Deposition of airborne fungal diaspores on special agar plates in Finland 1967-1968

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Abstract. — The deposition of fungal diaspores was investigated from samples collected mainly from airfields throughout Finland in the period from June 7, 1967 to May 29, 1968. The number of colonies which had grown from the diaspores after 10 days in the laboratory were counted from agar plates, but no attempt was made to identify the fungi. In 1968, samples were taken in three spruce (*Picea abies* (L.) Karst.) stands of South Finland throughout 24 hour periods at weekly or fortnightly intervals. The correlation of diaspore deposition with weather elements was statistically analysed.

Introduction

Aerial distribution of fungi has been studied by e.g. HANSEN (1882), ROSTRUP (1908), GREGORY (1945, 1952 a, b, 1961), and HIRST etal. (1967 a, b). Especially the airborne spores of fungi causing allergy in man have been intensively studied in the last decades (FEINBERG and LITTLE 1936, DURHAM 1938, BERNSTEIN and FEINBERG 1942). RISHBETH (1951) found that the fungus causing root and butt rot (*Fomes annosus* (Fr.) Cooke) was aerially distributed to the cut surfaces of healthy and fresh stumps of the pine (*Pinus silvestris* L.), and thense into roots and other trees. After RISHBETH'S report, intense research was started in several countries into the aerial distribution of *F. annosus* (MOLIN 1957, MILLER 1960, YDE-ANDERSEN 1961, DIMITRI 1963, SINCLAIR 1964, KALLIO 1965, 1970, REYNOLDS and WALLIS 1966, DRUMMOND and BRETZ 1967).

The present study was carried out in connection with another study into the aerial distribution of *F. annosus* in 1967—68 (KALLIO 1970). The purpose was to study the aerial diaspore deposition of unidentified fungi capable of growing on certain growth substrates, and the correlations of this deposition with weather elements.

Materials and methods
Growth substrates

The study was divided into two parts: a preliminary and a main study. The preliminary study used two growth substrates, both developed for *F. annosus* studies. KUHLMAN and HENDRIX (1962) developed a substrate containing streptomycin and PCNB; this substrate — working name K-agar — was one of the two used in the present study. The other was the agar substrate developed by KUHLMAN (1966) for studies of the root rot fungus — working name H-agar. The latter contains penicillin and PCNB. The main study used only H-agar substrates. According to studies by the above authors who developed the substrates, a number of fungi will not grow on these substrates.

Observation places and times
Preliminary study

The substrates weres exposed on six open sites in various parts of Finland (Fig. 1) in the period from June 7, 1967 to May 29, 1968. The observation places on open sites were the meteorological stations of Ivalo, Oulu, Jyväskylä, Lappeenranta and Turku airfields, and a meteorological station in Helsinki (Viikki). An observation place in a forest site was located in Helsinki (Viikki). On these the substrates were exposed to aerial deposition of diaspores on given days at hours which the staff of the meteorological stations found to be convenient. The substrates were exprosed simultaneously. On each observation site, there were three H-agar and three K-agar substrates which had been prepared in palstic dishes, diameter 88 mm (surface area 61 sq. sm), and been inserted into plastic bags. Ome H-agar and one K-agar substrate were controls and were never exposed. The lids of the other two dishes were opened simultaneously. One was exposed for 5 minutes, the other for 10 minutes. After exposure the substrates from the outlying sites were airmailed to Helsinki and all samples were kept in the laboratory there.

