THE SIGNIFICANCE OF SOIL MICROORGANISMS AS A LIMITING FACTOR IN INFECTION OF CLOVER BY *SCLEROTINIA TRIFOLIORUM* ERIKSS. AT DIFFERENT TIMES OF THE YEAR

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Antibiotic microorganisms limiting the growth of fungi are found very frequently in the soil (4, 5, 27). Some of these microorganisms are also presumed to restrict the mycelial growth in the soil of the causal agent of clover rot (*Sclerotinia trifoliorum* ERIKSS.) (9, 21). Microbes with such an antibiotical effect on artificial culture media have been isolated from various parts of Finland (21, 22, 23, 24). It was observed during these studies that environmental factors, such as temperature and the quality of the growth medium, have a marked effect on the antibiotical capacity of the microbes (10, 22). The present paper describes investigations on the antagonistic effectivity of the soil — due to microorganisms (cf. POHJAKALLIO, 20) — on the extent of clover rot infection in the soil. The principal object of these studies was the variation in the antagonistic effectivity of the soil at different times of the year. A second point investigated was the effect of the numbers of living microbes in the soil on clover rot infection.

Experimental methods

The trials were begun each year in April and continued until November or December. During this time, when the ground was not covered by snow, soil samples were taken about once a week from cultivated land. The samples were put in sterilized petri dishes (Ø 9 cm); 5 dishes per sample were used in 1961 and 6 in the other years. In addition, soil from each sample was autoclaved and put into 3 petri dishes. The soil was moistened with sterilized water. Mycelia of *S. trifoliorum* were transferred to the surface of the soil in the centre of the dish. Observations were subsequently made on the spreading of the mycelia on the soil surface. Since the mycelia of the clover rot fungus are often invisible to the naked eye, clover seed was sown in each dish so that the antagonistic capacity of the soil could be determined on

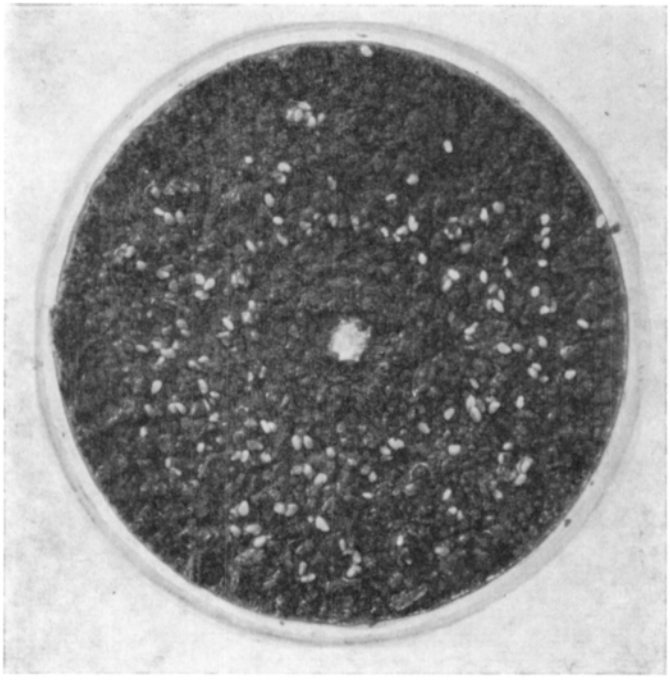

Fig. 1. The growth of S. trifoliorum mycelia in the soil samples was determined by sowing clower in the petri dishes; the smallest distance from the seeds to the transferred mycelia in the centre of the dish was 1.2 cm.

the extent of the infection of the clover seedlings. Just before seeding, the seed was dressed with a solution of Ceresan and carefully rinsed with sterilized water. In sowing the nearest seeds were located at least 1.2 cm from the transferred mycelium of *S. trifoliorum* (Fig. 1).

In the years 1957—61 the petri dishes were kept in a thermostat at a temperature of 7—10°C and in 1961—63 they were kept outdoors in a shaded place. Accordinlyg, within one year (1961) similar trials were carried out both in the thermostat and outdoors.

The extent of infection of the clover seedlings by *S. trifoliorum* was evaluated after the two-week test period. If the fungus had infected the seedlings this was evidently caused by a reduction in the antagonistic effectivity of the soil. If the fungus did not produce infection, this was held to indicate an increased antagonistic capacity of the soil. In the tables showing the results of the trials, the percentages of the petri dishes in which the clover seedlings were infected by clover rot are indicated.

Soil samples were taken from a depth of 0—5 cm from the following fields: a) fallow, b) limed fallow, c) winter rye, d) soil where clover has been sown (with spring wheat as nurse crop) in the year of the test, as well as soil where clover had been sown in the previous year, e) unlimed area, and f) limed area. The main soil type was sandy clay with a pH of 5,5—6,0. Ground limestone was applied to the

surface layer of the soil (0—10 cm) in the spring before the commencement of the trials. In 1962 an amount of 12 000 kg/ha was applied, with the result that the soil became alkaline. In the following year, 1963, only 6 000 kg/ha were used. In 1962 the pH of the fallow was 5.4—5.8, that of the limed fallow 7.3—8.0 and that of the clover field 6.0—6.5. In 1963 the pH values were: fallow 5.1—5.5, limed fallow 6.2—7.0, clover field 5.6—6.3 and limed clover field 6.3—7.5. The pH of the soil samples was not determined in the other years.

