Evidence for Longevity of Seeds and Microorganisms in Permafrost

The problem of whether seeds and microorganisms are able to remain viable in permanently frozen ground during a very long span of years was recently considered by Porsild *et al.*[1] who germinated *Lupinus arcticus* seeds from fossil rodent burrows in permanently frozen silt deposited in a placer mining area in the Yukon Territory. Based on the geology of the locality, Porsild *et al.* estimated the age of the seeds to be at least 10,000 years. Moreover, in that and in previous papers[2,3] Porsild suggests that seeds of *Rorippa barbareaefolia, Descurainia sophioides,* and *Senecio congestus* may be able to survive in permanently frozen silt as these species apparently are restricted to freshly disturbed soil within the placer mining districts.

Discussing in general the problems of dating viable seeds in old deposits Godwin[4] questions the dating of the *Lupinus arcticus* seeds because of the indirect method applied.

In view of these studies and the investigations made by Ødum[5] on the presence and age of viable seeds in Danish soils, Ester Creek placer mine west of Fairbanks, Alaska, was visited 16 to 19 July 1968. Some observations on the vegetation on the exposed silt and disturbed soil were made and soil samples were taken for further examination.

At the time of the visit an 8 m. to 10 m. section of the permanently frozen silt had recently been exposed (Fig. 1). The silt is rich in organic material, and thick deposits of this type of sediment are present at several localities around Fairbanks. Detailed descriptions of geology and faunal remains of these silt deposits in the region have been published by Péwé[6], by Repenning *et al.*[7], and by Guthrie[8,9]. The exposed section through the Ester Creek deposit showed a great amount of buried wood and roots of small diameter mainly concentrated at more or less horizontal strata. In the upper part there were a few large masses of almost pure ice. The original unfrozen top soil had earlier been partly removed.

Exposed silt (20 m. to the right of the section shown in Fig. 1) had been left undisturbed since the fall of 1966. Some of the silt had slid down forming a southeast-facing, rather steep slope. The vegetation of this slope was dominated by *Descurainia sophioides* (Fig. 2). Within 10 different m.^{2}, all plants were counted and the result of this vegetation analysis gave a total of: 18 *Agrostis* sp., 117 *Betula* sp., 467 *Descurainia sophioides*, 24 *Epilobium adenocaulon*, 25 *Equisetum arvense*, 1 *E. silvaticum*, 5 *Polygonum alaskanum*, 1 *P. buxiforme*, 2 *Rorippa barbareaefolia*, 2 *Salix* sp., 3 *Senecio congestus*, 1 *Taraxacum* sp., 2 undetermined grasses.

On the disturbed gravel and sand beneath the slope the very open vegetation consisted mainly of common widespread anthropochorous species; of scattered *Rorippa barbareaefolia* and *Senecio congestus;* of species with airborne seeds present in the surrounding landscape; and of perennial herbs transported in with fragments of turf. A few

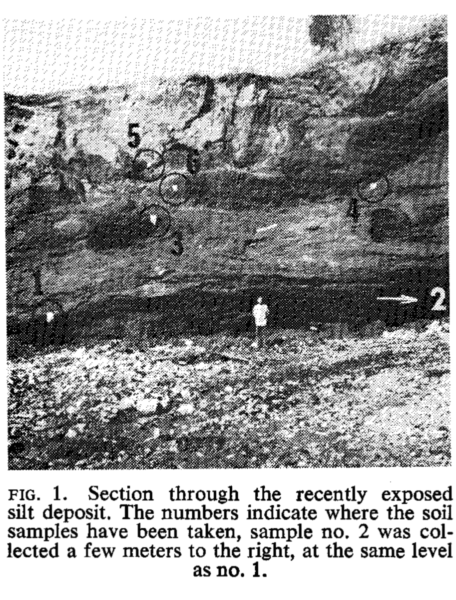

FIG. 1. Section through the recently exposed silt deposit. The numbers indicate where the soil samples have been taken, sample no. 2 was collected a few meters to the right, at the same level as no. 1.