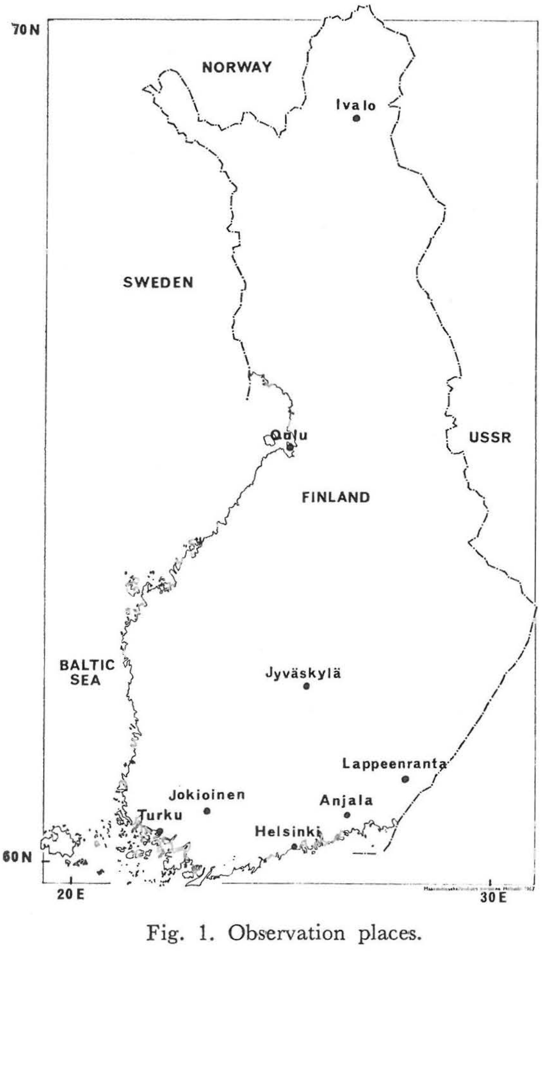


Fig. 1. Observation places.

Main study

The principal observation places were *Picea abies* (L.) Karst. stands in Helsinki (Viikki), Anjala and Jokioinen (Fig. 1). The dishes were exposed on a plate lying on the ground. Observations were made throughout the year 1968. The 24-hour observation day started on Wednesday at 13.15 and ended on Thursday at 13.15. In Helsinki the observation day between March 13 and December 5 occurred once a week, otherwise once a fortnight. The H-agar substrates, from January 3 to June 20, and from October 2 to December 19, were exposed for 5 minutes beginning on Wednesday at 13.15, 15.15, 17.15 and so on at 2-hour intervals until the 5-minute period beginning on Thursday at 11.15. From June 26 to September 26 the duration of exposure alternated between one and 5 minutes, the substrates being exposed for 5 minutes durink tre day and for 1 minute during the night.

At Anjala and Jokioinen, the 24-hour observation day from January 3 to December 19 occurred once a fortnight. The substrates were exposed for 5 minutes at 2-hour intervals beginning at 13.15 on Wednesday and ending with the 5minute period that started at 11.15 on Thursday. The substrates were mailed to and from the observation places. Due to irregularities in transportation the observation day at Anjala fell on June 20—21 while at Helsinki and Jokioinen it was June 19—20. For the same reason the observation day at Jokioinen fell on December 19—20 while at Helsinki and Anjala it was on December 18—19.

The main study collected a number of supplementary observations on diaspore deposition in the various places, and they will be reported in detail in the relevant contexts.

Weather elements

Weather observations for the main study were carried out in Helsinki alone. From the records of the Meteorological Institute's stations at Viikki and at Malmi airfield, data concerning air temperature, precipitation, sunshine sum, the difference between the maximum and minimum atmospheric pressures during the observation day, and wind velocity were used for the study. They were obtained from tables calculated by the Meteorological Institute. A recording thermometer and hygrometer (model Lambrecht No. 252, weekly graph) were used to measure temperatures and air humidities in the forest in Helsinki in the immediate neighbourhood of the site of diaspore deposition, at a height of 2 metres from ground level. The Viikki meteoroligical station was about 1 km and Malmi airfield about 4 km from the place where the diaspore deposition was measured.

Results

Preliminary study

After exposure, the H-and K-agar substrates were kept in the laboratory in plastic bags for 10 days. After this the colonies on fungal growth were counted from the substrates, excluding the *F. annosus* colonies. The number of colonies was indicated per 100 sq.cm. of exposed surface per hour.

