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ROOT ROT AS A CAUSE OF RED CLOVER DECLINE IN LEYS IN FINLAND

Selostus: **Juurilaho puna-apilan hävittäjänä Suomen niittonurmissa**

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To be presented, with the permission of the Faculty of Agriculture and Forestry of the University of Helsinki, for public criticism in Auditorium V on May 31, 1967, at 12 o'clock

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PREFACE

The present study was carried out in the years 1960—66 as a part of the research programme of the Department of Plant Pathology of the Agricultural Research Centre. It belongs to a series of studies on the thriving of overwintering plants under conditions prevailing in Finland.

I wish to express my sincere thanks to Professor E. A. Jamalainen, D. Agric. and For., Head of the Department of Plant Pathology, for his active interest and encouragement during the investigation as well as for placing technical assistance at my disposal. I am also very grateful to him for many helpful discussions, criticism and advice during the preparation of the manuscript.

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The agricultural societies and their personnel deserve my sincere thanks for kind help in collecting red clover root samples.

For the financial help given by the Emil Aaltosen Säätiö (Emil Aaltonen Foundation) I wish to express my deep gratitude.

To the Agricultural Research Centre I extend my thanks for including this study in their series of publications.

Tikkurila, February 7, 1967.

Aarre Ylimäki

CONTENTS

INTRODUCTION

Grasslands play a very important role in crop production in Finland. In recent years 53—55 % of arable land, i.e. 1.4 million hectares, has been occupied by grassland (ANON. 1962—65). Most of this area, or about 1.15 million hectares, was harvested for hay or silage, while pastures made up some 230 000—240 000 ha and seed fields 30 000—37 000 ha.

The yields obtained from leys in this country are on an average rather low and the extensive grasslands constitute one of the weak points in Finnish crop production. The low yields of leys are due mainly to their inadequate content of legumes. Since red clover, being the most valuable protein source for livestock, is the most important fodder legume cultivated in this country, the poor yields obtained from leys are a consequence of many factors hindering the successful growth of clover as well as poor management practices on leys (e.g. PAATELA 1953 a, b).

Because of the large total yield and high protein content of red clover, as well as its beneficial effect on soil fertility, leys containing only red clover would be better than mixed leys with grasses. However, owing to the uncertain persistence of this crop, pure clover leys are not cultivated — not even for seed production. Most of the leys in Finland are mixtures of different herbage plants, with the dominant plant being either red clover, timothy or sometimes weeds, depending on the conditions. If red clover grows well, the leys are dominated by this plant in the first and second years, and sometimes even in the third year, before other plants take over.

As the leys become older, their yields decrease, which is due chiefly to the rapid decline in clover beginning in the second year. Poor persistence in the stand is the biggest drawback of red clover.

The growth and persistence of clover, as of other crops, is dependent on many factors such as soil, availability of nutrients, weather conditions, characteristics of the species, cultivation practices, plant diseases and pests.

Under the conditions prevailing in Finland, survival over the winter is often particularly difficult. The long summer day has an unfavourable effect on the winter hardiness of clover (e.g. POHJAKALLIO et al. 1960). In addition, clover is commonly subject to the adverse effects of surface water, ice scorch, frost heaving and sometimes excessively low air temperatures (YLIMÄKI 1962 b). Clover rot, *Sclerotinia trifoliorum* Erikss., may in some winters be very destructive to clover (POHJAKALLIO 1939, YLIMÄKI 1956).

In connexion with studies carried out at the Department of Plant Pathology on clover rot and its control, the author repeatedly observed another disease occurring in clover. This disease caused the disappearance of clover in leys where clover rot was not destructive nor were there any unfavourable abiotic factors present, and the growing conditions appeared to be suitable in all respects. Examination of diseased clover plants in such fields showed that their roots were decayed and contained micro-organisms, chiefly certain fungal species which occurred repeatedly. As more root samples were being studied, it

became obvious that this was a disease similar to the so-called »root rot» of ley legumes reported in Canada and the U.S.A. (e.g. YOUNG 1923, FERGUS and VALLEAU 1926, KILPATRICK et al. 1954 a). The disease was given the Finnish name »apilan juurilaho» by the author in a preliminary report (YLIMÄKI 1962 a).

The present work deals with investigations carried out in order to determine the prevalence and destructiveness of root rot in Finnish grasslands, the symptoms and effects of the disease, as well as the kinds of micro-organisms isolated from diseased clover roots. In addition, pathogenicity tests were carried out with the fungi discovered and studies were made on the conditions favouring infection of clover plants.

MATERIAL AND METHODS

Origin of material

The studies on diseases of red clover roots and the determinations of the micro-organisms found in diseased roots have been carried out in the years 1960—65.

The material studied was obtained partly on collecting trips throughout the country and partly from growers on request. In 1962 a circular was sent to all the agricultural societies in Finland, requesting their help in collecting samples of clover roots. Samples taken at random from leys of all ages were requested, each sample to be accompanied by a reply form giving, in addition to the origin, information on age and size of ley, type of soil, variety of clover, persistence, and the most common causes of destruction. The major part of the material investigated in the present study was also obtained in 1962 and 1963 as samples collected by agricultural advisers. Of the samples, 80 % were from first- and second-year leys and only 4 % from leys older than three years. The leys were mostly 1.1—5 hectares in size, although smaller leys, 0.5—1.0 ha, comprised 37 %. Finnish red clovers made up 82 % of the clover in the leys, many of them (38 %) were local strains. As regards soil type, 90 % of the samples were taken from mineral soils and only 10 % from organic soils.

The material comprised a total of 429 red clover samples containing 8 838 roots which were received from 188 communes, as indicated in the adjoining list where they are arranged according to agricultural societies (cf. Table 1, Fig. 2).

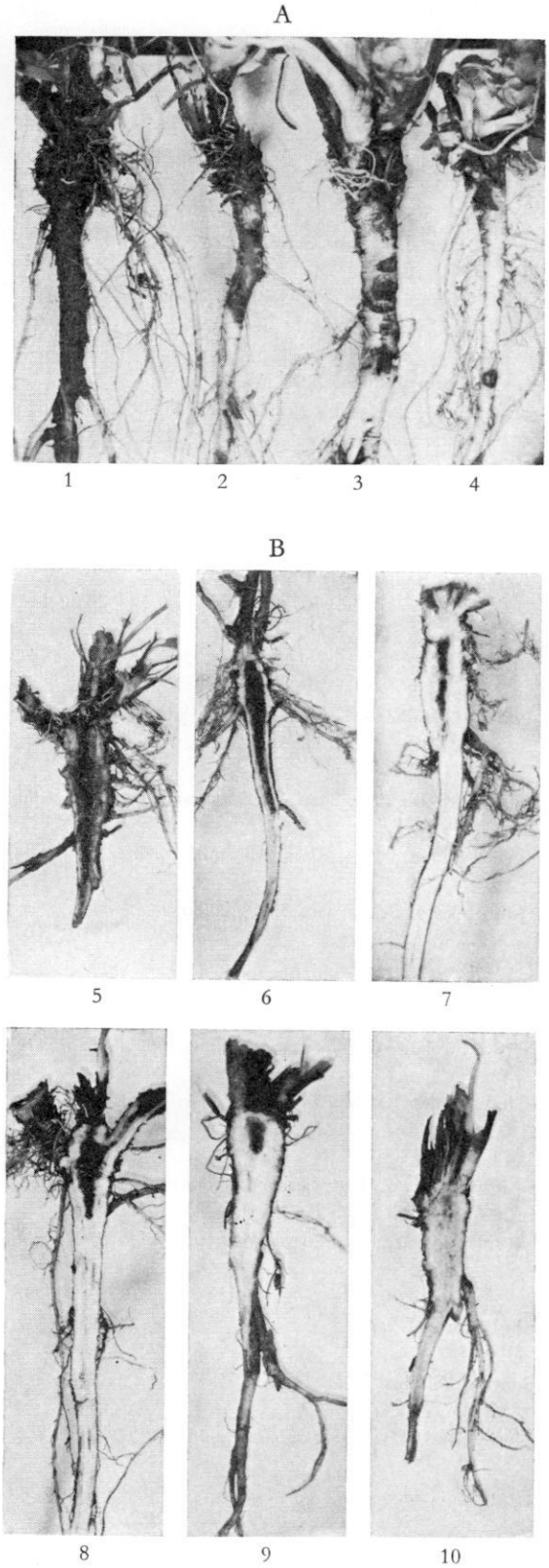
Analysis of material

The roots to be analysed were first washed under running water, after which their health condition was judged both on the surface and internally on a longitudinal section, as shown in Fig. 1.

For the isolations of micro-organisms, pieces of roots were disinfected by immersing them for about 2 minutes in a 0.3 % solution of oxykinolinsulphate and alcohol (3 g oxykinolinsulphate in 200 ml distilled water + 800 ml 96 % ethyl alcohol). Small bits of these disinfected root pieces were then taken from the margin of diseased and healthy tissue and placed on nutrient medium in Petri dishes. Isolations were made from many different parts of both the tap root and the secondary roots. The preliminary determinations of the fungi were made on the Petri dish cultures by means of a stereomicroscope. For the final identifications as well as for the pathogenicity tests, single-spore cultures were made either by hand or with a micromanipulator (Zeiss). In some cases hyphal tip transfers were made from 3—4 day old Petri dish colonies.

Agricultural societies, communes and number of samples

	samples	communes
1. UUDENMAAN LÄÄNI Askola 1, Lapinjärvi 3, Lohja mlk. 1, Mäntsälä 1, Nummi 4, Orimattila 2, Pusula 1, Ruotsinpyhtää 3, Tuusula 4, Vihti 2	22	10
2. NYLANDS SVENSKA Borgå—Porvoo 1, Helsinge—Helsingin mlk. 90, Ingå—Inkoo 2, Karis—Karjaa 2, Pojo—Pohja 1, Sjundeå—Siuntio 1, Tenala—Tenhola 1	98	7
3. VARSINAIS-SUOMI Kiikala 1, Loimaa 2, Mietoinen 3, Muurla 1, Mynämäki 1, Paimio 1, Pertteli 1, Suomusjärvi 2	12	8
4. FINSKA HUSHÅLLNINGS Dragsjärd 1, Hitis—Hiittinen 1, Houtskär—Houtskari 1, Jomala 1, Kimito—Kemiö 2, Korpo—Korppoo 2, Nagu—Nauvo 2, Pargas lk.—Paraisten mlk. 3, Vestanfjärd 1	14	9
5. SATAKUNTA Ahlainen 1, Eura 1, Eurajoki 2, Hinnerjoki 1, Honkilahti 1, Ikaalinen 2, Kankaanpää 2, Kihniö 2, Kokemäki 6, Lappi T.l. 2, Luvia 1, Merikarvia 1, Mouhijärvi 2, Noormarkku 1, Parkano 1, Pomarkku 1, Siikainen 1, Vampula 2	30	18
6. HÄME-SATAKUNTA Eräjärvi 1, Kuorevesi 1, Kuru 1, Längelmäki 1, Pohjaslahti 1, Pälkäne 4, Ruovesi 1, Vesilahti 1, Vilppula 2	13	9
7. HÄMEEN LÄÄNI Hattula 7, Hausjärvi 1, Humppila 1, Janakkala 7, Jokioinen 1, Kalvola 2, Koijärvi 1, Loppi 1, Somero 2, Tammela 2, Vanaja 1	26	11
8. ITÄ-HÄME Joutsa 1, Jämsä 2, Kärkölä 1, Padasjoki 1	5	4
9. KYMENLAAKSO Anjala 3, Elimäki 2, Kymi 2, Kuusankoski 1, Miehikkälä 4, Pyhtää 1, Valkeala 1, Vehkalahti 1, Virolahti 3, Sippola 1	19	10
10. LÄNSI-KARJALA Imatra 2, Joutseno 1, Lappee 1, Lemi 1, Luumäki 1, Nuijamaa 1, Ruokolahti 1, Saari 1, Taipalsaari 1	10	9
11. MIKKELIN LÄÄNI Enonkoski 3, Haukivuori 1, Heinävesi 2, Juva 1, Mikkelin mlk. 12, Pieksämäki 1, Ristiina 3, Sulkava 1	24	8
12. KUOPIO Hankasalmi 1, Iisalmi mlk. 1, Kuopio mlk. 2, Lapinlahti 1, Maaninka 4, Rautavaara 3, Sonkajärvi 3, Tuusniemi 2, Tervo 2, Vieremä 2	21	10
13. POHJOIS-KARJALA Kesälahti 2, Kitee 1, Kontiolahti 1, Liperi 1, Nurmes 2, Rääkkylä 3, Tohmajärvi 4, Valtimo 1, Juuka 1	16	9
14. KESKI-SUOMI Kannonkoski 1, Korpilahti 2, Laukaa 17, Pihtipudas 3, Saarijärvi 2, Suolahti 1, Toivakka 1, Viitasaari 1	28	8
15. ETELÄ-POHJANMAA Alajärvi 1, Alavus 1, Evijärvi 1, Isokyrö 1, Karijoki 1, Laihia 1, Lappajärvi 1, Nurmo 1, Töysä 1, Ylihärmä 1, Ylistaro 4	14	11
16. ÖSTERBOTTENS SVENSKA Esse—Ähtävä 1, Karleby—Kaarlela 1, Korsnäs 1, Kronoby—Kruunupyy 2, Lappfjärd—Lapväärtti 1, Larsmo—Luoto 1, Malax—Maalahti 1, Munsala 1, Nedervetil—Alaveteli 1, Nykarleby lk.—Uudenkaarlepyyn mlk. 1, Närpes—Närpiö 2, Pedersöre—Pietarsaaren mlk. 1, Purmo 1, Pörtom—Pirttikylä 1, Terjärv—Teerijärvi 1, Öja 1, Övermark—Ylimarkku 1	19	17
17. KESKI-POHJANMAA Halsua 2, Kalajoki 3, Kannus 3, Kaustinen 3, Reisjärvi 1, Toholampi 2, Ullava 2, Veteli 2, Ylivieska 1	19	9
18. OULUN LÄÄNI Alavieska 2, Nivala 1, Oulu 1, Pyhäjoki 2, Rantsila 2, Revonlahti 4, Ylikiiminki 1	13	7
19. KAJAANI Kajaani mlk. 1, Paltamo 1, Sotkamo 2, Suomussalmi 1, Vaala 4, Vuolijoki 2	11	6
20. PERÄPOHJOLA Kemi mlk. 1, Rovaniemi mlk. 5, Simo 1, Tervola 4, Ylitornio 1	12	5
21. LAPPI Pelkosenniemi 1, Savukoski 1, Utsjoki 1	63	3
	429	188



The fungi were cultured at a temperature of 18—20°C the adjacent windows providing an illumination of 1 500—2 000 lux. The nutrient medium was mainly oatmeal agar (50 g oatmeal, 14 g agar, 5 ml glycerin, 1 000 ml distilled water; bacterial contamination was prevented by adding lactic acid, in the first phase the pH was 4.5, later 5.5). Particularly in culturing *Fusarium* fungi, potato-dextrose agar was also used for comparison (200 g potato, 20 g dextrose, 30 g agar, 1 000 ml distilled water).

The microscopic identification of the fungi was performed on preparations made from the oatmeal agar cultures. In many cases the slide culture method was also employed. At least 20 conidia, usually 50, were measured on each of the isolates. Species of *Fusarium* were determined according to the system of GORDON (1952, 1960). The *Cylindrocarpon* fungi were identified according to WOLLENWEBER (1928). In the other cases, AINSWORTH (1961) and MOORE (1959) were followed in the classification of the fungi and their terminology. The colour of the fungi was determined on the basis of the colour tables of RIDGWAY (1912), and KORNERUP and WANSHER (1961).

