COMMUNICATIONES INSTITUTI FORESTALIS FENNIAE 121

BACTERIA AND THEIR EFFECT ON THE MICROFLORA IN WOUNDS OF LIVING NORWAY SPRUCE (PICEA ABIES)

ANNA-MAIJA HALLAKSELA

SELOSTE

BAKTEERIT JA NIIDEN VAIKUTUS ELÄVIEN KUUSIEN VAURIOIDEN MIKROBILAJISTOON

HELSINKI 1984

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Cover (front & back): Scots pine (*Pinus sylvestris* L.) is the most important tree species in Finland. Pine dominated forest covers about 60 per cent of forest land and its total volume is nearly 700 mil. cu.m. The front cover shows a young Scots pine and the back cover a 30-metre-high, 140-year-old tree.

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Approved on 10.2.1984

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The interaction between micro-organisms in wound rot was studied in one fertilized and one unfertilized spruce stand. The most common group of micro-organisms isolated from the Norway spruce *(Picea abies* (L.) H. Karsten) wounds was bacteria (71 % of the trees). *Stereum sanguinoletum* (Alb. & Schw. ex. Fr.) Fr. was the most common decay fungus (34 % of the trees). *Heterobasidion annosum* (Fr.) Bref. was isolated from 15 % of the wounds. Other micro-organisms isolated in considerable amounts were: *Ascocoryne* spp., *Graphium* spp., *Nectria fuckeliana* (Booth) and *Nectria* sp. *S. sanguinolentum* was the most frequent decay fungus in the unfertilized stand. Fertilization had no effect on the occurence frequency of *H. annosum*. Only *Graphium* spp. increased as a result of fertilization.

The capacity of a mixed bacterial population to prevent the passage of decay fungi into Norway spruce wounds was investigated also in this study. The bacterial population was antagonistic towards *H. annosum* and *S. sanguinolentum* under laboratory conditions. The bacterial population used in this study had no effect on the microflora under field conditions.

Mikrobien keskinäisiä vuorovaikutussuhteita analysoitiin vauriolahoissa lannoittamattomassa ja lannoitetussa kuusikossa. Yleisin kuusen *(Picea abies* (L.) H. Karsten) vaurioista eristetty mikrobiryhmä oli bakteerit (71 %:ssa puita). *Stereum sanguinolentum* (Alb. & Schw. ex. Fr.) Fr. oli yleisin (34 %:ssa puista) lahottajasieni. Myös *Heterobasidion annosum* (Fr.) Bref. tavattiin 15 %:ssa vaurioista. Muita huomattavassa määrin eristettyjä mikrobeja olivat *Ascocoryne* spp. (25 %:ssa puita), *Graphium* spp. (31 %), *Nectria fuckeliana* (Booth) (23 %) sekä *Nectria* sp. (11 %). Lahottajasienistä *S. sanguinolentum* oli yleisempi lannoittamattomassa metsikössä. Lannoitus ei vaikuttanut *H. annosum* -sienen esiintymisrunsauteen. Ainoastaan *Graphium* spp. määrä kasvoi lannoituksen vaikutuksesta.

Tutkimuksessa selvitettiin lisäksi bakteeriseoksen kyky estää lahottajasienen pääsy kuusen vaurioihin. Bakteeriseos oli laboratorio-olosuhteissa antagonistinen *H. annosum* ja *S. sanguinolentum* sienille. Tämä bakteeriseos ympättiin vaurioitettuihin kuusiin *(Picea abies)*. Neljän kasvukauden jälkeen puut kaadettiin. Tutkimuksessa käytetyllä bakteeriseoksella ei näissä olosuhteissa ollut vaikutusta mikrobilajistoon.

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PREFACE

This study was carried out at the Department of Forest Protection, the Finnish Forest Research Institute. I wish to thank Professor Tauno Kallio for the support he has given me in my work over a long period of time and for his constructive criticism of the manuscript. Most of the laboratory work was carried out at the Department of Plant Pathology, the University of Helsinki. I wish to express my sincere gratitude to my teacher, Professor Eeva Tapio, Head of the Department.

Special thanks are due to the personnel of the Department of Forest Protection, especially Mr. Matti Kaivos and Mrs. Eeva Aho for the field work and to my excellent laboratory assistants Mrs. Hillevi Nieminen and Miss Eija Juntunen. I would also like to thank Mr. Risto Häkkinen for planning the VAX table programmes and for providing statistical advice.

Doctors Seppo Niemelä, Finn Roll-Hansen, Lalli Laine and Timo Kurkela have read the manuscript and have given much of their valuable time in refining the final manuscript.

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Helsinki, January 1984

Anna-Maija Hallaksela

1. INTRODUCTION

11. Microbial succession in living trees

A diverse microflora infects a tree after it is damaged. Micro-organisms appear simultaneously and successively in the wood. Wood-decaying hymenomycetous fungi may bring about a first stage discolouration of the wood and, at the same time, activation of the defence mechanisms of the tree. Non-hymenomycetous fungi and bacteria also immediately colonize the damaged point. They are capable of growing in living wood without causing any real changes (Huse 1978b, Shain 1971, Shortle and Cowling 1978). These pioneer microbes live, independent of the other microbes, of sugars. According to Shortle and Cowling (1978), decay fungi may initially live for quite a long period of time in a semi-dormant state in the wood. Microbes which are resistant to phenolic compounds in the wood gradually become the dominant species. After a time they reduce the amount of toxic compounds in the wood to such a level that decay fungi finally become dominant.

The reactions of the tree and the behaviour of the microbes in wounds in Norway spruce *(Picea abies)* have been studied in Germany (Schönhar 1975, Aufsess 1978, Bonnemann 1979). Trees start to react already within a few hours after being damaged. Yeasts and bacteria, as well as blueing fungi, are usually the first to penetrate the wood tissues around the wound. The decay fungi may infect the wound tissue immediately, or within a few months or even years after the damage has occurred. Damage caused during the wintertime remains free from infection by decay fungi for a longer period of time. In the initial stages, the fungal mycelia spread rather rapidly in the damaged part of the tree and the development of the fungi is not checked or slowed down until the tree starts to excrete the compounds which constitute the defence mechanism of the tree. Sometimes the defence mechanisms of trees are able to destroy the mycelia of the fungi which have penetrated the tree. In most cases, however, spruce is not able to prevent attack by pathogens and, if it is damaged, rot starts to develop.

12. Biological control

In this study the term biological control refers to methods in which the effect of a living organism is used to try to prevent a pathogen from infecting the host plant (Garrett 1965).

Bacteria have also been used as controlling agents against pathogens. Treating the leaf scars of apple trees in the autumn with a bacterial suspension has been found to be an effective method of controlling canker. During the summer time, fungicide treatment was also required (Swinburne 1978). Willow cuttings treated with a bacterial suspension remained healthy under controlled conditions, but not in the field (Spiers 1980).

13. The aim of the study

In addition to *Heterobasidion annosum* (Fr.) Bref., another serious causal agent of rot developing from wounds in spruce is *Stereum sanguinolentum* (Alb. & Schw. ex Fr.) Fr. The colour changes and rot caused by these two fungi in wood usually result in very serious financial losses in practical forestry. For this reason, damaged trees left along strip roads should be removed from the forest or the wounds treated with a micro-organism or a chemical which protects against infection by rot fungi.

The interaction between the fungi and bacteria in the discoloured columns spreading from wounds in the spruce trees was analysed. The effect of fertilization on the microflora which infected the wounds was also studied.

In one case Kallio (1973) found a mixed bacterial population that was antagonistic towards *H. annosum* and *S. sanguinolentum* (Kallio 1974). The antagonistic ability of this bacterial population against decaying fungi was studied in the present work.

2. THE STUDY STANDS AND THE EXPERIMENTAL DESIGN

The stands used in the study were situated in forests owned by the Agricultural Research Centre at Jokioinen (60°48'N, 23°29'E). The experiment was carried out in two approximately 80-year-old Norway spruce stands growing on sites of the MT site type. One of them had been fertilized with urea (300 kg/ha) in 1973, and the other had not been fertilized at all. The trees in both stands were damaged by striking and rupturing the surface of the stem once a month during the period May — October 1974. In May, June and September the wounding was carried out in the middle part of the month and in July, August and October in the first part of the month. The area of the wounds was about 10 cm^2 (Fig. 1). Eight trees in each stand were damaged each month at a height of 1,3 m above the ground and eight other trees at a height of 0,5 m.

Four of the wounds in each group were treated with a suspension obtained from the fresh bacterial culture and the other four were left untreated as controls (Table 1). The density of the suspension was determined on a spectrophotometer each morning before spraying was carried out. The number of cells in the bacterial suspension varied from $8,3 \times 10^6 - 9,3 \times 10^6$/ml (in 0,9 % NaCl). Inoculation with the bacteria was done by spraying immediately after the trees had been damaged (Fig. 1).

At the start of the study the material consisted of 192 damaged spruce trees. By the end of the study, 23 of them had severe butt rot and 2 of them had been felled by storms. There were thus 167 damaged trees at the end of the study (Table 1).

Fig. 1. Spraying a spruce wound with the bacterial suspension.
Kuva 1. Kuusen vaurion ruiskutus bakteerisuspensiolla.

Table 1. Description of the treatments used in the study, including the number of spruce trees treated. Trees treated with the bacterial suspension (b), control trees (k).

Taulukko 1. Tutkimusaineiston puut ryhmitettynä metsän ja vaurioiden käsittelymenetelmien ja vauriokorkeuksien perusteella. Bakteerisuspensiolla käsitellyt puut (b), kontrollipuut (k).

Month wounded, 1974 *Vaurioituskuukausi, v. 1974*	Fertilized *Lannoitettu* — Height of wound — *Vauriokorkeus*: 0,5 m — Wound treatment — *Vaurion käsittely*: b	k	1,3 m: b	k	Unfertilized *Lannoittamaton*: 0,5 m: b	k	1,3 m: b	k	Total, No *Yhteensä, kpl*
May — *toukokuu*	3	4	4	3	4	2	4	4	28
June — *kesäkuu*	4	3	4	2	4	3	4	4	28
July — *heinäkuu*	4	3	4	3	2	4	3	3	26
August — *elokuu*	2	4	4	4	4	4	3	3	28
September — *syyskuu*	4	4	4	4	4	4	3	3	30
October — *lokakuu*	4	3	3	4	3	2	4	4	27
Total — *Yhteensä kpl*	21	21	23	20	21	19	21	21	167

3. SAMPLING

The trees were felled in November — December 1977, after four growing seasons. In the spruce trees where the wound was at the height of 1,3 m, sample discs were cut from the stem starting from the middle point (0) of the wound and continuing to a distance of ± 60 cm from the end point (± E) of the wound (Fig. 2). If the colour changes extended more than 60 cm, then additional discs were cut at distances of 80 cm, 100 cm and subsequently every half metre until the colour changes were no longer evident. The last disc was always taken from completely healthy wood. Sample discs were taken down to a distance of only 100 cm below the wound. The discs for the trees wounded at the root collar were taken in the same way as for trees with a wound at 1,3 m, expect that no discs could be taken below the wound point.