The diaspore deposition varies according to the time of day or night (Nilsby 1949, Gregory and Sreeramulu 1958, Harvey et al. 1969, Pady et al. 1969). Since the substrates were not exposed simultaneously, a comparison of the depositions at different hours is not feasible. Table 1 gives the monthly mean values on the different observation places; the mean values were calculated from the total depositions on the H- and K-agar substrates. Some substrates carried so many colonies that their exact number could not be counted. In these cases the table indicates that colonies were more numerous than is shown by the numeral of the table. In January-March the diaspore deposition was scantier than in the other seasons. The finding agrees with those reported by many authors (e.g. Hyde and Williams 1946, 1953, Rennerfelt 1947, Nilsby 1949, Richards 1954, a, 1954 b). The deposition was particularly light during this period at the Ivalo and Oulu airfield meterological stations, in Ivalo scantier than anywhere else in the country.

Main study

The diaspore deposition is illustrated by the graph of Fig. 2. The minimum deposition was in January-March, and it was also relatively scanty in the autumn, November-December. The result agrees with that of the preliminary study. The deposition was most profuse from late May to mid-September. Figs. 3—6 specify the depositions of a few observation days at the various observation places by hours of day and night. At the end of April (Fig. 3) no distinct diurnal rhythm could be observed, even though Helsinki and Jokioinen showed distinct deposition peaks at night and in the morning. In late June (Fig. 4) a distinct maximum was noted in Helsinki in the middle of the night and another time of profuse deposition in the early hours of the morning (cf. Rich and Waggoner 1962). At Anjala and Jokioinen also the deposition increased in the middle of the night. The middle of August (Fig. 5) the deposition in the early morning hours was profuse at Helsinki and Jokioinen.

Table 1. Total diaspore settling 1967—1968, spores/100 cm^2/hr.

	1967							1968				
Place	June	July	August	September	October	November	December	January	February	March	April	May
Helsinki, forest	>361	>456	>208	>468	>361	>198	>164	37	15	5	>558	239
Helsinki, field	>292	>270	>491	>298	>315	109	180	58	2	14	>410	103
Turku	124	170	381	161	180	>150	4	71	2	36	305	150
Jyväskylä	>240	>380	>445	>282	152	70	170	12	12	25	161	125
Lappeenranta	191	>347	>325	116	>208	48	>199	25	23	102	88	288
Oulu	>259	>198	>181	>267	43	81	192	69	1	3	10	>85
Ivalo	> 93	150	>171	89	2	13	7	0	2	9	8	28

