Peridermium bethelii: A Rust Associated with Lodgepole Pine Dwarf Mistletoe

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ABSTRACT

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The rust fungus *Peridermium bethelii*, which is associated with dwarf mistletoe (*Arceuthobium americanum*) in lodgepole pine (*Pinus contorta*), has generally been considered synonymous with *Cronartium comandrae*. *P. bethelii*, however, differs in its smaller, less-pyriform aeciospores, apparent lack of spermagonia, later aecial sporulation period, and constant association with dwarf mistletoe. Attempts to infect mistletoe-infected pines were either unsuccessful or inconclusive and potential alternate hosts have not been found. Field evidence suggests the rust may be autoecious.

For many years, there have been reports of pine rust (*Cronartium comandrae* Pk.) associated with dwarf mistletoe (*Arceuthobium americanum* Nutt. ex Engelm.) on lodgepole pine (*Pinus contorta* Dougl.) (7,15,19) (Fig. 1). *C. comandrae* is a widespread parasite of many North American pines that alternates to *Comandra* spp., the obligate telial host (16,18).

In the late 1960s, it was observed that "*Cronartium comandrae*" associated with dwarf mistletoe near Red Feather Lakes in the Roosevelt National Forest, CO, seemed to be spreading directly from pine to pine. Thus, a comparative study of the morphology and life history of the "mistletoe rust" and *C. comandrae* was undertaken.

Peridermium bethelii Hedgcock & Long (8) was described from pine specimens collected in Colorado by Ellsworth Bethel in 1913. Hedgcock and Long (8) considered *P. bethelii* different from *C. comandrae* because its spores were less pyriform. In 1925, Bethel (*unpublished*) noted that the new rust was almost always associated with dwarf mistletoe, but this association was not mentioned in the description of the rust by Hedgcock and Long (8). We examined 10 of Bethel's Colorado *P. bethelii* collections in the National Fungus Collections and found all were associated with dwarf mistletoe. Most were associated with *A. americanum* on lodgepole pine; however, two were with *A. vaginatum* subsp. *cryptopodum* (Engelm.) Hawksw. & Wiens on ponderosa pine. Hedgcock and Long (9,10) attempted to infect *C. umbellata* (L.) Nutt. with aeciospores of *P. bethelii* but the inoculations were unsuccessful. In 1915, however, Bethel (*unpublished*) reported that spores from the type tree of *P. bethelii* did infect comandra.

The objectives of this study were to 1) determine the geographic distribution and host range of *P. bethelii*, 2) determine the life cycle of *P. bethelii*, 3) compare aeciospore germination and morphology of *P. bethelii* and *C. comandrae*, 4) make histological studies of *P. bethelii* and its association with dwarf mistletoe and lodgepole pine, and 5) determine the taxonomic status of *P. bethelii*.

MATERIALS AND METHODS

Geographic distribution and host range. Herbarium and field studies were conducted to determine the geographic distribution of *P. bethelii* and its associated pine and dwarf mistletoe hosts.

Inoculations. Inoculation tests were conducted at the Forestry Sciences Laboratory of the Intermountain Forest and Range Experiment Station in Logan, UT, in 1968–1970 and at the Rocky Mountain Station in Fort Collins, CO, in 1977.

The Utah inoculation trials included *Pinus contorta* naturally infested with *A. americanum* transplanted 1 yr before inoculations from the north slope of the Uinta Mountains, *C. umbellata* transplanted 1–3 yr previously from the Bear River Mountains, and 2- to 3-yr-old *P. contorta* grown from seed. Seven sources of *P. bethelii* aeciospores with viability ranging from about 20 to 70% were used separately for inoculum. Inoculation techniques included dusting dry spores over aerial plant surfaces, painting spore suspensions on plant parts, and inserting dry spores into fresh scalpel wounds in mistletoe-infested bark tissue. During the 3 yr, inoculations were attempted on 50 dwarf mistletoe-infested pines and about 20 comandra plants. In parallel trials, five dwarf mistletoe-infested lodgepole pines were inoculated with pedigreed aeciospores of *C. comandrae* and five with *C. comandrae* sporulating telia in situ. In all trials, an equal number of plants was treated similarly, but without exposure to the rust fungus, to serve as controls. Initial incubation was 48 hr in darkness in mist chambers with controlled temperatures of 18 ± 2 C. Plants were then moved to a greenhouse for observation. The plants overwintered outdoors in a lathhouse cold frame. Observations were continued periodically for 3 yr.