The isolated fungi were grown on malt agar (Nobles 1948). The bacteria isolations were prepared by paring off thin strips of wood from the discs and then homogenizing them in 0,9 % NaCl. The homogenate was then cultivated on Taylor's media for bacteria using the surface spreading technique (Kallio 1973).

4. RESULTS

41. Occurrence of micro-organisms

Bacteria formed the most common group of micro-organisms isolated from all over the spruce wounds (Table 2). They were found in 71 % of all the trees, half of which were control trees. The second rather large group consisted of yeast-like fungi, which were isolated from 64 % of the trees. All the white and black, extremely slow-growing fungi were classified as yeast-like fungi (Table 2).

As expected, *Stereum sanguinolentum* was the most common decay fungus, occurring in 34 % of the trees. In addition, *Heterobasidon annosum* was isolated from 15 % of the trees, although this fungus is not considered to be a proper wound decay fungus. A number of other decay fungi were isolated in small numbers from the wounds, such as *Panellus* mitis (Pers. ex Fr.) Sing., *Peniophora pithya* (Pers.) John Erikss., *Sistotrema brinkmannii* (Bres.) John Erikss. (Table 2).

42. The microbial composition at different wounding heights

Root collar wounds (0,5 m) were more susceptible to infection by *S. sanguinolentum* (χ^2-value 9,71**, level of significance 1 %) and *H. annosum* (χ^2-value 5,49*, level of significance 5 %) than stem wounds. *S. sanguinolentum* infected root collar wounds considerably more frequently than stem wounds in May, June and July (χ^2-value 8,33**), there was no difference during the late summer and the autumn (Fig.3). This may be due to differences in the moisture content at different heights along the tree stem, especially in the middle of summer.

Infection by *H. annosum* was clearly more common at a distance of 0,5 m during May — October (Fig. 3). Owing to the small number of observations, however, it was not possible to test the material statistically. Among the other micro-organisms, *Graphium* spp. (χ^2-value 5,47*) were more frequently isolated from root collar wounds. As regards the monthly variation, these fungi were especially abundant at a distance of 0,5 m in July (χ^2-value 5,15*) (Fig. 3). Only the bacteria (χ^2-value 4,09*) and yeast-like fungi (χ^2-value 9,14**) were able to infect the wounds on the stem (1,3 m) in June.

43. Growth of the micro-organisms in a vertical direction within the spruce stems

All the micro-organisms showed the strongest growth in the damage area (0-point and ± E cf. Fig. 4 and Table 3) in this study. The number of micro-organisms isolated from outside these areas fell considerably. Micro-organisms were found at distances of 5—20 cm from stem wounds in only 50 % of the trees (Table 3). Micro-organisms were isolated from the root-collar wounds upto the point +E cm in almost 100 % of the trees. Micro-organisms were isolated upto a distance of one metre in about 30 % of the trees. The number of micro-organisms isolated fell considerably at a distance of more than one meter from the wounds (Table 3).

The decay fungi, were present also in the wood in some part above the wounded area

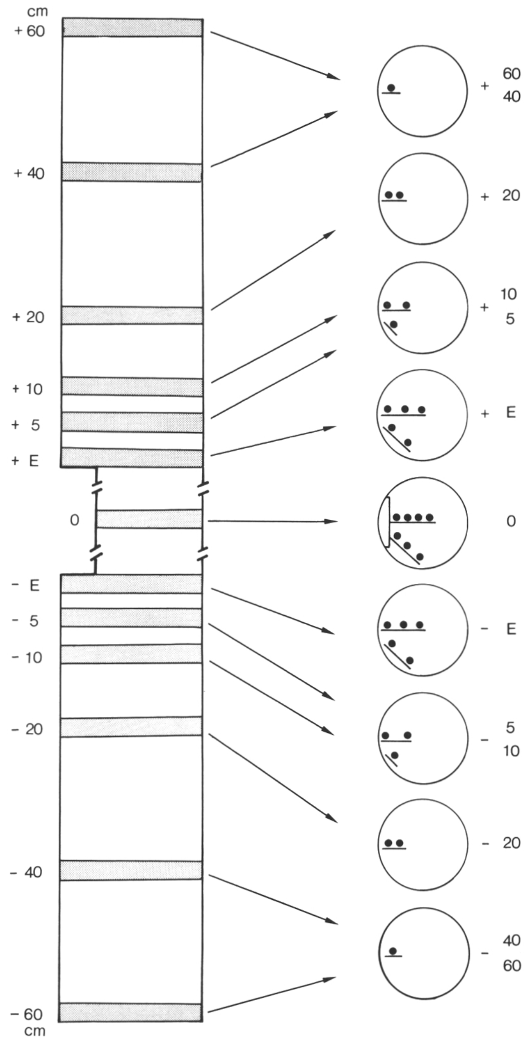

Fig. 2. Sample discs ( ) cut from the damaged spruce trees and the wood samples (●) removed from the discs for microbial cultivation.

Kuva 2. Vaurioitetuista kuusista sahatut näytekiekot ( ) sekä näistä mikrobien viljelyyn irroitetut näytepalat (●).

(Fig. 4). *Stereum sanguinolentum* was isolated most frequently from the wound area and upto a distance of + 40 cm (χ^2-value 6,07*) in the case of the stem wounds (Fig. 4). The corresponding distance in the case of the root-collar wounds was + 80 cm (χ^2-value 5,4*). The maximum spread of *S. sanguinolentum* after four growing seasons was one meters in the stem wounds and two meter in the root-collar wounds (Fig. 4). The corresponding yearly rate of spread was estimated as 25 cm and 50 cm.

Table 2. Micro-organisms found in damaged trees. Trees treated with the bacterial suspension (b), control trees (k).

Taulukko 2. Mikrobien esiintyminen vaurioitetuissa kuusissa. Bakteerisuspensiolla käsitellyt puut (b), kontrollipuut (k).

Micro-organism *Mikrobi*	Fertilized *Lannoitettu*				Unfertilized *Lannoittamaton*				Infected trees *Saastuneet puut* Total *Kokonaismäärät*	
	Height of wound — *Vauriokorkeus*									
	0,5 m		1,3 m		0,5 m		1,3 m			
	Wound treatment — *Vaurion käsittely*								No.	
	b	k	b	k	b	k	b	k	*kpl*	%
Heterobasidion annosum	2	6	2	1	4	9	1		25	15
Panellus mitis	1			1				2	4	3
Peniophora pithya	1		1				1		3	2
Sistotrema brinkmannii		1	1	1	1				4	3
Stereum sanguinolentum	10	6	3	5	12	9	4	7	56	34
Ascocoryne spp.	3	3	5	3	6	6	8	7	41	25
Aureobasidium pullulans						2	1		3	2
Graphium spp.	9	12	7	4	6	5	3	5	51	31
Nectria fuckeliana	7	6	3	3	3	6	4	7	39	23
Nectria sp.	4	1	3	2	2		3	3	18	11
Phialophora sp.	3	3	5	1	2	5	4	4	27	16
Penicillium spp.	3		2		1	1	2	1	10	6
Streptomyces spp.	1		3	4	2	1	1		12	7
Trichoderma viride	3	4	3	1	3	1			15	9
Yeast-like fungi *Hiivamaiset sienet*	16	12	16	15	12	10	13	12	106	64
Unidentified fungi *Tunnistamattomat sienet*	14	16	12	10	8	10	14	14	98	59
Bacteria — *Bakteerit*	14	16	15	13	15	14	16	15	118	71
Number of trees, total *Puita yhteensä kpl*	21	21	23	20	21	19	21	21	167	

Table 3. Proportion of trees where micro-organisms were growing in a vertical direction. 85 of the trees had stem wounds, and 82 had root-collar wounds.

Taulukko 3. Pystysuunnassa kasvavien mikrobien lukumäärä ja prosenttiosuudet puiden kokonaismäärästä (85 runkovauriopuuta, 82 juurenniskavauriopuuta).

Height of microbial growth, cm *Mikrobien etäisyys vauriosta cm:nä*	Stem wound (1,3 m) *Runkovaurio (1,3 m)* Upwards, no. (%) *Ylöspäin, kpl (%)*	Stem wound (1,3 m) *Runkovaurio (1,3 m)* Downwards, no. (%) *Alaspäin, kpl (%)*	Root-collar wound (0,5 m) *Juurenniskavaurio (0,5 m)* Upwards, no. (%) *Ylöspäin, kpl (%)*	Total, no. (%) *Yhteensä, kpl (%)*
0	82 (97)		81 (99)	163 (98)
E	73 (86)	77 (91)	77 (94)	154 (92)
5	41 (48)	51 (61)	49 (60)	100 (60)
10	47 (55)	50 (59)	65 (79)	115 (69)
20	34 (40)	44 (52)	57 (70)	101 (60)
40	26 (31)	30 (35)	45 (55)	75 (45)
60	23 (27)	28 (33)	33 (40)	61 (37)
80	14 (17)	8 (9)	24 (29)	38 (23)
100	9 (11)	6 (7)	22 (27)	31 (19)
150	9 (11)		18 (22)	27 (16)
200	6 (7)		8 (10)	14 (8)
250	3 (4)		7 (9)	10 (6)
300	1 (1)		5 (6)	6 (4)
350	1 (1)		5 (6)	6 (4)
≥400	25 (2)		8 (1)	33 (1)

Heterobasidion annosum spread in small amounts to a distance of + 10 cm in the case of stem wounds (Fig. 4). This decay fungus spread considerably more easily from the root-collar wounds, the maximum distance being + 2,5 m (Fig. 4). *H. annosum* was at its most abundant in the wounded area and upto a distance of + 40 cm (χ^2-