Fig. 1. Analysis of red clover roots. A = surface; 1 = completely brown, 2 = upper half brown, 3 = numerous lesions, 4 = few, mild lesions; B = internally, 5 = completely decayed, 6 = almost completely decayed, 7 = slightly decayed, 8 = upper part hollow, 9 = upper part brown, 10 = lower part decayed.

Kuva 1. Juurien analysointi A pinnalta: 1 = kokonaan ruskea, 2 = puoleksi ruskea, 3 = useita syöpymiä, 4 = lievästi syöpynyt; B sisästä: 5 = kokonaan lahonnut, 6 = enin osa lahonnut, 7 = vähän lahonnut, 8 = yläosa ontto, 9 = yläosa ruskea, 10 = alaosa lahonnut.

Experimental methods

Pathogenicity tests of the fungi were conducted in the greenhouse.

Several different inoculation methods were initially tested, of which the most suitable and reliable was found to be the following modification of the procedure of HALPIN et al. (1952).

The containers employed were ordinary drinking glasses with an upper diameter of 65—70 mm and a height of 85 mm, or in some trials 110 mm. The growth medium was sand, which was sieved and sterilised at 180—200°C in a drying oven, and subsequently saturated with a 0.3 % Hiltner nutrient solution (2.25 g KCl, 2.10 g KH_2PO_4, 2 g $CaSO_4$, 9.60 g NH_4NO_3, 1.80 g $MgSO_4$, 100 ml aq. dest.); in some trials sterilised distilled water alone was used.

Using a cork borer, discs were taken from 10- to 14-days old fungal cultures growing on oat agar in the Petri dishes, and equal numbers of them were placed on the sand in each glass and covered with a thin layer of sand. In each trial, consisting of 3 or 4 replicates, all the cultures were of the same age. Germinated clover seeds, either 10 or 50 in number, were put on the surface of the sand. The clover seed had previously been disinfected for 2 minutes in a 0.1 % sublimate solution, rinsed in sterilised water and allowed to germinate on filter paper. The glasses were tightly covered with transparent plastic film. The test period was 21 days, after which the seedlings were analysed. Isolations were made from diseased seedlings in order to ascertain the cause of infection and its severity. Two or more parallel tests were usually perfomed on each of the fungal isolates.

Tests were furthermore carried out with older clover plants (2—10 months old), which were grown in steam-sterilised soil in plastic pots. Inoculations of the plants with fungi growing on oat agar were made by transferring fungal-agar discs or a mycelial-conidial suspension in distilled water to the base of the plant, or in other cases, by placing mycelia in or near a cut made in the root of the plant with a sterile scalpel.

The greenhouse was illuminated for 7—9 hours a day, depending on the time of year, by mercury vapour lamps, which provided 2 100—2 500 lux.

The clover seedlings and plants in these tests were analysed in the same way as the previously mentioned root samples. On the basis of these analyses, the health condition of the roots was estimated on a scale of 5—0 (5 = healthy, 0 = completely decayed). The significance of the difference in pathogenicity between the isolates was determined either by the χ^2 test or by analysis of variance (MUDRA 1958, STEEL and TORRIE 1960). In connection with the descriptions of fungi, the degree of pathogenicity is defined as follows: high (100—75 % dead and diseased seedlings), above average (75—50 %), below average (50—25 %), weak (25—0 %).

ROOT ROT OF CLOVER

Historical notes

The destruction of ley legumes as a result of damage to their roots has long been recognized. Reports of such diseases have been known in Europe since at least 1669 and in America since 1747 (FERGUS and VALLEAU 1926).

In the early and middle parts of the last century, *Rhizoctonia violaceae* (DC.) Tul., syn. *R. crocorum* (Pers.) DC. was found to destroy the roots of alfalfa in both France and Germany (DE CANDOLLE 1815, FUCKEL 1861). Later, the disease caused by this fungus was known to be injurious to alfalfa roots in Italy, Austria, Sweden and Denmark, and to the roots of clovers in at least Denmark and Germany (ROSTRUP 1902, ERIKSSON 1926).

MCCALLUM (1907) was the first to refer to *Fusarium* fungi as causing rotting in the roots of alfalfa in Arizona. About the same time Selby

and Manss determined the species in Ohio as *Fusarium roseum* Lk. (SELBY 1910). In Russia JACZEWSKI (1916) found a species of *Fusarium* which injured clover roots and which he named *Fusarium trifolii* Jacz. In most subsequent investigations, different species of *Fusarium* have generally been found to be dominant in samples of root rot (see p. 26).

In North America, first in the United States and later in Canada, extensive and thorough investigations have been made on root rot of ley legumes. In addition to *Rhizoctonia* and *Fusarium*, many other fungi have been discovered in diseased roots (see pp. 18—37). Most of the early authors in the U.S.A. (e.g. SELBY 1910, YOUNG 1923, PIETERS and HOLLOWELL 1924, FERGUS and VALLEAU 1926) realised the importance of root rot. YOUNG (1923) considered the disease to be more frequent and widespread in Ohio than clover rot *(Sclerotinia trifoliorum)*. The disease has later been found to be injurious in numerous regions in the U.S.A. and Canada. WEIMER (1928) mentions receiving information about root rot of alfalfa in almost every state in the U.S.A. where this crop is grown; he isolated a very pathogenic *Fusarium* fungus from nearly every sample he had collected. CORMACK (1937 a, b) demonstrated that species of *Fusarium* and *Cylindrocarpon* were harmful to alfalfa and sweet clover in Alberta. CHEREWICK (1941) found that *Rhizoctonia solani* was the cause of root rot over an extensive area in Minnesota, U.S.A. and Manitoba, Canada. The same fungus was observed by BENEDICT (1954) to be the reason for considerable economic losses to clovers in Ontario. MCDONALD (1955) determined that in Manitoba root rot was caused by a combination of several fungi, which together caused such a steady decline in alfalfa after the second year that it became a serious problem for the growers.

KREITLOW and HANSON (1950) state that the cultivation of red clover in Pennsylvania and other states in the U.S.A. has become difficult as a consequence of the poor persistence of this crop resulting from root rot. SMITH (1950) found in Wisconsin that 60 % of the plants that survived their first summer failed to survive their first winter, and that a large proportion of those remaining died during the following summer. KILPATRICK and HANSON (1950) estimated losses of 39—52 % in first-year stands in Wisconsin. ELLIOT (1952) mentions that although red clover is actually a perennial plant, it cannot be grown in West Virginia for more than two years, owing to damage caused by root rot. According to KREITLOW et al. (1953), root rot can be regarded as one of the chief factors limiting legume production. KILPATRICK et al. (1954 a) as well as FULTON and HANSON (1960) state that rotting of roots is common already in the first year, but it becomes so serious in the second year that in damp regions of the U.S.A. only few stands remain productive for over two years. Recent investigations have shown that root rot occurs every year in red clover in Prince Edward Island, Canada, and in that region it is the most harmful disease of clover (WILLIS 1965).

As far as is known, no extensive studies have as yet been carried out in Europe on root rot of clover and its causal agents. The reports in the literature appearing in recent decades on rotting diseases of roots in ley legumes have been few in number and mostly generalised. In England, WARE (1923), MOORE (1943), and SAMPSON and WESTERN (1954) consider the principal agent to be *Rhizoctonia crocorum*, whereas various species of *Fusarium* are held to be chiefly responsible for the disease by ERIKSSON (1926) in Sweden, GRAM and THOMSEN (1927) in Denmark, NEUWEILER (1928) in Switzerland, TOMSON (1934) in Estonia, WOLLENWEBER and REINKING (1935) in Germany, TVERSKOI et al. (1950), KOROBEINIKOVA (1956 a), VINITSKAYA (1963) and ŠNEJDER (1965) in the U.S.S.R., and YLIMÄKI (1962 a, 1966) in Finland.

TVERSKOI et al. (1950) mention that in 1947 over 80 % of the stands were destroyed in the region of Moscow as a consequence of *Fusarium* infection. In 1949, 85 % of the plants in northern, damp regions were killed, while the losses in southern, drier areas were only 18 %. KOROBEINIKOVA (1956 a) found that *Fusarium* was one of the causes of the destruction of

clover in the Urals, where the amount of infected roots in 1950 in first-year stands was 22.5 % and in second-year stands 36 %. VINITSKAYA (1963) observed that in the regions of Moscow, Rostov and Altai 64 % of the plants, or even more, were infected with *Fusarium*.

All of the above authors, as well as many others, consider fungi or in certain cases also bacteria (e.g. SAMPSON and WESTERN 1954, p. 62), to be the primary initiators of clover root rot, even though most of the micro-organisms are known to be chiefly parasites of weakened plants. Certain investigators believe that the micro-organisms encountered in the diseased roots are only secondary phytopathogens and are very weakly parasitic or even saprophytic (WEIMER 1930, FERGUS 1931, FEENE 1947, ZACHOS and PANAGOPOULOS 1960).

A disease called »Internal breakdown» is a common disease of clover in the U.S.A. It begins to appear in plants about 3 months old as an internal discolouration of the crown, resembling in this respect the symptoms of root rot (GRAHAM and NEWTON 1959, GRAHAM et al. 1960). According to present knowledge, this disease, whose cause is unknown, is nevertheless a non-parasitic condition. It appears to be related in some way to the growth of the plant, since a positive correlation has been noted between the appearance of the disease and the thickness of the tap root. Environmental factors, such as fertilisation of the soil, apparently do not have a direct effect on the initiation of this condition (LEFFEL and GRAHAM 1966).

Persistence of clover in the leys studied

According to data and samples received in 1962 and 1963 the persistence of clover in the stands from where the samples were taken was as follows: good (at least 3 years) 28 %, satisfactory (2 years) 50 % and poor (1 year) 22 % of the cases.

On the basis of these data it is evident that the clover persisted quite well since 78 % of the farms reported at least satisfactory results. The most common causes of decline in clover reported by the growers were as follows:

Excessive wetness	1.5	% of the replies
Insufficient fertilization	2	» » »
Acidic soil	13	» » »
Late cutting or grazing	4	» » »
Poor winter survival (frost, soil heaving, ice scorch)	58.5	» » »
Plant diseases or pests	21	» » »

These replies indicate that over half of the growers attributed the destruction of clover to winter injuries caused by unfavourable weather conditions.

Health condition of clover roots and prevalence of root rot

Observations made on leys over a number of years suggested that root rot was a prevalent disease of red clover. The material collected in the present study confirmed these observations (Table 1). Of the nearly 9 000 clover roots examined, an average of only 10.8 % were completely healthy. Since the samples were derived from various parts of the country (Fig. 2), covering 188 communes (34.3 % of all the communes), the material can be considered to be relatively representative of leys throughout the whole country. It also reveals that root rot occurs in all parts of Finland. Since nearly 80 % of the root samples came from first- or second-year stands (p. 8), this fact helps to explain the rapid disappearance of clover from grasslands in this country. According to the material investigated, clover roots in northern Finland are healthier than those in central and southern Finland (Tables 1 and 2).

Table 1. Red clover root samples, according to agricultural societies
Taulukko 1. Puna-apilan juurinäytteet maanviljelysseuroittain

Agricultural society *Maanviljelysseura*	Communes — *Kuntia*		Number of samples *Näytt. lukum.*	Roots — *juuret*		
	total no. *yht.*	from which samples *joista näytteitä*		total no. *yht.*	healthy *terveitä* %	diseased *sairaita* %
1. Uudenmaan lääni	26	10	22	546	4.8	95.2
2. Nylands svenska	22	7	98	436	16.1	83.9
3. Varsinais-Suomi	60	8	12	174	17.8	82.2
4. Finska Hushållnings.	26	9	14	544	11.8	88.2
5. Satakunta	47	18	30	798	6.3	93.7
6. Häme-Satakunta	24	9	13	297	16.2	83.8
7. Hämeen lääni	26	11	26	533	5.3	94.7
8. Itä-Häme	18	4	5	95	5.3	94.7
9. Kymenlaakso	15	10	19	539	3.5	96.5
10. Länsi-Karjala	18	9	10	373	3.5	96.5
South Finland — *Etelä-Suomi*	282	95	249	4 335	8.2	91.8
11. Mikkelin lääni	26	8	24	461	8.7	91.3
12. Kuopio	31	10	21	534	4.7	95.3
13. Pohjois-Karjala	21	9	16	419	11.5	88.5
14. Keski-Suomi	28	8	28	476	24.4	75.6
Central Finland — *Keski-Suomi*	106	35	89	1 899	12.1	87.5
15. Etelä-Pohjanmaa	29	11	14	462	10.8	89.2
16. Österbottens svenska	36	17	19	487	8.4	91.6
17. Keski-Pohjanmaa	16	9	19	613	11.4	88.6
Ostrobothnia — *Pohjanmaa*	81	37	52	1 562	10.3	89.7
18. Oulun lääni	41	7	13	368	21.5	78.5
19. Kajaani	11	6	11	297	6.4	93.6
20. Perä-Pohjola	16	5	12	301	28.6	71.4
21. Lappi	8	3	3	85	35.3	64.7
North Finland — *Pohjois-Suomi*	76	21	39	1 051	20.4	79.6
Entire country — *Koko maa*	545	188	429	8 838	10.8	89.2

Table 2. Analysis results of clover roots from samples taken in 1962—63

Region *Alue*	No. of samples *Näytteiden lukumäärä*	Roots — *Juuret*			Percentages of diseased roots —							
					Primary roots —							
					Surface — *Pinta*					Internally —		
		total no. *yht.*	healthy *terveitä* %	diseased *sairaita* %	completely brown *kokonaan ruskeita*	partially brown *osaksi ruskeita*	numerous lesions *useita syöpymiä*	few mild lesions *muut. lievä syöpymiä*	Total *Yhteensä*	completely decayed *kokonaan lahonnut*	mostly decayed *enin osa lahonnut*	slightly decayed *vähän lahonnut*
South Finland — *Etelä-Suomi*	135	3 933	7.6	92.4	16.3	7.0	58.7	0.5	82.4	1.2	7.8	11.6
Central Finland — *Keski-Suomi*	63	1 555	7.5	92.5	20.0	6.7	68.0	0.6	95.3	0.8	11.1	14.2
Ostrobothnia — *Pohjanmaa*	52	1 562	10.3	89.7	16.8	6.8	67.7	0.2	91.5	0.4	9.4	15.6
North Finland — *Pohjois-Suomi*	32	996	21.3	78.7	18.6	8.3	57.7	0.4	85.1	1.1	10.8	25.9
Entire country — *Koko maa*	282	8 046	9.8	90.2	17.4	7.0	62.1	0.5	87.0	1.0	9.1	14.4

Characteristics of the root rot disease of clover were studied by examining both external and internal features of the roots collected in 1962—1963 on the basis of the system given in Table 2 (cf. also Fig. 1). Only 9.8 % of these samples, comprising the bulk of the material studied, were found to be entirely healthy. Externally discoloured roots were most often internally damaged as well. In some cases seemingly healthy roots were internally discoloured. The limited amount of damage to secondary roots in the material examined was due to the fact that the secondary roots were often broken off when the plants were lifted, despite the careful performance of the lifting operation. The external injuries on both the primary and secondary roots consisted mainly of lesions (cf. Fig. 1). The internal discolouration and decay occurred most often, in nearly 60 % of the cases, in the crown of the plant (Table 2, Fig. 1), where the disease apparently usually originated. Holes made by insect pests were rarely encountered and the pests themselves even more rarely.