Mycelia of *S. trifoliorum* for the trials were cultured at a temperature of 7—10°C on Henneberg agar (dist. water 100 ml, glucose 10 g, CaCl 0.01 g, $MgSO_4$ 0.05 g, KNO_3 0.2 g, $NH_4H_2PO_4$ 0.2 g, peptone 1 g). After two weeks of growth the mycelia were transferred in the form of agar tablets 1—2 mm thick (Ø 0.5 cm) to the surface of the soil in the petri dishes.

The clover rot fungus degenerated when continually cultured on artificial medium and had to be replaced twice with a more vigorous growth. In 1957—58 a single-spore strain isolated at Viik was used, in 1959—60 a saltant of this strain was used, and in the years 1961—63 the fungus employed had been isolated in the autumn of 1960 from mycelia growing in an infected red clover plant in the field. During several years a sporadic decline in growth and also in the infective capacity of the clover rot fungal mycelia was observed. No reason for this could be found.

Counts of microorganisms in the soil samples

Directly before the antagonistic effectivity of the soil was determined, microbe counts were made of the soil samples. These counts were made by means of the dish dilution method (14), which is based on the development of living cells into colonies when growing on artificial media. The substrate used was soil extract agar prepared in the following manner: The soil extract consisted of soil and water in a ratio of 1:1 which was autoclaved for 30 min; a small amount of $CaCO_3$ was added and mixed; the solution was clarified by centrifuging and water was added to the original volume. The final agar medium consisted of 1000 g soil extract, 0.2 g K_2HPO_4, 15 g agar; pH adjusted to about 6.0 with NaOH. Since the substrate was soil extract, the colonies consisted principally of native (autoctonic) soil microorganisms (14). Before the counting was undertaken, the soil samples were diluted by adding 1 g soil to 99 ml 0.05 % peptone solution, which was further diluted (1:10). The most suitable dilution proved to be 1:100 000. An 1-ml portion of it was added to a petri dish of liquid soil extract agar (+45°C). The dishes (2 replicates per the soil sample) were held for 14 days at a temperature of about +20°C, after which the number of colonies was visually counted. Among the various microorganisms in the soil samples, the bacteria proper comprised about 82—93 %, the actinomycetes about 1—6 % and the fungi about 5—16 %.

Correlation calculations for the trial results were performed according to MUDRA (15). In the outdoor trials determinations were made of the

Table 1. Infection of red clover seedlings by *S. trifoliorum* in sterilized and unsterilized soil samples.

Year	Soil sample	% of petri dishes with infected seedlings		Total no. of dishes		Trial period
		Sterilized soil	Unsterilized soil	Sterilized soil	Unsterilized soil	
	A. THERMOSTAT (7—10°C)					
1957	Fallow	100	39	93	186	17. 4.—13. 12.
1958	Winter rye field	99	57	138	276	14. 4.—10. 12.
1959	Fallow	100	45	189	378	2. 4.—17. 12.
	Clover field (year of sowing)	99	38	189	378	2. 4.—17. 12.
1960	Fallow	100	22	135	270	21. 4.—20. 12.
	Clover field (1st year)	98	23	135	270	21. 4.—20. 12.
	Clover field (year of sowing)	99	20	126	252	29. 4.—20. 12.
1961	Fallow	91	15	90	150	17. 4.— 9. 12.
	Clover field (1st year)	88	15	90	150	17. 4.— 9. 12.
	Clover field (year of sowing)	86	6	90	150	17. 4.— 9. 12.
	Average	97	31	Total 1275	Total 2460	
	Av. temp. during 4-day period before start of trial:					
	<10°C	99	32	720	1392	
	10°C $<$	95	30	555	1068	
	B. OUTDOORS					
1961	Fallow	98	24	84	140	17. 4.—22. 11.
	Clover field (1st year)	87	27	84	140	17. 4.—22. 11.
	Clover field (year of sowing)	86	26	84	140	17. 4.—22. 11.
1962	Fallow	100	29	96	192	17. 4.—28. 11.
	Limed fallow	*78*	*6*	93	186	30. 4.—28. 11.
	Clover field (1st year)	99	22	96	192	17. 4.—28. 11.
1963	Fallow	90	33	72	144	22. 4.— 7. 11.
	Limed fallow	*79*	*15*	72	144	22. 4.— 7. 11.
	Clover field (1st year)	92	29	72	144	22. 4.— 7. 11.
	Limed clover field (1st year)	*85*	*3*	72	144	22. 4.— 7. 11.
	Average	90	21	Total 825	Total 1566	
	Av. temp. during 2-week trial period:					
	<10°C	99	44	402	759	
	10°C $<$	81	7	423	807	

correlation between the infection of clover by *S. trifoliorum* (scale 0—6; 6 = infection in each of the 6 replicate dishes; in 1961 the scale was 0—5), the average temperature during the 2-week trial period, and the number of microorganisms in the soil at the beginning of the trial. In the thermostat trials (7—10°C) the correlation was determined between the extent of infection by *S. trifoliorum* and the number of microorganisms in the soil at the start of the trial.