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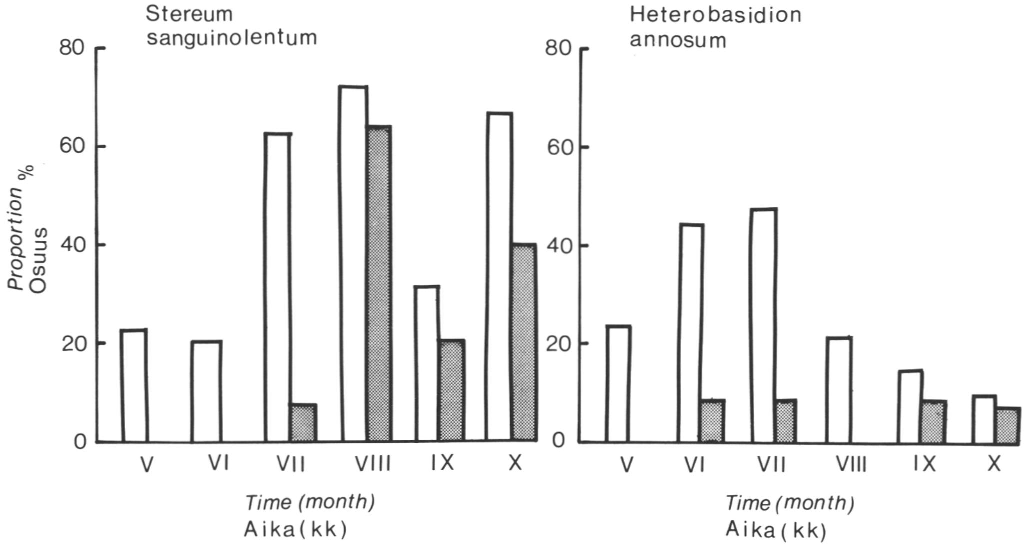


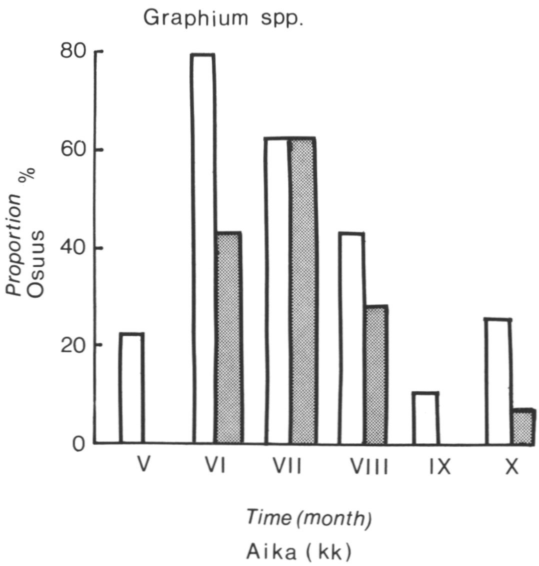


Fig. 3. Effect of wound height on the microbial population each month. Half of the trees were wounded on the root collar (0,5 m ☐) and half on the stem (1,3 m ▒). Month when wounding and treatment carried out, May—October (V—X).

Kuva 3. Vauriokorkeuksien vaikutus mikrobilajistoon kuukausittain. Puolet puista vaurioitettiin juurenniskakorkeudelle (0,5 m ☐) ja puolet runkokorkeudelle (1,3 m ▒). Vaurioitus- ja käsittelykuukaudet toukokuun—lokakuun (V—X).

value 32,5*** level of significance 0,1 %) (Fig. 4).

The yeast-like fungi which occurred in large numbers were clearly concentrated in the damaged area (0, +E) in both the stem (χ^2-value 86,79***) and the root-collar (χ^2-value 32,06***) wounds (Fig. 4). The yeast-like fungi occurred in small numbers throughout the stem outside the damage areas (Fig. 4).

Bacteria were the most common group of micro-organisms at almost all distances from the wounds (Fig. 4). Bacteria also showed the fastest rate of spread in the case of both the stem (+ 15,6 m) and the root-collar (+ 7,5 m) wounds (Fig. 4). There were four different zones apparent in the location of the bacteria. The greatest number of bacteria were found in the damage area (χ^2-value 29,72***). The next zone occurred upto + 60 cm from the stem wounds (χ^2-value 18,15***). Bacteria formed a third zone at a distance of between + 80 cm and + 2 m (χ^2-value 63,48***). The number of bacteria then fell considerably. However, bacteria were still isolated to some extent upto a distance of + 15,5 m from the stem wounds (Fig. 4). In the case of root-collar

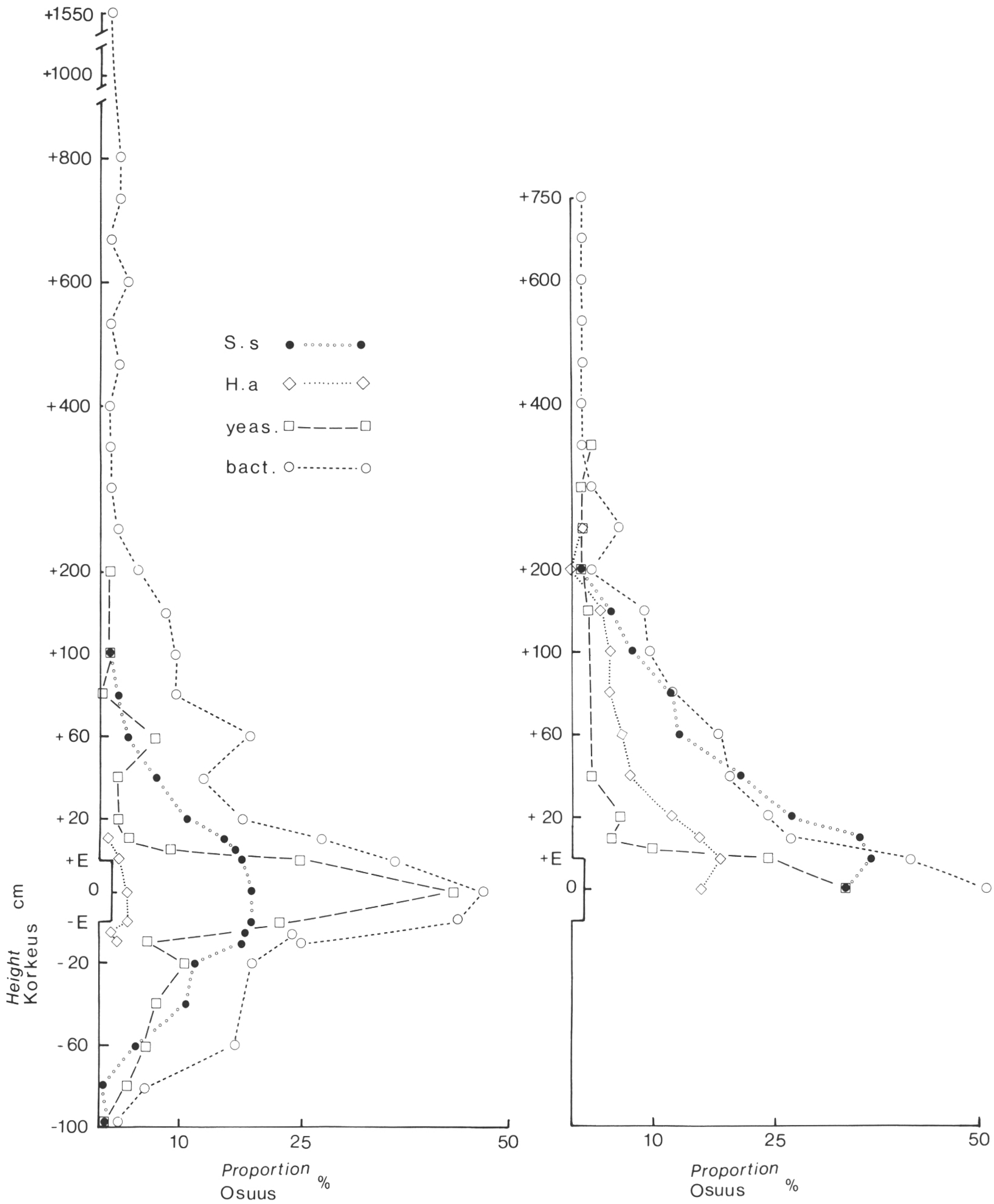


Fig. 4. Growth of the micro-organisms (% of total number of trees) in a vertical direction (cm) from the stem and root-collar wounds. Stem wounds on the left, root collar wounds on the right. S.s. = *Stereum sanguinolentum*, H.a = *Heterobasidion annosum*, yeas. = Yeast-like fungi, bact. = Bacteria.

Kuva 4. Mikrobien (%-osuudet puiden kokonaismäärästä) kasvu kuusen runko- ja juurenniskavaurioista pystysuunnassa (cm:nä). Runkovauriokuva vasemmalla, juurenniskavauriokuva oikealla. S.s. = Stereum sanquinolentum, H.a = Heterobasidion annosum, yeas. = hiivamainen sieni, bact. = bakteerit.

wounds the next zone occurred upto + 80 cm (χ^2-value 28,36***) and the third upto + 3 m (χ^2-value 36,75***) (Fig. 4). The rate of spread of bacteria cannot be directly compared with that of fungi since bacteria flow along the water conduction channels inside the tree. Fungi usually have to penetrate the cell walls, which is a much slower process than passing through the pores in the water conducting canals.

All the other micro-organisms which occurred to a significant extent in the wood material were concentrated in the damage area and in the immediate vicinity of the wound. These micro-organisms included: *Ascocoryne* spp. (χ^2-value 25,25***), *Graphium* spp. (χ^2-value 29,98***), *Nectria fuckeliana* (Booth) (χ^2-value 14,94***) and *Nectria* sp. These fungi spread to a greater distance from the root-collar wounds than from the stem wounds. *Ascocoryne* spp. spread in small numbers upto a distance of + 3,5 m. The maximum distances for the other fungi were + 60 cm for *N. fuckeliana,* + 40 cm for *Nectria* sp. and + 80 cm for *Graphium* spp.

431. The spread of the micro-organisms and colour changes in the wood

In most cases the colour changes had spread to a distance of about 5 m. The extent of the colour change exceeded this value in 17 of the trees with stem wounds and 10 with root-collar wounds.

