Symptomology on Spruce Trees and Spore Characteristics of a Bud Rust Pathogen

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ABSTRACT

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Spruce bud rust (caused by *Chrysomyxa woroninii*) attacked both the leaf (needle) buds and ovulate strobili of *Picea glauca* and *P. mariana*. The infected needle buds produced extremely stunted shoots with bright yellow-colored needles. Infected cones became most conspicuous when aeciosori began to sporulate. Spermogonia were found exclusively at the apical region of infected needles, and also on the rims of scales on the dorsal side of infected cones. The nucleus was the most prominent feature of the small spermatia. Aeciosori, emerging from the stomatal bands, covered the entire length of the needle except for the apex region. On the cone, blisterlike aeciosori were produced on the dorsal and ventral sides of scales. Aeciosori were covered with a single layer of white, thick-walled, peridial cells. No viable seeds were produced by diseased cones. Aeciospores varied greatly in size and shape and were covered entirely with annular ornamentation. Most aeciospores that germinated produced a simple germ tube, but multiple germ tubes were also common. Germ tubes were non-septate and unbranched, and either anucleate, uninucleate, or multinucleate. There was evidence of secondary cycle infection caused by aeciospores.

Spruce bud rust (*Chrysomyxa woroninii* Tranz.) infects the buds of spruce trees (*Picea* spp.), from which are produced extremely stunted shoots. In Alaska, bud rust has become increasingly important in recent years, and interest increased following an outbreak on white spruce seedlings (*Picea glauca* (Moench) Voss) at the site of a natural regeneration study at Bonanza Creek Experimental Forest in 1977. The discovery in 1979 (15) of the ability of *C. woroninii* to infect ovulate strobili of both white and black spruce (*P. mariana* (Mill.) B.S.P.) further indicated the potential economic importance of this disease.

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Although bud rust was first found in the 19th century, relatively little is known about this disease. The pathogen was first described by Fries (12) in 1824 as *Aecidium coruscans* Fries. In 1904, Tranzschel concluded that *A. coruscans* was the aecial stage of a newly discovered rust on wild rosemary (*Ledum palustre* L.); he based this on his field observation of rusts on *L. palustre* and *P. excelsa* Link (12). Tranzschel called this new rust pathogen *Chrysomyxa woroninii* Tranz. Later, similar observations of the relationship between *A. coruscans* on spruce and *C. woroninii* on *Ledum* sp. were recorded by Savile in his studies on the rusts caused by *Chrysomyxa* in North America (17,18). Repeated attempts to prove experimentally the association between *A. coruscans* and *C. woroninii* have not been successful, but Tranzschel's conclusion is considered valid (17).

The purpose of the current study was to gain a more thorough

Figs. 1–4. Symptoms of bud rust on spruce. **1,** An apical and two lateral shoots of a white spruce seedling displaying typical symptoms of bud rust infection. **2,** Current year's needles of *Picea glauca*. Two needles infected by *Chrysomyxa woroninii* showing large sporulating aeciosori and tiny black scars of spermogonia at the tip (*left*). A needle with secondary infection by *C. woroninii* showing the smaller aeciosori and absence of spermogonia (*right center*). A healthy needle of similar age (*right*). **3,** A branch of *Picea mariana* showing three *C. woroninii*-infected cones (one of them with only part of the cone infected) and one heavily infected apical shoot (S). **4,** Secondary infections of needles on a lateral shoot adjacent to a terminal shoot (S) heavily infected with *C. woroninii*. (Note the reddish-purple pigmentation, swelling of tissues [arrow], small aeciosori, and the absence of spermogonia).

understanding of the symptomology, etiology, and morphology of this rust pathogen on *P. glauca* and *P. mariana*. Morphological characteristics of aeciospores of the bud rust fungus collected in interior Alaska and the aeciospores of *C. woroninii* collected by Tranzschel (in Russia) and Savile (in N. America) were compared to confirm the conspecificity of these collections. The unique ability of this fungus to affect only the leaf (needle) bud and female flower (cone) bud of spruce trees indicates that "spruce bud rust" would be a logical common name. Part of the results has been published elsewhere (13–15).

