatrix* uredospores and spread of coffee leaf rust. J Coffee Res 13:120–124.

Sass JE. 1958. Botanical microtechnique. 3rd ed. Ames, Iowa: The Iowa State University Press. 228 p.

Savary S, Janeau JL. 1986. Rain-induced dispersal in *Puccinia arachidis,* studied by means of a rainfall simulator. Neth J Pl Pathol 92:163–174.

Savile DBO. 1950. North American species of *Chrysomyxa.* Can J Res C 28:318–330.

Thirumalachar MJ. 1946. A cytological study of *Uromyces aloës.* Bot Gaz 108:245–254.

Weir JR. 1923. The genus *Chrysomyxa.* Mycologia 15:183–187.

Zadoks JC. 1988. A salty act. Notes on the dispersal of *Uromyces limonii.* Z Pflanzenkrankh Pflanzenschutz 95: 384–391.

Ziller WG. 1974. The tree rusts of western Canada. Publ no 1329. Victoria, BC: Canadian Forestry Service. 272 p.