S. sanguinolentum was the micro-organisms which had spread the furthest (36 %) of the trees from which the fungus was isolated. However, the colour change had already developed ahead of the fungus in all the cases. The micro-organism had spread to the apex point or above the colour change in twenty one (37 %) of these trees. Bacteria were isolated from above the colour change in eight trees (14 %) and in three cases they were the apex microbe. Other micro-organisms which had grown the furthest in trees infected with *S. sanguinolentum* were *Ascocoryne* spp. (7 %), yeast-like fungi (7 %) and in one case *H. annosum.*

In one case the colour change reached a distance of 15 m and bacteria were isolated at a distance of 15,5 m, and *S. sanguinolentum* of 1 m. In the other case the colour change reached a distance of 12,5 m, the micro-organism present at the apex of the colour change being *Sistotrema brinkmannii* (12,55 m) *S. sanguinolentum* had spread to a distance of 40 cm only.

H. annosum was the furthest micro-organism in seven of the trees (28 %) from which the fungus was isolated. The extent of spread of the fungus varied from 5—150 cm. The colour change had spread to a much greater height in these trees. The micro-organism had spread to a point above the colour change in four of these trees (16 %). Bacteria were present in the apex of the colour change in three (12 %) of these trees. Other apex micro-organisms present in trees with a colour change that had been infected by *H. annosum* were *Ascocoryne* spp. (12 %), *S. sanguinolentum* (8 %) and in one case (4 %) both *S. brinkmannii* and a yeast-like fungus.

Trees from which bacteria were isolated (55 %), but not *S. sanguinolentum* or *H. annosum,* are discussed in the following. Bacteria isolated from near to the damage area (0—20 cm), were in most cases associated with a yeast-like fungus (25 %). The yeast-like fungus was usually lying above the apex of the colour change and the bacteria below the apex in such trees. The colour change in trees infected with a bacteria usually extended for 2 m. In one of the trees the light coloured wood extended up to a distance of 18 m. The apex micro-organisms in this case were bacteria lying, at a distance of 14 m. In those cases where bacteria had reached a distance of 40 cm — 3 m, they were generally present as the sole apex micro-organisms (28 trees). Bacteria were present in most of the trees at a distance of about 20 cm above the colour change.

44. Growth of the micro-organisms from the sapwood into the heartwood

The number of micro-organisms isolated from the wood samples was highest in the area ranging from the wound into the wood to a depth of 30 mm. Rather a large number of isolations were obtained down to a depth of 60 mm. The maximum depth at which a micro-organism was isolated was 90 mm.

The decay fungi *S. sanguinolentum* and *H. annosum* grew most extensively at a depth of 10—40 mm in the spruce wood after four growing seasons (Fig. 5). The yeast-

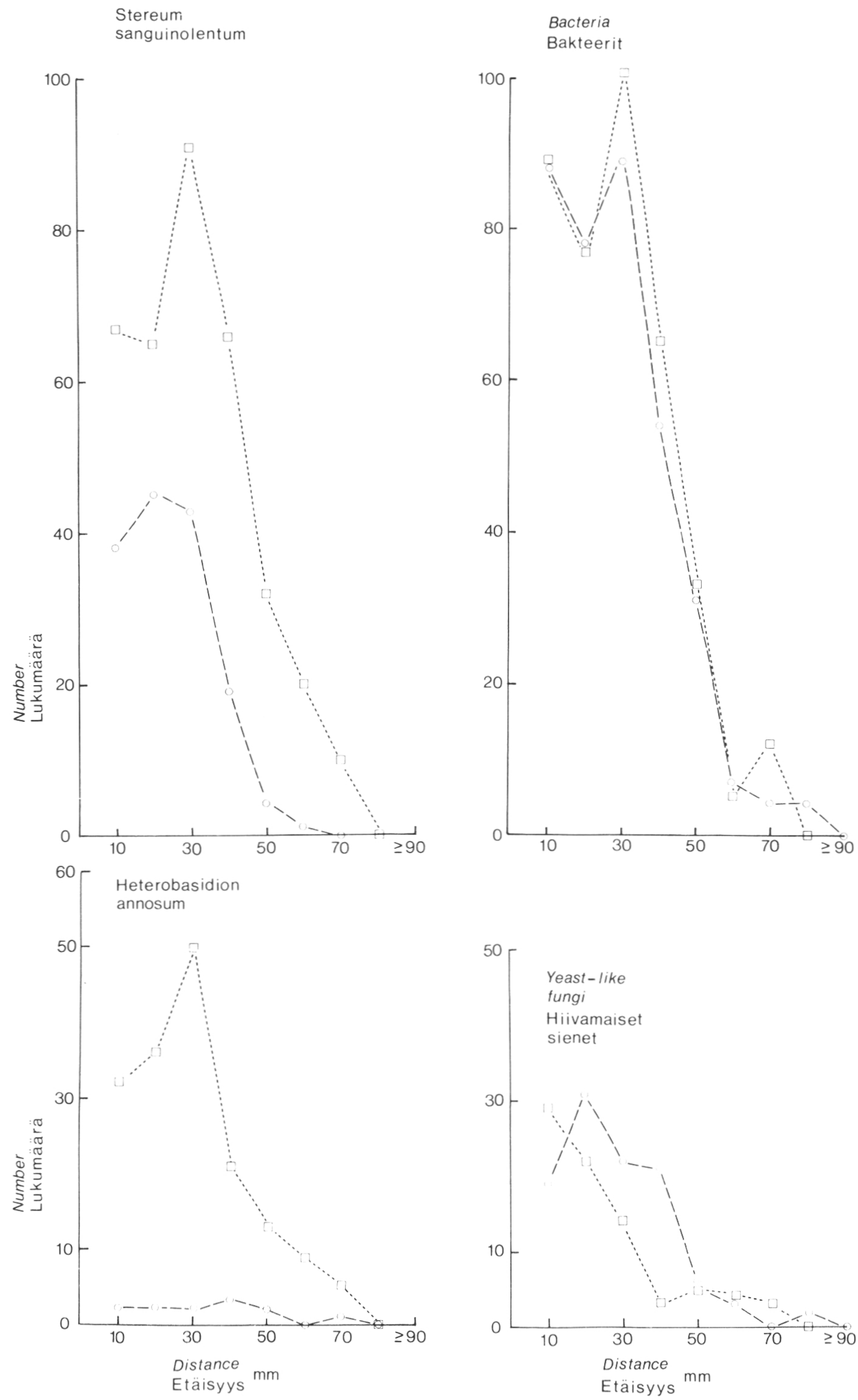


Fig. 5. Growth of micro-organisms from the sapwood inwards in stem wounds (o--o) and in root collar wounds (□······□). Distance from the wound in mm.

Kuva 5. Mikrobien kasvu puussa mannosta sydänpuuhun runko- (o--o) ja juurenniskavaurioissa (□......□). Etäisyys vaurioista mm:nä.

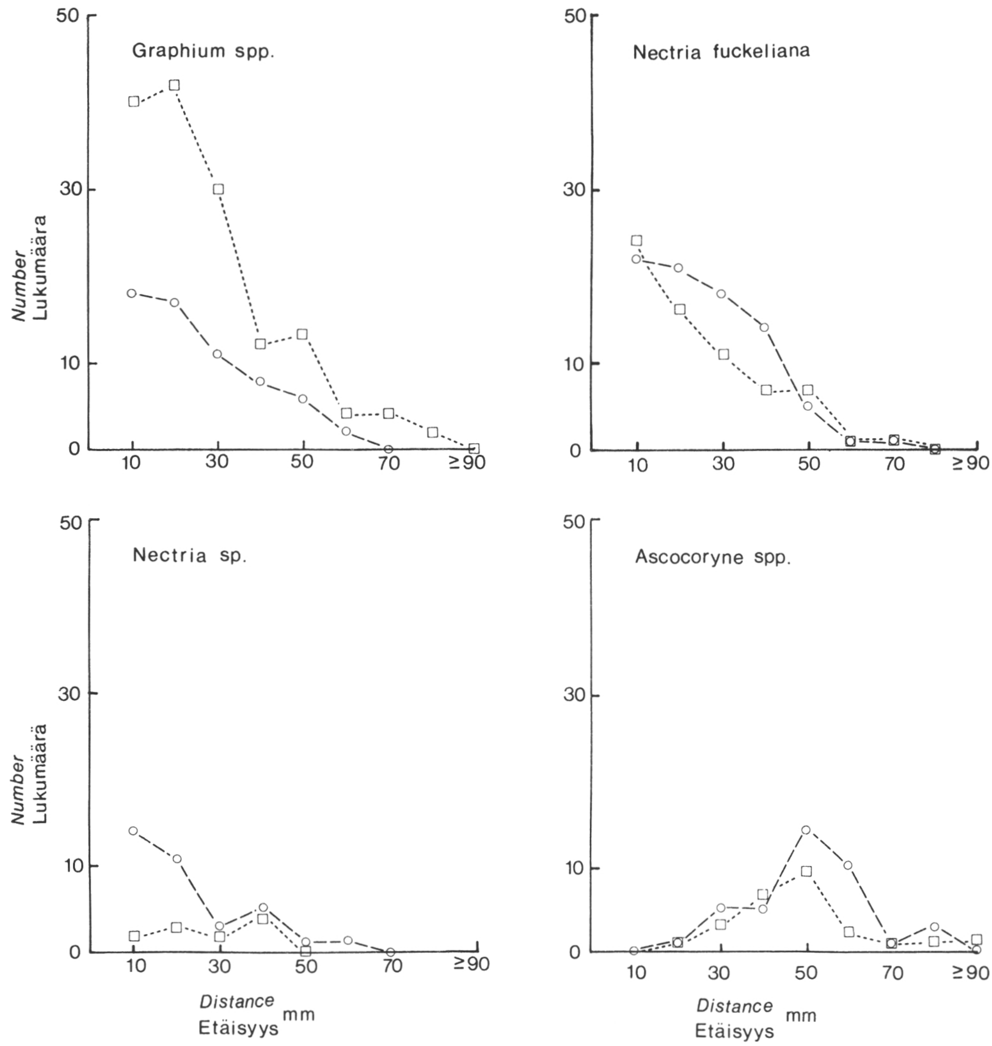
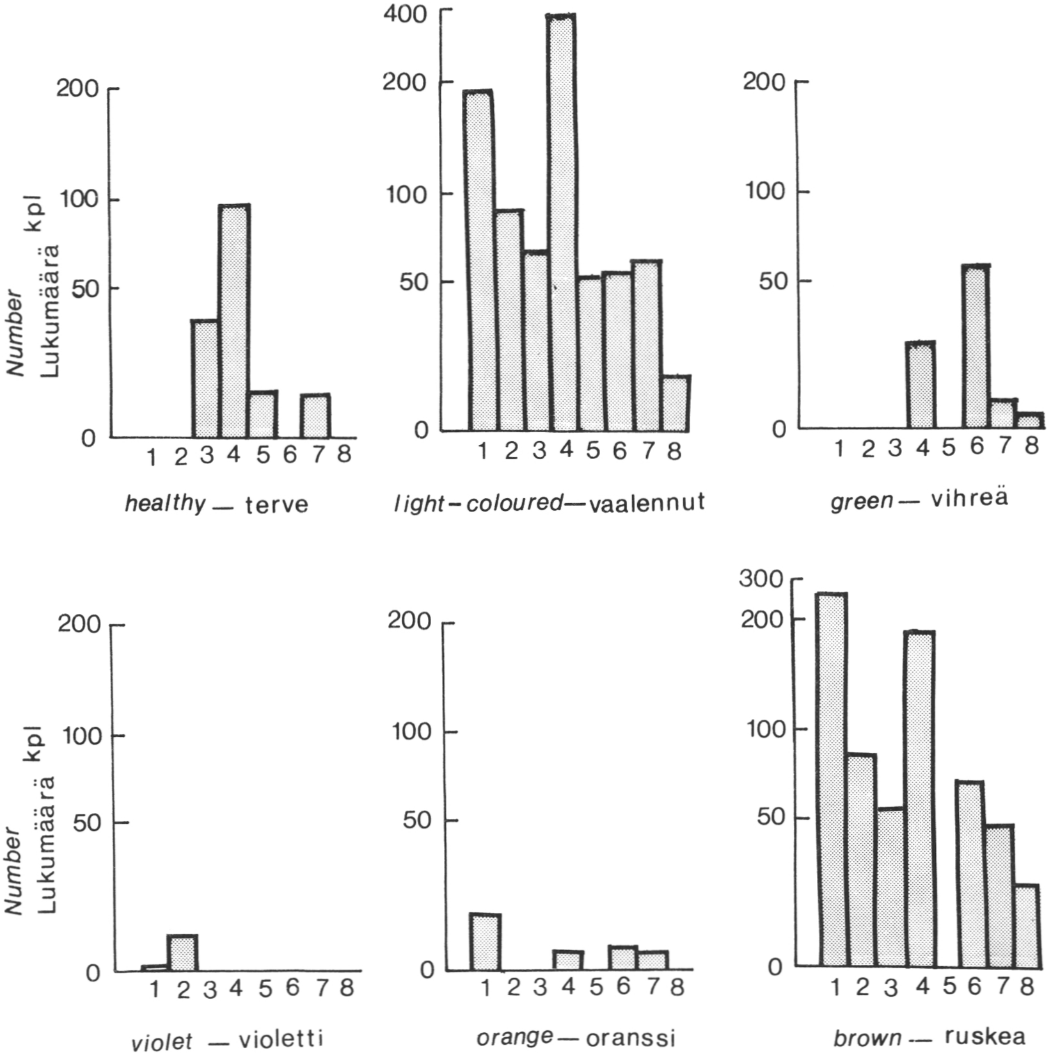