MATERIALS AND METHODS

Field observations. Samples were collected from infected and healthy trees in the Bonanza Creek Experimental Forest and in the Wickersham Dome area near Fairbanks, in interior Alaska. Spruce trees showing symptoms of infection were marked in early spring (May). The number of infected buds and their location on each tree were recorded. The development of symptoms on spruce trees (*P. glauca* and *P. mariana*) was closely followed during the entire growing season. The following spring, infections on marked trees were checked, and the sites of infection were compared with the previous year's record.

Light and scanning electron microscopy. From late May through late July, diseased and healthy samples of buds, shoots, and cones were collected weekly and examined with a stereoscopic microscope for gross morphological changes. Spermatia and aeciospores of *C. woroninii* were collected from diseased tissues and examined with a phase-contrast microscope. The shape and size of these spores were recorded and measured.

Detailed external morphology of spermatia and aeciospores was determined using a JEOL JEM 35 scanning electron microscope. For spermatia, spruce tissues that contained actively sporulating spermogonia were excised and fixed in 2.5% glutaraldehyde in 0.1 M sodium phosphate buffered saline, pH 7.3, for 30 min. After rinsing in 0.1 M sodium phosphate buffered saline, with two changes at 20-min intervals, the tissues were dehydrated in a gradient alcohol series (25, 50, 75, 95% for 30 min each, and absolute alcohol, three changes at 10 minutes each). They were then dried in a Bomar SPC-900 Critical-Point Dryer (The Bomar Co., Tacoma, WA 98401), mounted on stubs, sputter-coated with gold-platinum alloy in a model 12121 SPI Sputter Coater (Structure Probe, Inc., Westchester, PA 19380), and examined under the scanning electron microscope.

Aeciospores from actively sporulating aeciosori were dusted onto a piece of 3-M double-stick tape attached to a stub, sputter-coated with gold-platinum alloy, and examined under the scanning electron microscope.

Collections of *C. woroninii* made by Tranzschel and Savile were obtained from the National Fungus Collection and examined under phase-contrast and scanning electron microscopy. Comparisons of the size and shape of the aeciospores and ornamentation of the spore surface were made with samples collected from the field. Collections examined included US0101438 on *P. excelsa* collected by W. Tranzschel in 1893; US0141039 on *P. glauca*, collected by D. B. O. Savile in 1949; US0141040 on *P. glauca*, collected by D. B. O. Savile and J. Vaillancourt in 1951; and US0141042 on *P. mariana*, collected by D. B. O. Savile in 1951.

RESULTS

Symptoms on needle buds. The symptoms of bud rust on *P. glauca* and *P. mariana* were very similar. *Chrysomyxa woroninii* attacked apical buds primarily, but occasionally also one or two lateral buds (Fig. 1). Generally, all needles on shoots arising from rust infected buds showed signs of the fungus, although some shoots showed infection of only some of the needles. The pathogen did not appear to be perennial—infected buds were scattered randomly on the branches, and it was uncommon to find buds infected by *C. woroninii* on branches that had been infected the previous year.

The period of infection of *C. woroninii* on spruce trees is not completely understood. The fact that some unopened buds were infected and destroyed indicates that infection occurred at a very early stage of bud development.

Symptoms of infection by *C. woroninii* became clearly visible in mid- to late May, when the needle buds of spruce trees started to unfold. Normal shoots had green needles, but infected shoots had bright-yellow needles and were severely stunted. Small, papillate, yellowish spermogonia were found exclusively at the apical region of the needles. In late May mature spermogonia started to sporulate. Sporulation was accompanied by the secretion of an aromatic, viscous honeydew. The spermogonia usually remained active for ~5–7 days and then gradually shriveled into tiny black scars (Fig. 2).