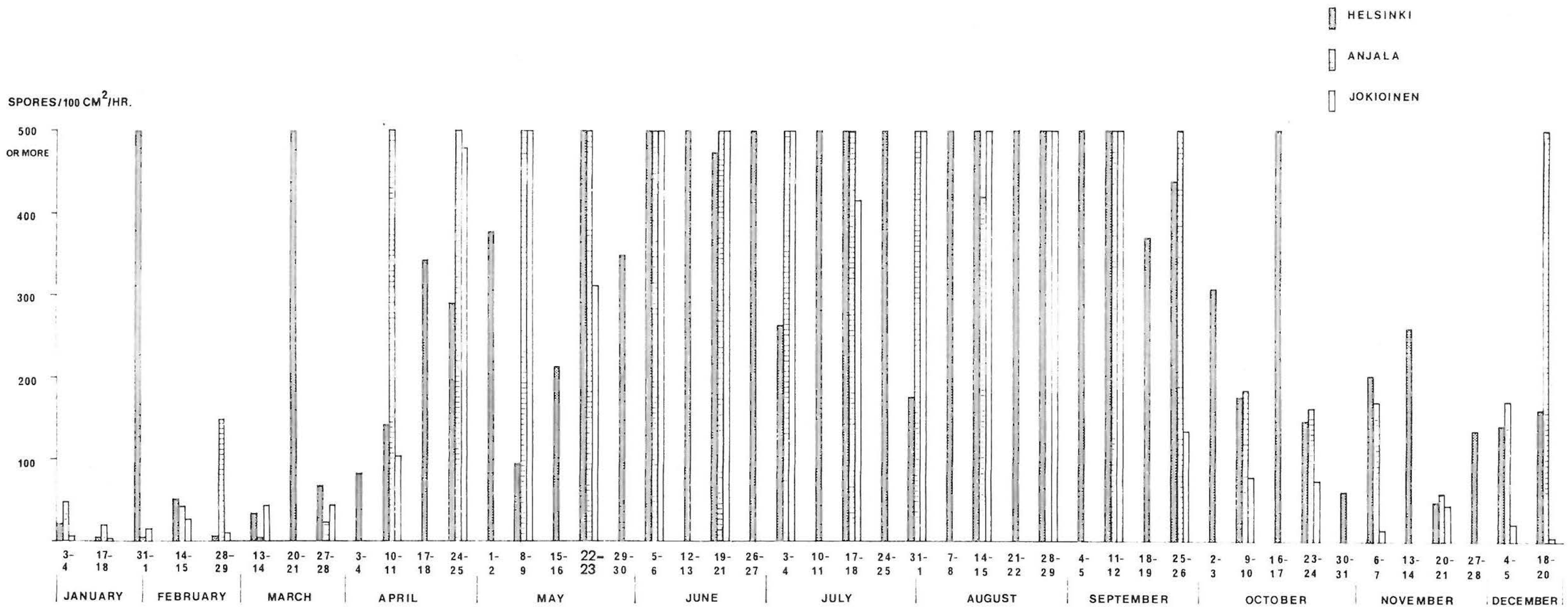
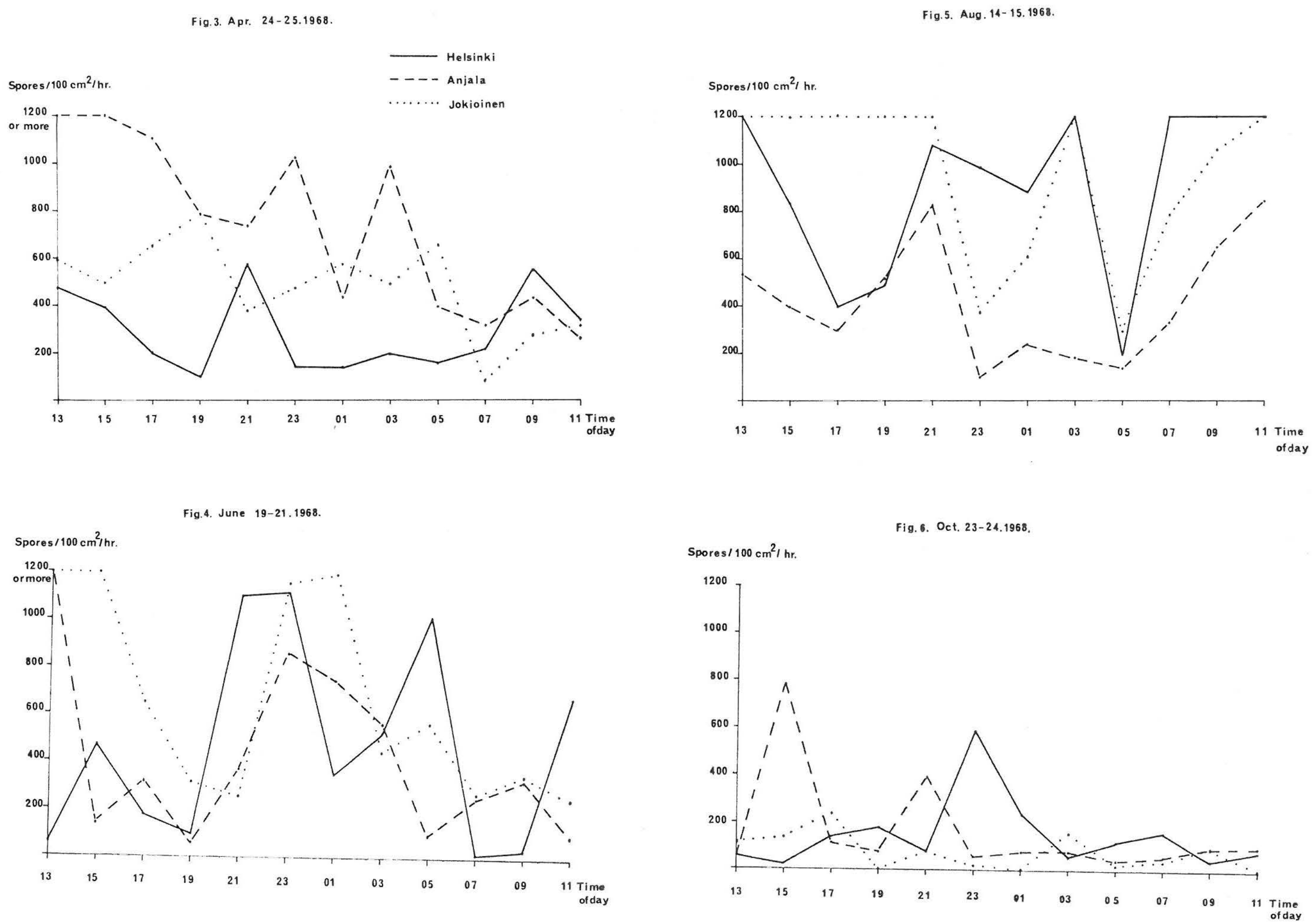