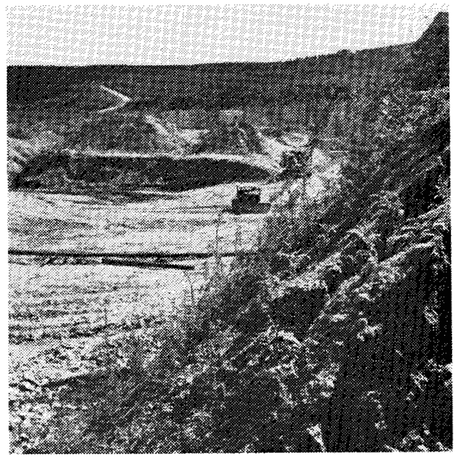

FIG. 2. Numerous plants of *Descurainia sophioides* growing on thawed silt 20 m. north of Fig. 1.

plants of *Descurainia sophioides* were also found, most of them growing on displaced pieces of silt.

List of flora: *Achillea sibirica, Agrostis* sp., *Alnus* sp., *Alopecurus alpinus, Betula* sp., *Blitum capitatum, Calamagrostis* sp., *Chamaenerium angustifolium, Chenopodium album, Descurainia sophioides, Epilobium adenocaulon, Equisetum arvense, Hordeum jubatum, Matricaria suaveolens, Plantago major, Polygonum Alaskanum, P. buxiforme, Potentilla chamissonis, Rorippa barbareaefolia, R. palustris, Salix* spp., *Senecio congestus, Silene williamsii.*

Above the slope the vegetation was composed partly of a dense cover of perennial herbs, mainly grasses, a few trees *(Betula* and *Populus),* and on disturbed soil an open vegetation including most of the annual species found below the slope.

Herbarium specimens of most of the species present were collected. Soil samples of 3 kg. to 5 kg. fresh weight were taken from the exposed silt at the places shown in Fig. 1. The outer surface soil was removed and the recently-thawed silt was put into plastic bags to be examined for viable seeds. Samples nos. 1 to 6 were collected here, and sample no. 7 was collected from unfrozen subsoil just outside the placer mine area. At the sites of samples 1, 2, 4, 5 and 6, pieces of wood were collected for C^{14}-dating and for microscopic examination.

At the same 7 sites soil was scraped directly into sterile Petri dishes to be examined for possible microorganisms. Four mammal bones collected at the level of samples 1 and 2 by E. Clark, a miner at the place, were added to the collections.

TABLE 1. Soil analyses of samples 1 and 7. Methods as described by Kjeld Hansen[12] whose laboratory made the analyses.

	Sample No. 1	Sample No. 7
Loss of weight by drying, %	27.5	2.6
pH	6.8	6.3
Conductivity, μ mho	96	170
P, ppm	331	361
Na exchangeable cont., meg/100 g.	0.10	0.42
K " " "	0.16	0.17
Mg " " "	3.1	5.0
Ca " " "	9.2	6.2
Mn " " "	0.032	0.021
Kation exchange capacity "	335	253
Fe, ppm	6220	703
Cu, "	25.8	16.8
Zn, "	11.4	7.2
L "	2.0	1.5

From 1 August to 1 October the big soil samples were placed in a greenhouse. No plants germinated from the soil. Soil analyses of samples 1 and 7 were carried out, and the results are given in Table 1. Wood-remains from samples 2 and 4 have been C^{14}-dated to $>$ 35,000 years. Eighty pieces of wood were all from angiosperm species.

U. Möhl at the Zoological Museum, University of Copenhagen, identified the bones as femur of *Mammuth primigenius* and parietale, scapula, and humerus of *Bison* sp., and he drew attention to the fact that the bones were frayed and had obviously been transported.

