The Fungus Geniculodendron pyriforme in Stored Sitka Spruce Seeds: Effects of Seed Extraction and Cone Collection Methods on Disease Incidence

Jack R. Sutherland and T. A. D. Woods

Department of Fisheries and the Environment, Canadian Forestry Service Pacific Forest Research Centre, Victoria, British Columbia, V8Z 1M5.

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ABSTRACT

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Isolations were made to determine the incidence of the fungus *Geniculodendron pyriforme* in stored Sitka spruce, *Picea sitchensis,* seeds and the effects of seed extraction and cone collection methods on disease incidence of extracted seeds. The fungus was present in 35% of the 60 stored seedlots that were assayed; fungus incidence ranged from 0.2 to 26%. Diseased seeds occurred in seedlots collected over a 9-yr period throughout British Columbia. Fungus incidence was not related to seed collection year. Diseased seeds were present in either all or in only some cones within collections. The fungus was isolated more frequently from seeds that had been hand-extracted from cones than from seeds that had been commercially (machine) extracted. *Geniculodendron pyriforme* was isolated more frequently from filled than from empty seeds. More filled than empty seeds were diseased. We concluded that seed extraction procedure may affect variation in disease incidence of extracted seeds as much as does variation of diseased seeds within cones. Seeds from cones collected from the ground beneath trees yielded the fungus, but seeds from cones picked from trees did not.

RÉSUMÉ

Des isolats ont servi à determiner l'incidence du champignon *Geniculodendron pyriforme* dans les graines d'Epinette de Sitka (*Picea sitchensis*) sorties (extraites) des cônes et a l'interieur des cônes. Le champignon fut observé dans 36% des 59 lots de graines examinés; l'incidence de la maladie a varie de 0.2 à 26%. Les graines contaminees furent manifestes dans des lots de graines receuillies au cours d'une période de 9 ans dans toute la Colombie-Britannique. L'incidence du champignon n'était aucunement reliée à l'année de récolte des graines. On décela des graines contaminées parfois dans tous les cônes et parfois dans quelques cônes d'une même récolte. Le champignon fut isolé plus souvent de graines extraites des cônes manuellement que de graines extraites commercialment (à la machine). *Geniculodendron pyriforme* fut isolé plus souvent de graines remplies que de graines vides. Les graines montrèrent une incidence du pathogène plus elevée que les graines vides. Nous avons conclu que les méthodes d'extraction de graines peuvent faire varier l'incidence de la maladie chez les graines extraites autant que la variation des graines contaminées à l'intérieur des cônes. Les graines des cônes récoltés sur le sol au dessous des arbres ont produit le champignon, mais les graines des cônes récoltés à-même les arbres ne l'ont pas.

In 1964, Epners (3) isolated a psychrophilic fungus from fall-sown conifer seeds which had failed to germinate in Ontario forest nurseries. He demonstrated that the fungus kills seeds of several conifer species and studied its growth on various media; he also determined that fungicide treatment of seeds provides disease control. Epners referred to this endophytic fungus as the S-fungus (seed fungus) but proposed no scientific name. Shortly afterward, Salt (6) isolated the fungus from Sitka spruce [*Picea sitchensis* (Bong.) Carr.] seeds imported into Britain from western North America. Subsequently, he confirmed pathogenicity of the fungus, expanded its host range on conifer seeds, and studied disease control (7, 8, 9, 11). Salt (10) and Gordon et al. (4) showed that this seed-borne fungus spreads from infected seeds and kills other seeds during stratification (moist-chilling treatment to break seed dormancy) or in nursery seedbeds during cool, moist weather. In 1974, Salt (10) described the fungus and proposed the name *Geniculodendron pyriforme* Salt (Deuteromycetae, Moniliales). Salt's findings suggested that *G. pyriforme* was present in, and was killing, domestic Sitka spruce seeds in British Columbia (B.C.). Thus, our first objective was to determine the incidence of *G. pyriforme* in locally stored Sitka spruce seeds. Since Salt (10) and Epners (3) had studied the disease only on extracted seeds, information was unavailable for that portion of the disease cycle occurring before seeds were extracted from cones. Also, it was not known how much variation in disease incidence among seedlots was caused by the process of extracting diseased seeds from cones or eliminating them during seed cleaning. Therefore, our other objectives were to determine: (i) if diseased seeds occur in all or in only some cones in a collection; (ii) if disease incidence of extracted seeds might vary with

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method of seed extraction, and (iii) the fungus incidence in seeds from cones picked from trees or from the ground beneath trees.