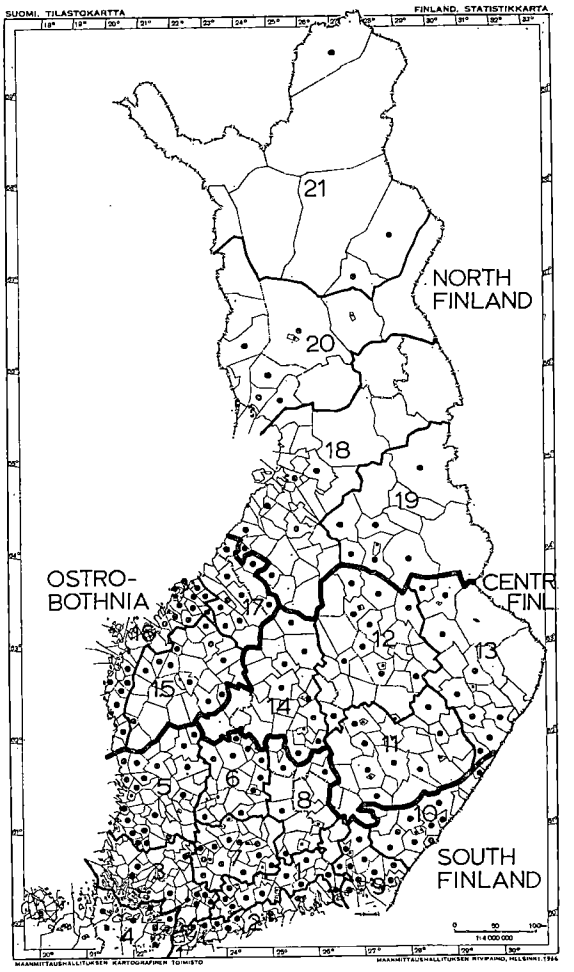


Fig. 2. Origin of red clover root samples according to agricultural societies. The black dots indicate the communes from which samples were taken.

Kuva 2. Apilan juurinäytteet maanviljelysseuroittain. Kunnat, joista on saatu juurinäytteitä, merkitty mustalla pyöryllällä.

Taulukko 2. Apilan juurien analysointitulokset vuosien 1962—63 näytteistä

Sairaista juurista %

Pääjuuret				Secondary roots — *Juurihaarat*												
Sisus				Insects *Hyönteiset*		Surface — *Pinta*					Internally — *Sisus*					
upper part hollow *yläosa ontto*	upper part brown *yläosa ruskea*	lower part decayed *alaosa lahonnut*	Total *Yhteensä*	tunnels *käytäviä*	larvae *toukkia*	completely brown *kokonaan ruskeita*	partially brown *osaksi ruskeita*	numerous lesions *useita syöpymiä*	few mild lesions *muut. lieviä syöpymiä*	Total *Yhteensä*	completely decayed *kokonaan lahonnut*	mostly decayed *usin osa lahonnut*	slightly decayed *vähän lahonnut*	upper part hollow *yläosa ontto*	upper part brown *yläosa ruskea*	lower part decayed *alaosa lahonnut*	Total *Yhteensä*
20.4	32.7	1.3	74.9	4.7	3.2	0.8	2.1	25.6	0.2	28.8	0.0	0.2	3.5	0.0	0.0	0.0	3.8
19.2	41.3	1.6	88.2	2.9	1.6	0.5	1.9	29.3	0.1	31.8	0.0	0.1	5.7	0.0	0.0	0.0	5.8
16.8	44.0	1.1	87.2	5.6	3.1	0.9	4.6	22.6	0.1	28.3	0.0	0.5	5.1	0.0	0.0	0.0	5.4
15.4	28.4	2.4	84.2	2.8	1.8	0.1	3.8	12.2	0.5	16.7	0.0	0.5	2.7	0.0	0.0	0.0	3.2
18.9	36.1	1.4	80.9	4.3	2.7	0.7	2.8	24.3	0.2	28.0	0.0	0.3	4.2	0.0	0.0	0.0	4.5

Symptoms of root rot

The primary root of red clover develops into a tap root which branches profusely, especially in its upper part, and penetrates already in the first year deep into the soil. Consequently, red clover is tolerant to drought. At the same time the rest of the root system grows rapidly and extensively in soil which is fertile, adequately drained and well-aereated. In healthy plants the roots are sound and light-coloured both externally and internally during their entire lifetime (FERGUS and HOLLOWELL 1960).

The term »*root rot*» as used in the present study denotes the disease complex which injures and destroys the roots of clover and other legumes beginning already at the seedling stage. The decline in mature stands usually takes place progressively, starting from the first growing season (cf. HAWN and CORMACK 1952, KILPATRICK et al. 1954 a, MCDONALD 1955).

When infection occurs at the seedling stage, the micro-organisms kill the plant either immediately before it emerges to the soil surface or just after emergence. These forms of the disease are called »*pre-emergence killing*» and »*post-emergence damping off*» (HANSON and KREITLOW 1953). Remnants of seedlings which were killed before emergence are extremely difficult to find in the soil, while plants injured or killed by post-emergence damping off are usually easy to discover and identify. The hypocotyl of such plants is brown or nearly black above and below the level of the soil surface, and is usually heavily constricted. Very frequently only a dark stump is all that remains of the primary root (Fig. 18). The leaves are chlorotic and twisted. Observations made by the present author in drill-sown clover stands directly after emergence showed that it is often possible to find many seed remnants killed before emergence as well as seedlings destroyed by post-emergence damping off. In contrast, such dead plants are very difficult to discover in broadcast-sown stands, particularly when a nurse crop has been used.

When root rot attacks older stands of clover, it may result in various kinds of symptoms, depending on the causal agent of the disease, the kind of host plant and the environmental conditions. It may appear as a general rotting of the whole root system or may be localized in certain parts of it. The disease most frequently occurs in the primary and secondary roots located 5—7 cm below the crown. In mild cases, and often in the early stages of infection, the symptoms may be limited to lesions and discolouration of only the cortical region (Fig. 1, A), but as infection progresses the internal vascular cylinder is damaged and finally destroyed (Fig. 1, B). Infection of the cortex and vascular core may take place simultaneously, or in some roots mainly the vascular region may be discoloured while in others the rotting is only in the cortex. The injured tissue is brown, reddish brown or blackish brown, depending on the type of micro-organism causing the disease. Bacteria cause discolouration of the root immediately below the cortical region, but in other respects the symptoms are similar to those produced by fungi (WILSON and MELTON 1962).

The primary root of a diseased clover plant usually shows initial discolouration in varying degrees of its upper part (Fig. 1, B), later the discolouration spreads along the vascular tissues both upward and downward into all parts of the root (Fig. 26). The discolouration is accompanied by a progressive rotting of the tissues which leads to a hollowing of the root and ultimately to its total destruction (Fig. 3). As the tap root becomes destroyed, new and profuse secondary roots rapidly grow in its upper part in order to replace the functions of the decaying tap root Fig. 29). However, often they do not carry on the uptake and transport of water and minerals for very long before being destroyed by fungi and bacteria.

Occasionally the tap root may be completely healthy, while most of the secondary roots are destroyed. According to HARDISON (1952), decay of the secondary roots is the most characteristic symptom of root rot. His observations showed that decay stopped at the base of the youngest

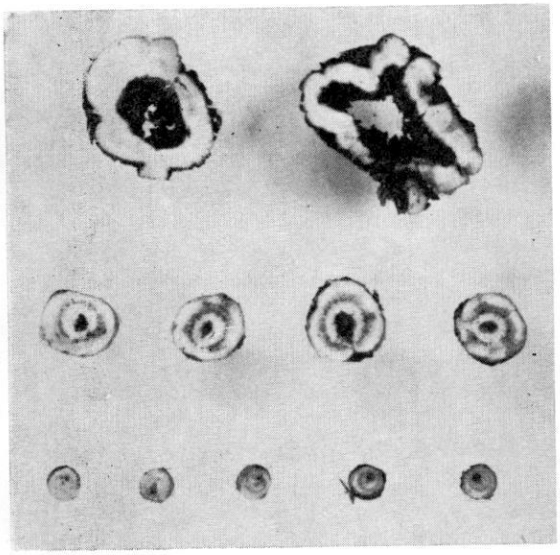


Fig. 3. Cross sections of decayed red clover tap roots at different levels.

Kuva 3. Poikkileikkauksia lahonneen puna-apilan pääjuuren eri kohdista.

secondary roots and only rarely extended into the older branch roots or the tap root. When the infection is extremely severe, all the secondary roots may be killed.

The destruction of particularly the smallest secondary roots as a result of root rot is very difficult to determine, since they are readily broken off when the plant is lifted from the ground. This aspect was studied on a second-year ley by lifting clover plants according to the method of SALONEN (1949, p. 14). By employing this method of lifting and rinsing, the root system remained almost undamaged and spread out, so that observations could be made on the extent and symptoms of the disease in all the secondary roots. It subsequently became apparent that the secondary roots had suffered much greater damage than revealed by e.g. the previously described material (Table 2).

As the micro-organisms clog the vascular tissues and ultimately destroy them, the growth and metabolism of the plant are seriously disturbed. As a result, there is a retardation in growth, the stems remain shorter than normal and their leaves are small and greyish or yellowish green in colour. When metabolism is finally completely suppressed, the edges of the leaflets become curled and the whole plant withers. Consequently, as one stem after the other dies, the vegetative production of the plant slowly declines. Especially in dry and warm periods, such plants succumb completely.

In general, root rot destroys clover plants relatively slowly. In mature plants discolouration of the roots appears at the earliest about 18—20 days after infection. Lesions in the root cortex, however, do not appear until about 1½ months later. The rapidity of infection depends to a great extent on the pathogen and its mode of infection (see p. 22). According to observations of the author on the field, it is not uncommon that a clover plant infected with root rot may live for even a year. Another characteristic of the disease is that it increases progressively as the stand ages. The most striking damage caused by root rot is usually not seen until in the second year, even though the disease appears also in first-year stands as is evident from the analysis results of the root samples obtained in 1962—63:

	Age of ley			
	1 year	2 years	3—years	total
Roots	3 479	3 122	1 445	8 046
Healthy %	15.3	6.5	1.4	9.8

Although root rot of clover occurs at all times of the year, the damage is usually greatest in the spring directly after the disappearance of snow, since at the end of the dormant period, the clover plants are in a weakened state and highly susceptible to attack by pathogens. At this juncture it is difficult visually to distinguish plants injured or killed by root rot from those infected with clover rot *(Sclerotinia trifoliorum)*. Up to the present, root rot injuries have in consequence generally been considered to be due to clover rot or abiotic factors.

Micro-organisms isolated from the roots

A total of 1 441 isolates of micro-organisms were made from the root samples collected in 1960—63. Most of the isolates were made from the internal root tissues and only a few from surface lesions. Bacteria made up 5 % of the isolates and various fungi 95 %. According to these studies, there were no noteworthy differences in the occurrence of the various fungal species in different parts of the country (Table 3).

Phycomycetes

According to reports in the literature, the following *Phycomycetes* fungi have been found in clover roots as a cause of root rot:

OOMYCETIDAE: *Pythium arrhenomanes* Drechs., *P. debaryanum* Hesse, *P. irregulare* Buism., *P. paroecandrum* Drechs., *P. rostratum* Butler, *P. splendens* Braun, *P. ultimum* Trow (HALPIN et al. 1952), *Polymyxa graminis* Lodingh. (GERDEMANN 1955), ZYGOMYCETIDAE: *Mucor* spp., *Rhizopus nigricans* Ehrenb., *Absidia* sp. (KILPATRICK et al. 1954 a, KILPATRICK and DUNN 1961).

Table 3. Micro-organism isolates, according to region
Taulukko 3. Pieneliöisolaattien lukumäärän alueellinen jakaantuminen

Micro-organism *Pieneliö*	Number of isolates and percentage of total *Isolaattien lukum. osuus koko määrästä* %					
	Entire country *Koko maa*		South Finland *E-Suomi*	Central Finland *K-Suomi*	Ostro-bothnia *Pohjanmaa*	North Finland *P-Suomi*
	total no. *yht.*	%	%	%	%	%
Phycomycetes						
Mucorales	50	3.5	4.0	3.6	3.6	0.6
Peronosporales	52	3.6	3.5	3.6	0.9	7.6
Ascomycetes	41	2.8	2.3	2.6	5.9	1.7
Basidiomycetes	9	0.6	0.8	0	0.9	0.6
Fungi imperfecti						
Sphaeropsidales	83	5.8	4.3	4.6	7.7	11.6
Melanchoniales	1	0.1	0.1	0	0	0
Moniliales						
Moniliaceae	57	3.9	4.2	3.9	4.1	2.9
Dematiaceae	18	1.2	1.5	1.0	0.9	1.2
Tuberculariaceae						
Fusarium poae	21	1.5	1.5	2.0	1.4	0.6
» *arthrosporioides*	144	10.0	9.4	8.9	14.4	8.7
» *avenaceum*	144	10.0	10.5	12.5	6.8	7.6
» *acuminatum*	162	11.2	11.2	14.8	7.2	10.5
» *culmorum*	10	0.7	0.9	1.0	0	0
» *graminearum*	9	0.6	0.4	0.7	0.9	1.2
» *sambucinum*	19	1.3	1.7	1.3	0	1.2
» *oxysporum*	38	2.6	3.9	2.3	0.5	0.6
» » v. *redolens*	8	0.6	0.3	2.0	0	0
» *solani*	17	1.2	1.2	1.0	0.5	2.3
Cylindrocarpon radicicola	233	16.2	19.4	14.1	14.0	8.7
» *ehrenbergi*	6	0.4	0.3	0.3	0.5	1.2
» *obtusisporum*	3	0.2	0.1	0.7	0	0
Mycelia sterilia						
Rhizoctonia solani	99	6.9	5.0	6.6	9.5	12.2
» *engophytica*	59	4.1	4.4	3.3	6.8	0.6
» *crocorum*	51	3.5	2.6	3.0	6.3	5.2
Bacteria	73	5.1	3.9	4.9	5.9	9.3
Others, unidentified — *Muut, määrittämättömät*	34	2.4	2.6	1.3	1.8	4.1
Total — *Yhteensä*	1 441	—	—	—	—	—
Average — *Keskimäärin*	—	—	51.6	21.1	15.4	11.9

In the present study only two of the above species were isolated from the roots of red clover: *Pythium debaryanum* and *Rhizopus nigricans*.

Pythium debaryanum Hesse 1874

The fungus occurs both as a parasite of higher plants and as a soil saprophyte in all parts of the world (MIDDLETON 1943). In many kinds of plants it causes damping off and root rot disease. BUCHHOLZ and MEREDITH (1938) demonstrated the pathogenicity of this and other *Pythium* species to alfalfa. The susceptibility of red clover to the fungus was shown by e.g. KILPATRICK and HANSON (1950) and HALPIN et al. (1952). In Finland *Pythium debaryanum* has been found to be a common causal agent of damping off as well as a rather serious pathogen of vegetables and ornamentals (LINNASALMI 1952). LIRO (1924) reported that the fungus destroyed clover seedlings.

In culture, the mycelium was hyaline, branched and septate; the hyphae had a diameter of 3—5 μ, oogonia 18.6—26.3 μ, antheridia ovoid 5.4—8.7 $\times$ 6.4—13.8 μ, sporangia spherical or often elongated 18—25 μ in diameter.

In clover plants the fungus caused pronounced and typical damping off, which was both pre- and post-emergence. It proved to be one of the most pathogenic fungi to red clover seedlings, as was also demonstrated experimentally by e.g. TVERSKOI et al. (1950) and KILPATRICK et al. (1954 b). The species was found in 3.7 % of all the isolates.

