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Epiphyllic cover on spruce needles in Denmark

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Epiphyllic microbial cover on Norway spruce (*Picea abies*) needles has increased in recent years in Denmark and in neighbouring countries. Based on a survey of 2 445 needles from seven localities in Denmark and Poland, the development of the microbial crust through 11 needle generations was analysed. An algal crust consisting of mainly *Apatococcus lobatus* (Chodat) Boye-Petersen and *Desmococcus viridis* Brand is built up during the first four years, peels off during the subsequent three years whereafter a new crust starts accumulating. These crusts are frequently colonized by two fungi, *Scolecotheca cornuta* Søchting & Sutton and *Capronia* sp. The amount of microbial cover on the needles did not reflect the varying levels of nitrogen deposition within the area.

Key words: epiphyllic vegetation, eutrophication, foliicolous vegetation, lichens, *Scolecotheca*, spruce needles

INTRODUCTION

Tropical rain forests have been shown to host an astonishing diversity of epiphyllic (foliicolous) lichens (Farkas & Sipman 1993). In subtropical and temperate regions this flora is much poorer, although a number of species do occur, particularly in humid regions (Serusieaux 1993). In drier parts of temperate and boreal forests epiphyllic microorganisms have largely been overlooked due to the low species diversity and their poor development. In recent decades, however, the leaves and needles of evergreens in such climatic regions are becoming colonized by a significant, mainly algal, vegetation (Göransson 1988, 1990, Søchting *et al.* 1992). Microbial colonization on individual spruce needles was illustrated by Jahns *et al.* (1979) and Steffens (1987). Tenberge and Peveling (1991) and Peveling *et al.* (1992) also studied the ultrastructural relationships between the microorganisms and their influence on the needles.

The needle surface is a specialized habitat where nutrients are provided partly by organic and inorganic exudates that are dissolved in throughfall from the above canopy. Algal growth on the needles may also be highly dependent on deposition in the canopy of inorganic compounds derived from human activities such as farming and burning of fossil fuel. However, the microbial vegetation may be limited by low humidity of the ambient air and the specific light regime on the needle surface, e.g. the intensity of ultraviolet light (Preece & Dickinson 1971, Andrews & Hirano 1991).

Based on information from old foresters and the lack of information in the old literature, there

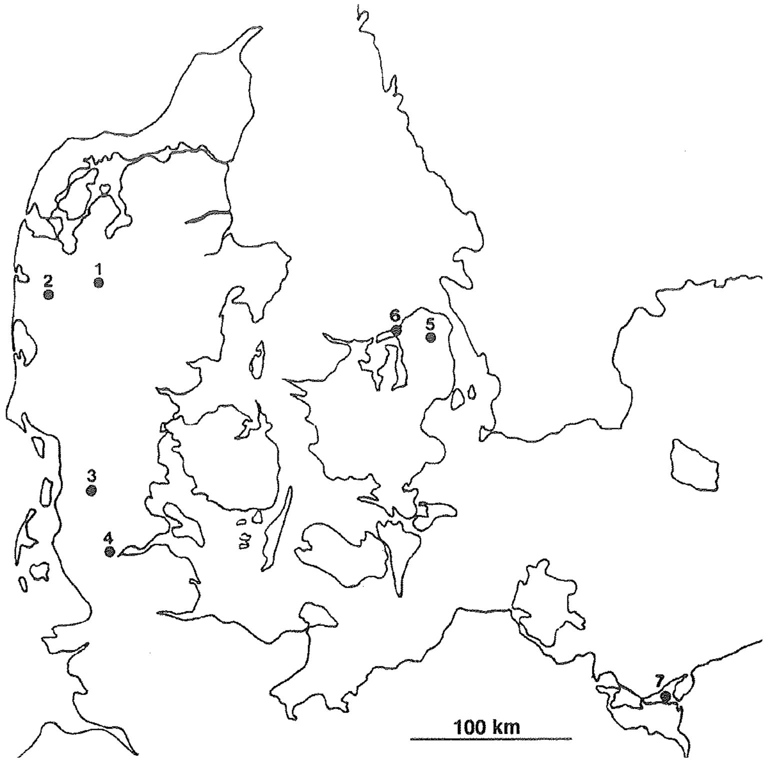

Fig. 1. Localities where needles were collected. 1. Feldborg; 2. Ulborg; 3. Frøslev Plantage; 4. Lovrup Skov; 5. Stenholt Vang; 6. Asserbo Plantage; 7. Wolin National Park.