By early June, four large aeciosori—each covering the entire length of the needles except for the apical region—emerged from the four stomatal bands (Fig. 2). Aeciosori of *C. woroninii* conformed to the peridermioid type described by Cummins (6). The blisterlike aecia were covered with a peridium composed of a single cell layer of thick-walled, white, peridial cells. As the aeciosori matured, ruptures occurred randomly on the peridium and numerous bright yellow aeciospores were released (Fig. 2). The aeciosori remained active until mid- to late July, when the infected needles and shoots gradually became dehydrated and turned dark brown. Infected buds remained on the branches for several years before disintegrating.

One or two of the lateral buds adjacent to an infected apical bud frequently produced lateral shoots. Many of the lateral shoots appeared healthy and had dark green, normal-sized needles. However, some of the lateral shoots were small and twisted with pale green, thin needles. Occasionally, more than one of these abnormal shoots was produced by a lateral bud and gave the diseased branch the appearance of a small witches' broom. In the fall, needles on the abnormal-looking lateral shoots and, occasionally, those on apparently normal lateral shoots turned yellow and fell from the tree.

Symptoms on ovulate strobili. Infection by *C. woroninii* was found on ovulate strobili (Fig. 3) but not on male strobili. Although in late May the diseased cones possessed a somewhat shiny, wet look due to the actively sporulating spermogonia, infection on cones was not readily detectable from afar until the aeciosori emerged in June.

Tiny, papillate spermogonia of *C. woroninii* on the diseased cones formed primarily at the rims of the scales on the dorsal (outer) side (Fig. 5). Aeciosori of *C. woroninii* were found on both dorsal and ventral (inner) sides of the scale (Fig. 6); they were round, blisterlike structures covered with a discrete white peridium, of one cell layer thickness, beneath the cuticular tissues of the cone scale. As matured aeciosori emerged, the cuticle and peridium above the sori were pushed outward and became detached, exposing the aeciosori (Figs. 5 and 6). Massive amounts of mature, powdery, bright yellow aeciospores were released. The bright colored aeciospores and aeciosori made the diseased cones very conspicuous (Fig. 3). No viable seeds were produced in infected spruce cones.

Secondary infection. Generally, aeciosori and other signs of infection were found only on shoots and cones arising from diseased buds. Other parts of the trees appeared to be free of the disease. Various authors have thought that *C. woroninii* is heteroecious (10,15,16) and that reinfection of spruce by aeciospores does not occur. However, observations first made in 1979 and repeated several times since suggest that aeciospores may be capable of infecting spruce. On apparently healthy shoots (lateral shoots mostly) adjacent to heavily infected shoots, needles in close proximity to diseased needles were those later found infected while the rest of the needles on the lateral shoots remained free of disease symptoms. The new infections were characterized by reddish-purple discoloration and occasional swelling (Fig. 4). Aecial sori, covered by white peridia, soon appeared along the stomatal region of these needles. Sori of the later infection were smaller than the large sori formed earlier in the growing season (Figs. 2 and 4). Often a number of sori formed on one stomatal band (Fig. 4). Spores and peridial cells taken from the sori of the later infection were morphologically indistinguishable from aeciospores and peridial cells from the earlier infection.

Generally, spermogonia were not formed on needles from the later infection (Figs. 2 and 4). The very few spermogonia formed were either sterile or sporulated at approximately the same time as aeciosori.

Spermatia. Spermatia of *C. woroninii* produced from spermogonia at the apex of the needle (Fig. 7) were hyaline to slightly yellow, oval, ovate, or ellipsoid (Fig. 8). The size ranged from 1.5 to 2.0-μm wide by 2.0 to 3.8-μm. The nucleus was the most prominent feature in the spermatia, and occupied most of the cellular space (1).