MATERIALS AND METHODS

Geniculodendron pyriforme in stored Sitka spruce seeds.—Stored (−18 C, 6-9% moisture content, wet-weight basis) Sitka spruce seeds were obtained from the B.C. Forest Service seed inventory at Duncan. Five hundred seeds from each of 60 seedlots, sensu Dobbs et al. (1), were surface sterilized with 1% sodium hypochlorite solution for 5 min, rinsed twice with sterile distilled water, and plated (25 seeds per petri dish) onto 2% water agar, as recommended by Salt (10). Seeds were incubated at 15 C and examined for the characteristic mycelium (10) of *G. pyriforme* every 3 days for 3 wk; percentage of infested seeds was based on the cumulative data.

Diseased seed incidence among cones.—To determine if diseased seeds occurred in all or in only certain cones in a collection, isolations for *G. pyriforme* were made from hand-extracted, filled seeds from 10 cones each of three commercial cone collections made in mid-October, 1975, at Port Clements, Ship Island and Tlell, Queen Charlotte Islands (Q.C.I.), British Columbia (B.C.). X-ray radiography (2) was used to select filled seeds.

Effect of seed extraction procedure on disease incidence in extracted seeds.—To determine if the method of seed extraction might affect disease incidence of extracted seeds, *G. pyriforme* isolations were made from 1,000 (500 filled, 500 empty) hand-extracted and 1,000 commercially extracted seeds from each of the three above-mentioned cone collections. The data (percentage of seeds with *G. pyriforme*) were subjected to analysis of variance and the means were compared by using the Student-Newman-Keuls test (12).

Fungus incidence in seeds from cones picked from trees or from the ground beneath trees.—This experiment was made to determine if fungus inoculation occurs (i) while cones are on the tree or (ii) after they fall to the ground. If inoculation occurred when the cones were on the tree, we wanted to know the distribution of diseased seed-bearing cones among trees; e.g., do all cones on all trees contain some diseased seeds or do only certain trees bear cones with diseased seeds? To answer the first question, 10 cones per tree were hand-picked during the fall of 1976 from each of three, three, six, 16, three, and four Sitka spruce trees at Kitimat, Sooke, Campbell River, Q.C.I., B.C., and at Brookings and Gold Beach, Oregon, USA, respectively. To answer the second question, 10 cones each were collected in early January 1977 from the ground beneath Sitka spruce trees at each of five locations near Queen Charlotte City, Q.C.I. Seeds were hand-extracted and isolations were made to determine the percentage of seeds yielding *G. pyriforme*.

TABLE 1. *Geniculodendron pyriforme* incidence in hand-extracted, filled seeds from cones of three Sitka spruce cone collections from British Columbia

	Seeds yielding *G. pyriforme* in each of 10 cones collected at:		
	Tlell (%)	Ship Island (%)	Port Clements (%)
	35.0	0.0	0.0
	47.0	0.0	0.0
	49.5	0.0	2.7
	50.4	0.0	4.0
	57.0	0.0	9.0
	64.0	0.0	10.7
	66.9	0.0	21.8
	89.6	0.0	22.9
	92.0	0.0	38.0
	92.4	1.0	52.0
Filled seeds per cone (range)	77 to 143	92 to 233	79 to 249

RESULTS

Geniculodendron pyriforme in stored Sitka spruce seeds.—*Geniculodendron pyriforme* was isolated from 35% of the seedlots. The fungus was found in seedlots originating from throughout the B.C. range of Sitka spruce (Fig. 1). Within infested seedlots, 0.2 to 25.8% of the seeds contained *G. pyriforme*. Total seedlots collected each year and percentage of the total (in parentheses) containing *G. pyriforme* were: 14 (36%), 6 (33%), 13 (69%), 3 (0%), 6 (0%), 1 (0%), and 17 (29%) from 1966, 1968, 1970, 1972, 1973, 1974, and 1975, respectively. No relationship existed between collection year or length of storage, and either *G. pyriforme* incidence levels in individual seedlots or percentages of total seedlots infested for each year. Seeds from which the fungus was

TABLE 2. Incidence of *Geniculodendron pyriforme* in Sitka spruce seeds collected in British Columbia and removed from cones by hand or by a commercial seed-extraction plant