The contents of microorganisms in the soil samples were examined by using the dilution plate technique: 10 g. of each sample were mixed with 90 ml. of sterile water and placed on a shaking table for 20 minutes. Serial dilutions were made, and 0.1 ml. of each of 10^{-1}, 10^{-2}, 10^{-3}, and 10^{-4} suspensions were plated on Bengal Rose-, Sabouraud-, Cook-, and V-8-agar. Besides 1 ml. of each suspension was added to test tubes with NIH.

After one week of incubation at 4°C., 24°C., and 37°C. the colonies were counted and the different strains isolated on slants with V-8-agar.

The sample from the top soil showed *c.* 10^3 fungi per gr. soil at 24°C., representing the following genera, known from similar localities: *Cladosporium herbarum, Mortierella* sp., *Mucor circinelloides, Penicillium* spp., *Trichoderma viride,* and unidentified, sterile imperfects and phycomycetes.

The permafrost samples nos. 1, 3, 5, and 6 showed no growth at all.

No. 2 showed on Sabouraud-agar at 24°C. growth of red-orange and yellow, shiny colonies, 10^4 per g. soil.

No. 4 showed on Sabouraud-agar at 37°C. growth of yellow shiny colonies, 2 x 10^3 per g. soil, and at 24°C. growth on all substrates, averaging 2.6 x 10^4 per g. soil. Besides the same organism grew in NIH at 37°C.

The two isolates from no. 2, and that from no. 4 showed the same morphology, and were identified as an actinomycete, *Nocardia* sp. Unfortunately the red-orange strain later died; the two yellow strains were kept on slants with V-8 agar covered with paraffin-oil at 4°C.

The two organisms have been tested against 20 bacteria and fungi in order to determine their antibiotic activity, but with negative result. Furthermore the investigations have shown that they produce an enzyme, fucidinase, destroying the antibiotic, fucidin.

Nocardia sp. was only found in 2 samples of permanently frozen silt. No actinomycetes were present in the topsoil sample. Furthermore the collection of the samples and the analyses were carried out with extreme care without contamination of the plates. Therefore it seems probable that living microorganisms are present in soils that have been frozen for many years.

Only a few investigations have been carried out to find living microorganisms in permanently frozen soil, and generally only the upper layers have been studied, because of the difficulties of sampling through permafrost. However Becker and Volkmann[10] recovered 8 bacteria at 20 to 60 feet below the surface in permanently frozen soil near Fairbanks. Boyd and Boyd[11] also studied permafrost soils from Barrow, Alaska, near Elson Lagoon, and found living bacteria at 8 to 15 feet.

The distribution of *Descurainia sophioides* within the placer mine area and its extremely high frequency on the exposed muck does strongly support Porsild's theory. However, the fact that the seeds of the species are lacking in the investigated samples may lead to the alternative possibility that the ecology of the species is rather specific. Which factor or combination of factors is responsible for the germination and growth of the species on that particular substratum, cf. Table 1, cannot be determined without field experiments at the locality and further analyses; the high water content of the silt may appear to be a factor of importance.

The investigation does not prove that viable seeds of *Descurainia sophioides, Rorippa barbareaefolia, Senecio congestus* or any other species are not present in permanently frozen soil; the negative result of the investigation, however, indicates that on this locality it is not very likely that they are present.

A dating of the sediment and of the viable *Nocardia* sp. in particular is problematic. The mammal bones have been transported and may be older than the wood-remains dated 35,000 years. According to the discussion of age of the sediments in Repenning *et al.*[7] even the sediments including wood may have been reworked and replaced in certain periods. Still referring to Repenning *et al.*, the silt at this locality has probably been left undisturbed for about 7,000 years as coniferous wood is absent, which supports the assumption that the *Nocardia* sp. is at least as old as that.

Too little is known about extent and significance of survival of organisms in permanently frozen soils. Further and more detailed investigations should be made on this subject.