is good circumstantial evidence that epiphyllic vegetation on spruce needles has changed from being almost nonexistent in the early part of this century to the thick cover seen today. This phenomenon is significant, especially in the temperate part of Northwest Europe, viz. Norway, Sweden, Denmark, Germany and The Netherlands (Göransson 1988, 1990, Peveling *et al.* 1992, Thomsen 1992), and it has become a problem in some locations for commercial production of ornamental conifers. It is hypothesized that the recent increase of epiphyllic cover is due to increased nitrogen deposition from the atmosphere. This study deals qualitatively and quantitatively with the formation of epiphyllic covers on needles of Norway spruce (*Picea abies*) in Denmark and Poland. The results are compared with local levels of nitrogen deposition as calculated from models and as expressed in nitrogen levels of the lichen *Hypogymnia physodes*.

LOCALITIES

Seven forests in Denmark and Poland were chosen to represent varying levels of nitrogen deposition. The localities are indicated in Fig. 1. All forests grew on rather dry soil. Sampling took place between July 5th and September 5th, 1991.

METHODS

Sampling of twigs

Twigs were sampled from 8–10-m tall Norway Spruce growing either along forest roads or in fairly open situations to ensure uniform light and humidity conditions.

At each location up to 12 primary branches at breast height were chosen, from which secondary branches about one metre from the tip of the primary branch were cut.

The branches were cut into annual units in such a way as to discard the nodes. For each branch, internodes of corresponding age were placed in paper bags to dry. Fifteen needles were randomly chosen to represent each needle generation. Up to 11-year-old needles were sampled, although generally it was only possible to sample up to 6 or 7 generations of needles. A total of 2 445 needles was examined.

Quantification of microbial cover

The selected needles were examined under a dissecting microscope to estimate percentage cover of microorganisms, thickness of cover, fungal colonization, and occurrence of specific microorganisms. Only the needle side with the highest coverage of microorganisms was examined.

The degree of microbial cover was estimated visually and recorded as the following percentage states: 0, 1, 5, 10, 20, 30 etc.

The thickness of microbial cover was assessed following a relative three-point scale, with one representing the thinnest cover and three representing a cover about three times as thick.

Fungal infection was rated according to the following infection states: 0 = no infection; 1 = sparse infection/only few hyphae visible; 2 = medium infection/conspicuous mycelium present; 3 = fungal infection over the entire algal crust.

Biomass of cover

The biomass of the epiphyllic cover was estimated based on a sampling of 100 needles, 2.5 years of age from 10-m high spruces. The cover and the thickness was estimated for each needle and the needles were weighed before and after removal of the epiphyllic cover.

Presence of organisms

The presence on the needles of two fungi (*Scolecotheca cornuta* Søchting & Sutton and *Capronia* sp.) and one alga (see below) was noted.

Nitrogen content in lichen thalli

The nitrogen content in thalli of the lichen *Hypogymnia physodes* on Norway Spruce branches that had received through-fall was analysed following Søchting (1995).

RESULTS AND DISCUSSION

Microbial cover

The percentage of cover, thickness of cover and the fungal infection of the microbial crust for the seven localities are shown in Table 1. Only results from 3–6-year-old needles are included in the table. The estimated level of total nitrogen deposition modelled for each locality (Asman & Janssen 1987, Asman & Runge 1991, W. A. H. Asman pers. comm.), and the nitrogen content in *Hypogymnia* at the locality are also listed in the table.

On the needles used to estimate microbial biomass, the percentage cover was 66% and the thickness was 1.5. The average needle dry weight was 3.97 mg and the average cover weight was 0.45 mg. The biomass of the cover thus represented 11.6% of the needle weight. The material used is assumed to be quite typical for Danish localities.

There appears to be little variation in the cover thickness and the fungal infection between the localities. The percentage cover and the thickness of the cover does not appear to be related to the modelled deposition of nitrogen or to the percentage total nitrogen in *Hypogymnia physodes*.

Within each locality microbial cover varied between the forest parts. Conditions such as light and humidity seemed to be more important for the formation of algal cover than the differences in nitrogen deposition within the studied region.