Rhizopus nigricans Ehrenberg 1818

The fungus is known throughout the world as a common soil saprophyte (FISCHER 1892). It has been found in different parts of many kinds of plants, among others, in the roots, as well as in many different kinds of plant products. KILPATRICK et al. (1954 a) isolated the fungus from the roots of red clover and KILPATRICK and DUNN (1961) from white clover roots, demonstrating at the same time its pathogenicity to these plants. In Finland MUKULA (1957) showed the fungus to be one of the causes of decay in stored carrots.

The fungus grew rapidly in culture, forming a white, glossy mycelium. The rhizoids were profusely branched and later became dark in colour. The sporangiophores were unbranched, 1—2 mm long. The sporangia were initially light brown, later dark brown, with a diameter of 100—225 μ. The spores, which were polymorphous, spherical, elongated, or irregularly shaped, had streaks and a diameter of 8.5—13 $\times$ 7—8 μ.

R. nigricans made up 3.4 % of the total number of all isolates. It was weakly pathogenic.

Ascomycetes

According to the literature, the following *Ascomycetes* fungi have previously been isolated from roots of clover species infected by root rot:

Thielavia sp., *Gibberella zeae* (Mont.) Petch, *Chaetomium* sp. (KILPATRICK et.al. 1954a).

In connection with the present investigation, the following species were found in roots of clover:

Chaetomium aureum Chivers 1912

The genus *Chaetomium* comprises a great number of species, and like most of them, *C. aureum* occurs as a saprophyte on a wide variety of substrata, but especially on seeds and in the soil (GILMAN 1959, SKOLKO and GROVES 1953).

On oat agar *C. aureum* produced colonies which were initially pale yellowish, later becoming greenish brown or olive green. The perithecia were globose with an average cross-sectional diameter of 86 $\times$ 116 μ, mostly in the range of 58—112 $\times$ 68—146 μ, with extreme values of 46—116 $\times$ 50—150 μ. They were yellowish when young, turning dark greenish brown or olive green when old. The young hairs were yellowish grey and straight, later becoming pale olive greenish brown, smooth, slightly curved at their tips (Fig. 4, A). The ascospores were at first hyaline, later olive green, asymmetrically fusiform with dimensions of 4.2—4.7 $\times$ 10.2—11.2 μ. This fungus made up 1.2 % of the isolates and proved to be a weak pathogen to red clover seedlings.

Chaetomium cochliodes Palliser 1910

The fungus has been found in the soil (GILMAN 1959) as well as in the seeds of numerous plants, including red clover and alfalfa (SKOLKO and GROVES 1953).

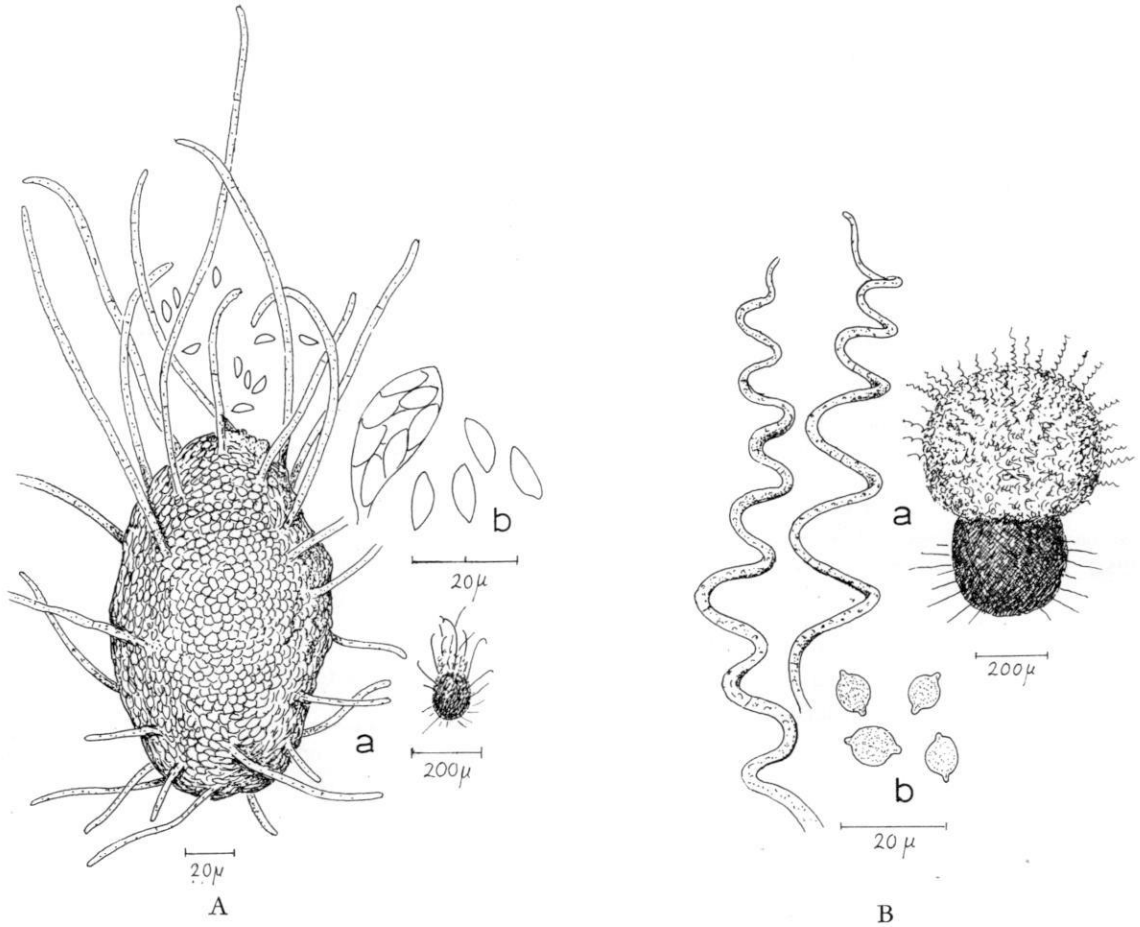


Fig. 4. *Chaetomium* species: A. *C. aureum*, B. *C. cochliodes*. a. perithecia, b. ascospores.
Kuva 4. Chaetomium-lajit: A. C. aureum, B. C. cochliodes. a. kotelopullo, b. koteloitiöitä.

The colonies in culture were initially pale yellowish green soon turning dark green or brown. The perithecia had an average diameter of 263 × 339 μ, most of them in the range 200—340 μ and with extreme values of 160—450 × 250—550 μ. The lower part of the hairs was straight, the upper part sharply bent and spirally curved (Fig. 4, B). The ascospores were olive green, broad, with nodules at both ends, and with dimensions of 6.5—7.4 × 8.4—10.2 μ. This species was found in 0.5 % of the isolates made from the roots. It was moderately pathogenic to clover seedlings.

Sclerotinia trifoliorum Eriksson 1880

The fungus is known as a destructive agent of clover rot in all places where this crop is grown. Besides clover, it parasitizes many other legumes (Pape 1937). In Finland the species occurs from the south of the country to Lapland (Pohjakallio 1939). Clover rot causes destruction of clover in the autumn and throughout the entire winter. The disease is especially damaging in areas with a heavy snow cover (Ylimäki 1962 b). Red and alsike clovers are more susceptible than white clover and alfalfa.

S. trifoliorum comprised 1.1 % of the fungal isolates investigated. When cultured on agar, it formed a characteristic cottony mycelium and sclerotia, from which arose apothecia with a diameter of 3—10 mm. These contained a large number of asci, 10—15 × 170—190 μ in size, each of which had eight ovoid, hyaline ascospores with dimensions of 5—8 × 14—17 μ. The fungus was highly pathogenic to red clover seedlings.

Basidiomycetes

According to the literature, the following *Basidiomycetes* fungi have been found in roots of clover:

Helicobasidium purpureum Pat. (see *Rhizoctonia crocorum* p. 37). *Pellicularia filamentosa* Pat.) Rogers (see *Rhizoctonia solani* p. 37), and an unidentified low temperature basidiomycete (LTB) fungus (BROADFOOT 1936).

In the present work 10 root samples of red clover were found to contain the following fungus belonging to the family *Agaricacea*.

Marasmium graminum (Lib.) Fries 1874

The large genus *Marasmium* is cosmopolitan and some of its many species are very harmful pathogens to tropical crops, such as tea, cocoa, sugar cane, banana, rubber and palm (WINTER 1884). In Europe the genus is known chiefly by its species found as saprophytes in the branches, leaves and needles of coniferous and deciduous trees, in forest litter, and in the stems, leaves and roots of grasses (BUCH 1952, MOSER 1955). Perhaps the most well known species of this genus is *M. oreades* (Bolt. ex Fr.) Fr., which causes »fairy rings» on fields and lawns in both Europe and America (e.g. SAMPSON and WESTERN 1954). The fungus, originally named *Agaricus graminum* by Libert in 1837, is now known by the name *Marasmius graminum*, assigned by FRIES (1874). Its synonyms are *M. graminum* Berk. (BERKELEY 1860) and *M. graminum* (Lib.) Berk. (MIGULA 1912), as well as *Androsaceus graminum* (Lib.) Pat., which was used by KARSTEN (1889) in Finland. *M. graminum* has been encountered in the roots of many crops (PHILIPP 1959, VIGOROV 1961), and in infection experiments it has been found to attack numerous additional plants; for example, a fungus isolated from maize was pathogenic to red clover, among others (PHILIPP 1959).

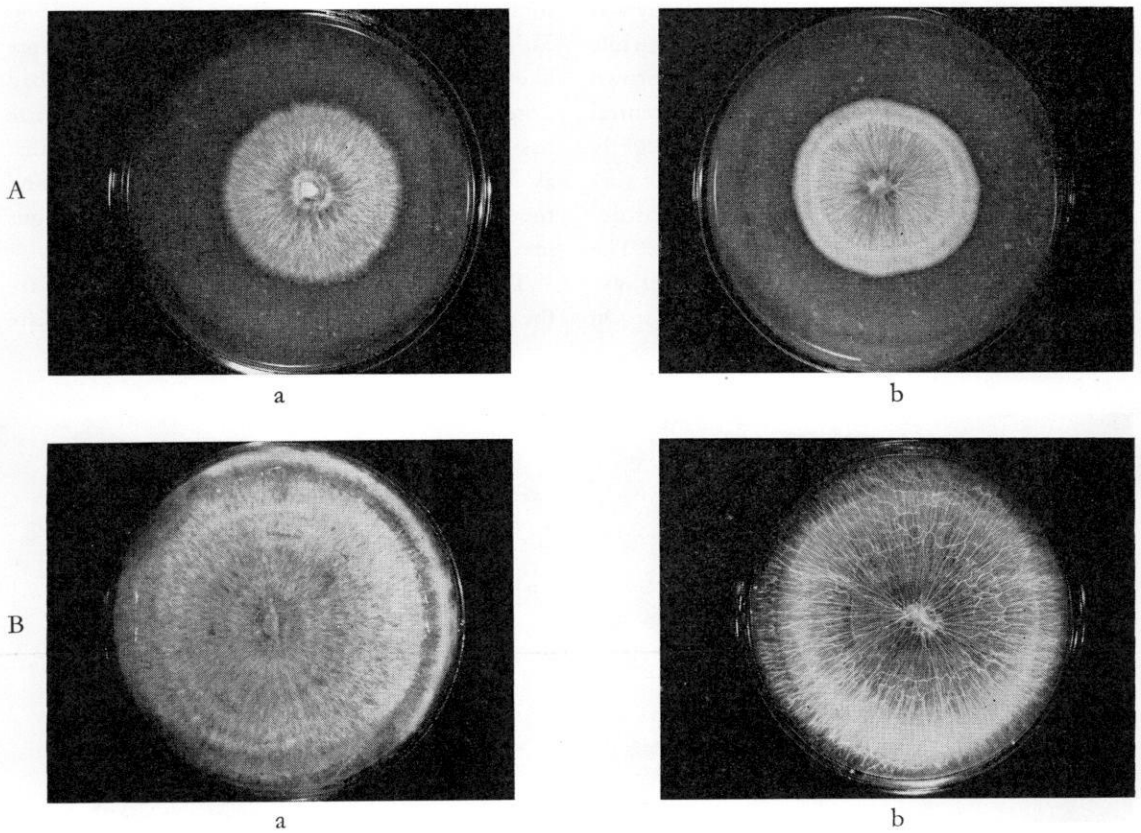


Fig. 5. Mycelia of *Marasmius graminum*, A = 14 days old, B = 60 days old; a = cottony isolate 6046-k, b = rhizomorphic isolate 62140-8.

Kuva 5. Marasmius graminum -sienen rihmastoa, A 14 vrk:n, B. 60 vrk:n ikäisenä, a. pumpulimainen isolaatti 6046-k, b. jänteinen isolaatti 62140-8.

M. graminum produced a white, rather dense cottony growth mainly on the surface of the culture medium. The hyphae were septate and either thin (diam. 1.9—2.3 μ) or thicker (2.8—5.1 μ). The fungus produced zoned colonies in which there were often white rhizomorphs. In some of the isolates the mycelium was so compactly intergrown that the colonies appeared rather rough (Fig. 5). The hyphal tips growing at the borders of the colonies were profusely branched. In some of the older isolates, white or pale yellowish stromata were produced (Fig. 6). There were many clamp connections in all the isolates (Fig. 7). Hydrocyanic acid (HCN) was produced in the mycelium of some isolates (see p. 23). The stipe of the fruiting body was 2—4 cm long, about 0.5 mm thick, very hard, smooth and glossy, dark brown in its lower part, light brown in the upper part for a distance of about 10 mm. The leathery cap (pileus) of the fruiting body was initially club-shaped and reddish brown, while later it was 4—9 mm in diameter and light brown in colour, especially at the edges. The central portion of the young cap was usually slightly elevated (i.e. umbonate) and darker than the surrounding portion, later it became somewhat depressed, remaining, however, dark brown. The dichotomous lamellae, usually 13 in number, extended to the stipe in the form of a collar. On the upper surface of the cap there were furrows at the sites of the lamellae with convex elevations in between. The basidiospores were oviform, hyaline, with dimensions of 3.6 × 5.4 (2.8—5.6 × 3.7—7.0) μ.

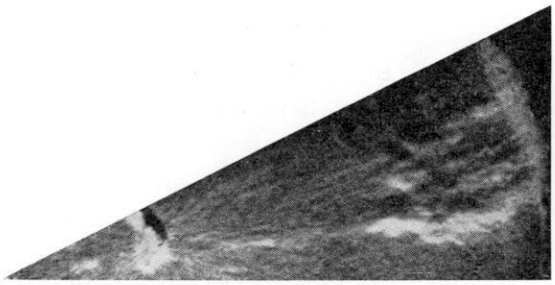
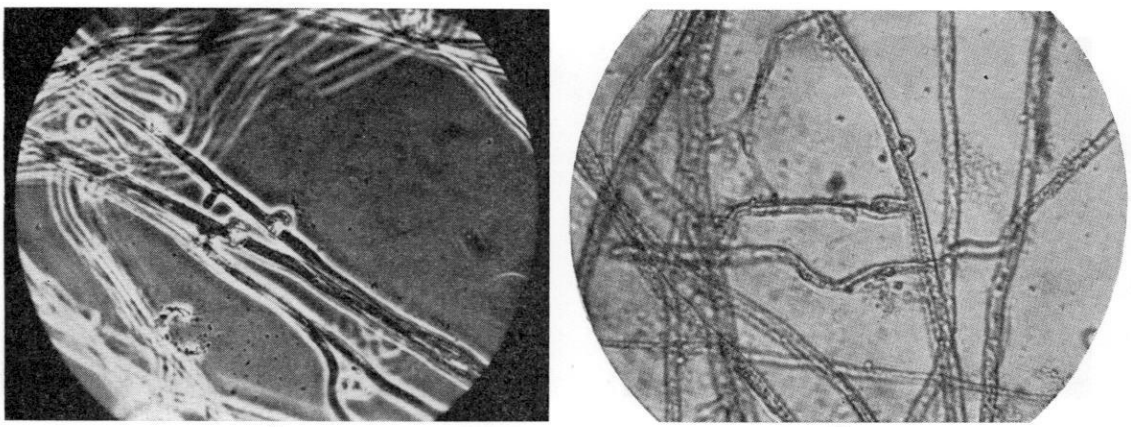


Fig. 6. Stroma-like formations in the mycelium of *Marasmius graminum*.
Kuva 6. Stromamaisia muodostumia Marasmius graminum -sienen rihmastossa.