Fig. 6. Growth of micro-organisms from the sapwood inwards in stem wounds (o--o) and root collar wounds (□······□). Distance from the wound in mm.
Kuva 6. Mikrobien kasvu puussa mannosta sydänpuuhun runko- (o--o) ja juurenniskavaurioissa (□······□). Etäisyys vaurioista mm:nä.

like fungi and bacteria had reached the same depth. Both of these micro-organisms reached a depth of up to 90 mm (Fig. 5). This suggests that the bacteria and yeast-like fungi travel ahead of the decay fungi into the healthy wood. *Ascocoryne* spp. grew at a depth of 50—60 mm (Fig. 6). These fungi were also clearly located in the innermost parts of the wood. *Graphium* spp., on the other hand, were concentrated in the surface wood layer close to the damage area (Fig. 6). *N. fuckeliana* and *Nectria* sp. also grew most extensively in the surface layers, although without showing any clearly defined reduction in the deepest parts of the wood (Fig. 6).

45. Colour changes produced by micro-organisms in the wood

Colour changes were considered to be all those colours or shades which differed from the normal colour of the sapwood or heartwood of spruce. The most common change



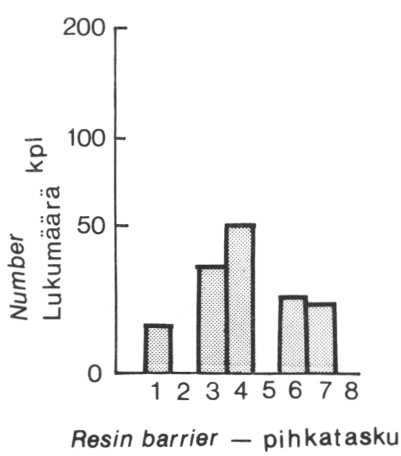


Fig. 7. Variation in the number of micro-organisms in wood of different colour. 1. *Stereum sanguinolentum*, 2. *Heterobasidion annosum*, 3. Yeast-like fungi, 4. bacteria, 5. *Ascocoryne* spp. 6. *Graphium* spp., 7. *Nectria fuckeliana*, 8. *Nectria* sp.

Kuva 7. Mikrobien määrän (kpl) vaihtelu eri värisessä puuaineksessa 1. Stereum sanguinolentum, 2. Heterobasidion annosum, 3. hiivamainen sieni, 4. bakteerit, 5. Ascocoryne spp., 6. Graphium spp., 7. Nectria fuckeliana, 8. Nectria sp.

was a lightening in the colour of the wood, which may have been caused by a decrease in the moisture content of the wood cells. The other wood colours observed in this study were orange, brown, green and violet.

Bacteria and *S. sanguinolentum* were isolated from the wood samples which were either brown or light coloured. Bacteria were predominant in light-coloured wood (381 samples), while the greatest number of *S. sanguinolentum* (260 samples) were found in brown wood (Fig. 7.) *Ascocoryne* spp. (52 samples) were isolated almost exclusively from light-coloured parts of the wood (Fig. 7). *H. annosum,* the yeast-like fungi, *Graphium* spp. *N. fuckeliana* and *Nectria* sp. occurred almost as commonly and in the same ratio in both these wood colour groups (Fig. 7). Bacteria, yeast-like fungi, *Ascocoryne* spp. and *N. fuckeliana* were isolated to some extent from wood which appeared to be perfectly healthy.

The other colours which occurred in the wood of spruce were green, violet and orange. These colours were found rather infrequently. *Graphium* spp. were commonly isolated from green coloured wood (55 samples) as well as a few bacteria (25 samples) (Fig. 7).

The onset of *H. annosum* rot (9 samples) was most frequently associated with the violet coloured wood (Fig. 7). Orange rot was rather rare and the most common fungus in this type of colour change was *S. sanguinolentum* (14 samples) (Fig. 7). Resin barriers were an important growing site for bacteria. The micro-organisms isolated from such sites, which are important defence mechanisms of trees, included bacteria (49 samples), yeast-like fungi (32 samples), *Graphium* spp. (21 samples), *N. fuckeliana* (19 samples) and even *S. sanguinolentum* (12 samples) (Fig. 7). Under certain conditions the micro-organisms are thus able to penetrate the resin barrier of the tree.

46. Effect of fertilization on the microbial composition

Fertilization affected the occurrence frequency of *Graphium* spp. (χ^2-value 4,12*) only. As far as the monthly distribution of these fungi were concerned, these fungi were most abundant in this area in August (χ^2-value 5,6*) (Fig. 8) compared to the unfertilized stand.

Of the decay fungi, *S. sanguinolentum* (χ^2-value 11,83***) occurred more frequently in the unfertilized stand (Fig. 8). According to the results of this study, fertilization had no effect on the occurrence frequency of *H. annosum* (χ^2-value 0,559). In addition, *Ascocoryne* spp. (χ^2-value 6,10*) were more common in the unfertilized stand (Fig. 8). In this area, *Ascocoryne* was at its most abundant in May (χ^2-value 9,96**) and the bacteria in October (χ^2-value 5,04*).

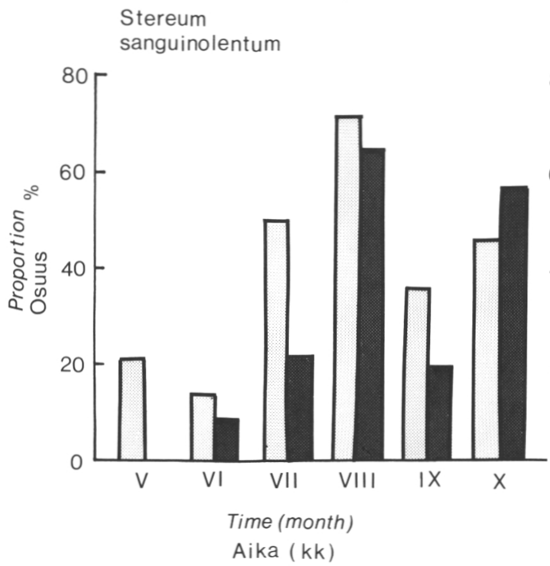
47. Effect of the bacterial treatment on the microbial composition

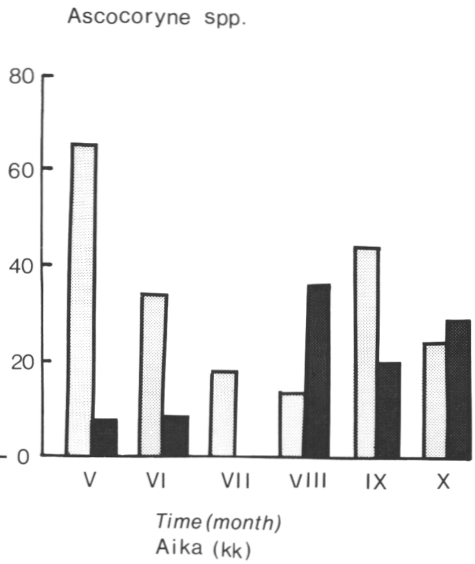
The mixed bacterial population used in this study did not have any effect on the microbial composition of the wounds under these conditions. *S. sanguinolentum* and *H. annosum,* which are both very important from the point of view of practical forestry, were as common in the spruce wounds treated with the bacterial suspension as in the control wounds. The mixed bacteria population thus did not exhibit the expected controlling effect under natural conditions.