Fig. 2. Total diaspore settling 1968 in Helsinki, Anjala, and Jokioinen.



Figs. 3—6. Diurnal settling of airborne spores in some days 1968.

Table 2.

Correlation coefficients between total diaspore settling and some weather elements in Helsinki 1968.

Weather element	1	2	3	4	5	6	7
The hole year	0.274 + + +	0.264 + + +	0.108 +	0.220 + + +	0.064	0.047	0.071
Jan. 3 — Mar. 28	0.071	0.129	0.216 +	0.279 + +	0.000	0.000	0.000
Apr. 3 — May 30	0.126	0.027	0.070	0.088	0.268 +	0.033	0.300 + +
Jun. 5 — Sep. 26	0.219 + +	0.094	0.056	0.310 + + +	—0.009	—0.054	0.065
Oct. 2 — Dec. 19	0.178 +	0.109	0.100	0.250 + +	—0.057	—0.011	—0.002

Weather element	8	9	10	11	12	13
The hole year	0.111 + +	0.019	0.274 + + +	0.257 + + +	—0.010	0.035
Jan. 3 — Mar. 28	0.000	0.000	0.099	0.127	—0.134	0.232 +
Apr. 3 — May 30	0.202	0.360 + +	0.216	0.042	—0.378 + + +	—0.123
Jun. 5 — Sep. 26	0.077	—0.183 + +	0.185 + +	0.106	0.021	0.042
Oct. 2 — Dec. 19	0.167	0.281 + +	0.184 +	0.024	0.098	0.141

1. Mean air temperature on open site (°C, at 2 meters above ground level) of the 5 calendar days preceding the day of observation.
2. Mean air temperature (measured as above) of the calendar days on which the 24-hour observation period was divided.
3. Total precipitation (mm) during the 5 calendar days preceding the day of observation.
4. Total precipitation (mm) of the 2 calendar days on which the 24-hour observation period was divided.
5. Hour of bright sunshine during the 5 calendar days before observation day on open site.
6. Hours of bright sunshine during 24-hour observation day on open site.
7. Wind velocity (m/sec.) during the 24-hour observation, by recording periods at the Malmi aeronautical meteorological station.
8. Wind velocity (m/sec.) mean of the 24-hour observation, at the Malmi aeronautical meteorological station.
9. Difference between the maximum and minimum atmospheric pressure readings on the observation day at the Malmi aeronautical meteorological station.
10. Air temperature by 2-hour periods of the 5 preceding days, at 2 meters above ground level in the forest.
11. Air temperature (measured as above) of 24-hour observation.
12. Air huminity (measured as above) of the 5 preceding days.
13. Air humidity (measured as above) of 24-hour observation.