Cone collections (all Queen Charlotte Islands)	Seeds yielding fungus[z]			
	Filled seeds		Empty seeds	
	Hand-extracted (%)	Commercially extracted (%)	Hand-extracted (%)	Commercially extracted (%)
Tlell	41.8 a	7.8 b	2.0 x	0 y
Ship Island	3.4 a	0 b	0 x	0 x
Port Clements	42.0 a	24.2 a	3.6 x	0 y

[z]Percentages based on 500 seeds. Reading across, mean comparisons only valid within empty and filled seed categories. The two means within a category differ significantly ($P = 0.05$) when followed by a different letter.

isolated never germinated.

Incidence of diseased seed among cones.—All Tlell cones contained *G. pyriforme*-infested seeds, while the Ship Island and Port Clements collections contained cones with and without diseased seeds (Table 1). When present, the fungus occurred in 1 to 92.4% of the seeds within individual cones.

Effect of seed extraction procedure on disease incidence in extracted seeds.— In two of the three cone collections, significantly more *G. pyriforme*-infected seeds were obtained from hand-extracted than from commercially extracted seeds (Table 2). The fungus was isolated much less frequently from empty than from filled seeds, and it never was isolated from empty, commercially extracted seeds.

Fungus incidence in seeds from cones picked from trees or from the ground beneath trees.—*Geniculodendron pyriforme* was not isolated from the 27,939 seeds