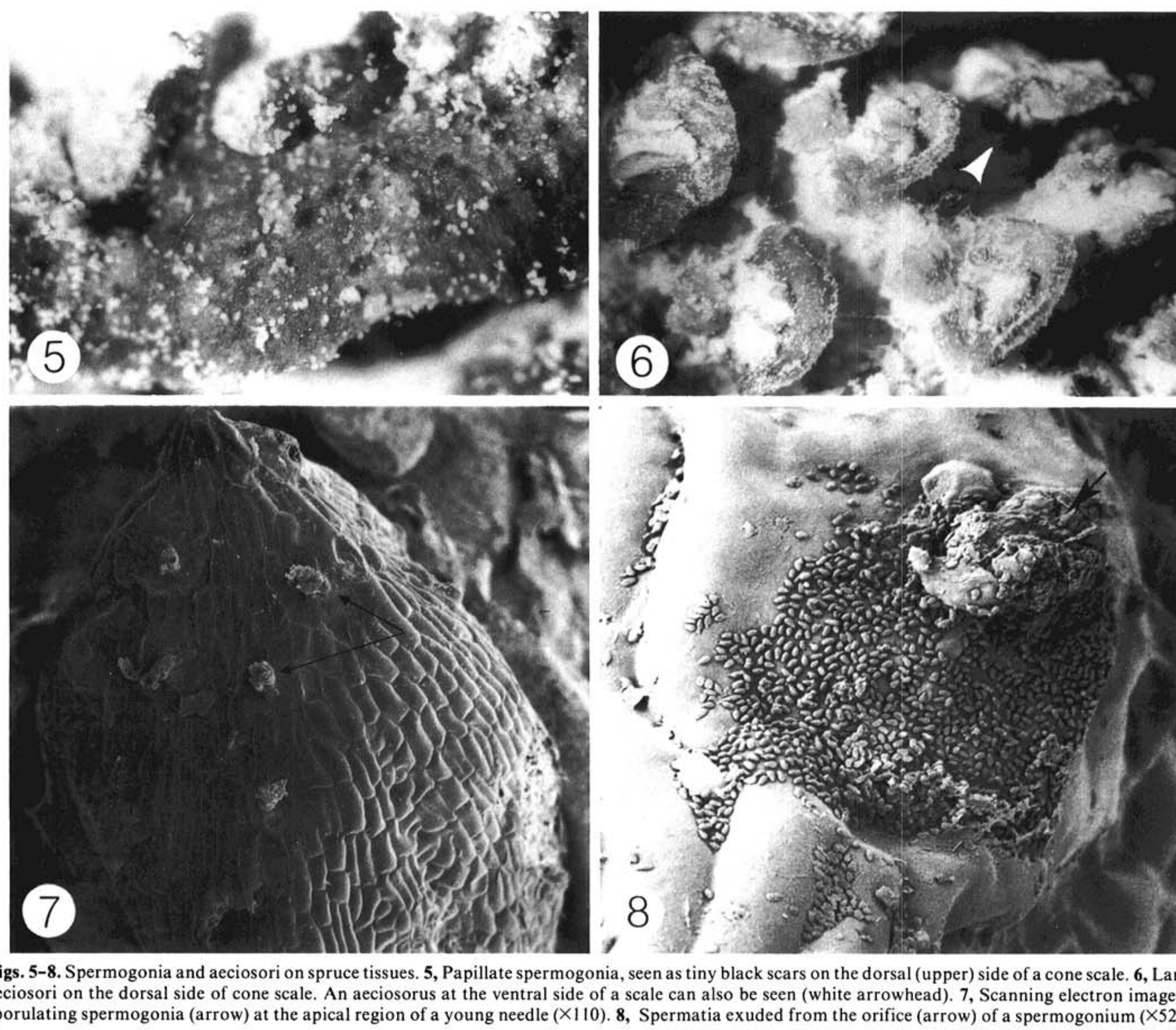
Aeciospores. Aeciospores of the pathogen were bright yellow and globose or oval to elliptical. Some aeciospores were pyriform, obclavate, or cylindrical (Fig. 9), and a few were fusiform, ventricose, or clavate. The size ranged from 15 to 36 μm in width and 26 to 72 μm in length. Giant aeciospores, ranging from 38–55 μm in width to 86–136 μm in length and ventricose or clavate in shape, were observed occasionally. The great variation in the size and shape of the aeciospores seemed to be a distinctive characteristic of this fungus—a feature that was also observed by Kuprevich and Tranzschel (12) and Savile (18).