Even at the localities with the lowest nitrogen deposition, the deposition is sufficient for formation of a substantial microbial cover on spruce needles.

Table 1. Microbial covers on spruce needles (3–6 years old), and their thickness and fungal infection for each forest, compared with nitrogen content in *Hypogymnia physodes* and modelled total nitrogen deposition.

Locality	Cover (%)	Thickness	Infection	Needles examined	Nitrogen in *H. physodes* (%)	Nitrogen deposition (kg N/ha/year)
Feldborg	40.5	1.3	2.0	735	2.3	20.5
Ulborg	49.2	1.4	2.2	435	2.1	16.4
Frøslev Plantage	58.5	1.7	2.3	480	2.7	25.9
Lovrup Skov	51.6	1.5	2.4	330	2.3	39.8
Stenholt Vang	59.1	1.2	2.6	180	1.8	15.0
Asserbo Plantage	69.3	1.5	2.2	120	1.6	13.3
Wolin National Park	58.0	1.7	2.4	165	1.7	18.6

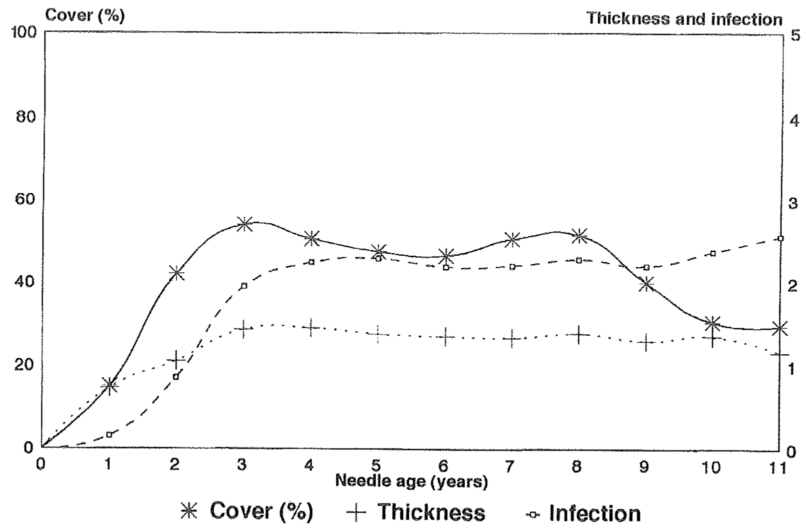

Fig. 2. Microbial crusts on needles of different ages. Average for all trees (2 445 needles). Thickness and infection based on specific scales.

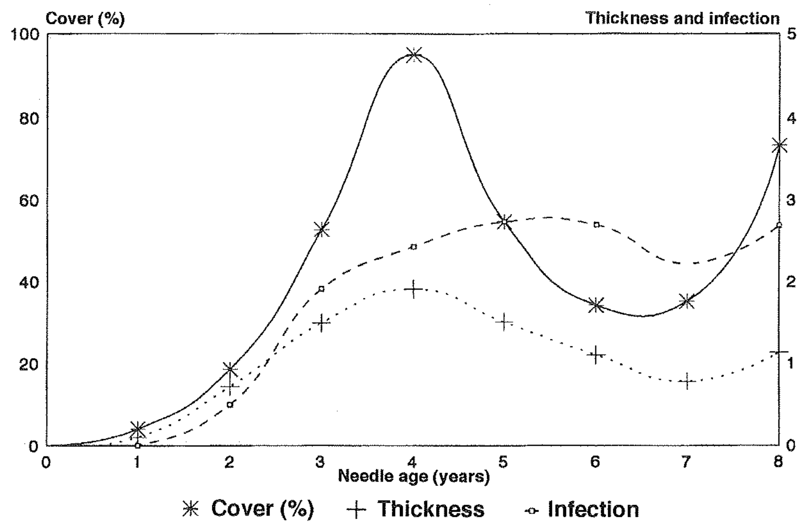

Fig. 3. Microbial crusts on needles of different ages. One tree in Frøslev Plantage. 18 August 1991.

Development of the cover

The development of the cover on needles of increasing age is illustrated in Figs. 2 and 3.

Up to 11-year-old needles are included, but data from needles older than 8 years was limited.

