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Caloscypha fulgens (Ascomycetidae, Pezizales): The Perfect State of the Conifer Seed Pathogen Geniculodendron pyriforme (Deuteromycotina, Hyphomycetes)

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Caloscypha fulgens (Ascomycetidae, Pezizales): the perfect state of the conifer seed pathogen *Geniculodendron pyriforme* (Deuteromycotina, Hyphomycetes)

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Results of comparative cultural, conidiophore and conidia morphology, growth–temperature, and pathogenicity studies proved that *Geniculodendron pyriforme* Salt is the imperfect state of *Caloscypha fulgens* (Pers.) Boudier. The results are discussed in relation to the etiology and ecology of the disease of conifer seeds caused by the fungus.

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Les auteurs concluent que *Geniculodendron pyriforme* Salt est le stade imparfait de *Caloscypha fulgens* (Pers.) Boudier, après des études culturelles comparatives, de morphologie des conidiophores et conidies, de croissance, température et pathogénicité. Ils discutent des résultats par rapport à l'étiologie et l'écologie de la maladie des graines de résineaux que cause ce Champignon.

Introduction

In 1974, Salt (7) described the psychrophilic fungus *Geniculodendron pyriforme* (Deuteromycotina, Hyphomycetes) from Sitka spruce, *Picea sitchensis* (Bong.) Carr., seeds imported into Britain from western North America. Earlier, Epners (4) had isolated this seed-borne fungus from fall-sown conifer seeds which had failed to germinate in Ontario forest nurseries. The studies by Epners and Salt determined fungus pathogenicity, host range, and optimum media and temperature for fungus growth. These studies also showed that the fungus is unable to infect seeds once germination has begun, but it spreads and kills dormant seeds during stratification (cold treatment) and in cool, moist seedbeds. Recently, *G. pyriforme* was found in stored Sitka spruce seeds in British Columbia (B.C.) and it was shown that infected seeds occur only in cones collected from the ground or from squirrel caches (9).

In September 1976, one of us (J.W.P.) examined some of the B.C. isolates of *G. pyriforme* and recognized the fungus as the imperfect state of *Caloscypha fulgens* (Pers.) Boudier (Ascomycetidae, Pezizales). *Caloscypha fulgens* is a large discomycete, up to 4 cm in diameter (broad), sessile, occasionally split down one side, hymenium bright orange, exterior darker orange with a somewhat evident olive-green band at the margin. Apothecia occur in coniferous forests on litter in early spring or following snow melt. Ginns (5) provides a description of *C. fulgens* and its distribution and ecology in Canada. The purpose of this study was to demonstrate that *G. pyriforme* is the imperfect state of *C. fulgens*.

Materials and Methods

Sources of Geniculodendron pyriforme and Caloscypha fulgens Isolates

The six fungus isolates (designated *i* to *vi*) studied were as follows: (*i*) isolated May 1977 from stored Sitka spruce seed, collected at Knight Inlet, B.C.; (*ii*) isolated May 1977 from stored white spruce, *P. glauca* (Moench) Voss seed, collected at Babine Lake, B.C.; (*iii*) Salt's (7) isolate T387 which had been isolated from Sitka spruce seeds exported from coastal B.C.; (*iv*) grown from ascospores from a *C. fulgens* ascocarp collected March 1968 at the University of Victoria campus, Victoria, B.C.; (*v*) grown from ascospores from a *C. fulgens* ascocarp collected July 1976 in the Olympic National Park, Washington; and (*vi*) grown from ascospores from a *C. fulgens* ascocarp collected May 1970 at Naden Harbor, Graham Island (Queen Charlotte Islands). Stock cultures of these fungi were maintained on either potato–dextrose agar, PDA (1), or carrot–potato agar, CPA (2).