The entire surface of the aeciospore was covered with annulated processes (Fig. 10). The attachment of these processes to the aeciospore wall did not appear to be firm. On degenerating aeciospores, patches of smooth, denuded wall area were often found. The annulated processes resembled those of *Coleosporium* spp. (8,11), *Cronartium* spp. (10), and *Endocronartium harknessii* (10). The processes were cylindrical and 1.0 to 2.0 μm high. A stack of four to five disks could be detected on these processes. The spacing between processes ranged between 0.3 and 0.8 μm. On giant aeciospores, the processes were shorter (0.7 μm high) and farther apart (1.0 to 2.0 μm) (Fig. 11).

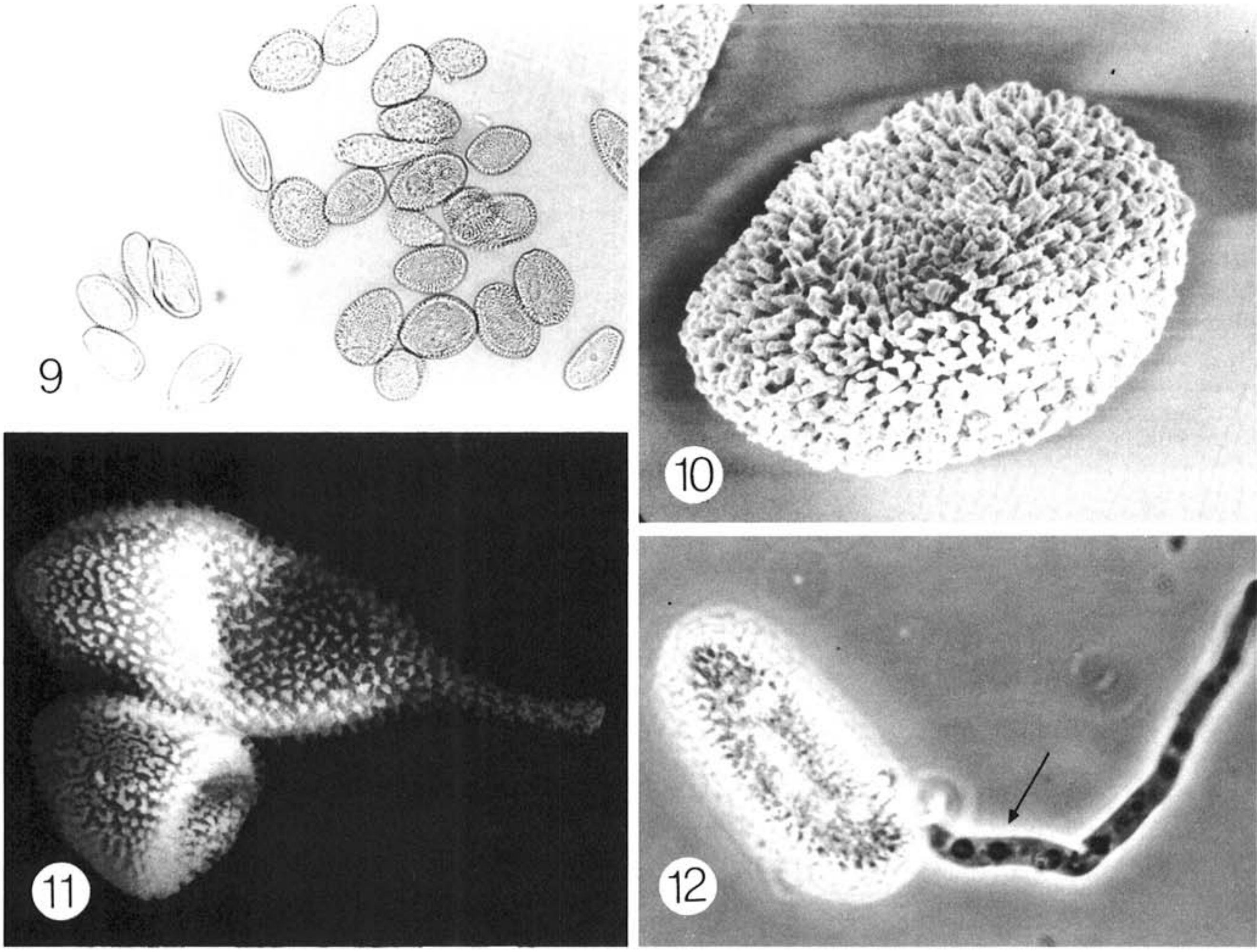
One characteristic of *C. woroninii* not reported for other *Chrysomyxa* spp. was the high frequency of aeciospores germinating prior to dissemination. Evidence of aeciospore germination was also found in specimens collected by Savile or Tranzschel. Most spores formed a single germ tube (Fig. 12), but spores with two or three germ tubes were also observed (Fig. 13). Germ tubes were anucleate, uninucleate or multinucleate (up to 12 nuclei). The tube was simple, nonseptate, and mostly unbranched (Fig. 12). Basidiosporelike or sterigmatalike structures were not found. The diameter of these germ tubes (4.2 μm in average) was similar to the diameter of intercellular hyphae (2.4 to 6.4 μm). Nuclear division was often observed in germ tubes. A structure resembling an appressorium formed at the tip of a germ tube (Fig. 14).