Judging by the rhizomorphs, there appeared to be two morphologically distinct types (Fig. 5). As regards the stromata, differences were likewise noted, just as WARD et al. (1961) had observed in the mycelium of the low-temperature basidiomycete (LTB), which is known, inter alia, as a cause of root rot of legumes in Canada and the U.S.A. and which resembles the fungus investigated in the present study.

The author had the opportunity to compare the *M. graminum* isolates with the LTB isolate



A B

Fig. 7. A. Hyphae of *Marasmius graminum* showing clamp connections, isolate 6046-k, B. isolate LTB 218 (Canada). 1000.
Kuva 7. A. Marasmius graminum -sienen rihmastoa sinkilöineen, isolaatti 6046-k, B. LTB isolaatti 218, Kanada.

No. 218 which was brought from Lethbridge, Canada by Professor E. A. Jamalainen in 1956.

When grown on both oat agar and PDA culture mediums, the mycelium of *Marasmium graminum* grew relatively slowly at temperatures under 15°C. Its optimum was close to 30° and the minimum evidently slightly below 0° (Fig. 9). The response of this fungus to temperature was thus different from that of LTB, which grows best at comparatively low (6—15°C) temperatures (BROADFOOT 1936, WARD et al. 1961, Fig. 9, isolate 218).

The acidity of the substrate had no appreciable effect on the mycelial growth of *M. graminum*, at least not in the range pH 3.6—6.5 (Fig. 10), while the optimum range of the LTB isolate was more limited. Usually, however, LTB has a wider optimum pH range (WARD et al. 1961).

The capacity of the mycelium to produce HCN was tested qualitatively using sodium carbonate + picric acid on fungus grown in a mixture of ground soybeans and soil (LEBEAU and DICKSON 1953, WARD et al. 1961). The results are given below:

Species and isolate			Colour (KORNERUP and WANSCHER 1961)
Marasmius graminum		62 140—8	4 A 8
»	»	6 221—2	»
»	»	6 046—k	1 A 8 — 2 A 8
»	»	6 227—6	1 A 8
»	»	62 245—8	»
LTB		218	»
Control, without fungus			»

The most rapid and abundant production of HCN occurred in the rhizomorphic isolates 6 221—2 and 62 140—8, while it was moderate in 6 046-k, which had a more delicate mycelium, and extremely weak, being only barely visible, in the isolates 6 227—6, 62 245—8 and LTB 218.

Since exact identification of the fungus on the basis of its mycelium was not possible, several methods were used to induce the fungus to produce fruiting bodies. The following method gave satisfactory results.

Five *Marasmius graminum* isolates were allowed to grow for 10 days on oat agar at 28—30°C,

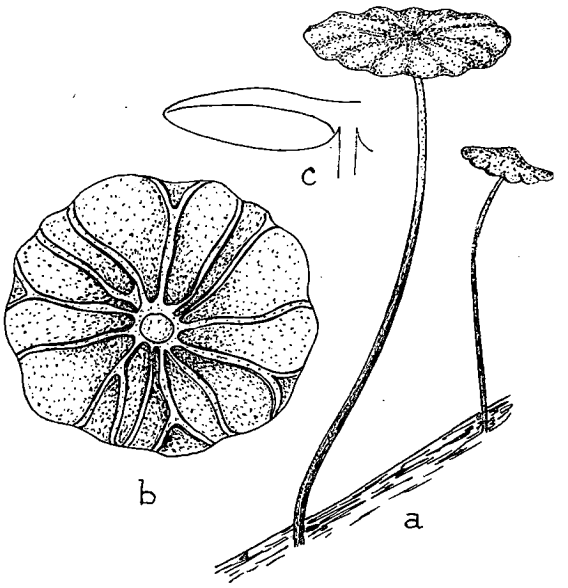


Fig. 8. Fruiting body of *Marasmius graminum;* a. fruiting bodies 3 ×, b. underside of a pileus 5 ×, c. longitudinal section.

Kuva 8. Marasmius graminum -sienen itiöemä. a. itiöemiä 3 ×, b. lakki alapuolelta 5 ×, c. pitkittäisleikkaus.

after which their mycelia were mixed with autoclaved soil at about 50 % water capacity. The mixture was then stored in closed glass jars for 65 days at a temperature of 4°C. In the spring (21. 5. 1965) the jars were transferred to the greenhouse, and on the surface of the soil were sown red clover seeds which had been disinfected with 0.1 % mercuric chloride and carefully rinsed with distilled water. The jars were kept closed, but air was allowed to enter. When necessary, the soil was moistened with sterilised water. The temperature in the greenhouse varied from 12° to 33°C. After 38 days, fruiting bodies appeared in two of the jars (isolates 6 046-k and 62 245—8) and were found to belong to the family *Agaricacea* (Fig. 8). Later, further fruiting bodies were produced both in the above two isolates and in 6 227—6, so that a total of 17 were obtained. Single basidiospore cultures were made, and the resulting mycelia were used according to the above-described method to produce further fruiting bodies, of which a total of 54 were obtained in the early summer of 1966.

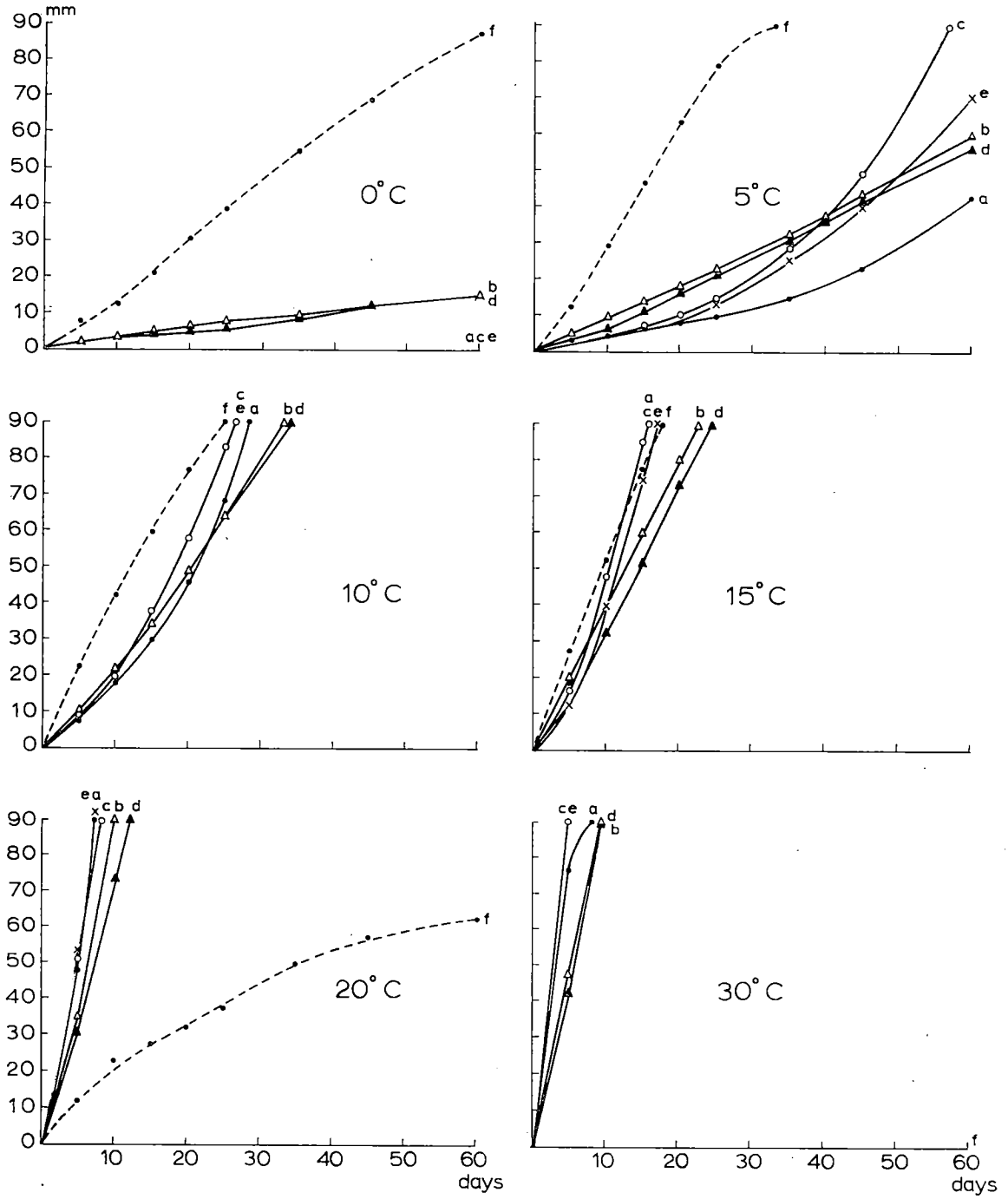


Fig. 9. The effect of temperature on the mycelial growth of *Marasmius graminum*. Isolates: a = 6046-k, b = 6221-2, c = 6227-6, d = 62140-8, e = 62245-8, f = LTB 218 (Canada).

Kuva 9. Lämpötilan vaikutus Marasmius graminum -sienen rihmaston kasvuun. Isolaatit: a = 6046-k, b = 6221-2, c = 6227-6, d = 62140-8, e = 62245-8, f = LTB 218 (Kanada).

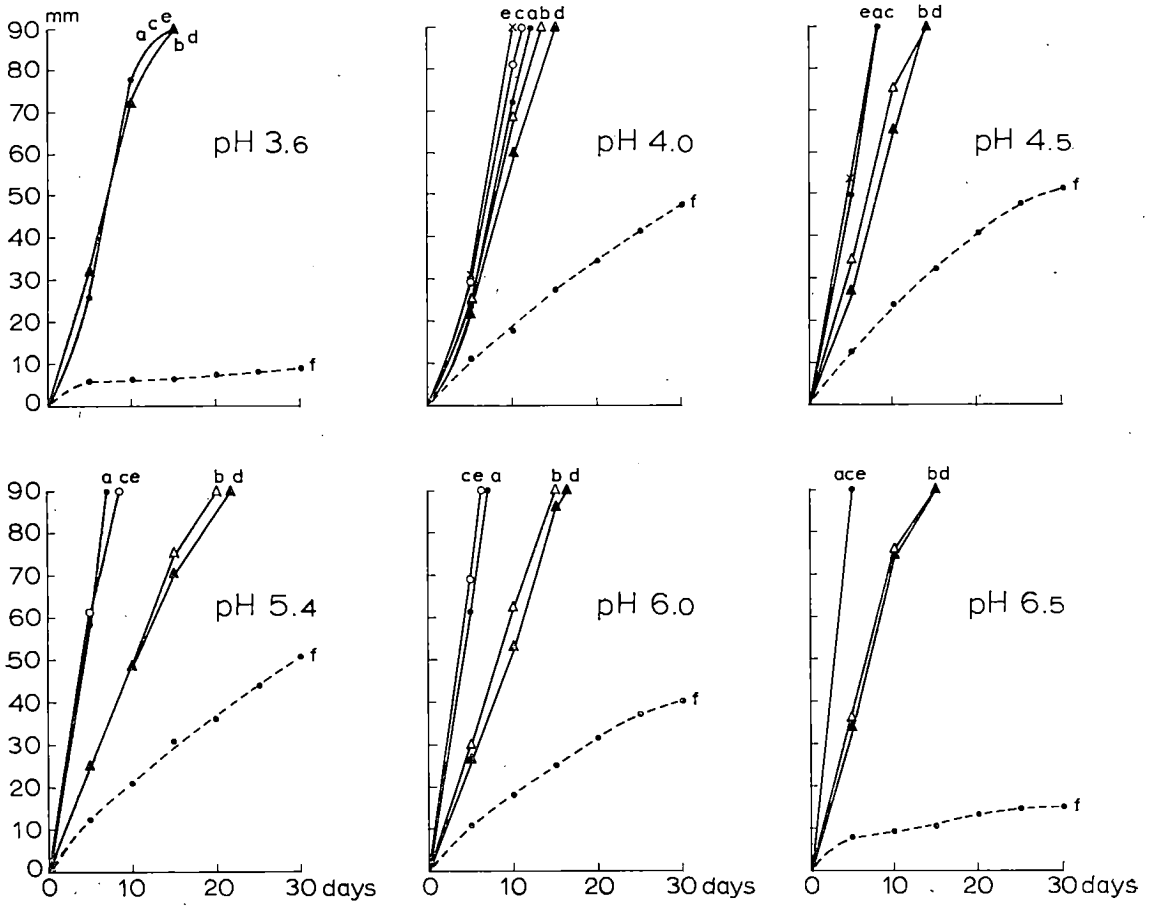


Fig. 10. The effect of pH of the culture medium on the mycelial growth of *Marasmius graminum*. Isolates: a = 6046-k, b = 6221-2, c = 6227-6, d — 62140-8, e = 62245-8, f = LTB 218 (Canada).

Kuva 10. Ravintoalustan happoisuuden vaikutus Marasmius graminum -sienen rihmaston kasvuun. Isolaatit: a = 6046-k, b = 6221-2, c = 6227-6, d = 62140-8, e = 62245-8, f = LTB 218 (Kanada).

On the basis of the fruiting bodies, it was found that the fungus in question belonged to the genus *Marasmium* of the family *Agaricacea* and was most likely the species *M. gramium* (Lib.) Fr., which comprises a considerable variation of forms; this diagnosis agrees with that of Buch (1952) in his identification of the species. Furthermore, this species is apparently identical with that described by Karsten (1889) under the name *Androsaceus graminum* (Lib.) Pat., which was a synonym of *M. graminum* (Lib.) Fr.

Marasmius graminum was slow-acting but its pathogenicity to red clover seedlings was above average. The pathogenicity of the fungus was not greatly affected by the temperature, although seedlings growing under warm conditions were more severely infected than those in cool conditions, as demonstrated below by the results of a 25-day test carried out with red clover seedlings:

	Cool conditions (2—13°C)		Warm conditions (14—35°C)	
Fungal isolate	% diseased	% dead	% diseased	% dead
6 046—k	100.0	0.0	98.8	1.2
6 221—2	100.0	0.0	92.5	7.5
62 140—8	100.0	0.0	90.0	10.0
62 245—8	90.0	10.0	81.3	18.7
LTB 218	96.2	3.8	86.3	13.7
Control, without fungus	13.8	0.0	22.5	0.0

There was consequently no essential difference between the *Marasmius graminum* isolates and LTB.