Fig. 2 presents data from all trees, a total of 2 445 needles. Microbial cover increases during the first three to four years to about 55%, then decreases slightly and has another peak at 8-year-old needles. Thickness of the microbial crust increases during the first three years then remains unchanged. Fungal infection increases more slowly and reaches a maximum after 4–5 years.

The pattern described above reflects average development taking place within the study area. However, as the speed of development of the cover can vary between branches and sites, it obscures the more dramatic changes taking place on the individual needle. In order to show this, a representative set of data from a single branch is shown in Fig. 3, where 15 randomly chosen needles represent each needle age. After the initial colonization of over 90% of the needle surface, the microbial cover starts to peel off reducing the cover to less than 40% before a new cover starts to build up again. The increasing level of fungal infection corresponds to a decreasing thickness of the cover.

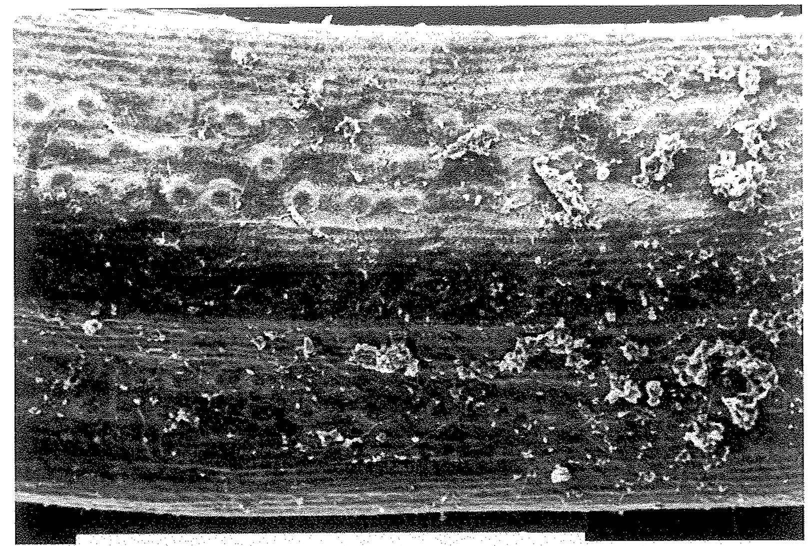

Fig. 4. Microbial crust on 0.5-year-old needle. Storskoven, Central Zealand. January 1992. Scale 1 mm.

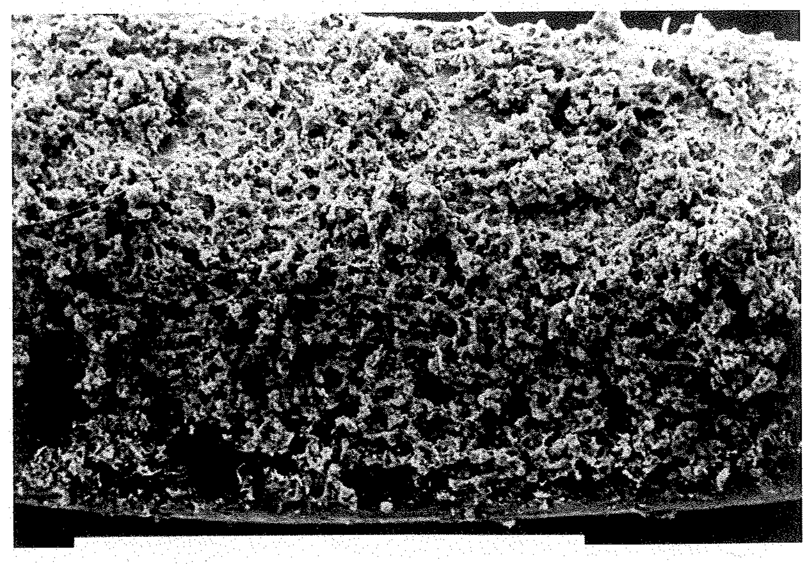

Fig. 5. Microbial crust on 1.5-year-old needle. Storskoven, Central Zealand. January 1992. Scale 1 mm.

The surface of the needles at different ages is illustrated in Figs. 4–8. The data shown in Fig. 3 are in agreement with the results of Peveling *et al.* (1992), who found that needles in Northwest Germany had an algal cover of 65–80% after four years.

Organisms on the needles

The first organisms to colonize the needle surface are algae and fungi as shown with SEM on Fig. 4. The early colonizing fungi were not identified.