Cultural Characteristics, Morphology of Conidiophores, and Size of Conidia of Geniculodendron pyriforme and Caloscypha fulgens Isolates

To compare colony morphology, the isolates were grown on PDA in petri dishes for 25 days at 20°C. Asexual sporulation of the isolates was obtained by growing them at 20°C on pieces of

TABLE 1. Comparison of *Geniculodendron pyriforme* and *Caloscypha fulgens* cultures, conidiophores, conidia, and hyphae

Characteristics observed	*G. pyriforme*	*C. fulgens*
Cultures*	Surface: texture loose to low cottony; color varying from solid beige or whitish gray to pale yellowish brown with lighter lines radiating from center to margin; margin definite, even or finely serrated. Reverse: color varying from yellowish with bluish patches to solid pale yellowish orange or with slight greenish center and remainder whitish; no diffusion zone	Surface: texture loose cottony, sometimes tufted; color orange in the center and fading to hyaline at the margin or multicolored (blue–green–grayish) throughout; margin somewhat diffuse or uneven. Reverse: pale orange or blue-green; center fading to cream at margin; no diffusion zone
Conidiophores, conidia, and hyphae†	Conidiophores: from aerial hyphae 150–450 μm high; smooth or verrucose below, smooth above; hyaline to pale yellow–brown below, hyaline above; 8–12 μm broad below, tapering to 4–5 μm above; unbranched up to 250 μm, then more or less dichotomously branched	Conidiophores: from aerial hyphae, 200–550 μm high; smooth pale yellow to yellow–brown below; in diameter 8–17 μm broad below, tapering to 3.6–6 μm above, unbranched up to 220 μm, then more or less dichotomously branched; hyaline above
	Conidiogenous cells: sympodulae; in verticils of three or four or paired, rarely single; 34–48 μm long; 3.2–4.4 μm broad below, tapering to 2–2.7 μm	Conidigenous cells: sympodulae; in verticils of three or four, or paired, rarely single; 16–50 μm long; 3.2–5.2 μm broad below; tapering to 2.4–8 μm
	Conidia: 4.3–6.4 × 3.1–4.0 μm; holoblastic; smooth, hyaline obovate, dry	Conidia: 4.6–7.6 × 3.2–4.0 μm; holoblastic; smooth, hyaline, obovate, dry
	Hyphae: hyaline when young; older hyphae with greenish–blue tinge, granular, right angle branching, septum near base of each branch, cells 55–350 × 5–115 μm	Hyphae: hyaline when young; older hyphae with greenish–blue or brownish tinge, granular, right angle branching, septum near base of each branch, cells 71–190 × 10–18 μm

*Based on *G. pyriforme* isolates *i* to *iii* and *C. fulgens* isolates *iv* and *v*; isolate origins in Materials and Methods. All cultures dark grown on PDA for 25 days at 20°C.

†Based on *G. pyriforme* isolates *i* to *ii* and *C. fulgens* isolates *iv* to *vi*; isolate origins in Materials and Methods. Conidiophore and conidia data from *G. pyriforme* culture growing from Sitka spruce seeds on 2% water agar and for *C. fulgens* isolates growing on potato–carrot agar. Hyphal characteristics of all isolates from 45-day-old cultures on PDA at 20°C.

Sitka spruce seeds incubated on 2% water agar in petri dishes or deep-well slides or on CPA in petri dishes. The morphology and sizes of conidiophores and conidia were determined with the light microscope (12.5–1250 ×).

Growth of Geniculodendron pyriforme and Caloscypha fulgens Isolates at Different Temperatures

Growth of fungus isolates *i* to *v* was compared at 1, 5, 10, 15, 20, 25, and 30°C. Ten petri dishes (containing PDA) for each isolate and temperature were center inoculated with a 4-mm disk cut from the margin of young fungus cultures. Colony diameters were measured (the mean of two measurements at right angles less original inoculum diameter) weekly for 3 weeks. These procedures are the same as Salt (7) used to determine the optimum temperature for *G. pyriforme* growth. The data were subjected to analysis of variance and the means were compared, using the Student–Newman–Keuls' test (8).