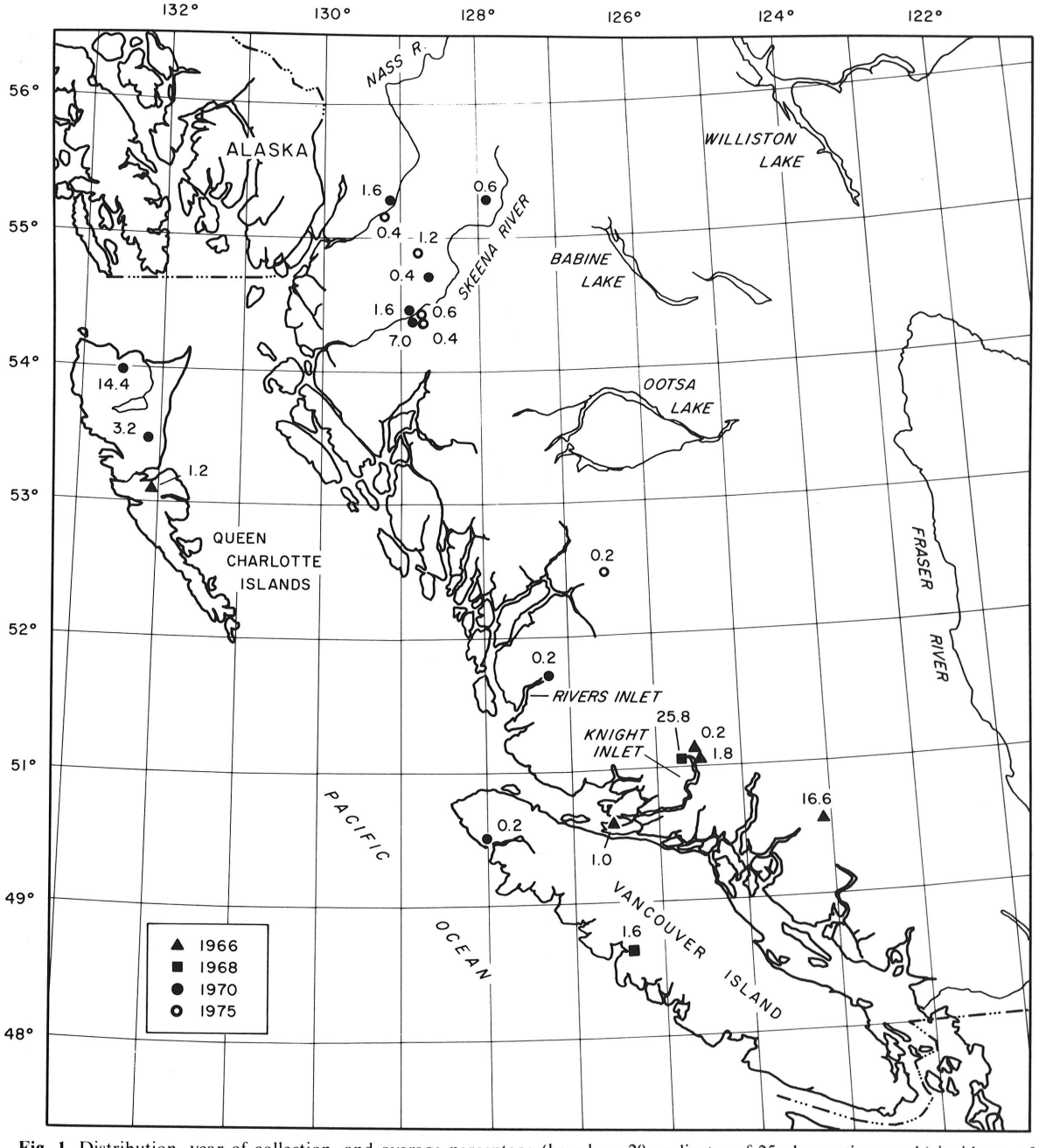

Fig. 1. Distribution, year of collection, and average percentage (based on 20 replicates of 25 observations each) incidence of *Geniculodendron pyriforme*-infested Sitka spruce seedlots in British Columbia. Fungus isolations were made in 1976.

extracted from the 350 cones picked from trees, but it was present in seeds from two of five cone collections made beneath Sitka spruce trees. The fungus occurred in 24.5 and 0.6% of the 1,645 and 903 full seeds from those two collections.

DISCUSSION

Isolations showed that *G. pyriforme* is present in 35% of the Sitka spruce seedlots stored by the B.C. Forest Service. Apparently, disease incidence is unrelated to collection year or location because infested seedlots have been collected over a 9-yr period and from throughout the B.C. range of Sitka spruce (Fig. 1). The presence of *G. pyriforme* in domestic seedlots is important because it could account for some of the pre-emergence seed losses in our nurseries. Even seedlots with low fungus levels can suffer significant losses because the fungus spreads during stratification (moist chilling) or in seedbeds during cool, moist weather (10). In B.C., additional fungus spread probably occurs after stratification when seeds are cold-stored at the nurseries for 2 to 3 wk before sowing. Recently, for infested seedlots, we have recommended adding a fungicide (4, 10) to the water-soak used during stratification.

Since incidence of *G. pyriforme*-infected seeds varied among cones within a collection (Table 1) and hand-extracted seeds always contained more diseased seeds than did commercially extracted seeds (Table 2), we conclude that seed extraction and cleaning procedures may contribute as much to variation in disease incidence of extracted seeds as does variation of diseased seeds within cones. Additional studies are needed to investigate why fungus incidence is higher in hand-extracted than in machine-extracted (commercial) seeds (Table 2). Perhaps some diseased, filled seeds weigh less than healthy seeds and are removed during cleaning, or machine extraction may not remove all diseased, filled seeds from cones. As *G. pyriforme* is not very abundant on empty seeds (Table 2), we feel that the fungus does not increase numbers of empty seeds.

We established that inoculation of seeds occurs only after cones contact the ground. Recent studies (J. W. Paden and J. R. Sutherland, *unpublished*) demonstrate that *G. pyriforme* is a soil-borne fungus. Not all cones contain diseased seeds (Table 1) because cone collections are usually mixtures of tree- and ground-picked (individual or squirrel-cache) cones. Whether or not ground-picked cones contain diseased seeds probably depends upon the length of time they are on the ground or in squirrel caches and the availability of fungus inoculum. Studies are needed to determine how these factors and the length of post-harvest cone storage affect disease incidence. However, disease incidence can be reduced or eliminated according to numbers of cones picked from the ground.

To date, the importance of *G. pyriforme* has centered on seed losses in bare-root nurseries (3, 10). However, the fungus may be an important pathogen in container nurseries where seeds are seldom treated with fungicides. Losses could be especially severe in seedlots with low germination capacities caused by *G. pyriforme* or other factors.

Results from prechilling treatment to break dormancy of *Picea* seeds have been contradictory (5, 13), perhaps owing to the presence of *G. pyriforme*, which spreads and kills seeds under prechilling treatment conditions. The confusion between the harmful effects of prechilling and *G. pyriforme* could be eliminated by the use of disease-free seeds from tree-picked cones or those certified by isolation (10) as being disease free.

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