No morphological differences could be detected among aeciospores collected by Tranzschel, Savile, and the author. Aeciospore size varied among the specimens which were examined, but these differences are probably not significant.

Peridium. The peridium enveloping the aeciosorus was



Figs. 5-8. Spermogonia and aeciosori on spruce tissues. **5,** Papillate spermogonia, seen as tiny black scars on the dorsal (upper) side of a cone scale. **6,** Large aeciosori on the dorsal side of cone scale. An aeciosorus at the ventral side of a scale can also be seen (white arrowhead). **7,** Scanning electron image of sporulating spermogonia (arrow) at the apical region of a young needle (×110). **8,** Spermatia exuded from the orifice (arrow) of a spermogonium (×520).



Figs. 9-12. Morphology and characteristics of aeciospores of spruce bud rust. **9,** Aeciospores of *Chrysomyxa woroninii*. Note the variation in size and shape (×270). **10,** A scanning electron image of an aeciospore with annulated processes on the surface (×2,940). **11,** A scanning electron image of a giant clavate-shaped and a small aeciospore (×1,380). **12,** A germinating aeciospore with a nonseptate, unbranched germ tube. Approximately 10 nuclei (arrow) can be seen in the germ tube (×1,000).

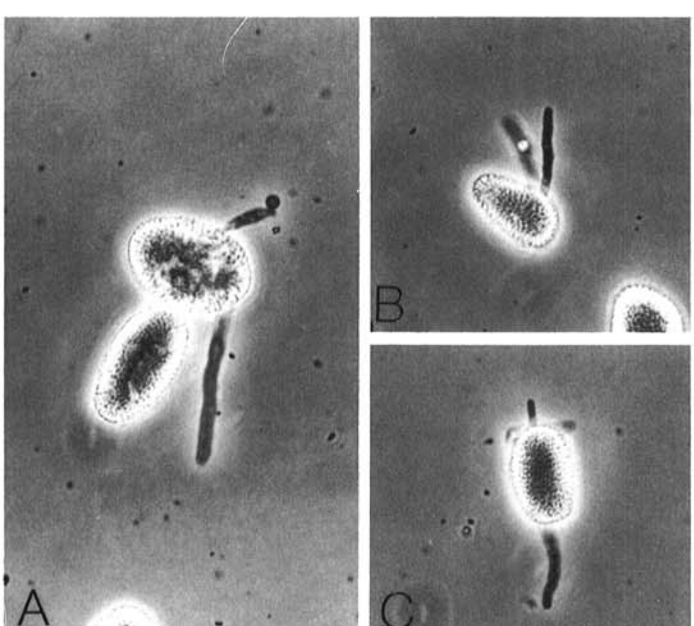


Fig. 13. Aeciospores of *Chrysomyxa woroninii* with multiple germ tubes. **A,** Two germ tubes originated from different sides of an aeciospore. **B,** A spore with two adjacent germ tubes. **C,** A spore with three germ tubes, two originating from the polar region and one from the side of the spore (shown as bended). (×400).

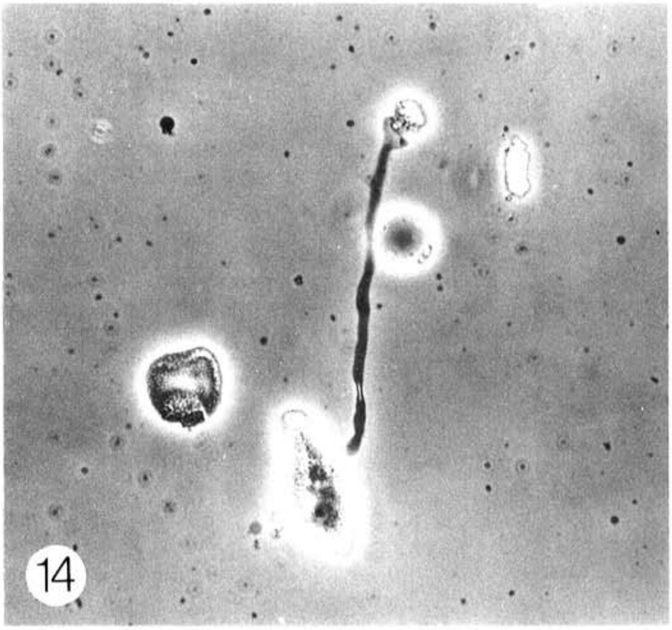
composed of a single layer of thick-walled, white peridial cells (Fig. 15). The shape of the peridial cells varied, but pentagonal and hexagonal shapes were most common. As with many rust fungi, peridial cells of *C. woroninii* also possessed two types of surface markings—a clavae-covered ventral surface (facing the aeciospores) and a compact rugose, dorsal (outer) surface. The different structures of the outer and inner peridial wall surface and their apparently different responses to humidity changes may facilitate rupture of the peridium.