Significant at the level: + $P \leqq 0.05$, + + $P \leqq 0.01$, + + + $P \leqq 0.001$.

However, almost the only feature shared by all three places of observation was that the lightest falls occurred late in the evening and early in the morning. At the end of October (Fig. 6) the deposition was scantier than in the summer and early autumn. At Anjala the maximum was noted in the afternoon and in Helsinki at midnight. The diurnal rhythm of deposition in the different seasons of the year was therefore different. Generally, however, in May-September more diaspores were caught during the night than the day. A similar result has been reported e.g. by Hirst (1953), Gregory and Sreeramulu (1958), Pathak and Pady (1965), Walkey and Harvey (1966), Hodgkiss and Harvey (1969), Pady and Kramer (1969), and Pady et al. (1969a).

Table 2 presents correlation coefficients between the diaspore deposition and a few weather elements in Helsinki. Mainly on the basis of the profusion of diaspore depostition, the year was divided into four parts. Correlation coefficients were separately calculated for the whole year and for each part. The diaspore deposition for the whole year was a highly significant positive correlation with air temperature recorded at a 2-metre height on open sites and in the forest, and with the precipitation sum measured on open sites. A similar correlation with air temperatur was reported by Sinclair (1964). In January-March the most pronounced positive correlation (significance below 1 per cent) was noted with the precipitation sum. In April-May the diaspore deposition was highly significantly negatively correlated with air humidity recorded in the forest at 2 metres from the ground. Pady et al. (1969b) reported that the spore liberation of the *Cladosporium* fungi was correlated positively with temperature rise and negatively with reduction in the relative air humidity. In Helsinki, in the season of the most profuse diaspore deposition from June to September, the diaspore deposition was highly significantly positively correlated with the precipitation sum of the 24-hour observation day. A similar result was reported e.g. by Hodgkiss and Harvey (1969) who studied the rhythm of spore liberation from sporophores. Hyde and Williams (1946) found that the spore amount of some fungi of genus *Alternaria* was at its highest in England in June-August, and that the number of airborne spores was correlated with air temperature. Of the weather elements measured in Helsinki, air humidity and precipitation in April-September seemed to be those most strongly affecting the diaspore deposition. In October-December, diaspore deposition was positively correlated (significance below 1 per cent) with the precipitation sum and the difference between maximum and minimum atmospheric pressures.

Table 3 gives the results of a sequential multiple regression analysis of the interdependence of weather elements and diaspore deposition in Helsinki. The weather elements for the analysis were selected on the basis of a correlation analysis. The mean air temperature of the last 5 days before the moment of measurement, at 2 metres above ground level in the forest, best explained the variations in the diaspore deposition during the whole year. The next best independent variables were precipiation and the difference between the maximum and minimum atmospheric pressure during the observation day. But all three weather elements together explained only 12.9 per cent of the variation in diaspore deposition. In January-March the best explanation was given by precipitation. In April-May air humidity, the differences between the maximum and minimum atmospheric pressure, the precipitation and wind velocity of the observation day together explained 47.3 per cent of the variations in diaspore deposition. In June-September the precipitation sum, air temperature on open site at a height of 2 metres, and the difference between the maximum and minimum atmospheric pressure together explained 14.1 per cent of the variation. In October-December the difference between maximum and minimum air pressure, air humidity and wind velocity provided the most important explanations.