The bulk of the biomass of microbial needle cover consists of aerial coccoid algae. The specific coccoid algae were not quantified, but the main species were *Apatococcus lobatus* and *Desmococcus viridis* (synonym: *D. vulgaris*). *Desmococcus* frequently produces characteristic spiny cysts. Both species are also common on trunks and on the cortex of twigs.

Filamentous alga

A characteristic filamentous alga was found with low frequency at some localities. Its identity is

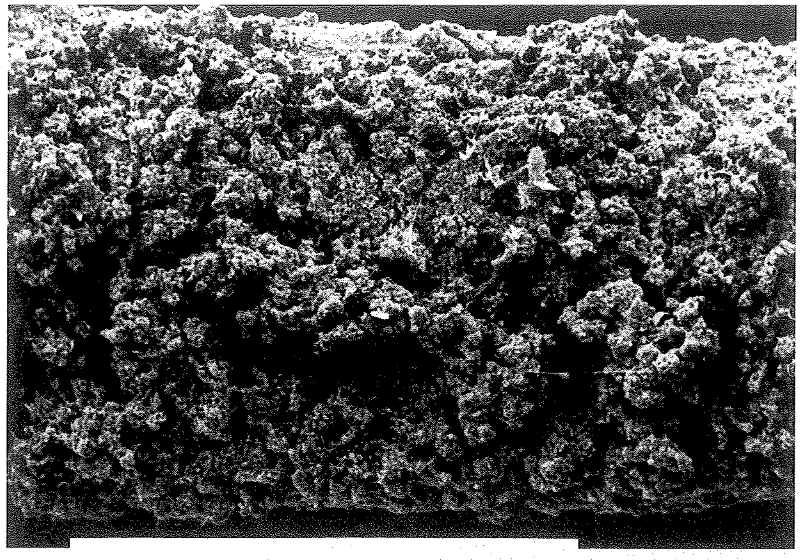

Fig. 6. Microbial crust on 2.5-year-old needle. Storskoven, Central Zealand. January 1992. Scale 1 mm.

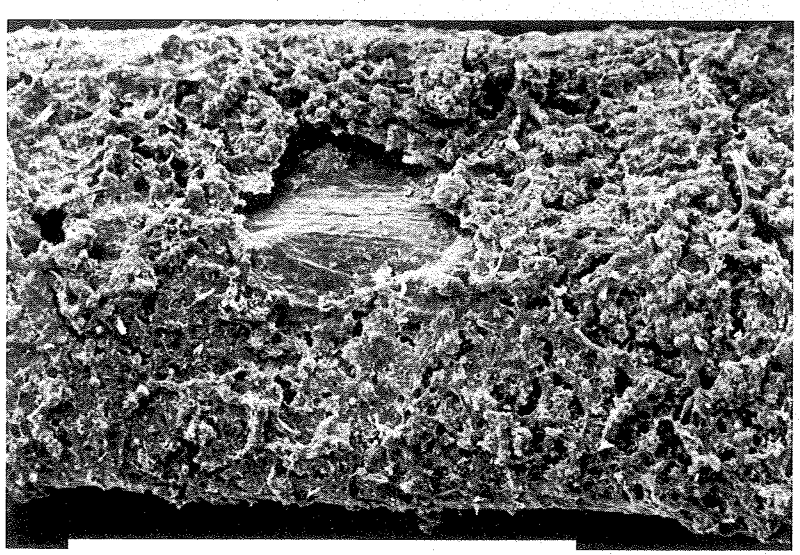

Fig. 7. Microbial crust on 3.5-year-old needle. Storskoven, Central Zealand. January 1992. Scale 1 mm.

presently unknown, and it may be undescribed (Søchting *et al.* 1992).

This alga is rather common on the bark of twigs and trunks in regions of Denmark with high deposition of ammonia and ammonium. Formerly unknown in Denmark, it was first observed in central Jutland in 1985, but is now known from many localities in Jutland and northern Germany, but only from one locality in Zealand (Søchting *et al.* 1992). Its occurrence on the needles seems to reflect high deposition of nitrogen, and it is supposed to be a reliable indicator of elevated deposition of nitrogen in farmland.

This filamentous alga is a late colonizer as seen from Fig. 9, where a frequency above 10% is only reached on the oldest needles.