Pathogenicity of Geniculodendron pyriforme and Caloscypha fulgens Isolates to Spruce Seeds

Pathogenicity of fungus isolates *i* to *v* to *Geniculodendron*-free Sitka (92% germination capacity) and white spruce seeds (90% germination capacity) seeds was determined, using Salt's (7) procedure wherein seeds are sown in sand, in petri dishes,

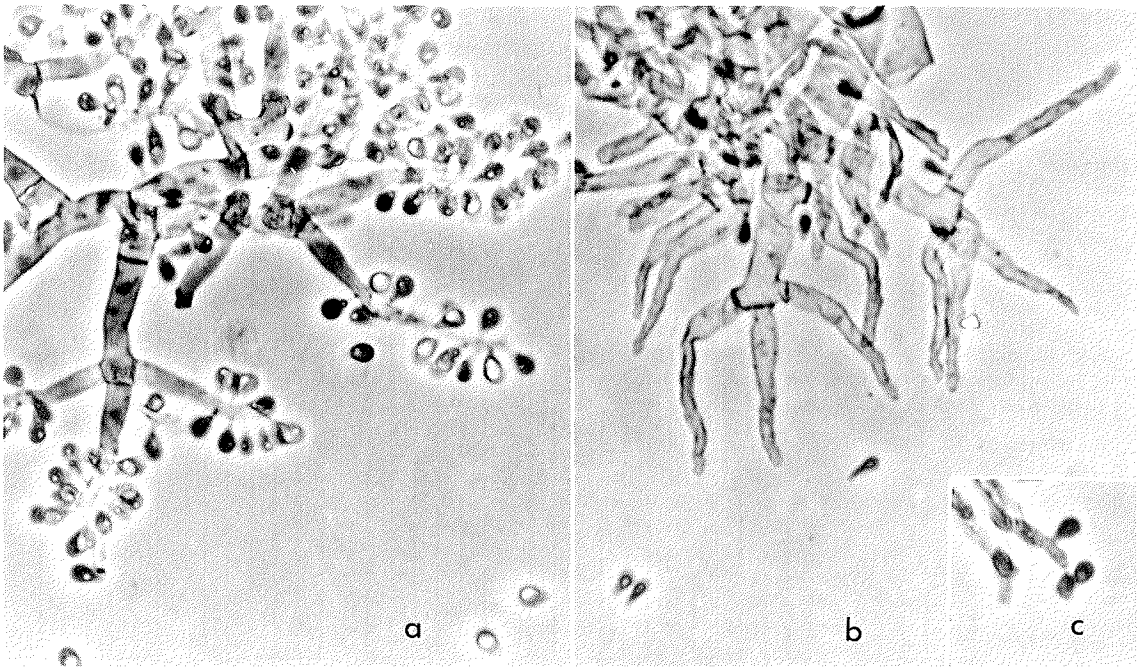

FIG. 1. Conidiophores and conidia of *Geniculodendron pyriforme* and *Caloscypha fulgens* isolates. (*a*) *G. pyriforme* (isolate *ii*) conidiophores and conidia. (*b*) *C. fulgens* (isolate *v*) conidiophores (*c*) *C. fulgens* (isolate *v*) conidia.

inoculated with the fungus, and then held at 10°C for 1, 2, or 4 weeks before being germinated. Salt (7) used 20°C for germination, whereas we used 20°C for 16 h (no light) and 30°C for 8 h (2260 lx). Seeds which failed to germinate were plated on 2% water agar and incubated at 20°C for isolation of *G. pyriforme*. The data were subjected to analysis of variance and the means compared, using the Student–Newman–Keuls' test (8).