In connection with the main study, the diaspore deposition in the forest in Helsinki was investigated on July 31 to August 1 at ground level and at a height of 10 metres. From 21 to 01 o'clokc in the night the diaspore deposition at 10-metres was greater, but during the other hours of the observation day smaller, than at ground level. On June 26—27 the diaspore deposition around the Myllypuro water tower in Helsinki was considerably more profuse at ground level than at a height of 56 m, and on July

Table 3. Combinations of the weather elements best correlated with the total settling of diaspores in Helsinki 1968.

Time of the year	Weather element or combination	R^2	t-value and its significance	
The whole year	10	0.075		6.37 + + +
	10,4	0.113	1	6.05 + + +
			4	4.63 + + +
	10, 4,9	0.129	1	6.76 + + +
			4	5.24 + + +
			9	—3.03 + +
Jan. 3 — Mar. 28	4	0.078		2.81 + +
Apr. 3 — May 30	12	0.143		—3.56 + + +
	12,9	0.236	12	—3.23 + +
			9	3.02 + +
	12, 9,4	0.357	12	—3.59 + + +
			9	4.76 + + +
			4	3.72 + + +
	12, 9, 4,8	0.473	12	2.75 + +
			9	6.00 + + +
			4	5.62 + + +
			8	—4.01 + +
Jun.5 — Sep. 26		0.096		4.55 + + +
	4,1	0.135	4	4.40 + + +
			1	2.93 + +
	4, 1,9	0.141	4	4.05 + + +
			1	2.65 + +
			9	—1.19
Oct. 2 — Dec. 19	9	0.079		3.34 + + +
	9, 13	0.107	9	3.54 + + +
			13	2.00 +
	9, 13, 7	0.121	9	3.81 + + +
			13	1.55
			7	—1.46

R^2 = coefficient of determination. For other explanations see Table 2.

17—18 it was again more profuse at ground level than at 56 metres. On July 3—4 and July 24—25 the diaspore depositions in Helsinki at ground level in the forest and on the open site were compared. On both occasions, the deposition was considerably more profuse on the open site than in the forest. On June 5—6 and 26—27 diaspore depositions were observed at the Kalbådagrund lighthouse in the Gulf of Finland. During both 24-hour observation days the total diaspore deposition on the sea in the Gulf of Finland was scantier than in the forest in Helsinki. Agar substrates were exposed on August 14—15 and October 2—3 in the forest in Helsinki some 40 cm above ground level so that diaspores were caught from above, from below, and from the north, south, east and west. The largest deposition was recorded from above, the smallest from below, but the amount of deposition varied greatly according to the time of day or night.

Discussion and conclusions

Agar substrates developed for studies of the *F. annosus* fungus were used in this investigation. The number of root rot fungus calonies was counted from these substrates for the *F. annosus* study (KALLIO 1970), and the number of other, unidentified fungal calonies for the present study. According to the authors who developed these agar substrates, a number of fungi will not grow on them at all. Therefore, in evaluating the results, the limitations of the method described above must be borne in mind. The results, however, probably provide a general idea of the diaspore deposition in Finland, on which subject no previous information seems to be available.

The following conclusions can be dreawn on the basis of the study:

1. Diaspore deposition was scantier in North Finland than in South Finland.

2. Deposition was most profuse in the late summer and autumn, scantiest in the winter.

3. Deposition was more profuse in an open place than in the forest. In the summer, it was often more profuse in the forest during the night than day. Deposition decreased from ground level upward. On open sea deposition was scantier than over land.

4. Weather elements and diaspore deposition had mutual correlations. The most distinct of these, over a deposition period of one year, were the positive correlations with air temperature and precipitation. During the period of profuse deposition in June-September the correlation with precipitation was highest. According to sequential multiple regression analysis the variation in diaspore deposition in the course of a whole year was best explained by the air temperature of the last 5 days precending the observation day, measured at 2 metres above ground level near the place of recording the diaspore deposition. The variations in deposition, during the season of profuse dispersal from June to September, were best explained by precipitation and after that by air temperature.

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