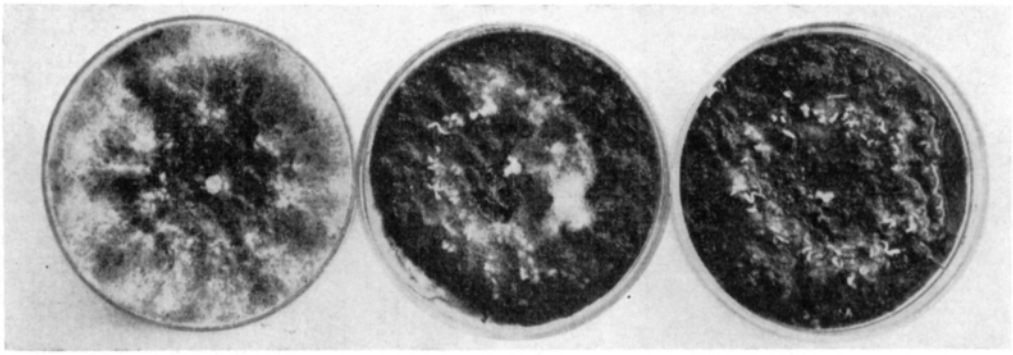

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Fig. 2. Infection of red clover seedlings by S. trifoliorum in the soil samples. In the sterilized sample (I) the fungus has completely destroyed the plants, In one of the unsterilized samples (II) tehere are mycelia of both S. trifoliorum and Mucor, in the other (III) only Mucor.

Table 2. Infection of red clover seedlings by *S. trifoliorum* in thermostat trials (7—10°C). Results grouped according to average temperatures during 4-day periods before start of trial.

Year	Source of soil sample (taken in April—December)	% of petri dishes showing clover infection													
		0—5.0°C				5.1—10.0°C				10.1–15.0°C		15.1–20.0°C		20.1°C <	
		Spring		Autumn		Spring		Autumn		Summer		Summer		Summer	
		%	(No. of dishes)	%	(No. of dishes)	%	(No. of dishes)	%	(No. of dishes)	%	(No. of dishes)	%	(No. of dishes)	%	(No. of dishes)
1957	Fallow	38	(24)	48	(48)	67	(24)	6	(18)	33	(66)	17	(6)		
1958	Winter rye field	71	(48)	83	(54)	63	(24)	46	(48)	48	(72)	25	(24)	0	(6)
1959	Fallow	67	(42)	33	(120)	77	(30)	28	(36)	65	(60)	32	(72)	33	(18)
	Clover field (year of sowing)	64	(42)	13	(120)	80	(30)	25	(36)	62	(60)	40	(72)	22	(18)
1960	Fallow	42	(24)	11	(66)	17	(6)	19	(36)	19	(54)	28	(72)	42	(12)
	Clover field (1st year)	8	(24)	8	(66)	50	(6)	31	(36)	33	(54)	43	(72)	8	(12)
	Clover field (year of sowing)	8	(12)	6	(66)			19	(36)	22	(54)	35	(72)	8	(12)
1961	Fallow	0	(10)	40	(25)	4	(25)	37	(30)	0	(15)	0	(40)	20	(5)
	Clover field (1st year)	0	(10)	48	(25)	12	(25)	27	(30)	0	(15)	0	(40)	0	(5)
	Clover field (year of sowing)	0	(10)	20	(25)	4	(25)	10	(30)	0	(15)	0	(40)	0	(5)
	Average (Total)	45	(246)	27	(615)	45	(195)	26	(336)	37	(465)	26	(510)	19	(93)

Results

Sclerotinia trifoliorum grew well in the soil samples which had been sterilized. In some cases it caused infection of clover in all of the petri dishes (Table 1).

On the other hand, in the unsterilized soil samples the infection of clover caused by *S. trifoliorum* varied considerably in the different years (Table 1)

Table 3. Infection of red clover seedlings by *S. trifoliorum* in petri dish trials performed o u t d o o r s . Results grouped according to average temperatures during the 2-week trial periods.

Year	Source of soil sample (taken in April—December)	% of petri dishes showing clover infection at different temperatures											
		0—5.0°C Autumn		5.1—10.0°C Spring		5.1—10.0°C Autumn		10.1–15.0°C Summer		15.1–20.0°C Summer		20.1/C < Summer	
		%	(No. of dishes)	%	(No. of dishes)	%	(No.of dishes)	%	(No. of dishes)	%	(No. of dishes)	%	(No. of dishes)
961	Fallow	60	(20)	16	(25)	53	(30)	5	(20)	0	(40)	0	(5)
	Clover field (1st year)	55	(20)	36	(25)	47	(30)	15	(20)	3	(40)	0	(5)
	Clocer field (year of sowing)	65	(20)	24	(25)	57	(30)	5	(20)	0	(40)	0	(5)
962	Fallow	61	(36)	57	(30)	10	(42)	14	(72)	17	(12)		
	Limed fallow	25	(36)	13	(24)	0	(42)	0	(72)	0	(12)		
	Clover field (1st year)	47	(36)	60	(30)	5	(42)	8	(72)	0	(12)		
963	Fallow	75	(18)	58	(18)	44	(18)	33	(42)	2	(48)		
	Limed fallow	42	(18)	25	(18)	17	(18)	12	(42)	0	(48)		
	Clover field (1st year)	71	(18)	67	(18)	56	(18)	17	(42)	0	(48)		
	Limed clover field (1st year)	17	(18)	0	(18)	6	(18)	0	(42)	0	(48)		
	Average	55		32		26		9		1		0	
	(Total)		(240)		(231)		(288)		(444)		(348)		(15)