Results and Discussion

The texture and pigmentation of *G. pyriforme* and *C. fulgens* cultures were very similar (Table 1). Although the pattern varied, isolates always produced some bluish-green pigment. The pigment intensified when the isolates were grown on water agar, a nutrient-deficient medium; Epners (4) found that his *G. pyriforme* cultures produced more blue–green pigment as the sugar content of the culture medium decreased. In all ways, the cultural and mycelial characteristics of our *G. pyriforme* and *C. fulgens* isolates (Table 1) agree with one another and with those characteristics given by Epners (4) and Salt (7) for *G. pyriforme*. The morphological and morphometric characteristics of the conidiophores, conidia, and hyphae of the *G. pyriforme* and *C. fulgens* isolates are essentially identical (Table 1, Fig. 1). These characteristics also agree with those given by Salt (7) for *G. pyriforme* and with Paden's (6) 1972 illustration (Plate 20, Fig. 3) of the imperfect stage of *C. fulgens*.

Isolates of both *G. pyriforme* and *C. fulgens* grew at temperatures between 1 and 25°C (Fig. 2); no growth occurred at 30°C. After 21 days on PDA at 1, 5, 10, 15, 20, and 25°C, mean colony diameters were 6, 11, 24, 42, 47, and 41 mm, all significantly ($P = 0.05$) different from one another; thus the minimum, maximum, and cardinal growth temperatures of our isolates were the same as those reported for *G. pyriforme* by Epners (4) and Salt (7). Within specific temperatures, growth rates of the various isolates differed significantly, but overall, *G. pyriforme* and *C. fulgens* isolates were indistinguishable.

Table 2 shows that none of the fungus isolates were pathogenic to white spruce seeds; however, one isolate each of *G. pyriforme* (number *i*) and *C. fulgens* (number *v*) caused significant preemergence losses of Sitka spruce seeds. These isolates were recovered from about two-thirds of the killed seeds. Lack of pathogenicity to white spruce is explained by the fact that these seeds broke dormancy and germinated much quicker than did the Sitka spruce seeds; i.e., 75% of the white spruce seeds germinated after 7 days at 20°C, whereas the Sitka spruce required 11 days to reach the same germination capacity. Indeed, some of the white spruce seeds even germinated during the 10°C pregermination treatment period. Since *G. pyriforme* only attacks dormant seeds (4, 7), any inherent or