The Colorado trials were conducted with dwarf mistletoe-infected lodgepole pine seedlings transplanted from the Roosevelt National Forest and potted and grown in a greenhouse in Fort Collins. Eighty trees were sprayed with a spore suspension (50,000–70,000 *P. bethelii* spores per milliliter) and 80 trees served as controls. Trees were then kept in moist chambers (100% RH) for 48 hr after inoculation. Aeciospore germination rates of 0–6% were observed on agar plate trials done at the time of inoculation.

Nine mistletoe-infected lodgepole pines were inoculated in the field in the Roosevelt National Forest in Colorado. Infected branches were sealed in plastic sleeves and sprayed with *P. bethelii* spores during a period of cool misty

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weather. The sleeves were removed after 5 days.

Aeciospore germination. *Agar plates.* Attempts to germinate aeciospores of *P. bethelii* were made in petri dishes on water agar, potato-dextrose agar (PDA), and water agar/mistletoe shoot (2 g dried mistletoe per 100 ml water) medium. Inoculated plates were incubated at room temperature and examined after 24, 48, and 72 hr. A series of phosphate-buffered water-agar plates of pH 6.0, 6.5, 7.0, 7.5, and 8.0 were prepared as described by Eppstein and Tainter (6) and were also used for aeciospore germination. Three plates of each pH were incubated at 10, 15, 20–22, and 15–25 C (alternating at 12-hr intervals) for 48 hr.

Collodion membranes. Aeciospores were placed onto collodion membranes (23) floating on distilled water in covered petri plates either as a water suspension or as a dust. The plates were either refrigerated at 5 C, placed under fluorescent light at room temperature (22 C), or placed in a darkened incubator at 18 C.

Agar-coated microslides. The technique described by Hiratsuka (11) was used to coat microslides with melted 4 and 0.3% water agar. Other microslides were also coated in a similar fashion with a single layer of 2% melted agar.

Fluorescent dye-treated spores. Aeciospores were stained with the fluorescent brightener Calcofluor M2R (American Cyanamid Co., Bound Brook, NJ) (5,13,17) by adding *P. bethelii* and *C. comandrae* aeciospores to a 0.5% water solution of brightener. After 15 min, spores were transferred to either agar-coated slides or to fresh lodgepole pine needles in petri dish moisture chambers and incubated at 17 and 25 C.

Fig. 1. Association of *Peridermium bethelii* with *Arceuthobium americanum* on lodgepole pine, Fraser Experimental Forest, Colorado.

Aeciospore morphology and cytology. Aeciospore shape and size was determined for 81 collections of *P. bethelii* and 36 collections of *C. comandrae*. After aeciospores were mounted in 25% lactic acid and 20 spores in each collection measured for length and width, 250 were classed in one of the six shape categories: I. subglobose, II. acuminate, III. pyriform, IV. strongly pyriform, V. teardrop-shaped, and VI. double-tailed (Fig. 2).

Scanning electron microscope (Hitachi HHS-2R) photographs were made of air-dried aeciospores of four *P. bethelii* and two *C. comandrae* collections to compare spore wall characteristics.

Several procedures for nuclear staining of germinated aeciospores on agar-coated microslides were studied: HCl-Giemsa (4), Feulgen's basic fuchsin (22), aqueous basic fuchsin (2), and Christenson's modifications of the HCl-Giemsa method (1,2).

Histology. The following lodgepole pine branch materials were used for histological studies: field samples naturally infected with *P. bethelii* and *A. americanum*, *A. americanum*-infected and uninfected branches from transplanted saplings inoculated with *P. bethelii*, and healthy saplings. Branch samples less than 1 cm in diameter and 1 cm long were boiled in water for 30 min to remove air and soften tissue, then cut on a freezing sliding microtome to a thickness of 18–22 μm.

Six staining techniques were attempted to determine which was most satisfactory for *P. bethelii*-infected tissue. These included safranin-light green (3), malachite green-acid fuchsin (24), orseillin-BB-aniline blue and safranin-*o*-aniline blue (14), Peterson's modification of Jewell's orseillin-BB-aniline blue (20), and safranin-*O*-aniline blue in lactophenol.