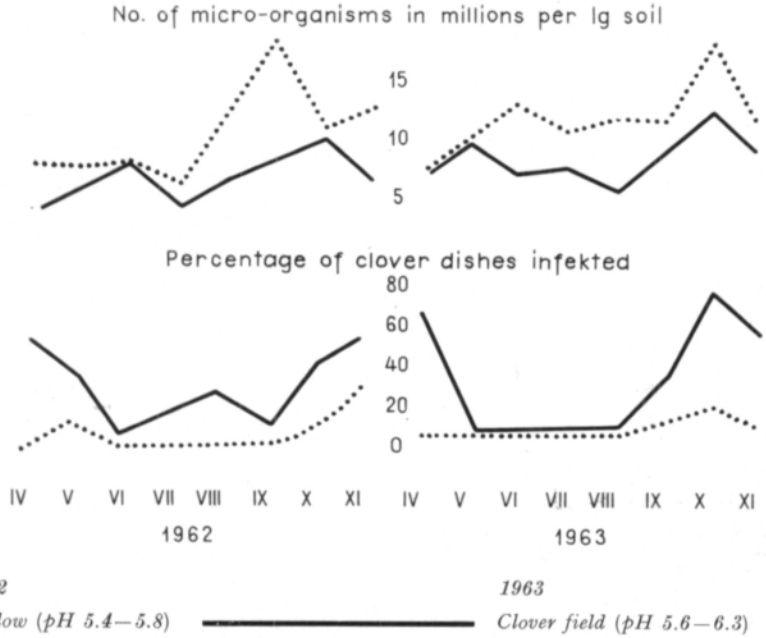


Fig. 3. The number of soil microorganisms as well as the percentage of infection of clover in petri dishes by S. t r i f o l i o r u m in soil samples taken from limed and unlimed fields between April and November (total number of dishes about 190 in 1962, 144 in 1963; cf. Fig. 4).

Table 4. Numbers of microorganisms in soil samples taken in April—December. Results grouped according to average temperatures during the 4-day periods before the counts were made. (The figures in brackets indicate the numbers of soil samples counted).

Year	Source of soil sample	No. of microorganisms (in millions per 1 g soil)													
		0—5.0°C				5.1—10.0°C				10.–15.0°C		15.1–20.0°C		20.1°C<		0–21°C Whole trial period	
		Spring		Autumn		Spring		Autumn		Summer		Summer		Summer			
1958	Winter rye field	2.3	(8)	16.2	(9)	10.7	(4)	18.0	(8)	12.4	(12)	7.7	(4)	36.0	(1)	12.3	(46)
1959	Fallow	6.3	(7)	7.0	(20)	7.0	(5)	7.2	(6)	6.5	(10)	6.7	(12)	6.6	(3)	6.8	(63)
	Clover field (year of sowing)	6.5	(7)	12.6	(20)	6.8	(5)	15.0	(6)	8.8	(10)	11.3	(12)	8.7	(3)	10.6	(63)
1960	Fallow	8.8	(4)	19.1	(11)	6.9	(1)	15.7	(6)	15.9	(9)	15.4	(12)	16.2	(2)	15.7	(45)
	Clover field (1st year)	8.3	(4)	14.3	(11)	7.1	(1)	14.3	(6)	13.4	(9)	12.3	(12)	9.9	(2)	12.7	(45)
	Clover field (year of sowing)	11.4	(2)	16.5	(11)			15.7	(6)	17.1	(9)	13.8	(12)	9.7	(2)	15.2	(42)
1961	Fallow	13.5	(2)	6.8	(5)	13.6	(5)	8.6	(6)	8.9	(3)	11.6	(8)	11.9	(1)	10.4	(30)
	Clover field (1st year)	12.2	(2)	7.1	(5)	19.4	(5)	8.2	(6)	13.0	(3)	12.0	(8)	11.4	(1)	11.7	(30)
	Clover field (year of sowing)	10.2	(2)	6.8	(5)	14.0	(5)	14.9	(6)	8.5	(3)	9.1	(8)	7.1	(1)	11.3	(30)
1962	Fallow	5.3	(3)	7.8	(4)	6.0	(2)	9.4	(7)	7.6	(12)	4.8	(4)			7.4	(32)
	Limed fallow	*7.7*	(2)	*12.1*	(4)	*9.3*	(2)	*13.5*	(7)	*11.5*	(12)	*5.9*	(4)			*10.9*	(31)
	Clover field (1st year)	5.7	(3)	10.6	(4)	10.8	(2)	15.1	(7)	10.7	(12)	8.8	(4)			11.0	(32)
1963	Fallow			8.8	(3)	5.9	(3)	10.6	(3)	6.4	(7)	5.6	(8)			6.9	(24)
	Limed fallow			*13.0*	(3)	*6.9*	(3)	*13.2*	(3)	*8.3*	(7)	*9.4*	(8)			*9.7*	(24)
	Clover field (1st year)			10.9	(3)	8.5	(3)	12.5	(3)	9.0	(7)	6.9	(8)			8.9	(24)
	Limed clover field (1st year)			*15.0*	(3)	*7.8*	(3)	*18.3*	(3)	*10.7*	(7)	*11.8*	(8)			*12.2*	(24)
	Average (Total)	6.9	(46)	12.0	(121)	10.2	(49)	13.2	(89)	10.6	(132)	10.2	(132)	11.5	(16)	11.0	(585)

and at different times of the year (Fig. 4). In general, however, there were relatively small variations in infection between the soil samples taken at the same time from the various areas of the field cultivated in different ways. Nevertheless, in some autumns infection was less marked in the samples taken from clover fields than in those from fallow (Fig. 4). Furthermore, liming appreciably reduced the extent of infection (Table 1; Figs. 3 and 4).