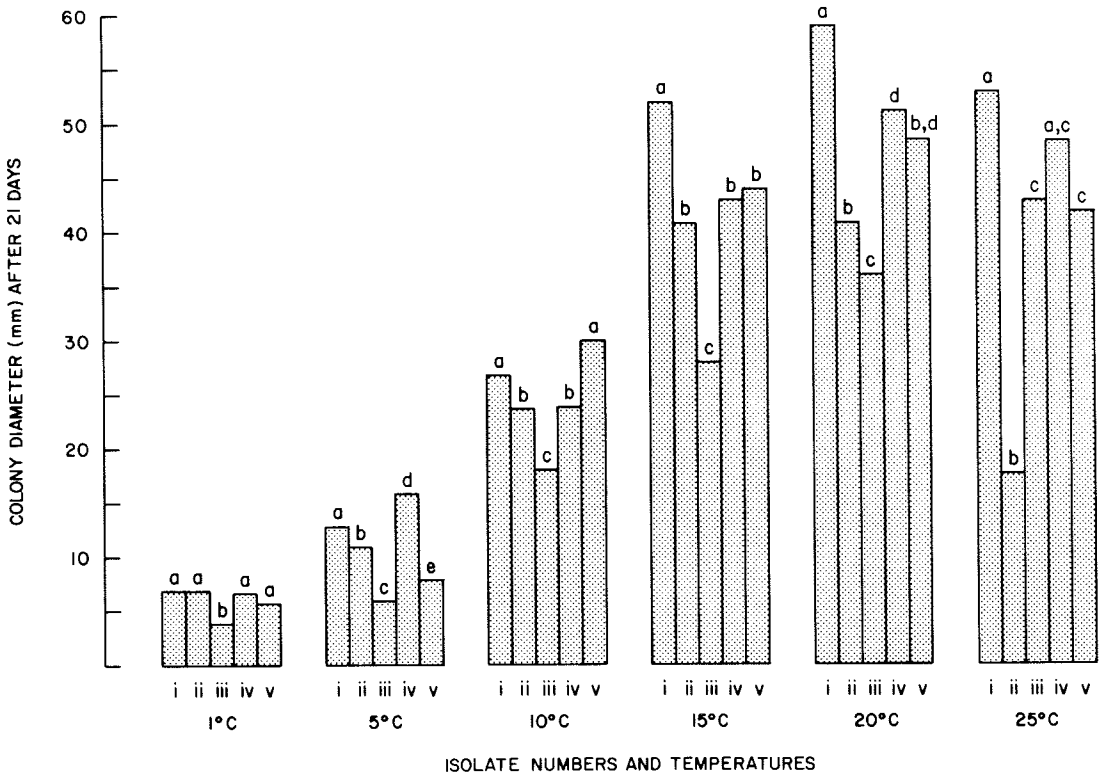

FIG. 2. Comparative growth of *Geniculodendron pyriforme* (isolates *i*, *ii*, and *iii*) and *Caloscypha fulgens* (isolates *iv* and *v*) cultures at different temperatures. No isolates grew at 30°C. Colony diameters are means of 10 replicates; within temperatures, means (bars) topped with the same letter are not significantly different ($P = 0.05$).

TABLE 2. Pathogenicity of *Geniculodendron pyriforme* and *Caloscypha fulgens* isolates to white and Sitka spruce seeds*

	Seed species and germination, %‡		Seed species and % killed seeds yielding fungus‡	
Treatments isolate†	White spruce	Sitka spruce	White spruce	Sitka spruce
i	82*a*	42*a*	15*ab*	69*a*
ii	88*a*	82*b*	0*a*	19*bc*
iii	86*a*	85*b*	0*a*	35*c*
iv	89*a*	91*b*	0*a*	0*b*
v	89*a*	66*c*	19*b*	66*a*
Control	84*a*	91*b*	0*a*	0*b*

*Data are for inoculated seeds kept at 10°C for 4 weeks before being germinated.
†Isolates *i* to *iii* were *G. pyriforme* from Sitka spruce, white spruce, and red pine, Salts (6) T387 isolate, seeds, respectively. Isolates *iv* and *v* were from *C. fulgens* ascospores. The control received a fungus-free agar plug.
‡Values are means of 10 replicates (50 seeds each); reading down, within seed species, means followed by the same letter do not differ significantly ($P = 0.05$).

environmental factor(s) that increases germination rate will decrease seed losses.

Results of our comparative morphological (e.g. cultures and conidiophore), growth–temperature, and pathogenicity studies provide conclusive evidence that *G. pyriforme* is the imperfect state of *C. fulgens*. Habitats and distribution of the two fungi are also identical (5; Sutherland and Woods, unpublished). Based on the above evidence and in accord with Article 59 of the International Code of Botanical Nomenclature, we now propose future use of the name *Caloscypha fulgens* for this seed pathogen as follows: *Caloscypha fulgens* (Pers.) Boud., Bull. Soc. Mycol. Fr. 1: 103. 1885. Stat. Conid. *Geniculodendron pyriforme* Salt, Trans. Br. Mycol. Soc. 63: 340. 1974. Knowing the perfect state of the fungus provides important information on etiology and ecology of both the disease and the pathogen. For example, ascospores are an unlikely source of inoculum for disease of commercially collected seeds because cones containing these seeds are collected in the autumn, while *C. fulgens* ascocarps only occur between March and July across the fungus' North American range (5; Paden, unpublished). More information is needed on the role of conidia in disease development. Their dry nature indicates that they are airborne, but they apparently do not serve as inoculum for seeds in tree-borne cones because these cones do not yield diseased seeds (9). Since only ground-picked cones

contain diseased seeds, conidia (in addition to soil-borne mycelium) may be important in disease spread there. Ascocarps of *C. fulgens* are unreported from Britain (3) but they may appear there because of importation of diseased seeds.

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