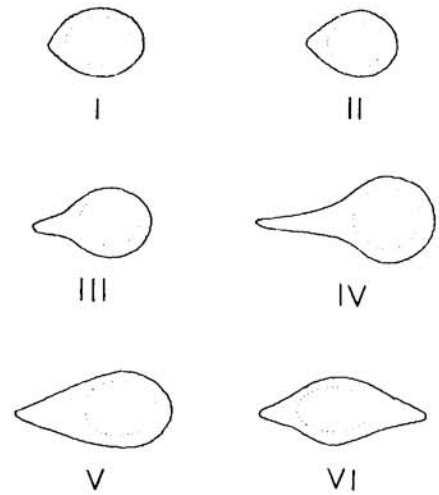

Fig. 2. The six shape classes used in classifying *Peridermium bethelii* and *Cronartium comandrae* aeciospores. I = subglobose, II = acuminate, III = pyriform, IV = strongly pyriform, V = teardrop-shaped, and VI = double-tailed.

After being stained, the sections were mounted in Permount for microscopic examination.

RESULTS

Geographic distribution and host range. *P. bethelii* has been collected in 29 localities in six western states (Idaho, Montana, Wyoming, Utah, Colorado, and California) and in Alberta, Canada (Fig. 3). Most aeciospore collections have been made from June through October but a few aecia with spores have been found as late as January.

Essentially all *P. bethelii* collections known are associated with *A. americanum* in lodgepole pine. The two exceptions are Bethel's 1913 collections from near Allenspark, CO, which were associated with *A. vaginatum* on ponderosa pine, but we have not found this rust on this host there or elsewhere.

P. bethelii is not usually common; however, in small areas, as many as 15% of the *A. americanum* infections are affected. Mistletoe-infected branches (and only rarely trees with bole infections) are eventually killed by the rust so some degree of biological control of the mistletoe is achieved. Although we have no quantitative data, it appears that the rates of girdling and branch killing by *P. bethelii* are much slower than for *C. comandrae* on nonmistletoed branches.

Inoculations. In the Utah tests, 50 dwarf mistletoe-infested pines were inoculated with *P. bethelii*. Aecia typical of *P. bethelii* developed on mistletoe-infected tissues of only one tree. This tree had been inoculated by dusting with aeciospores. No symptoms or signs of rust infection were observed in the controls, on the 10 pines inoculated with pedigreed *C. comandrae* aeciospores or telia, or on the 20 comandra plants. The rust source that provided the single infection was collected by J. G. Laut (3 July 1970) from Lynx Creek, CO. Aecia appeared 21 mo after inoculation on 28 August 1970.

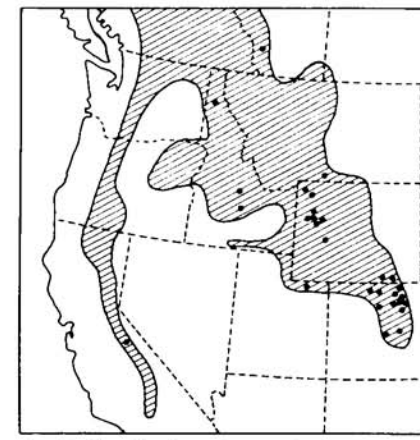

Fig. 3. Distribution of *Arceuthobium americanum* (crosshatched area) and known locations of *Peridermium bethelii* in western North America.

Neither the Colorado greenhouse nor field-inoculated pines showed visible symptoms of the rust during 2 yr of postinoculation observation.

Aeciospore germination. *Agar plates.* No germination was obtained on the water agar, PDA, or water agar/mistletoe media. Some aeciospore germination was obtained on the phosphate-buffered pH series agar plates. The highest germination rate (4.3%) was on plates incubated at 10 C with a pH of 8.0.

Collodion membranes. Germination rates with this technique were more consistent; in the Utah trials, they generally ranged between 10 and 60%, but in the Colorado trials, only 7% maximum was attained. The highest percentage was obtained for refrigerated dry spores dusted onto the membranes and incubated for 40 hr.

Agar-coated microslides. This technique yielded the best germination rates for the Colorado trials but the results were relatively low (0–18.0%). The highest germination was on 2% water agar at room temperature (20–22 C).

Fluorescent dye-treated spores. No germination was obtained with spores treated with the fluorescent brightener Calcofluor M2R, but brightened aeciospores inoculated onto agar-coated microslides and lodgepole pine needles fluoresced brightly when observed under a microscope illuminated with ultraviolet light, which showed they had either absorbed or adsorbed the Calcofluor dye.