In the outdoor trials, clover was more severely infected in the spring and autumn than in the summer; often it did not become infected at all in the summer (Fig. 4; Table 3). The maximum infection occurred late in the autumn, at a time when the temperature was lowest (0—5°C) (Table 3). In the thermostat trials (7—10°C) the clover rot fungus generally caused considerable infection also in the soil samples taken in the summer (Fig. 4; Table 2). A statistically significant negative correlation occurred between the infection of clover by *S. trifoliorum* and the temperature during the 2-week trial period (Table 5, B).

Table 5. The effect of the number of soil microorganisms and the temperature on the infection of red clover by *S. trifoliorum*, as determined by correlation calculations. (The infection was judged on a scale of 0—6 in which 6 = plants in all the 6 replicate dishes were infected; in 1961 the scale was 0—5).

Year	Soil sample taken in April—December	No. of trials	Regression coefficients	
			b_1 No. of microbes start of trial (mill. per 1 g soil)	b_2 Av. temp. during 2-week trial (°C)
	A. THERMOSTAT (7—10°C)			
1957	Fallow	31	—	
1958	Winter rye field	46	−0.05	
1959	Fallow	63	−0.04	
	Clover field (year of sowing)	63	−0.23***	
1960	Fallow	45	−0.06	
	Clover field (1st year)	45	−0.02	
	» » (year of sowing)	42	−0.08	
1961	Fallow	30	−0.10	
	Clover field (1st year)	30	−0.05	
	» » (year of sowing)	30	−0.04	
	B. OUTDOORS			
1961	Fallow	28	−0.12	−0.17**
	Clover field (1st year)	28	+0.01	−0.19***
	» » (year of sowing)	28	+0.03	−0.21***
1962	Fallow	32	−0.14	−0.22**
	Limed fallow	31	−0.04	−0.11**
	Clover field (1st year)	32	−0.09	−0.20**
1963	Fallow	24	+0.28	−0.25**
	Limed fallow	24	−0.05	−0.18***
	Clover field (1st year)	24	−0.08**	−0.48***
	Limed clover field (1st year)	24	+0.03	−0.05*

In the thermostat trials (7—10°C) (Table 2), as well as in the outdoor trials when the temperature was 5—10°C (Table 3), *S. trifoliorum* generally caused more severe infection of clover in the spring than in the autumn. In 1961, however, infection was much more severe in the autumn than in the spring (Tables 2 and 3).

As a rule, the maximum numbers of microorganisms in the soil were found in the autumn and the minimum numbers in the spring (Table 4). In 1961, however, they were more numerous in the spring. Larger amounts of soil microbes were often encountered in the samples taken from the clover field than in those from the fallowed area; this was especially evident in the autumn. Liming resulted in an increase in the numbers of microorganisms (Fig. 3).

In the thermostat trials (7—10°C) there was always a negative correlation between the infection of the clover plants and the numbers of microorganisms in the soil at the onset of the trial. However, only in one case was this correlation statistically significant (Table 5, A). In the outdoor trials the correlation between these factors varied from year to year (Table 5, B). Taking into account, however,