471. Effect of the treatment dates

Differences in the monthly occurrence frequency of the fungi are evident in the material. *S. sanguinolentum* clearly infected the spruce wounds more frequently at the end of the summer and the autumn (χ^2-value 22,809***) than in the spring and early summer (Fig. 9). There were no monthly differences between the control wounds and those treated with the bacterial suspension.

The bacterial treatment prevented *H. annosum* infecting the wounds in July (χ^2-value 4,89*) and in September. However, the number of trees from which this fungus was isolated in September was so low that it was not possible to calculate the statistical probability. The total monthly occurrence





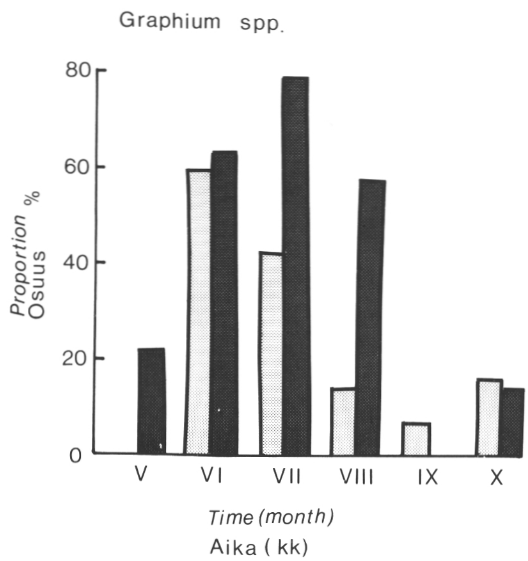


Fig. 8. Effect of fertilization on the microbial population in the wounds each month. Half of the trees were in an unfertilized stand (░) and half in a fertilized on (■). Month when wounding and treatment carried out, May—October (V—X).

Kuva 8. Lannoituksen vaikutus vaurioiden mikrobilajistoon kuukausittain. Puolet puista lannoittamattomassa (░) metsässä, puolet lannoitetussa (■). Vaurioitus- ja käsittelykuukaudet toukokuu—lokakuu (V—X).

of *H. annosum* was of the same order of magnitude (χ^2-value 7,66) throughout the period May — October (Fig. 9).

As regards the other micro-organisms, there were differences between the occurrence of *Graphium* spp. and *Nectria sp. Graphium* spp. were frequently isolated from the spruce wounds in June, July and August (χ^2-value 42,84***) and *Nectria* sp. in May and June (χ^2-value 12,06*) (Fig. 9).

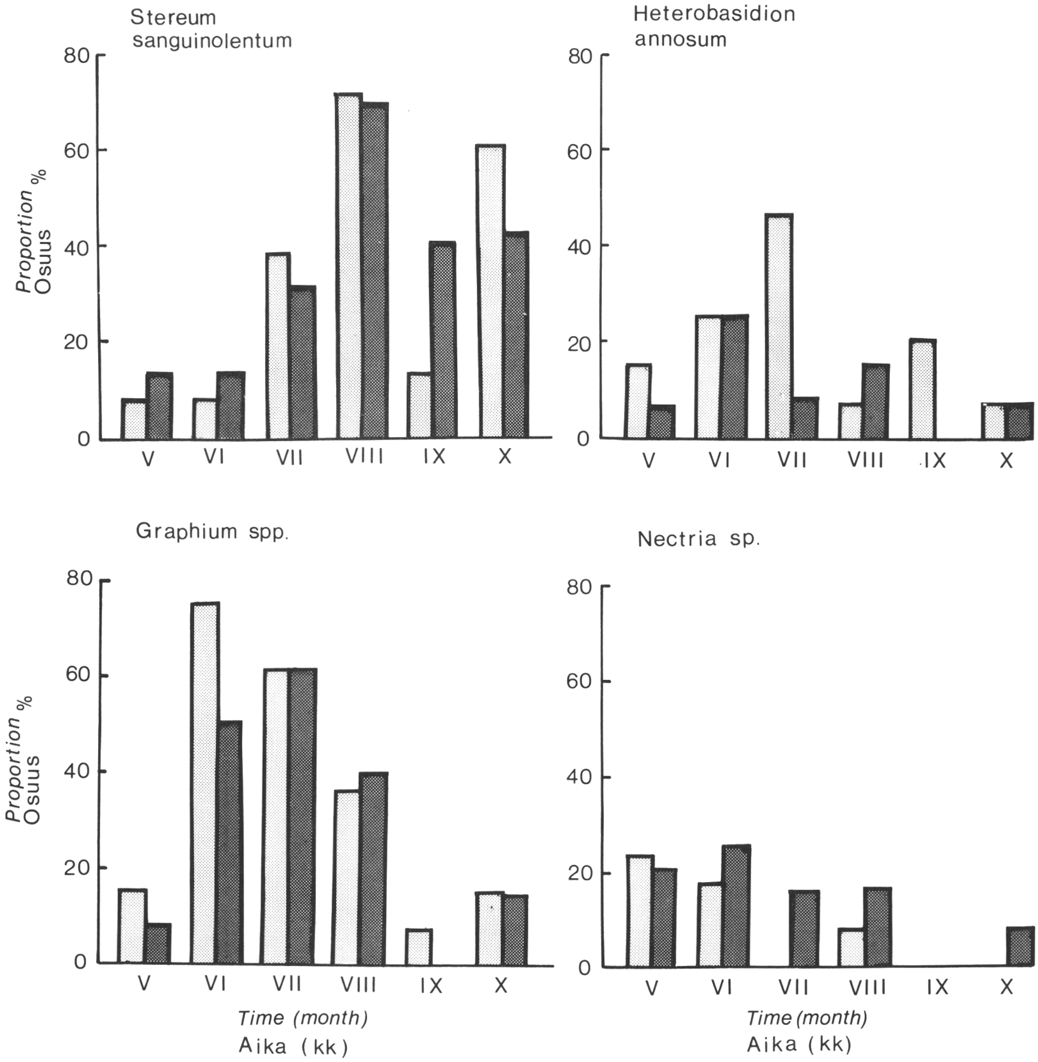


Fig. 9. Effect of treatment with the bacterial suspension on the microbial population present in the spruce wounds each month. Control trees ░ , trees treated with the bacterial suspension ▓ , month when wounding and treatment carried out May—October (V—X).

Kuva 9. Bakteerikäsittelyn vaikutus kuusen vaurioiden mikrobilajistoon kuukausittain. Merkinnät, kontrollipuut ░ , bakteerilla käsitellyt puut ▓ , vaurioitus- ja käsittelykuukaudet touko—lokakuu (V—X).

5. DISCUSSION

51. The micro-organisms in the spruce wounds

The number of micro-organisms observed in this study was slightly lower than that in a study carried out on spruce wounds in Norway over a corresponding period of time. The difference may be due to the sampling methods used. This study concentrated on analysing the colour changes, while in the Norwegian study (Roll-Hansen and Roll-Hansen 1980a) samples were taken from a considerably wider area in the wood. On the other hand, in this study micro-organisms were growing in less than 20 % of the samples already at a distance of 80 cm. In Roll-Hansen's (1980a) study the corresponding percentage level was not reached until a distance of 1,4 m. This shows that there was a greater number of fungi, such as *Ascocoryne* spp., in the wood. In other words, taking more samples from healthy wood on the one hand increases the number of sterile samples, and on the other hand gives a more accurate picture of the distribution of micro-organisms which do not change the colour of the wood. It is also impossible to know which micro-organisms are already present in healthy trees before wounding.

511. Bacteria

The number of bacteria in wood was actually much higher in this study than has been shown in most of the earlier studies. Considerably less bacteria have been found in corresponding studies (Kallio 1973, Roll-Hansen and Roll-Hansen 1980b). In addition to this, the only study in which the number of bacteria was found to be significant was the study carried out in Germany on well-advanced rot (Pechmann and Aufsess 1971). Bacteria are not even mentioned in many studies. In some studies they are considered to be of only slight importance (Pawsey and Stankovicova 1974b, Aufsess 1978). There are some references in the literature to the rate of spread of bacteria. Roll-Hansen and Roll-Hansen (1980b) observed bacteria at a distance of 60 cm within four years. Kallio (1973) estimated that bacteria spread the same distance (60 cm) in one year. It is primarily a question of how effective is the method used for isolating the micro-organisms.

As bacteria occur together with decay fungi in the living wood above the apex of the discoloured column in trees, the question is how strongly and at what stage do bacteria affect the decaying process brought about by fungi in the wood. Bacteria may have a synergetic effect, especially during the initial stage of the rot process. The presence of bacteria in the healthy living wood above the discoloured column may facilitate the spread of the decaying fungi in the wood. In any case the ability of bacteria to decompose the walls of the wood cells is much more restricted than that of the proper decay fungi (Liese and Greaves 1975). The bacteria may synthesize vitamins or other growth-promoting substances, for instance, for the decay fungi. The benefit which the bacteria gain from their association with the fungi is that they are supplied with the structural compounds of the cell walls broken down by the extracellular enzymes produced by the decay fungi (Henningsson 1967). The ability of bacteria living in the wood to fix atmospheric nitrogen may increase the rate of growth of the mycelia of the rot fungi and thus promote the break-down of wood material (Aho et al. 1974).

Answers to these questions can be obtained by identifying the composition of the mixture of bacteria present in the wounds of living spruces. In addition, the biochemical properties of these bacteria and the ability of fungi and bacteria to decompose the different components of trees should be determined.

512. *Stereum sanguinolentum*

Stereum sanguinolentum was the most common decay fungus in this study, as was the case in several earlier studies (Pawsey and Gladman 1965, Schönhar 1969, Pechman and Aufsess 1971, Kallio 1973, 1976, Isomäki and Kallio 1974, Aufsess 1978, Norokorpi 1980).

The wood is susceptible to attack by *S. sanguinolentum* immediately after the tree has been damaged and remains so for many years to come. Pawsey and Stankovicova (1974b) isolated *S. sanguinolentum* from stem wounds inoculated with the fungus already three months after the wounds had been made. On the other hand, the fungus infected 24 % of the trees within one year and the infection percentage increased to 37 during four years (Roll-Hansen and Roll-Hansen 1980a). In Germany, Schönhar (1975) isolated *S. sanguinolentum* from 27 % of the damaged spruce trees within one year and from 46 % after three years.