P. bethelii aeciospores typically produced more than one germ tube, and one usually became dominant after 48 hr. The germ tubes grew two to eight times the length of the spore and often branched. The germ tubes of *P. bethelii* aeciospores are very similar to those of *C. comandrae*.

Aeciospore morphology and cytology. Aeciospores of *P. bethelii* and *C. comandrae* differ significantly in spore shape and size (Tables 1 and 2, Figs. 4 and 5). In *P. bethelii*, 87.3% of the spores are in "subglobose" classes I and II, compared with 18.2% for *C. comandrae* (Table 1). On the other hand, 75.9% of the *C. comandrae* spores are in the "pyriform" classes III and IV, compared with only 10.0% for *P. bethelii*. A chi-square test indicates the difference in spore shape (groups I and II vs. groups III and IV) between the two rusts is highly significant ($P<0.01$).

Widths of *P. bethelii* and *C. comandrae* spores are about the same but *C. comandrae* spores average about 50% longer (Table 2). Spores of *C. comandrae* are strongly pyriform and teardrop-shaped with distinctly long tails, whereas *P. bethelii* spores are shorter, rounder, and without long tails.

Scanning electron microscope photographs of aeciospores of *P. bethelii* and *C. comandrae* are given in Fig. 6. In both taxa, the wall projections tend to be more pointed near the tail end of the spore and less pointed at the round end and at the center of the spore, a condition previously noted by Hiratsuka (12) for *C. comandrae*. In general, aeciospore surface features of the two taxa are quite similar. Germinating aeciospores appeared to be dikaryotic, but their nuclei were difficult to observe.

Histology. The best staining procedure used was Peterson's (20) modification of Jewell's orseillin-BB-aniline blue. Dwarf mistletoe sinkers and cortical strands stained light blue. Aecia stained a deep red and were several cells deep in the cortex. The rust hyphae and haustoria stained blue. Rust hyphae and haustoria were found among the rays and tracheids in the last one or two growth rings and in dwarf mistletoe sinker tissue.

DISCUSSION

Because inoculation tests with *P. bethelii* were largely negative, the taxonomic status of the rust remains uncertain. The one successful inoculation on dwarf mistletoe-infected pine may be interpreted as a latent field infection; however, because the rust differs from *C. comandrae* in so many respects, we suggest it should be regarded as a distinct taxon. We do not know whether *P. bethelii* is heteroecious or autoecious, but our field observations indicate it is a pine-to-pine rust. Bethel's unpublished notes indicate his inoculations on comandra were successful, but in this study and in those of Hedgcock and Long (9,10), *P. bethelii* did not infect comandra. In addition, inoculations of comandra with typical *C. comandrae* aeciospores, with

Table 1. Summary of shape of *Peridermium bethelii* and *Cronartium comandrae* aeciospores (250 spores per collection)

Rust	Collections (no.)	Spore shape (%) Classes I and II[a]	Classes III and IV	Classes V and VI
Peridermium bethelii	81	87.3	10.0	2.7
Cronartium comandrae	36	18.2	75.9	5.9

[a] Spore shape classes described in Figure 2.

Table 2. Summary of the sizes of *Peridermium bethelii* and *Cronartium comandrae* aeciospores (20 spores per collection)

Rust	Collections (no.)	Spore shape [minimum (mean) maximum] Length (μm)	Width (μm)
Peridermium bethelii	68	23.6 (35.3) 47.1	19.2 (22.0) 29.3
Cronartium comandrae	40	39.4 (54.2) 68.3	19.8 (24.0) 29.3