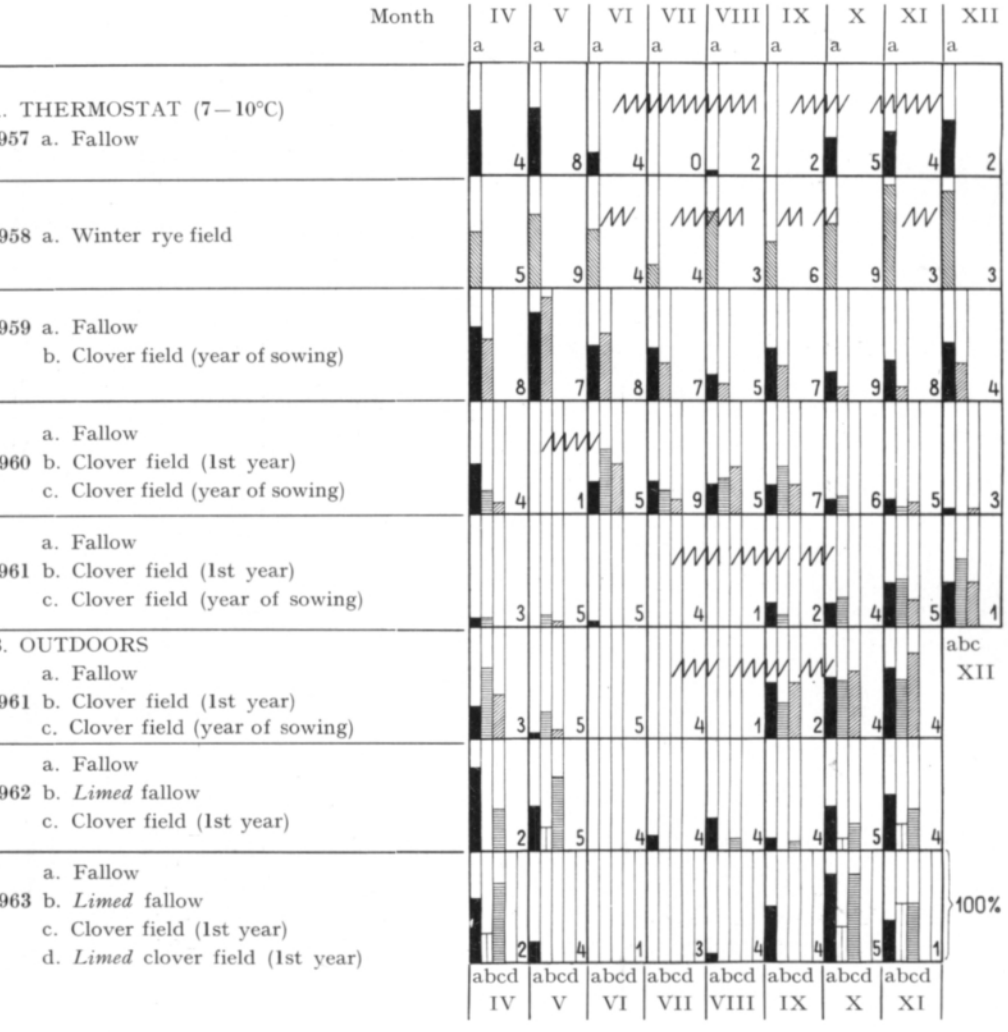


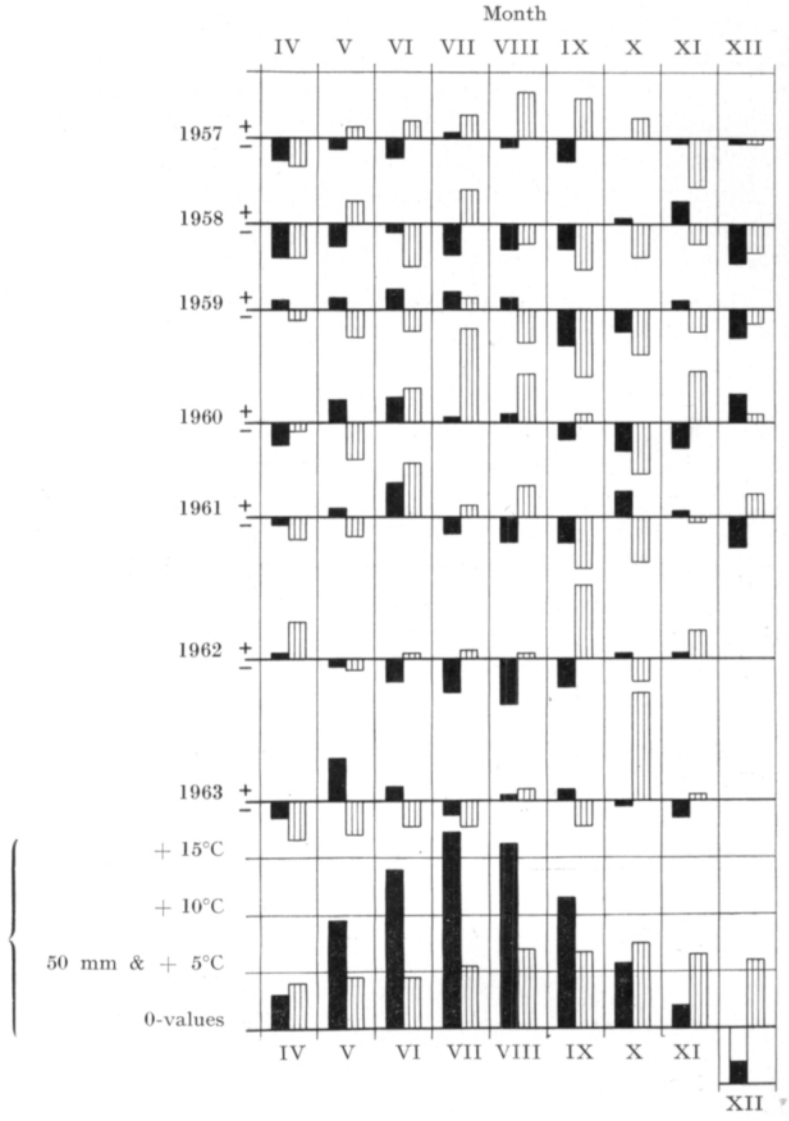
Fig. 4. The infection of red clover seedlings by S. trifoliorum in soil samples in different months. The amount of infection is denoted by the columns whose height indicates the percentage of petri dishes in which the seedlings were infected.

The number of trials in each month is shown by the numerals; in each trial there were 6 petri dishes (5 in 1961). WWWW *= the mycelia of S. trifoliorum used for inoculating the soil samples grew poorly* (*cf. p. 122*)

only those results obtained at the temperature range 5—10°C, it can be seen that when there were small numbers of microorganisms in the soil, *S. trifoliorum* generally grew better than when the numbers were large (Fig. 6).

Discussion

In general, clover plants become infected with the clover rot fungus (*Sclerotinia trifoliorum*)in the autumn. The damage may continue during the winter and spring, but in the middle of the summer the disease generally does not occur. This



Long-term temperature and precipitation values at Helsinki 1921—1950

Fig. 5. Long-term mean temperatures (■ °C) and precipitation (|||| mm) at Helsinki in the months April—December 1921—1950 as well as their deviations at Viik 1957—1963

has been attributed mainly to the fact that the dry conditions usually accompanying the high temperatures of the summer check the growth of the clover rot fungus (2, 3, 7, 13, 17, 18, 19, 25, 29). However, in the present trials, the infection was generally lightest in the middle of the summer also when the clover was grown in petri dishes where the soil was constantly moist. This is most clearly seen in the outdoor trials (Fig. 4, B) performed during the warmest period of the summer (Fig. 5) and indicates that the temperature had a preventive effect on clover rot infection especially during the actual time of the trial (Table 3). In checking infection the

temperature scarcely played a direct part, since it is known that the mycelia of *Sclerotinia trifoliorum* can grow throughout a temperature range of 0—33°C (16), the optimum being 13—20°C (7, 11, 16, 18).