Marasmius fungi were isolated from samples taken in the following localities (in parenthesis is the number of the agricultural society, cf. Fig. 2): Helsinki (2), Kemiö (4), Parkano (5), Virolahti (9), Saari (10), Sonkajärvi (12), Evijärvi and Laihia (15), and Rovaniemi (20). It was found that the fungus started to grow much more slowly than the other fungi studied. For this reason it is possible that during the early phases of collection, before this fact was noticed, some of the *M. graminum* material was overlooked. This may have been the case particularly when the same sample contained another micro-organism which grew more rapidly and thus prevented the appearance of *M. graminum*. Later, when isolation of the various organisms was carried out not only at about 20°C but also at lower (ca. 10—12°C) temperatures, there were better possibilities for *M. graminum* to appear, since under such conditions the growth of other fungi was likewise retarded.

Fungi imperfecti

In all countries where studies of clover root rot have been carried out, most of the micro-organisms isolated from diseased roots were fungi belonging to the group *Fungi imperfecti*. The following species have been mentioned in the literature:

SPHAEROPSIDALES. *Ascochyta imperfecta* Peck, *Phoma trifolii* Johns. & Vall. (JOHNSON and VALLEAU 1933), *Coniothyrium* sp., *Sphaeropsis* sp., *Chaetomella* sp., *Phoma* sp., *Trotteria* sp., *Zythia* sp. (KILPATRIC et al. 1954 a), *Plenodomus meliloti* Dearn. & Sandf. (SANFORD 1933), *Pyrenochaeta terrestris* (OSTAZESKI 1957).

MELANCHONIALES: *Colletotrichum graminicola* (Ces.) G. W. Wils. (GARREN 1955), *C. destructivum* O'Gara, *C. trifolii* Bain & Essary, *Leptodiscus terrestris* Gerdem. (HALPIN and MCCARTER 1961).

MONILIALES. Moniliaceae: *Aspergillus flavus* Link, *A. terreus* Thom, *Gliocladium roseum* (Link) Thom, *Geotrichum* sp., *Monilia* sp., *Penicillium* sp., *Trichoderma lignorum* (Tode) Harz, *Verticillium* sp. (KILPATRICK et al. 1954 a, KILPATRICK and DUNN 1961), *Botrytis cinerea* Pers. (MCCARTER and HALPIN 1962), *Cephalosporium* spp. (BALDWIN 1962), *Oedocephalum anthophilum* Jacz. (JACZEVSKI 1916), *Papularia* sp. (WILLIS 1965), *Spicaria* (FEZER 1961), *Verticillium albo-atrum* Reinke & Berth. (SACKSTON 1960), *V. dahliae* Kleb. (LEACH et al. 1961).

Dematiaceae: *Alternaria tenuis* Nees, *Curvularia trifolii* (Kauffm.) Boed., *Helminthosporium* sp., *Hormodendrum* sp., *Nigrospora* sp., *Stemphylium* sp. (KILPATRICK et al. 1954 a), *Cladosporium herbarum* (Link) Fr., *Thielaviopsis basicola* (Berk. & Br.) Ferraris (KILPATRICK and DUNN 1961).

Tuberculariaceae: *Fusarium roseum* Lk. (SELBY 1910), *F. trifolii* Jacz. (JACZEVSKI 1916), *F. oxysporum* Schl., *F. moniliforme* Sheld., *F. solani* (Mart.) App. & Wr. (FERGUS and VALLEAU 1926), *F. roseum* Lk. emend. Sn. & Hans., *F. moniliforme* Sheld. emend. Sn. & Hans., *F. oxysporum* Schlecht. emend. Sn. & Hans., *F. solani* (Mart.) App. & Wr. emend. Sn. & Hans., *Epicoccum* sp. (KILPATRICK et al. 1954 a), *Fusarium poae* (Pk.) Wr., *F. avenaceum* (Fr.) Sacc., *F. acuminatum* (Ell. & Ev.) Wr., (GORDON 1959), *F. redolens* Wr. (WOLLENWEBER and REINKING 1935), *F. arthrosporioides* Sherb., *F. culmorum* (W. G. Sm.) Sacc. (YLIMÄKI 1962 a), *F. bulbigenum* Cke & Mass. (MENDE 1954), *Cylindrocarpon* sp. (BENEDICT 1954).

MYCELIA STERILIA. *Rhizoctonia solani* Kühn (KREITLOW et al. 1953), *R. crocorum* (Pers.) D. C. (ROSTRUP 1902), *R. violacea* D. C. f. *trifolii* Erikss. (ERIKSSON 1926), *R. leguminicola* Gough & Elliot 1956), *Sclerotium bataticola* Taub. (HENSON and VALLEAU 1933), *S. rolfsii* Sacc. (VALDER 1954), *Macrophomina phaseoli* (Maubl) Ashby (HALPIN and MCCARTER 1961).

In the present study, 82 % of the isolates made from red clover roots were found to consist of fungi of the group *Fungi imperfecti*, and of these 68.8 % (or 56.5 % of all the isolates) were species of *Fusarium* and *Cylindrocarpon* (Table 3, Fig. 11). *Cylindrocarpon radicicola* Wr. was the most frequently occurring species, followed by *Fusarium acuminatum* (Ell. & Ev.) Wr., *F. arthrosporioides* Sherb., *F. avenaceum* (Fr.) Sacc. and *Rhizoctonia* spp.

Sphaeropsidales

Phoma medicaginis var. *pinodella* (L. K. Jones) Boerema 1965

Fungi of the genus *Phoma* are widely distributed throughout the whole world and occur as saprophytes or parasites in many different plants (e.g. MOORE 1959). Fungi of this genus have been commonly found in clover and other legumes in North America (HARDISON 1952,

KILPATRICK et al. 1954 a, BALDWIN 1962). Many different opinions have been expressed about the taxonomy of the *Phoma* species parasitising legumes. RABENHORST (1862) gave the name *Phoma herbarum* f. *medicaginum* West. to the species he isolated from the crown of alfalfa. Later the names *P. medicaginis* Malbr. & Roum. and *Ascochyta imperfecta* Peck., among others, have been used. The species encountered in red clover has generally been called by the name *Phoma trifolii* Johns. & Vall., originally applied by JOHNSON and VALLEAU (1933). In recent times certain authors have maintained that since the characteristics used for identifying species of the genus *Phoma* vary widely, it would be best to employ the single name *P. herbarum* v. *medicaginis* West. ex Rab. for both these species (ELLINGBOE and KERNKAMP 1957). On the other hand, EDMUNDS and HANSON (1960) suggest that both should be included in the species *Ascochyta imperfecta* Peck. However, according to recent studies, there is good justification for distinguishing between the species parasitising alfalfa, *P. medicaginis* Malbr. & Roum. 1886, and that parasitising chiefly red clover and pea, *P. medicaginis* var. *pinodella* (L. K. Jones) Boerema 1965 (BOEREMA et al. 1965).

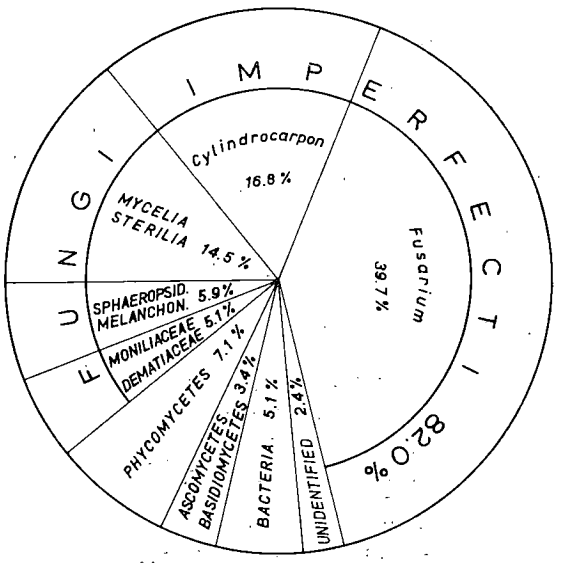


Fig. 11. Relative abundance of different micro-organisms in the root samples.

Kuva 11. Pieneliöiden suhteellinen runsaus juurinäytteissä.

When cultured on nutrient medium, this fungus produced a scanty, greenish grey mycelium which was partly aerial and partly imbedded in the medium and which had a diameter of 2.8—9.3 μ. Pycnidia were formed either on or somewhat within the surface of the substrate. They were 115—250 μ in size, black, mostly globose, containing an ostiole, and occurring either singly or in clusters. The pycnidiospores were released from the mature pycnidia as a pale grey slimy mass often with a slight reddish tinge. The conidia were mainly one-celled, hyaline, ovoid to elongate, with dimensions of 2.8—11.2 $\times$ 1.4—3.8 μ, and were often biguttulate (Fig 12).

Phoma cultures made up 5.8 % of the isolates made from the roots. Their pathogenicity to red clover seedlings was average (Table 5).

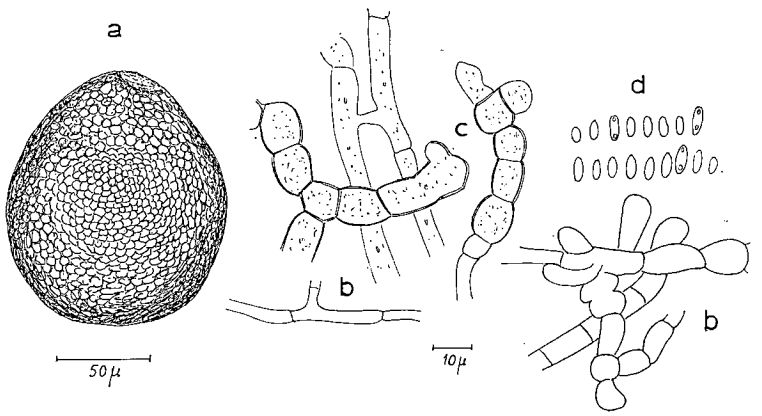


Fig. 12. *Phoma medicaginis* var. *pinodella*. a. pycnidium, b. vegetative hyphae, c. chlamydospores, d. conidia.

Kuva 12. Phoma medicaginis var. pinodella. a. kuromapullo, b. sienirihmoja, c. kätköitiöitä, d. kuromaitiöitä.

Melanchoniales

Pestalotia truncata Léveillé 1846

This species, belonging to the genus *Pestalotia*, which comprises several hundred species, has a very wide host range and many variations. It has been found in numerous deciduous trees as well as herbaceous plants, especially in their roots. The species is close to *P. hartigii* Tub. (GUBA 1961).

In cultures, the mycelium of *P. truncata* was first white, later slightly purplish grey, with a diameter of 4.7—5.1 μ. Acervuli, 200—600 μ in size, were initially brown in colour, later nearly black; they resembled pycnidia. Numerous conidia were produced, they were elliptical, fusoid, 4-celled, and had dimensions of 5.6—6.5 × 15.8—20.5 μ. The two innermost, dark brown cells had a combined length of 10.5—14 μ, while the two small, hemispherical terminal cells were hyaline and often gradually disappeared. The apical cell usually had two 15—30 μ long tufts, which were often branched (Fig. 13).

The fungus was one of the most pathogenic to red clover seedlings (Table 5).

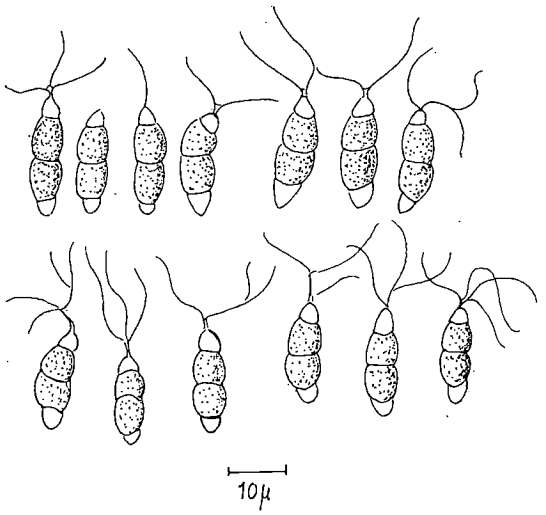


Fig. 13. Conidia of *Pestalotia truncata*.
Kuva 13. Pestalotia truncata -sienen kuromia.

Moniliales

Moniliaceae

Botrytis cinerea Persoon 1801

This is a very common fungus throughout the whole world (LINDAY 1907). It occurs as a saprophyte in many different kinds of plant remains as well as parasitically, most often, however, in plants which are in a weak condition. The fungus has been considered as the conidial stage of *Sclerotinia (Peziza) fuckeliana* (de Bary) Fuckel or *Botryotinia fuckeliana* (de Bary) Whetz. (GROVES and LOVELAND 1953). On the other hand, some investigators are of the opinion that *B. cinerea* should be considered an independent collective species relatively close to the genus *Sclerotinia* (BUCHWALD 1949). MCCARTER and HALPIN (1962) isolated *B. cinerea* from the roots of white clover suffering from root rot. In Finland the fungus has been found to be a saprophyte as well as one of the causal agents of damping off of certain vegetables and a cause of the spoiling of stored plant products (KARSTEN 1892, LINNASALMI 1952, MUKULA 1957).

In culture, the fungus grew rapidly, forming a dense, initially white but later greyish mycelium, from which arose sparsely branched, septate conidiophores having a diameter of 10—18 μ. The ovoid conidia had dimensions of 6.5—7.4 × 8.8—12.1 μ. Only few sclerotia were produced; they were black, 2—6 mm in diameter, and irregularly shaped. *B. cinerea* was not common in the samples investigated and its pathogenicity to red clover seedlings was below average.

In addition to the above species, the following *Moniliaceae* fungi were occasionally found in red clover roots: *Gliocladium roseum* (Link) Thorn, *Trichoderma lignorum* (Tode) Harz, *Verticillium albo-atrum* Reinke & Berth., *Aspergillus* sp. and *Penicillium* sp.

Dematiaceae

Alternaria tenuis Neergaard 1945

The fungus occurs in the whole world, commonly as a saprophyte in different plant parts and in the soil, but also as a parasite in many diverse plants and plant products, particularly in seeds (GROVES and SKOLKO 1944 b, NEERGAARD 1945).

The mycelium in culture was greenish grey and cottony, while the individual hyphae were brownish or olive green. The conidia were also olive green, located in long chains, and had both transverse and longitudinal septae. The size and shape of the conidia varied greatly, with dimensions of 6—16 × 11—36 μ, averaging 10.8 × 19.3 μ.

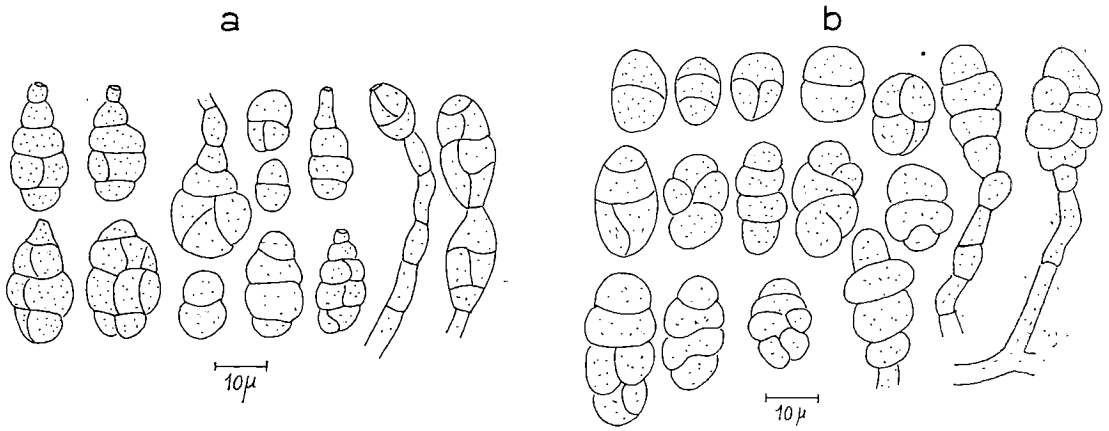


Fig. 14. a. Conidia of *Alternaria tenuis*, b. *Stemphylium botryosum*.
Kuva 14. a. Alternaria tenuis ja b. Stemphylium botryosum sienien kuromaitiöitä.