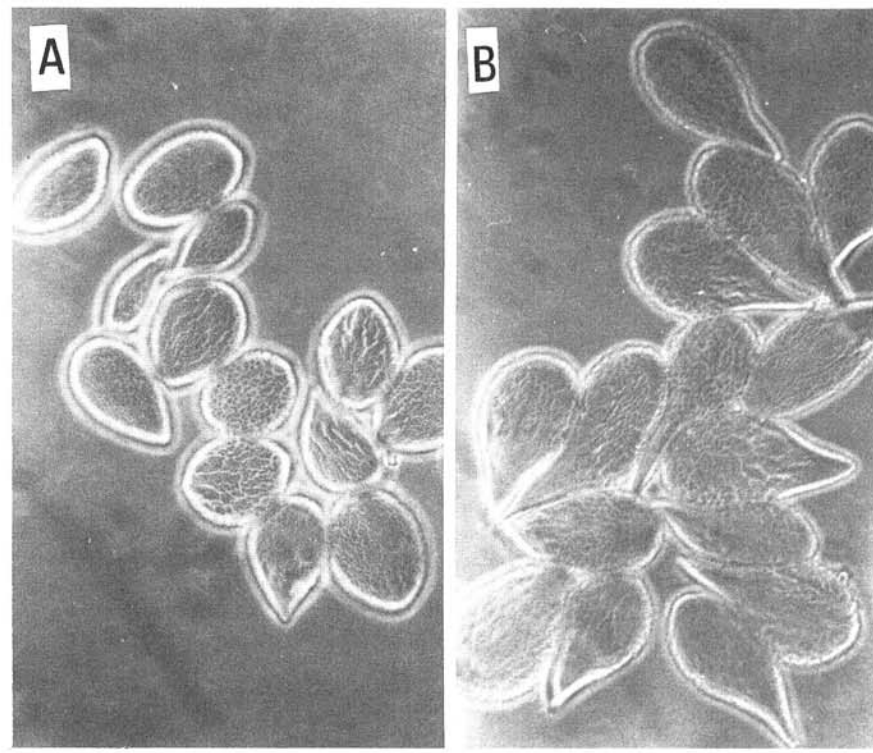

Fig. 4. Rust aeciospores (×400): **(A)** *Peridermium bethelii* and **(B)** *Cronartium comandrae*.

the same techniques and facilities used as in the Utah tests, consistently produced infection. No other potential plant hosts have been discovered. In some lodgepole pine stands where *P. bethelii* occurs, there are no understory plants and the closest known comandra habitats are many miles away. Because of the similar spore morphology of the two rusts, it is possible that *P. bethelii* is an autoecious microcyclic ecotype of *C. comandrae*. Germ tubes of *P. bethelii* are similar to those of *C. comandrae*, however, and do not have features characteristic of other short-cycle rusts (11,21).

Our histological studies confirm Peterson's (19) report of *Cronartium* mycelium parasitizing *Pinus* and *Arceuthobium* tissues simultaneously. Peterson reported the association of *C. comandrae*/*A. americanum* (Colorado, Wyoming, Montana) and *Peridermium filamentosum*/*A. campylopodum* (California). In addition, we have observed but not examined histologically these rust/mistletoe associations: *Endocronartium harknessii*/*A. americanum* (Colorado, Wyoming) and *P. filamentosum*/*A. vaginatum* (Arizona). We believe that these other associations are essentially random and only the *P. bethelii*/*A. americanum* is consistent, and that the "*C. comandrae*" studied by Peterson (19) was probably *P. bethelii*.

Some differences between *P. bethelii* and *C. comandrae* are summarized in Table 3. The distinctive features of *P. bethelii* are its smaller subglobose aeciospores, diminished spermagonial

Table 3. Comparison of *Peridermium bethelii* and *Cronartium comandrae*

Characteristic	*Peridermium bethelii*	*Cronartium comandrae*
Geographic distribution	Rocky Mountains, Colorado to Alberta; California	Widespread in North America
Hosts	*Pinus contorta*, very rare on *P. ponderosa*	At least 12 species of pines
Dwarf mistletoe association	Typically found with *Arceuthobium*	Very rarely associated with *Arceuthobium*
Alternate hosts	Unknown	*Comandra* spp.
Spore stages known	Aecial only	Spermagonial, aecial on *Pinus*; uredinial, telial on *Comandra*
Aeciospore sporulation period	June–October (to January)	May–August
Aeciospore shape	Subglobose, rarely pyriform	Strongly pyriform, rarely subglobose
Aeciospore size	35 × 22 μm	54 × 24 μm
Aeciospore color	Orange	Reddish orange