The maximum rate at which *S. sanguinolentum* had spread in this study was of the same order of magnitude (about 40 cm/year) as the rates reported in other studies (Pawsey and Gladman 1965, Kallio 1976, Roll-Hansen and Roll-Hansen 1980a).

S. sanguinolentum is not always the dominant species in spruce wounds. Huse (1978) studied 10-year-old wounds and found that the most common decay fungus was *Cylindrobasidium evolvens* (Fr. ex Fr.) Jülich (in 36 % of the trees). The proportion of *S. sanguinolentum* was 20 %. Roll-Hansen and Roll-Hansen (1980a) found *C. evolvens* in 19 % of the 2 to 4-year-old wounds. This fungus was found only once in the study in hand, and in this case the tree had been infected by *H. annosum* rot before it had been damaged. Both *C. evolvens* and *S. sanguinolentum* are primary rot fungi and their relationship varies from area to area in different aged wounds.

513. *Heterobasidion annosum*

H. annosum was of considerably less importance as a wound rot fungus than *S. sanguinolentum,* in this study although there was much *H. annosum* -butt rot in the area. This decay fungus is more successful in damaged trees in older spruce stands (Kato 1967, Pechman and Aufsess 1971, Isomäki and Kallio 1974, Kallio 1976) than in young stands subjected to thinnings (Pawsey and Gladman 1965, Schönhar 1975). In Norway, the fungus was isolated from 6 % of the artificially damaged 50 to 60-year-old spruce trees (Roll-Hansen and Roll-Hansen 1980a). In northern Finland (above the southern limit of the Northern Boreal Zone) *H. annosum* is not found at all in spruce wounds (Norokorpi 1980). According to the results of a number of other studies, *H. annosum* is of no importance as a wound rot fungus in younger forests (Hopffgarten 1933, Braun 1960, Pawsey and Stankovicova 1974a, Aufsess 1978).

Rennerfelt (1947) reported a growth rate of the same order of magnitude (50—60 cm/year) as found in this study from root-collar wounds. The rate of growth can vary and it slows down with time. The growth of *H. annosum* was 1 m in two years and 80 cm during four years (Roll-Hansen and Roll-Hansen 1980a). The size and depth of the wound has a decisive effect on the surface area and rate of spread of the rot. These factors make it difficult to compare the results of different studies.

514. *Other micro-organisms*

It was apparent in our study and in many other studies that *Ascocoryne* grows in the innermost parts of the wood, in the hertwood, and even in healthy trees (Etheridge and Carmichael 1955, Etheridge 1970, Kallio 1973, Roll-Hansen and Roll-Hansen 1979b, Huse 1981).

The fungus is a pioneer species which grows together with decay fungi in trees damaged by rot and in healthy wood (Etheridge and Carmichael 1955, Roll-Hansen and Roll-Hansen 1979b, 1980b, Huse 1981). In this study the combined name *Ascocoryne* spp. was used for *A. cylichnium* and *A. sarcoides.* The use of a group name is preferable because the differences between the species are not always clear (Roll-Hansen and Roll-Hansen 1979a, b).

Graphium species were common in the wounds examined in this study. As regards other studies in this field, Schönhar (1975) found large numbers of *Ceratocystis* (perfect state of *Graphium)* species in 46 % of the wounds. Roll-Hansen and Roll-Hansen (1980a) isolated *Ceratocystis* species from a total of 15 % of the wounds. *Graphium* spp. were present in the largest numbers in June — August. Warm and dry weather favour the dissemination of this fungus (Etheridge 1969). Roll-Hansen and Roll-Hansen (1980b) found *Ceratocystis* spp. in June and September. In the study presented here, *Graphium* spp. were concentrated in the area of the wound when growing upwards from the wound and into the wood. The fungus is a species which penetrates the wood strongly via wounds, primarily in the vicinity of the wound (Roll-Hansen and Roll-Hansen 1980b).

Roll-Hansen and Roll-Hansen (1980b) isolated *Nectria fuckeliana* in much greater numbers than in our study. This may be because they took many more samples. On the other hand according to both studies this fungus did not spread more than 60 cm upwards from the wounds.

Some of the microbes which occur in large numbers in the initial stage of the rot process in living spruces are able alone or together, to slow down the activity of rot fungi.

52. Effect of fertilization

According to the results of this study, fertilization (with urea) had only a slight effect on the occurrence frequency of the micro-organisms. On the other hand, nitrogen fertilization may, in the short term, considerably increase the number of fungi in pine stumps (Tsernyh 1978) or the rate of spread of rot in spruce wounds (Isomäki and Kallio 1974).

53. The controlling effect of the bacterial treatment

The bacterial treatment did not significantly reduce the numbers of *S. sanguinolentum* and *H. annosum* in the wounds of spruce during four growing seasons in this study. A similar result has been found in other studies with bacterial treatments (Swinburne 1978, Swinburne and Brown 1976, Spiers 1980).

The fundamental problem encountered in this study was making the bacteria stick onto the damage area. Excretions from the wound have a significant effect on the survival of the micro-organisms on the surface of the wound (Pawsey 1971). In the case of surface wounds, the micro-organisms are faced with the greatest number of competitors in the surface layers and they are not able to grow in the wood at the same rate as micro-organisms which have already passed deeper into the wood (Pawsey and Stankovicova 1974b). It is certain that some of the bacteria are washed away and that some are absorbed into the wood and are carried away from the damage area by the internal water circulation of the tree. Some sort of binding material which would absorb the bacteria should be developed. This type of "paste" could be used to cover the whole damage area. On the other hand, it is important how the bacteria is stored. In our study it had been kept more than one year in test tubes (+ 4°C).

The bacterial population used in this study presumably consisted of four species of bacteria. According to our most recent studies, the bacteria isolated from spruce are in fact mixtures of species. It may perhaps be a species mixture rather than only pleomorphism (Kallio 1974). Taylor and Guy (1981) observed that the bacterial-controlling effect was also based on a bacterial population.

6. SUMMARY

The interaction between micro-organisms in wound rot was studied by determining the location of the fungi and bacteria in the discoloured columns of the spruce trees.

The most common group of micro-organisms isolated from the spruce wounds were bacteria. Bacteria were most common at almost all heights. The maximum distance which they had spread was 15,5 m within four years after the trees had been damaged. The bacteria were often the apex micro-organism in trees infected by *Stereum sanguinolentum* and *Heterobasidion annosum*. A lightening of the colour of the wood was concentrated in the area where the bacteria were to be found. Bacteria were also isolated from healthy, green and brown coloured wood. Bacteria appeared to be present at all stages of the rot process.

Stereum sanguinolentum was the most common decay fungi in the spruce wounds after four growing seasons. The fungus infected root-collar wounds considerably more often in May — July than the stem wounds. *S. sanguinolentum* spread during the period of four years up to a distance of 1 m in the case of the stem wounds, and 2 m in the case of the root-collar wounds. *S. sanguinolentum* spread from the surface wood inwards at a rate of 4 cm in four years. *S. sanguinolentum* was mainly isolated from strongly coloured brown and orange wood. This fungus was quite often found as the wood was becoming lighter in colour.

Heterobasidion annosum was isolated from 15 % of the wounds. The fungus infected root-collar wounds considerably more frequently in May — October than stem wounds throughout the course of the study. The maximum distance which *H. annosum* had spread during four years was 2,5 m upwards from the root-collar wounds. In the case of the stem wounds, the fungus had spread only 10 cm. This fungus was also isolated from wood which had become brown or light in colour.

Other micro-organisms isolated in considerable amounts were: *Ascocoryne* spp. (in 25 % of the trees), *Graphium* spp. (31 %), *Nectria fuckeliana* (23 %) and *Nectria* sp. (11 %). These fungi were concentrated in the damaged areas. *Ascocoryne* reached a distance of up to + 3,5 m in small amounts. According to the results of this study, *Ascocoryne* was the only micro-organism which clearly grew in the innermost parts of the wood, even in the heartwood.

Samples were taken from the cambium behind the resin barrier (defence barrier) formed by the tree during the study. These "resin barrier" samples were used to detemine whether micro-organisms were able to penetrate the defences of the tree. Large numbers of bacteria, yeast-like fungi, *Graphium* spp. and *N. fuckeliana* were isolated from the resin barrier. The decay fungus *S. sanguinolentum* was also found (in 20 % of the trees). The micro-organisms are thus able to penetrate the resin barrier of the tree under certain conditions.

The effect of fertilizing forest stands on the microbial flora infecting the wounds was also investigated. *S. sanguinolentum* was the most common decay fungi in the unfertilized stand. Fertilization had no effect on the occurrence frequency of *H. annosum*. Only *Graphium* spp. increased as a result of fertilization.

The capacity of a mixed bacterial population to prevent the passage of decay fungi into spruce wounds was also investigated in this study. Under laboratory conditions the bacterial population was antagonistic towards *H. annosum* and *S. sanguinolentum* but not when inoculated in the wounds of spruces.

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Total of 39 references

SELOSTE

Bakteerit ja niiden vaikutus elävien kuusien vaurioiden mikrobilajistoon

Tutkimuksen tarkoitus

Tutkimuksessa analysoitiin mikrobien keskinäisiä vuorovaikutussuhteita vauriolahoissa, määrittämällä sienien ja bakteerien sijainti kuusten vauriolahoissa. Myös selvitettiin metsikön lannoituksen vaikutusta vaurioihin iskeytyvään mikrobilajistoon.

Eräässä suomalaisessa tutkimuksessa löydettiin bakteeriseos, joka yksin oli saanut jalansijaa vuoden vanhassa kuusen vauriossa. Tämä bakteeriseos osoittautui laboratoriossa suoritetuissa kokeissa antagonistiseksi mm. juurikääpää *(Heterobasidion annosum* (Fr.) Bref.) ja verinahakkaa *(Stereum sanguinolentum* (Alb. Schw. ex. Fr.) kohtaan. Tässä tutkimuksessa selvitettiin bakteeriseoksen kyky estää lahottajasienten pääsy kuusen vaurioihin.