Capronia sp.

This fungus belonging to the genus *Capronia* in the family Herpotrichiellaceae is presently undescribed. It seems to grow biotrophically on the algal cover, and apparently does not modify this in any way. Its ascomata are ± globular, naked pseudothecia, about 0.1 mm in size (Søchting *et al.* 1992).

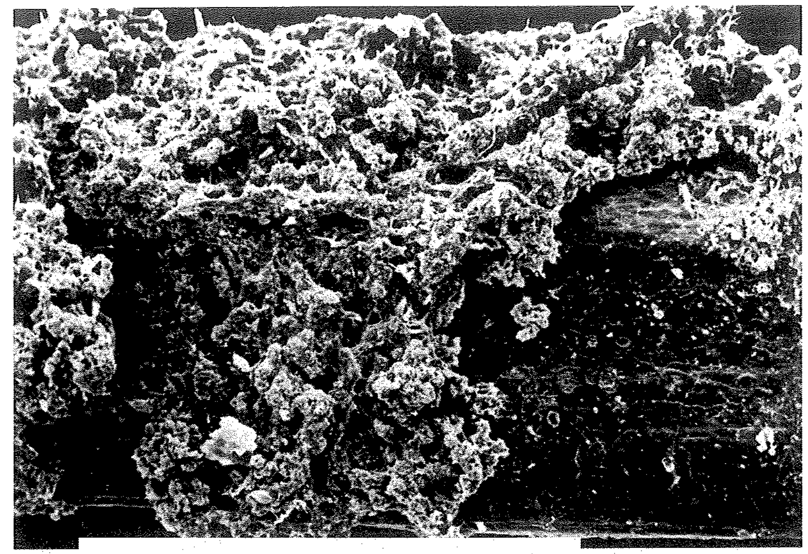

Fig. 8. Microbial crust on 5.5-year-old needle. Storskoven, Central Zealand. January 1992. Scale 1 mm.

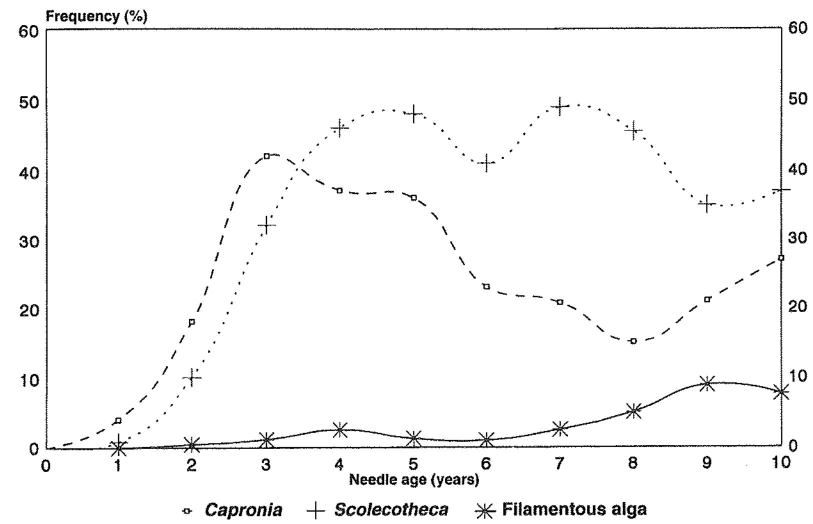

Fig. 9. Frequency of *Capronia* sp., *Scolecotheca cornuta,* and filamentous alga on needles of different ages. Average for all localities.

It occurs commonly at all localities with frequencies ranging from 30 to about 70% of the examined 3–6-year-old needles (data not presented). Its frequency on needles of different ages is related to the extent of the epiphyllic cover (Fig. 9).

Scolecotheca cornuta Søchting & Sutton

This coelomycete fungus appears to be a necrotrophic parasite on the algal cover, on which it causes greyish patches. The abundantly produced cornute conidiomata are 60–100 μm high and 30 μm thick. They contain filiform, 30–50-μm long, aseptate conidia (Søchting & Sutton 1997).

Scolecotheca is common at all localities, with its frequency on 3–6-year-old needles being 35–65%. No regional pattern seems to exist (Søchting *et al.* 1992). It colonizes early and also retains a high frequency on the older needles (Fig. 9).

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