Autoclaving of the soil had a marked effect in increasing the amount of infection of the clover seedlings growing in it (Table 1). This may show that the microorganisms normally occurring in soil limited the extent of clover rot infection in unsterilized soil. The formation of mycelia from sclerotia on the surface of autoclaved soil is also very abundant (6). Furthermore, it is known that autoclaving changes the soil nutrients into a form readily utilizible by fungal organisms (30), but at present it is not known whether this could lead to an increase in clover rot infection.

The number of soil microorganisms was not found, however, to be greater during the warm part of the summer — when clover rot infection was slight (Table 3; Fig. 4, B) — than in the spring and autumn (Table 4; cf. 28). On the other hand, at relatively low temperatures (5—10°C) the extent of infection decreased as the number of soil microbes increased (Table 5, A; Fig. 6). As a rule, there were less microbes in the soil in the spring than in the autumn (Table 4). Correspondingly, clover rot infection was usually (at 5—10°C temperatures) more severe in the spring than in the autumn (Tables 2, 3). In this case, accordingly, the number of microorganisms determined the antagonistic power of the soil. At other temperatures such a corre-

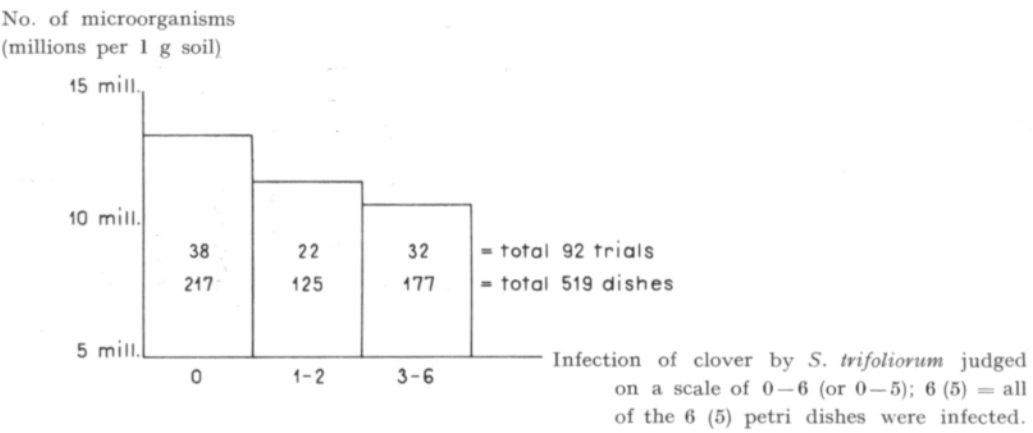


Fig. 6. The numbers of soil microorganisms and the amount of infection of red clover by S. trifoliorum in soil samples in trials performed outdoors at a temperature of 5—10°C.

lation was not observed (Table 5, B), which suggests that the effect of soil microorganisms in hindering clover rot infection (Table 5, B) is mainly due to their antagonistic influence. As is generally known, a rise in temperature increases the rate of metabolism of microbes (26) as well as the formation of many antibiotic substances (cf. 12). On certain artificial media, the infection-checking effect of the soil microorganisms was also found to be enhanced by an increase in the temperature (Fig. 7).

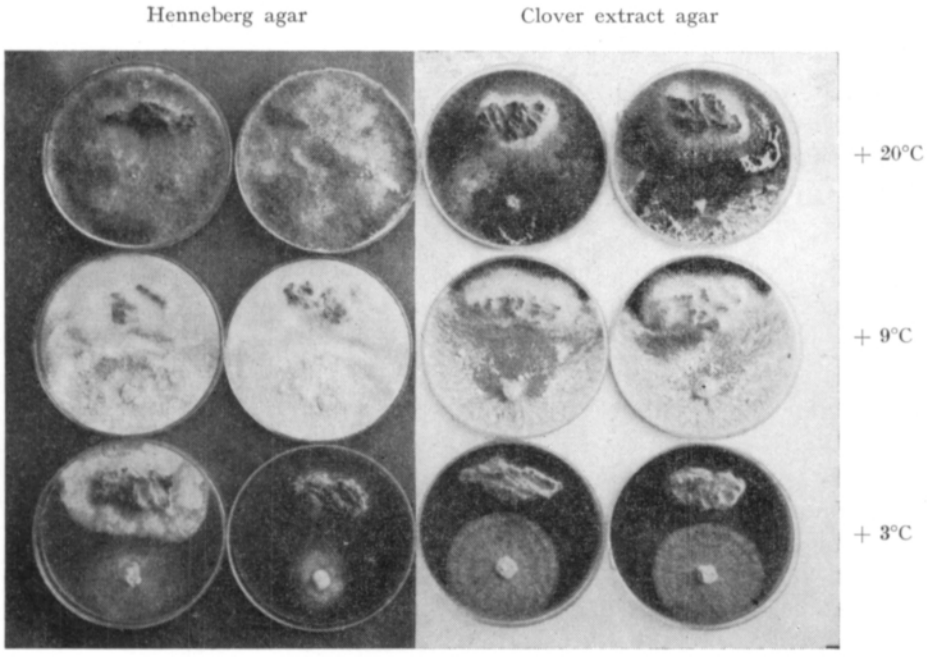


Fig. 7. The effect of a soil inoculum transferred to nutrient agar medium on the growth of S. trifoliorum at different temperatures.