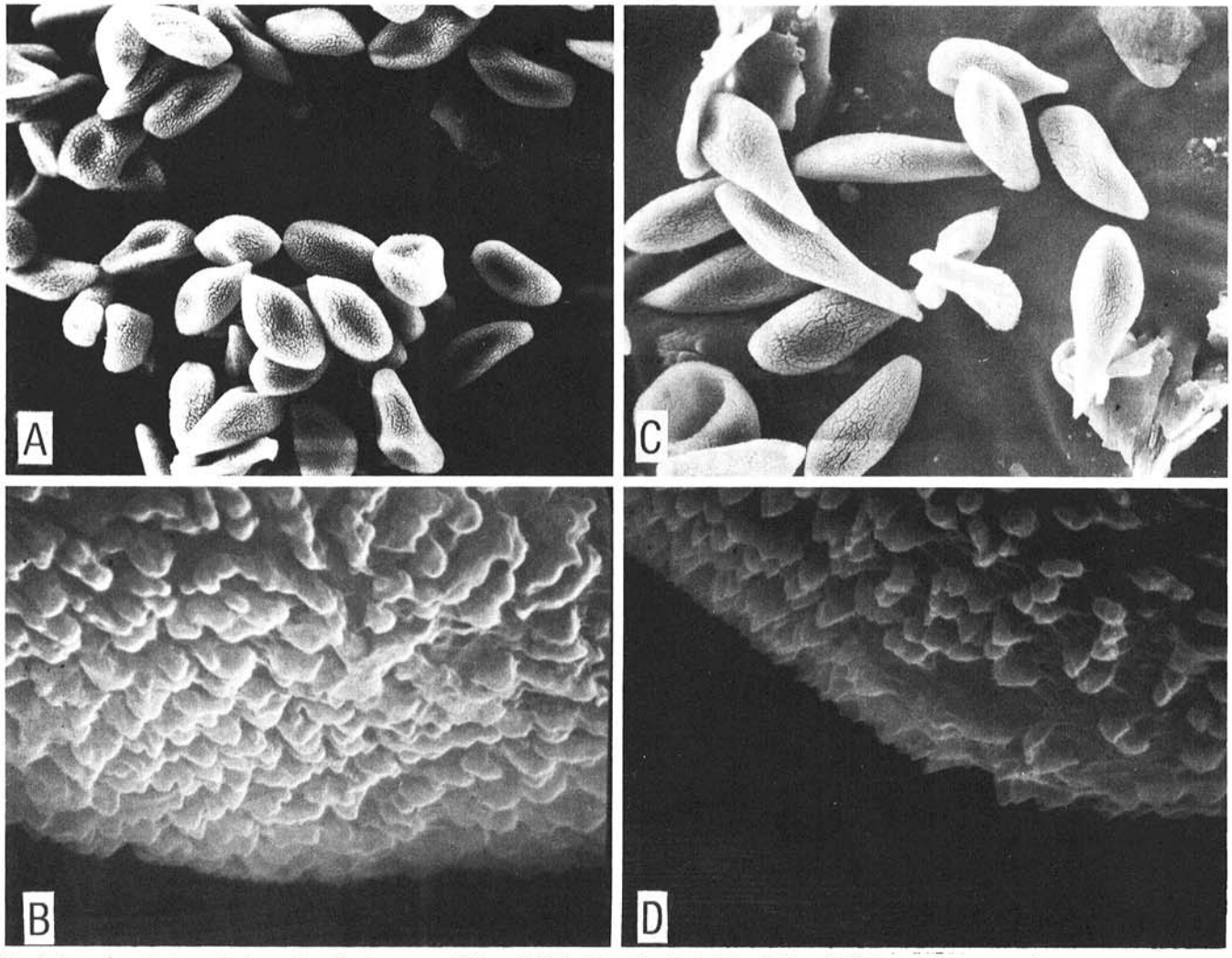

Fig. 5. Scanning electron photographs of aeciospores of **(A and B)** *Peridermium bethelii* and **(C and D)** *Cronartium comandrae*.

stage, later sporulation period, and association with *A. americanum*. The two rusts occur sympatrically in many stands in the Rocky Mountains and the differences noted are also consistent in these areas.

The basis for the association of *P. bethelii* with *Arceuthobium* was not established in this or in Peterson's (19) study. It is interesting that dwarf mistletoes are members of a parasitic family (Viscaceae) that is closely related to the parasitic family (Santalaceae), which contains the genus *Comandra*. It is tempting to conjecture analogous parasitism between *P. bethelii* and dwarf mistletoe, as between *C. comandrae* and comandra. Possibly, the mistletoe shoots or cracks between the shoots and host tissues provide the points of infection for *P. bethelii*.

Further studies are planned to elucidate the taxonomic status of *P. bethelii* and its relationship with *C. comandrae*. These include further inoculations of dwarf mistletoe-infected pines and determination of the nuclear status of germ tubes, mode of infection, and the role of dwarf mistletoe in the *P. bethelii* life cycle.

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