The fungus was not common in red clover roots and its pathogenicity to red clover seedlings was below average (Table 5).

Stemphylium botryosum Wallr. 1833

This is a cosmopolitan species, occurring in a wide range of plants and plant parts, especially in seeds, it is also found in rotting plant remains. It is a facultative parasite, which may attack healthy plants although it is generally found in weakened plants (GROVES and SKOLKO 1944 a, NEERGAARD 1945).

The colonies were very dark in colour; the mycelium was initially light brown changing later to dark brown. The conidia, occurring individually on the conidiophores, were dark brown, globose or usually slightly oblong, with both transverse and longitudinal septae. Their average size was 12.0 × 21.8 μ with ranges of 9.3—13.0 × 14.0—30 μ. Only a few isolates were obtained from the clover roots.

Tuberculariaceae

Fusarium poae (Pk.) Wr. 1913

The fungus, originally known to be a parasite of grassy plants, has subsequently been found parasitising many other kinds of plants (WOLLENWEBER and REINKING 1935, GORDON 1959, RAILLO 1950). It has often been isolated from the crown and roots of alfalfa and clovers. In Finland *F. poae* has earlier been found in timothy, spring wheat and in the buds of carnation (JAMALAINEN 1943 b).

When cultured, the cottony mycelium grew quite rapidly; its colour was initially white, changing rapidly to reddish pink. The stroma was carmine red, occasionally with a distinct violet hue. The microconidia were mostly one-celled, lemon- or pear-shaped, and arose in the mycelium. The size of the conidia (5 isolates) in μ was as follows:

0-septate	....	5.1 × 9.3 (2.3—6.5 × 6.5—13.0)
1- »	....	2.7 × 16.8 (2.2—3.0 × 10.2—23.3)
2- »	....	3.0 × 21.6 (2.8—3.7 × 15.8—27.9)
3- »	....	3.3 × 28.9 (3.0—4.0 × 21.0—49.0)

The brown chlamydospores were intercalary, occurring individually, in pairs, chains or clusters (Fig. 16).

F. poae was isolated in 1.5 % of the root samples investigated. The pathogenicity of its isolates was below average (Table 5).

Fusarium arthrosporioides Sherbakoff 1915

This species, originally isolated from decayed potatoes, has subsequently been discovered in cereals, vegetables and ornamentals (WOLLENWEBER and REINKING 1935, RAILLO 1950, GORDON 1959). GORDON (1959) isolated it from the stolons of ladino clover, among others. In Finland the species has been found in the grains of oats, barley and rye, the stem of wheat, potato tubers, sugar beets and spruce saplings (JAMALAINEN 1944) as well as in stored carrots (MUKULA 1957).

The dense aerial mycelium of the fungus in culture was initially white, later pink, often with a tinge of yellow. The stroma was carmine red or light brownish red. Conidia were produced both in the mycelium and pionnotes, and sometimes in the sporodochia; their size and shape varied widely and they were 0—7 -septate. The average conidial size (45 isolates) was as follows (μ):

0-septate	....	3.1 × 10.3 (2.0—4.7 × 6.5—14.0)
1- »	....	3.2 × 16.1 (1.9—5.7 × 8.0—38.1)
2- »	....	3.7 × 22.7 (2.0—5.7 × 12.1—40.1)
3- »	....	3.7 × 31.7 (2.0—5.5 × 10.0—66.0)
4- »	....	3.7 × 44.5 (2.8—5.1 × 24.2—82.0)
5- »	....	3.7 × 55.7 (2.0—5.0 × 28.0—82.0)
6- »	....	3.7 × 63.8 (2.0—5.0 × 44.0—80.0)
7- »	....	4.0 × 72.7 (3.0—4.7 × 50.0—85.0)

No chlamydospores were produced.

F. arthrosporioides was the third most frequently isolated fungus in the root samples, making up 10 % of all the isolates. Its pathogenicity to red clover seedlings was average (Table 5).

Fusarium avenaceum (Fr.) Saccardo 1886

The species is one of the most widely distributed fungi in the temperate zone, and nearly 200 species of host plants have been found. The fungus is a highly virulent pathogen to many plants and plant products (WOLLENWEBER and REINKING 1935, RAILLO 1950, GORDON 1959, 1960). WOLLENWEBER and REINKING (1935) and CORMACK (1937 b) were the first to demonstrate that the fungus was one of the causes of root rot in alfalfa and sweet clover. Later its role in causing root rot in other legumes has been shown by several authors. The fungus, under the name *Sarcopodium avenaceum* Fr., was found in oats in Finland already by KARSTEN (1892). It has later been isolated from cereals, fodder crops, vegetables, root crops, fruit and ornamental plants as well as forest nurseries (JAMALAINEN 1943 b, OLLILA 1947, LINNASALMI 1952, HÅRDH 1953, MUKULA 1957).

The fungus produced a rapidly growing, cottony mycelium which was white or faintly pink; the stroma was carmine red to brownish red. The conidia were borne on the mycelium as well as in sporodochia and pionnotes. They were slender, usually curved, with stalks at their basal ends. They were 5 (0—8)-septate. The average conidia size (μ) of 64 isolates was:

0-septate	....	3.0 × 9.1 (1.9—4.0 × 5.6—12.5)
1- »	....	2.9 × 16.5 (1.8—4.2 × 8.4—38.1)
2- »	....	3.3 × 22.9 (2.3—5.0 × 16.0—28.0)
3- »	....	3.4 × 36.1 (2.0—4.7 × 20.0—70.0)
4- »	....	3.5 × 48.0 (2.8—5.0 × 24.2—74.0)
5- »	....	3.5 × 59.7 (2.3—5.0 × 36.0—86.0)
6- »	....	3.8 × 63.6 (2.8—5.0 × 48.0—90.0)
7- »	....	4.0 × 77.3 (2.8—5.0 × 60.0—88.0)
8- »	....	4.0 × 68.0

No chlamydospores were formed.

About 10 % of the isolates consisted of *F. avenaceum*, and its pathogenicity was found to be above average to clover seedlings (Table 5).

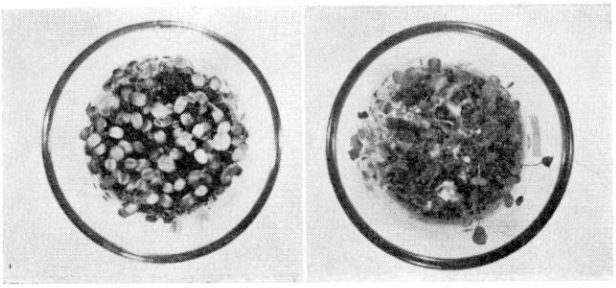
Fusarium acuminatum (Ell. & Ev.) Wollenweber 1914

The fungus, which WOLLENWEBER and REINKING (1935) later gave the name *F. scirpi* (Lamb. & Fautr.) var *acuminatum* (Ell. & Ev.) Wr., is a very cosmopolitan species occurring in numerous plant species. It causes, among other things, cereal foot rot, attacks many different plant parts, and destroys fruits and other stored plant products (WOLLENWEBER and REINKING 1935, RAILLO 1950, GORDON 1959, 1960). The fungus has also been found in the roots of ley legumes (p. 26). In Finland, JAMALAINEN (1944) isolated it from cereals, grasses, potatoes, tomatoes, onions and hawthorne, describing it under the name *F. scirpi* (Lamb. & Fautr.) var. *acuminatum* (Ell. & Ev.) Wr.

The mycelium was white or rose red, sometimes with a tinge of carmine red; the stroma was brownish carmine red, blood red, sometimes orange, and occasionally produced brown or dark blue sclerotia. The conidia, borne in the mycelium, sporodochia and pionnotes, were tapering at both ends, strongly curved, resembling a boomerang, the tip usually extending into a tuft, the base often with a stalk, 5 (0—7)-septate. The average conidia size (μ) of 83 isolates was:

0-septate	....	3.0 × 10.6 (1.9—4.7 × 5.5—15.8)
1- »	....	3.1 × 16.2 (1.9—5.0 × 7.5—32.0)
2- »	....	3.5 × 20.9 (2.0—4.7 × 12.0—34.0)
3- »	....	3.5 × 33.6 (2.0—6.0 × 14.9—70.0)
4- »	....	3.6 × 46.6 (2.0—5.0 × 26.0—74.0)
5- »	....	3.6 × 55.2 (2.0—5.0 × 28.0—80.0)
6- »	....	3.8 × 63.2 (2.8—5.0 × 48.0—82.0)
7- »	....	3.8 × 71.7 (3.0—5.2 × 62.0—88.0)

The chlamydospores were intercalary, in chains or clusters (Fig. 16).



a b

Fig. 15. Pathogenicity test with clover seedlings; a. healthy, b. injured by *Fusarium avenaceum*.

Kuva 15. Patogeenisuuskoe siementaimilla; a. terveitä, b. Fusarium avenaceum -sienen vioittamia puna-apilan taimia.

F. acuminatum was the second most common fungus in the material investigated (11.2 %). Its degree of pathogenicity was similar to that of *F. avenaceum*, i.e. above average (Table 5).

Fusarium culmorum (W. G. Sm.) Saccardó 1895

This is a species found in all parts of the world. It causes cereal foot rot diseases and decay of fruits and other plant products (WOLLENWEBER and REINKING 1935, RAILLO 1950, GORDON 1959, 1960). In Finland *F. culmorum* has been isolated from oats, barley, wheat, cabbage, potato, carnation and clover (JAMALAINEN 1943 a, LINNASALMI 1952, HÄRDH 1953).

The mycelium grew rapidly in culture; it was initially white, often partly yellowish, turning rapidly brownish red or carmine red; the stroma was carmine red, later brownish red. The conidia were borne in the mycelium, pionnotes and sporodochia; they were curved, thick-walled, strongly tapering at both ends, stalked, 5 (1—6)-septate. Their average size (μ) in 7 isolates was as follows:

1-septate	....	4.5 × 15.2 (3.3—6.0 × 10.0—18.0)
2- »	....	5.6 × 21.9 (4.0—6.0 × 15.8—26.0)
3- »	....	6.6 × 32.2 (4.0—8.0 × 18.0—38.0)
4- »	....	6.8 × 35.0 (5.0—8.0 × 28.0—40.0)
5- »	....	6.3 × 38.1 (4.7—8.0 × 28.3—50.0)
6- »	....	6.6 × 42.9 (6.0—7.0 × 36.0—54.0)

The chlamydospores were usually intercalary, single, in pairs, chains or clusters (Fig. 16).

The fungus was not very significant as a causal agent of clover root rot, since it made up only 0.7 % of the material. To red clover seedlings its pathogenicity was below average (Table 5).

Fusarium graminearum Schwabe 1838

This fungus is a parasite of numerous grassy plants, particularly cereals, invading all their parts, it also attacks many other kinds of plants throughout the world (WOLLENWEBER and REINKING 1935, RAILLO 1950, GORDON 1960). In Finland KARSTEN (1892) isolated the fungus from a number of plants naming it *F. roseum* Link or *F. heterosporum* Nees. The fungus has subsequently been found in oats, barley, rye, wheat and cucumber (RAINIO 1932, JAMALAINEN 1943 a, HÄRDH 1953).

The mycelium grew very rapidly in culture, producing a dense, floccose mat which was white or pink, sometimes with a tinge of yellow or brown. The stroma was carmine red, often with a yellowish tint. The conidia were formed in the mycelium, pionnotes or sporodochia; they varied considerably in size and shape, 5 (0—6)-septate. The average conidial size (μ) of four isolates was:

1-septate	....	3.6 × 17
2- »	....	3.6 × 22
3- »	....	4.1 × 37 (3.7—4.9 × 28—47)
4- »	....	4.6 × 44 (3.6—5.2 × 26—56)
5- »	....	4.8 × 48 (3.9—6.2 × 32—67)
6- »	....	5.1 × 56

The chlamydospores were either scarce, intercalary, or non-existent (Fig. 16).

The fungus had not previously been known as a causal agent of root rot, and in the present material it made up only 0.6 % of the isolates. It was highly pathogenic to red clover seedlings (Table 5).

Fusarium sambucinum Fuckel 1869

A cosmopolitan species occurring as a weak parasite or saprophyte in many wild and cultivated plants (WOLLENWEBER and REINKING 1935, RAILLO 1950, GORDON 1960). In Finland the fungus has been isolated from

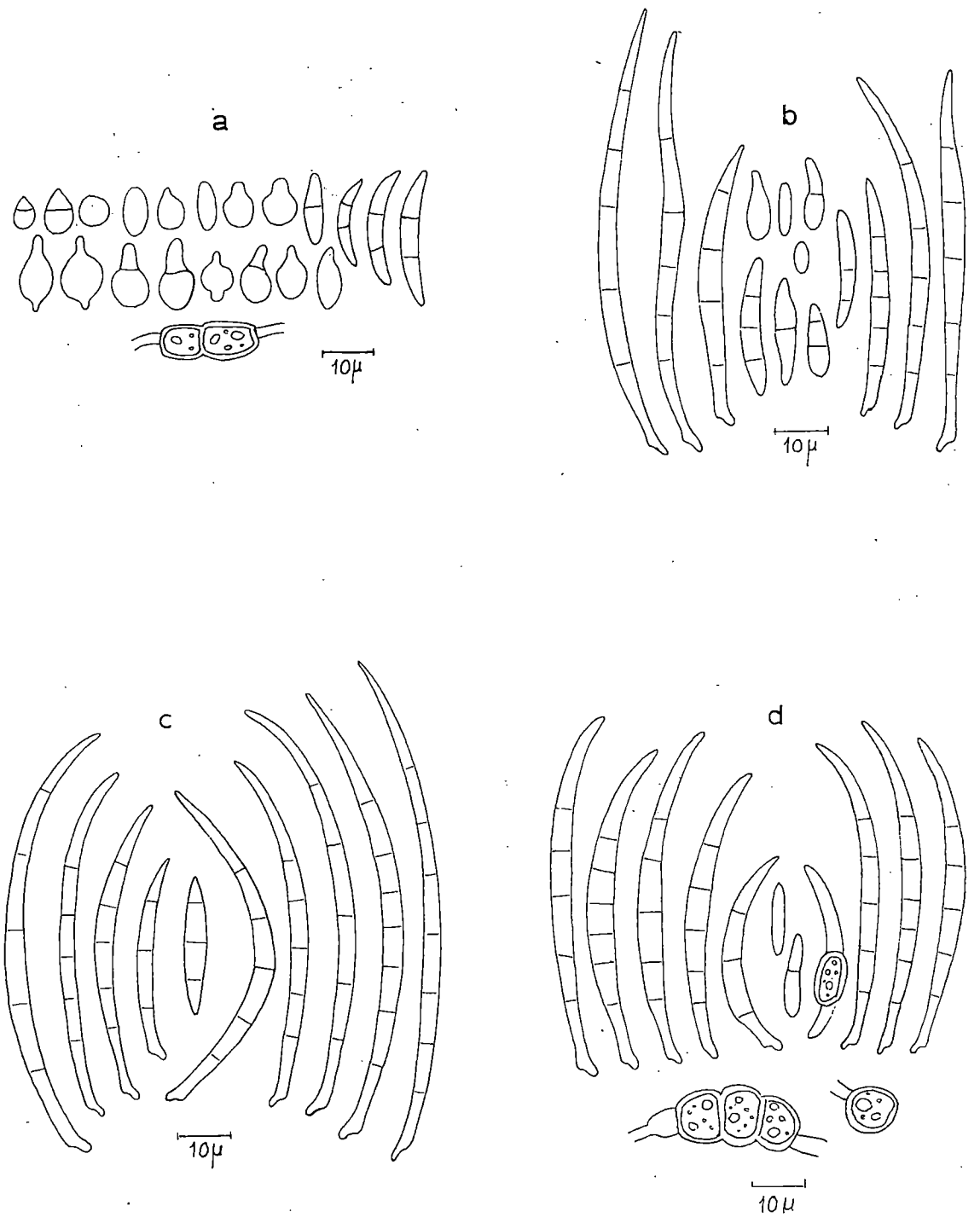
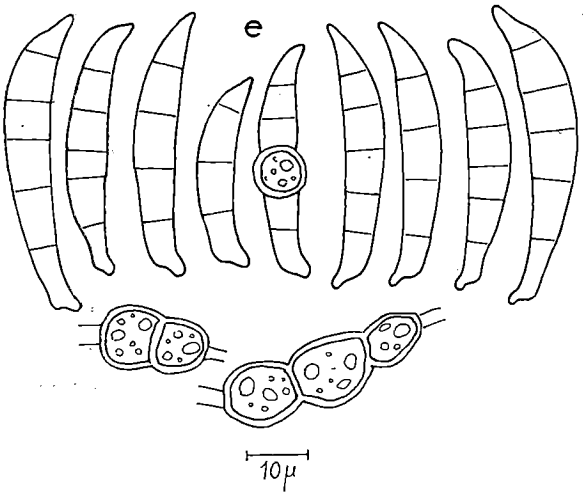


Fig. 16. Conidia of various *Fusarium* species: a. *F. poae*, b. *F. arthrosporioides*, c. *F. avenaceum*, d. *F. acuminatum*, e. *F. culmorum*, f. *F. graminearum*, g. *F. sambucinum*, h. *F. oxysporum*, i. *F. oxysporum* v. *redolens*, j. *F. solani*.