S. trifoliorum was inoculated to the lower edge of the dish, the soil to the upper edge. The growth is 2 weeks old. The soil contained microorganisms such as Mucor, Trichoderma and bacteria.

It is known (10, 16) that *S. trifoliorum* can grow in a wide range of pH concentrations, between 2.1 and 8.0; its optimum is pH 5.5—6.5 (16). In the current trials, treating fields with lime — which raised the soil pH to the alkaline side — appreciably increased the number of microorganisms and at the same time reduced the amount of clover rot infection (Fig. 3). In earlier studies (1, 16, 18, 19) the soil reaction was not known to have influenced the occurrence of clover rot on the field. This was possibly due to the fact that the fungus spread in the form of spores directly on the leaves of the clover plants (cf. 9, 13, 25). In the present trials, on the other hand, the clover became infected from the mycelia growing in the soil; such mycelial infection may also take place under normal field conditions (7, 8, 31).

The clover plants were less severely infected by the fungus in limed than in unlimed soil samples also in the cases when the soil was sterilized (Table 1). Likewise, the infection was somewhat weakened at high temperatures even in the sterilized samples (Table 1). This can be taken as being due to the fact that the autoclaving of the soil — although it killed the bacteria — did not completely destroy all the antibiotic substances in the soil. However, since the effect of soil sterilization in enhancing clover rot was even more distinct in the thermostat (7—10°C) trials than in those performed outdoors under varying temperature conditions, it appears that also other factors did to some extent modify the results. In this case attention can

be drawn to the fact that sometimes — due to unknown reasons — the mycelial growth of the fungus declined, a phenomen on which often occurred during the warm part of the summer (Fig. 4).

Conclusions

In the petri dish trials the mycelia of *Sclerotinia trifoliorum* in sterilized soil samples generally infected clover quite readily. In unsterilized samples the infection was less severe and showed marked variations in different years and at different times of the year. In the outdoor trials clover became less infected in the middle of the summer than in the spring and autumn. On the other hand, in the thermostat trials, where the temperature was constantly 7—10°C, the temperature at the time of taking the soil samples did not have a pronounced effect on the extent of clover infection.

Liming of the soil caused a marked decrease in the severity of clover rot infection. In some autumns clover plants growing in soil samples taken from fallow were more seriously infected than those growing in soil from a clover field. As a rule, however, there were only slight variations in the extent of infection in the soil samples taken at the same time from the various areas of the field cultivated in different ways.

In general, the highest numbers of microorganisms in the soil were found in the autumn and the lowest in the spring. In clover fields there were often more microorganisms — particularly in the autumn — than in fallow. Liming caused an increase in the numbers of soil microbes.

S. trifoliorum infected clover very severely at temperatures of 0—5°C; in the range 5—21°C the infection grew generally milder the more the temperature was found to be rising. At temperatures of 5—10°C an increase in the numbers of soil microbes resulted in a decrease in the infection of the clover. Still higher temperatures, which did not increase the numbers of soil microorganisms, nevertheless enhanced the antagonistic power of the soil.

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SELOSTUS:

MAAN PIENELIÖSTÖN MERKITYKSESTÄ APILAN *SCLEROTINIA TRIFOLIORUM* ERIKSS.-INFEKTIOTA RAJOITTAVANA TEKIJÄNÄ VUODEN ERI AIKOINA

ANNA-MARJA HALKILAHTI

Helsingin yliopiston kasvipatologian laitos

Petrinmaljakokeissa *Sclerotinia trifoliorum*in rihmasto saastutti steriloiduissa maanäytteissä apilaa yleensä verraten hyvin. Steriloimattomissa maanäytteissä sen saastutuskyky oli pienempi ja eri vuosina sekä eri vuodenaikoina varsin vaihteleva. Ulkona suoritetuissa kokeissa apila saastui lievemmin keskikesällä kuin keväällä ja syksyllä. Sen sijaan termostaattikokeissa, joissa lämpötila oli jatkuvasti 7—10°C, ei maanäytteen ottamisajankohdan lämpötilalla ollut selvää vaikutusta apilan saastumiseen.

Kalkitus heikensi apilamätäsienen saastutuskykyä huomattavasti. Eräinä syksyinä apilamätäsieni saastutti ankarammin kesannosta kuin apilapellosta otetussa mullassa kasvanutta apilaa. Yleensä ei apilamätäsienen saastutuskyky kuitenkaan kovin paljon vaihdellut saman peltolohkon eri tavoin viljellyistä osista keskenään samaan aikaan otetuissa maanäytteissä.

Mikrobeja oli maassa yleensä eniten syksyllä ja vähiten keväällä. Apilapellossa oli mikrobeja usein, etenkin syksyisin, enemmän kuin kesantomaassa. Kalkitus lisäsi mikrobien määrää.

S. trifoliorum saastutti apilaa hyvin ankarasti 0—5 °C:n lämpötiloissa ja 5—21°C:n lämpötiloissa yleensä sitä lievemmin mitä korkeampi lämpötila oli. 5—10°C:n lämpötiloissa apilan saastuminen heikkeni maan mikrobiluvun noustessa. Korkeammat lämpötilat, jotka eivät suurentaneet mikrobien lukua maassa, lisäsivät kuitenkin maan antagonistista voimaa.