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Changes in the Northern Limit of Spruce at Dubawnt Lake, Northwest Territories

Larsen[1] described the treeline west of Hudson Bay and particularly at Ennadai, Yathkyed and Dubawnt Lakes, as clumps of spruce, relict from a former more northerly distribution. He considered the distribution to be climate-controlled and suggested that the clumps were not re-establishing themselves. In contrast, Marr[2] reported that the treeline east of Hudson Bay is not climate-limited, but that the forest is migrating as soil develops and a measurable successional progression has occurred in less than half a century. Evidence collected by us in 1966 on the Dubawnt River system on the Mackenzie-Keewatin border, indicates that the spruce trees are re-establishing themselves and have moved northward and closer to Dubawnt Lake during the past one hundred years.

The Tyrrell brothers[3,4] on the first geological survey of the central barrens in 1893 recorded the last spruce grove at a point 5 miles upstream (southwest) from Dubawnt Lake (Fig. 1). J. Burr Tyrrell[3] describes the location: "On the north bank of the river, half way between the above lake and Doobaunt Lake, is the last grove of black spruce on the river, where the trees are so stunted that they are not as high as one's head . . . For five miles below this last spruce grove, the banks are rather low . . ." J. W. Tyrrell[4] describes these plants as "a little patch of stunted black spruce trees . . . not more than 4 or 5 feet high . . . We broke camp early, and bidding good-bye to the last vestige of growing timber to be seen, continued down the river . . ." In 1966, the last spruce tree (4 inch diameter at breast height) was within 2 miles of the shore of Dubawnt Lake, 1 mile north and 3 miles east of the last spruce plants visible to the Tyrrells. In addition, sight records of spruce of less than tree size were made north of Dubawnt Lake: at points 2 miles south of Loudon Rapids near the junction of the Dubawnt and Thelon Rivers, and on the northwest shore of Aberdeen Lake. These latter individuals are probably outliers from the large island of spruce further west on the Thelon River[5].

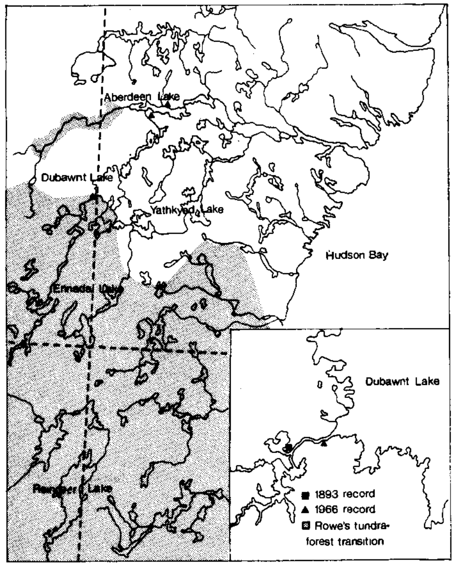

FIG. 1. Map showing locations of spruce plants on the Dubawnt River system, Northwest Territories. Inset shows locations of last spruce groves south of Dubawnt Lake.

The spruce trees at Dubawnt Lake appeared to be intermediate in form between white spruce *(Picea glauca)* and black spruce *(Picea mariana)*. In needle shape and number of rows of stomata they resemble black spruce, but in cone and twig characteristics they resemble white spruce. The absence of resin ducts in the needles indicates white spruce[6].

Examination of the annual growth rings of the largest spruce (14 feet high and a 5 inch basal diameter) shows clear increments for 79 years (back to 1888); prior to that time, there are perhaps 18 rings, 9 of which are extremely narrow. The existent trunk diameter of this tree in 1893 was approximately ¼ inch at a point 1 foot above the root. A plant of this size could not have been seen by the Tyrrells passing on the river as it was set between rocks and tussocks of a low swampy area.

A lone spruce, separated by 30 yards from the low-lying clump, was situated about 20 feet higher on the slope. It was 12 feet high with a basal diameter of 3 inches. The earliest ring dates from 1931 and all subsequent rings show good annual increments until 1960 —