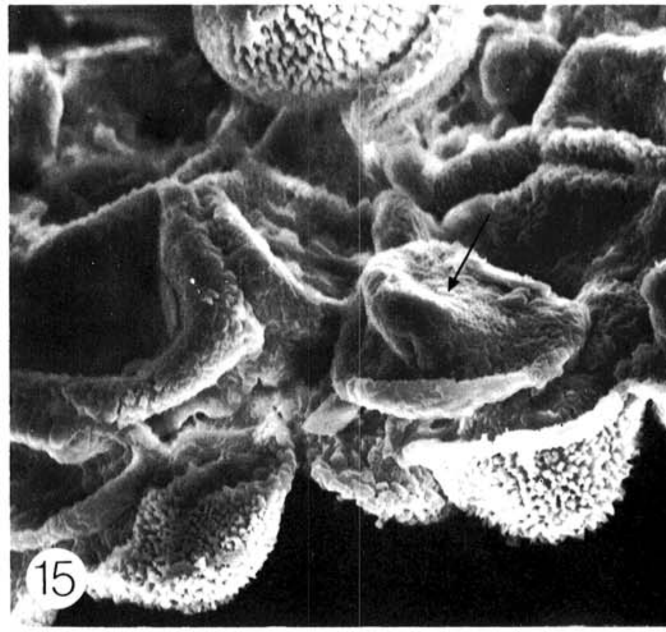
DISCUSSION

Spruce bud rust is a disease of the boreal forest, and had been found in Canada (Alberta (4), British Columbia (4,18), New Foundland (4,18), Northwest Territory (4), Quebec (17,18), Yukon Territory (4,7,18)); China (12); India (19); Japan (19); Sweden (17); European USSR and Siberia (3,12). In Alaska, infected trees were found mostly in the interior (15,18). *C. woroninii* was collected from *L. decumbens* at the Kenai Peninsula, southcentral Alaska, but spruce bud rust infection on spruce trees was not reported (18).

C. woroninii appears to be pathogenic on a wide range of spruce species, including: *P. ajanensis* (3); *P. excelsa* (12), *P. obovata* (12), *P. glauca* (4,7,15,17,20), *P. likiangesis* (12), and *P. mariana* (4,7,17,20).

Infection of spruce bud rust of up to 70% of the new shoots was occasionally observed (4), but generally infection was light and occurred principally on saplings or older trees. Thus, the disease is considered of little economic significance. The discovery of white spruce seedlings infected heavily and stunted by *C. woroninii*





Figs. 14–15. *Chrysomyxa woroninii*: **14,** An appressoriumlike structure at the tip of a germ tube (×400); **15,** Peridium. Note the two types of peridial cell wall surface (the compact rugose dorsal surface (arrow) and the fine clavae-covered ventral surface (×1,240).

indicates that spruce bud rust has a potential to become an important disease. Furthermore, infection of ovulate strobili resulted in the impairment of seed development and production. In this study, infection of spruces at Wickersham by *C. woroninii* was found primarily in areas near the treeline. At the treeline, an adequate seed source is of utmost importance for regeneration. Yearly occurrence of this rust on cone buds—even of low intensity—could have a serious impact on the ecosystem.

Autoecism has been well documented in several pine rusts (9,16). *Endocronartium harknessii* (9) and a short-germ-tube race of *Peridermium filamentosum* (5,16) are all capable of transmitting disease from pine to pine by aeciospores without the benefit of an alternate telial host. On spruce, autoecism of microcyclic species like *C. abietis* and *C. weirii* (2,20) is known. However, the life cycle of *C. woroninii* was believed to alternate between *Picea* (aecial host) and *Ledum* (telial host) (12,17,18). The apparent ability of *C. woroninii* aeciospores to infect spruce, observed repeatedly by the author, strongly suggests a possible alternative means in the completion of the life cycle of this rust. Studies involving inoculation of healthy spruce with aeciospores will be needed to provide additional information on the life cycle of this pathogen.

Comparisons of aeciospore germ tubes of *C. woroninii* with those produced by certain pine rusts revealed the close resemblance between *C. woroninii* and the autoecious race of *P. filamentosum* with short germ tubes (5,16). Aeciospores of *C. woroninii* and the autoecious *P. filamentosum* produced multiple germ tubes, although a single germ tube was most common. The large numbers of nuclei in the germ tube (up to 16 nuclei in *P. filamentosum* and up to 12 in *C. woroninii*) and the nonseptate, unbranching nature of the germ tubes were similar in both rusts. The absence of septation in the germ tube as well as the lack of sterigmatalike structures seemed to distinguish *C. woroninii* from endo-type rusts such as *E. harknessii* (5,9). Extensive branching of germ tubes, found in some of the heteroecious pine rusts (9), was not observed in *C. woroninii*. Other features of the cytology of aeciospores of bud rust, especially the nuclear processes during germination, await further investigation.

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