Tutkimusmetsiköt ja koejärjestelyt

Tutkimusmetsiköt sijaitsevat Maatalouden tutkimuskeskuksen metsissä Jokioisissa. Kokeessa oli kaksi noin 80 vuotiasta MT-kuusikkoa. Toinen oli lannoitettu urealla (300 kg hehtaaria kohden) vuonna 1973 ja toinen lannoittamaton. Vuonna 1974 kummassakin metsässä vaurioitettiin puita kerran kuussa touko-lokakuun aikana. Puut vaurioitettiin kivilekalla rikkomalla puun kuori ja pinta. Vaurion koko oli n. 10 neliösenttimetriä. Molemmissa metsissä vaurioitettiin kuukausittain kahdeksan kuusta runkoon 1,3 m:n korkedelle ja juurenniskan korkeudelle (0,5 m). Kummankin ryhmän neljä vauriota käsiteltiin antagonistisella bakteerisuspensiolla (kuva 1), toiset neljä jätettiin kontrolleiksi (taulukko 1). Bakteerimassasta (kasvatus THG-alusta 3 vrk, 24°C:ssa) valmistettiin tuore suspensio, jonka solutiheys mitattiin spektrofotometrisesti jokaisen saastutuspäivän aamuna. Bakteeritiheys vaihteli suspensiossa eri käsittelykertoina $8{,}3 \times 10^6$—$9{,}3 \times 10^6$ kpl/ml. Tutkimusaineisto koostui alunperin 192 vaurioitetusta kuusesta. Näistä puista oli kokeen päättyessä 23 pahasti tyvilahoisia ja 2 oli myrsky vuosien varrella kaatanut. Lopulliseen tutkimusmateriaaliin jäi näinollen 167 vaurioitettua puuta (taulukko 1).

Näytteenotto

Puut kaadettiin neljän kasvukauden jälkeen marras-joulukuussa vuonna 1977. Kuusista, joissa vaurio oli 1,3 m:n korkeudella, sahattiin näytekiekot vaurioalueelta lähtien ±60 cm:n korkeudelle (kuva 2). Mikäli värivika eteni 60 cm:n yläpuolelle otettiin näyte 80 cm:n, 1 m:n ja tämän jälkeen puolen metrin välein kunnes värivika loppui. Viimeinen näytekiekko sahattiin aina terveestä puuaineksesta. Juurenniskavaurioista edettiin kuten 1,3 m:n korkeudelta, mutta vain ylöspäin.

Sienet viljeltiin mallasagareille. Bakteerien eristystä varten jokaisesta näytepalasta vuoltiin lastuja, jotka homogenisoitiin 0,9 % NaCl:ssa ja viljeltiin pintalevitystekniikalla bakteereja suosiville Tayloralustoille.

Lahottajasienet kuusen vaurioissa

S. sanguinolentum (verinahakka) oli yleisin (34 %:ssa puista) lahottajasieni kuusen vaurioissa neljän kasvukauden jälkeen. Sieni saastutti toukoheinäkuussa huomattavasti useammin juurenniskavaurion (0,5 m) runkovaurioon (1,3 m) varrattuna (kuva 3). Verinahakka eteni neljän vuoden aikana runkovaurioissa aina 1 metriin ja juurenniskavaurioissa 2 metrin korkeudelle (kuva 4). Vuotta kohden arvioituna vastaavat luvut olivat 20 cm ja 50 cm. Näissä puissa värinmuutos eteni yleisesti viiteen metriin. Bakteerit olivat usein värin kärjessä. Verinahakka eteni pintapuusta sydämeen päin neljässä vuodessa 4 cm (kuva 5). Voimakkaasti värjäytyneestä ruskeasta ja oranssista puuaineksesta eristettiin suurin osa *S. sanguinolentum* -sienistä (kuva 7). Sieni on usein eristettävissä puuaineksesta ennen värinmuutosta.

H. annosum tavattiin 15 %:ssa vaurioista. Sieni saastutti koko tutkimusjakson ajan touko-lokakuussa huomattavasti yleisemmin juurenniskavaurion kuin runkovaurion (1,3 m) (kuva 4). *H. annosumin* maksimietäisyys neljässä vuodessa oli 2,5 m juurenniskavauriosta ylöspäin (kuva 4). Maksimietäisyys vuotta kohden oli 63 cm. Runkovauriossa sieni eteni vain 10 cm (kuva 4). Näissä puissa värinmuutos eteni 5 m. Juurikääpä oli usein kärkimikrobi etenemisnopeuden vaihdellessa 5—150 cm:iin. Tämä lahottajasieni eristettiin puun vaalenemista ja ruskeaksi värjäytyneestä puuaineksesta (kuva 7).

Bakteerit kuusen vaurioissa

Yleisin kuusen vaurioista eristetty mikrobiryhmä oli bakteerit. Niitä tavattiin 71 %:ssa puita (taulukko 2). Puolet näistä puista oli kontrollipuita toinen puoli bakteerisuspensiolla käsiteltyjä puita. Bakteerit olivat yleisin mikrobiryhmä lähes kaikilla korkeuksilla. Niitä esiintyi runsaasti aina 2 m korkeuteen. Maksimietäisyys oli 15,5 m neljän vuoden kuluttua vaurioittamisesta (kuva 4). Bakteerit olivat usein kärkimikrobeina *S. sanguinolentum*- ja *H. annosum* -puissa. Bakteerin seuralainen oli hidas hiivamainen sieni. Puuaineksen värin vaalentuma oli keskeisesti bakteerien sijoittumisalue. Myös terveestä, vihreästä ja ruskeasta puuaineksesta eristettiin bakteereja (kuva 7). Bakteerit näyttivät olevan mukana kaikissa lahovaiheissa.

Muut mikrobit kuusen vaurioissa

Muita huomattavassa määrin eristettyjä mikrobeja olivat *Ascocoryne* spp. (25 %:ssa puita), *Graphium* spp. (31 %), *Nectria fuckeliana* (23 %) sekä *Nectria* sp. (11 %). Nämä sienet keskittyivät vaurioalueelle. *Ascocoryne* eteni vähäisessä määrin aina + 3,5 m:n korkeudelle. *Ascocoryne* oli tämän tutkimuksen ainoa edellä luetelluista mikrobeista, joka kasvoi selvästi puun sisemmissä osissa jopa sydänpuussa.

Tämän tutkimuksen yhteydessä otettiin näytteitä nilasta puun muodostamien pihkavallien eli puolustusmuurin takaa. Näiden "pihkatasku" näytteiden perusteella selvitettiin pystyvätkö mikrobit tunkeutumaan puun puolustuksen taakse. Pihkataskuista eristettiin runsaasti bakteereja sekä hiivamaisia sieniä, *Graphium* spp. ja *N. fuckeliana* (kuva 7). Lisäksi löytyi lahottaja *S. sanguinolentum* (20:stä puusta) (kuva 7). Joissakin olosuhteissa mikrobit siis löytävät tiensä puuhun pihkabarrikaadin taakse.

Lannoituksen vaikutus mikrobilajistoon

Ainoastaan *Graphium* -lajit lisääntyivät lannoituksen vaikutuksesta (kuva 8). Lahottajasienistä *S. sanguinolentum* esiintyi runsaampana lannoittamattomassa metsikössä (kuva 8). Lannoitus ei vaikuttanut *H. annosumin* esiintymisrunsauteen.

Bakteerikäsittelyn torjuntavaikutus

Tutkimuksessa käytetyllä bakteeriseoksella ei näissä olosuhteissa ollut vaikutusta mikrobilajistoon. Metsätaloudellisesti merkittävät lahottajat *S. sanguinolentum* ja *H. annosum* esiintyivät sekä bakteerisuspensiolla käsitellyissä että kontrolleiksi jätetyissä kuusen vaurioissa yhtä useasti. Bakteereilla ei siis ollut odotettua torjuntatehoa luonnon olosuhteissa.

Jos materiaalia tarkastellaan kuukausittain, havaitaan sienten esiintymisrunsaudessa eroja. *S. sanguinolentum* iskeytyi kuusen vaurioihin selvästi yleisemmin loppukesällä ja syksyllä kevään ja alkukesän vaurioihin verrattuna (kuva 9). Kuukausittain ei ollut eroja kontrollivaurioiden ja bakteerilla käsiteltyjen vaurioiden välillä. Bakteerikäsittely torjui juurikäävän vaurioista heinä- ja syyskuussa (kuva 9). Sienien lukumäärä syyskuun vaurioissa oli kuitenkin niin alhainen, ettei tilastollista todennäköisyyttä voitu laskea.

Hallaksela, A-M. 1984. Bacteria and their effect on the microflora in wounds of living Norway spruce (*Picea abies*). Seloste: Bakteerit ja niiden vaikutus elävien kuusien vaurioiden mikrobilajistoon. Commun. Inst. For. Fenn. 121: 1 — 25.

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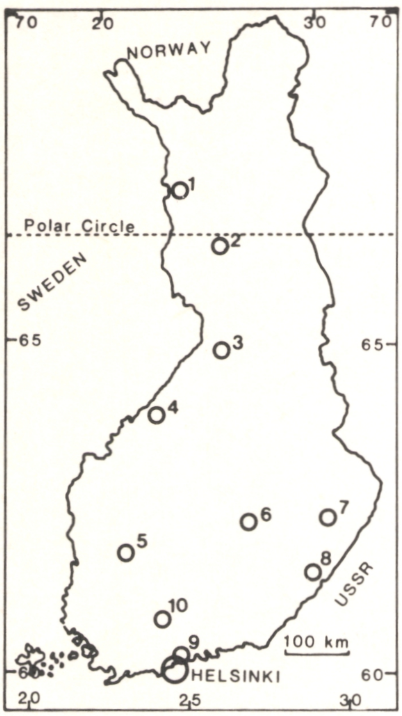

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FACTS ABOUT FINLAND

Total land area: 304 642 km^2 of which 60—70 per cent is forest land.

Mean temperature, °C:	Helsinki	Joensuu	Rovaniemi
January	-6,8	-10,2	-11,0
July	17,1	17,1	15,3
annual	4,4	2,9	0,8
Thermal winter (mean temp. < 0°C):	20.11.—4.4.	5.11.—10.4.	18.10.—21.4.

Most common tree species: Pinus sylvestris, Picea abies, Betula pendula, Betula pubescens

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121 Hallaksela, A-M. Bacteria and their effect on the microflora in wounds of living Norway spruce *(Picea abies)*. Seloste: Bakteerit ja niiden vaikutus elävien kuusien vaurioiden mikrobilajistoon.

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