Kuva 16. Fusarium-lajien kuromia.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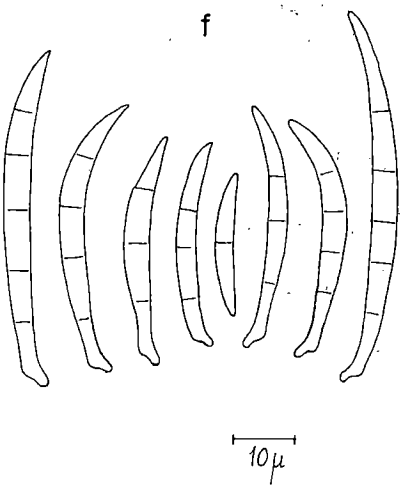
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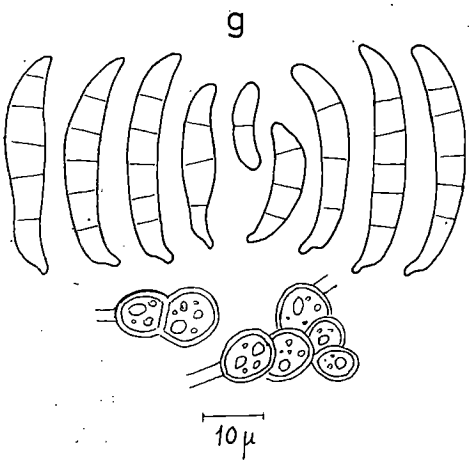
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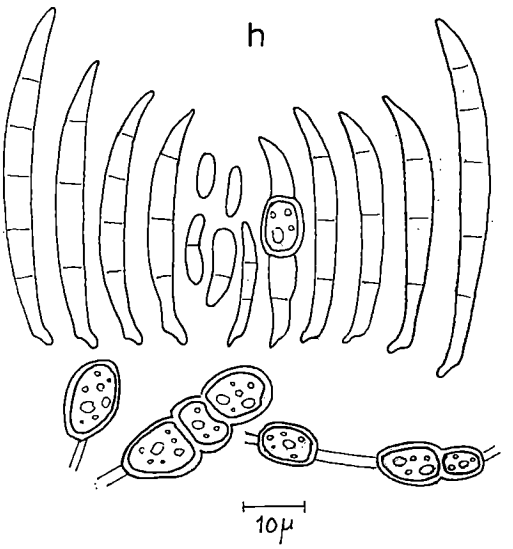
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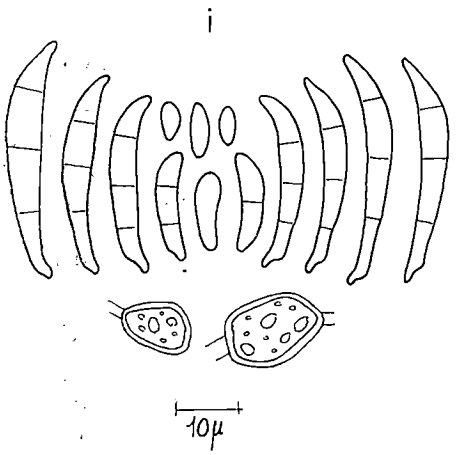
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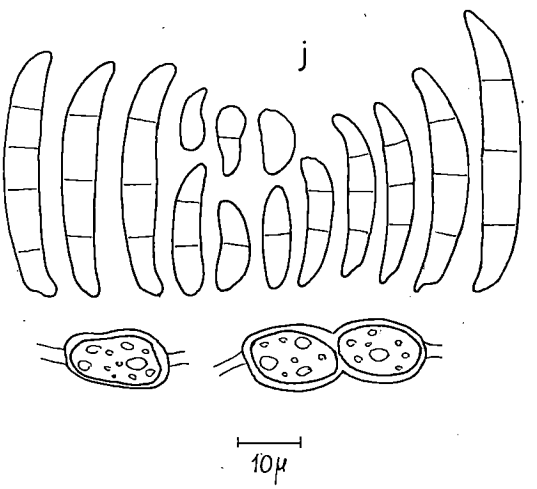
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all kinds of cereals, potato, tulips, clover leaves and spruce saplings (JAMALAINEN 1943 a, OLLILA 1947).

The mycelium grew slowly in culture, was white or pink, the stroma was pink, tan or pale brownish red, producing occasionally tan sclerotia. The conidia were borne in the mycelium, pionnotes and sporodochia and were 5 (0—8)-septate. The average conidial size (μ) of 18 isolates was:

0-septate	....	4.2 × 8.8	(2.8—6.0 × 5.6—14.0)
1- »	....	4.3 × 14.6	(2.8—6.0 × 9.3—20.0)
2- »	....	4.5 × 20.4	(4.0—6.0 × 16.0—26.0)
3- »	....	4.6 × 25.0	(3.0—6.0 × 10.0—32.0)
4- »	....	4.8 × 30.5	(4.0—6.0 × 24.0—36.0)
5- »	....	4.9 × 33.0	(4.0—7.0 × 24.0—52.0)
6- »	....	4.6 × 42.5	(4.0—5.0 × 36.0—60.0)
7- »	....	5.3 × 44.3	(5.0—5.6 × 34.0—58.0)
8- »	....	4.0 × 64.0	

The chlamydospores were intercalary occurring singly or more often in chains or clusters (Fig. 16).

This species made up 1.3 % of the isolates obtained. Since it was only weakly pathogenic, it is apparently not very important as a cause of clover root rot.

Fusarium oxysporum Schl. emend. Snyder & Hansen 1940

The species, belonging to the group *Elegans*, is very cosmopolitan and is found in many different host plants. Different forms of the fungus have become specialised and occur in certain host plants (WOLLENWEBER and REINKING 1935, SNYDER and HANSEN 1940, RAILLO 1950, GORDON 1959, 1960). *F. oxysporum* is one of the most commonly found fungi in clover and other legumes infected with root rot. It has earlier been isolated in Finland from root crops and onions under the name *F. oxysporum* Schl. v. *aurantiacum* Link. (JAMALAINEN 1944) and from carrots under the name currently used here (MUKULA 1957).

In culture, the fungus produced white mycelium which occasionally had a tinge of yellow or pink. The stroma was usually reddish violet or pale carmine red. Microconidia, 0—1-septate, were formed in the mycelium, while 3 (2—6)-septate macroconidia appeared in the sporodochia and sometimes also in the pionnotes. The size of the conidia (μ) in 21 isolates was:

0-septate	....	3.0 × 8.1	(2.0—5.1 × 4.0—14.0)
1- »	....	3.2 × 12.3	(2.0—5.0 × 7.5—17.7)
2- »	....	3.3 × 20.0	(2.8—3.7 × 11.2—28.0)
3- »	....	4.0 × 31.1	(2.3—5.0 × 22.0—46.0)
4- »	....	4.3 × 38.6	(3.7—6.0 × 32.0—50.0)
5- »	....	4.1 × 47.4	(3.0—5.0 × 40.0—54.0)
6- »	....	3.6 × 46.0	(3.0—4.0 × 42.0—50.0)

The chlamydospores were both intercalary and terminal, one- or two-celled (Fig. 16).

F. oxysporum occurred quite frequently in the clover roots studied (2.6 %). Since in the tests its pathogenicity proved to be below average to clover seedlings, it seems not to be so harmful in Finland as in other countries.

Fusarium oxysporum Schl. emend. Sn & Hans. var *redolens* (Wr.) Gordon 1952

This fungus, which GERLACH (1961 b) believes in an independent species *F. redolens* Wr., originally determined by WOLLENWEBER (1931), has been found as a saprophyte in many plants in both Europe and the U.S.A., (WOLLENWEBER and REINKING 1935, RAILLO 1950, GORDON 1959, 1960). It has also been discovered in red clover roots (WOLLENWEBER and REINKING 1935). In Finland JAMALAINEN (1944) isolated it under the name *F. redolens* Wr. f. *1* Wr. from pine saplings.

The mycelium of the fungus in culture was white, low-growing, finally quite powdery; many of the isolates had a distinct aromatic odour. In young colonies the stroma was pale brown, sand-coloured, occasionally with a violet tinge. Sometimes a few pale brown sclerotia were formed. The conidia, borne in the mycelium, pale brown sporodochia and pionnotes, were 0—3-septate, often stalked at the base, and with a beak-shaped tip. The conidial size (μ) of 3 isolates was:

0-septate	....	3.6 × 7.7	(2.8—4.7 × 5.5—11.2)
1- »	....	3.4 × 13.0	(2.8—4.7 × 6.5—23.3)
2- »	....	4.2 × 21.0	(3.7—4.7 × 15.8—26.0)
3- »	....	4.2 × 30.0	(3.7—5.1 × 22.3—36.0)

The chlamydospores were both intercalary and terminal (Fig. 16).

F. oxysporum var. *redolens* was relatively rare in the root samples (0.6 %). Its pathogenicity to seedlings was below average, indicating that it is one of the causes of red clover root rot.

Fusarium solani (Mart.) App. & Wr. emend. Snyder & Hansen 1941

This species, which has been described under many different names, occurs throughout the world chiefly as a saprophyte in numerous plants and plant products. It has also been encountered as a pathogen in many plants (WOLLENWEBER and REINKING 1935, RAILLO 1950, GORDON 1959, 1960). It is well known as an initiator of root rot of clovers and other legumes (p. 26). In Finland KARSTEN (1892) found it in potatoes and named it *F. solani* (Mart.) Sacc. Later the name *F. solani* (Mart.) App. & Wr. was applied to the fungus isolated from potatoes by JAMALAINEN (1943 b) and OLLILA (1947). JAMALAINEN (op. cit.) also found the species in young spruce, while LINNASALMI (1952) demonstrated that the isolates of *F. solani* (Mart.) App. & Wr. and *F. solani* (Mart.) App. & Wr. v. *minus* Wr. from cabbage, stock and carnation were also mildly pathogenic to seedlings of cabbage, tomato and cucumber. MUKULA (1957) observed that *F. solani* (Mart.) App. & Wr. emend. Sn. & Hans. caused decay in stored carrots.

When cultured, the fungus had a scanty aerial mycelium which was initially white, later pale yellow. The stroma was light tan or greyish yellow, in some isolates greenish blue formations resembling sclerotia appeared. The conidia, borne in the mycelium, or in pale brown to yellowish grey sporodochia and pionnotes, were slightly curved, shuttle-shaped, 3 (0—5)-septate. Their average size (3 isolates) was as follows (μ):

0-septate	....	4.2 × 9.0 (3.0—5.1 × 6.0—11.2)
1- »	....	3.8 × 14.7 (3.0—4.7 × 9.3—20.0)
2- »	....	4.2 × 21.4 (3.7—4.7 × 18.6—26.0)
3- »	....	4.9 × 28.0 (4.0—5.0 × 20.5—38.0)
4- »	....	4.8 × 40.6 (4.0—5.0 × 38.0—44.0)
5- »	....	5.0 × 48.0

The chlamydospores were mainly single, smooth, terminal or intercalary (Fig. 16). This fungus made up 1.2 % of all the isolates. It was a relatively weak pathogen to red clover seedlings (Table 5).

Cylindrocarpon radicicola Wollenweber 1928

This is a very widely distributed fungus, occurring in many plant species as a saprophyte and wound parasite. Similar to many other species of *Cylindrocarpon*, it is also a common soil fungus (WOLLENWEBER 1928, 1932). *C. radicicola* is one of the most frequently found fungi in the roots of forest trees and other plants and is presumed to be a normal inhabitant of the root surface (GARRET 1960, KUBIKOVA 1963). Data on the pathogenicity of *C. radicicola* are rather scant: CORMACK (1937 a) found it to be a weak parasite in the roots of alfalfa and sweet clover, while GERLACH (1961 a) observed it to be destructive and pathogenic to many ornamentals. In Finland the fungus has been encountered in decayed potatoes (OLLILA 1947) and carrots (MUKULA 1957).

When cultured, the isolates showed the following characteristics: the aerial mycelium was relatively scanty, often floccose, initially white, later pale yellow or tan, and finally dark brown. The hyphae had a diameter of 2.3—5.1 μ. The stroma was dark brown, occasionally reddish brown. The conidia, which were formed both in the mycelium and the pionnotes, were cylindrical, slightly curved, elliptical at the end, mostly 1-septate (0—4). The average sizes of conidia of 37 isolates were as follows (μ):

0-septate	....	4.0 × 10.2 (1.9—6.0 × 3.7—30.0)
1- »	....	5.2 × 19.6 (2.0—8.4 × 7.4—30.7)
2- »	....	5.8 × 27.6 (3.7—9.0 × 18.0—36.3)
3- »	....	5.9 × 29.8 (3.7—8.0 × 18.0—40.0)
4- »	....	6.0 × 30.0 (5.0—8.0 × 22.0—38.0)

Abundant chlamydospores were produced. They were dark brown, nearly spherical, usually intercalary, had a diameter of 11 μ (9.8—14.6 μ), and occurred individually, in chains or in clusters (Fig. 17).

C. radicicola made up 16.2 % of the isolates from the root samples. Its pathogenicity was below average as has also been established by other research workers (e.g. KILPATRICK et al. 1954 b).

Cylindrocarpon ehrenbergi Wollenweber 1928

The fungus is a common soil saprophyte both in cultivated and uncultivated land and is also a parasite in numerous different plant species (WOLLENWEBER 1928). Its role in destroying the roots of alfalfa and sweet clover was first established by CORMACK (1937 a), who also demonstrated its high pathogenicity to alfalfa and clovers. In Finland the fungus has earlier been encountered in decayed potato tubers (OLLILA 1947).

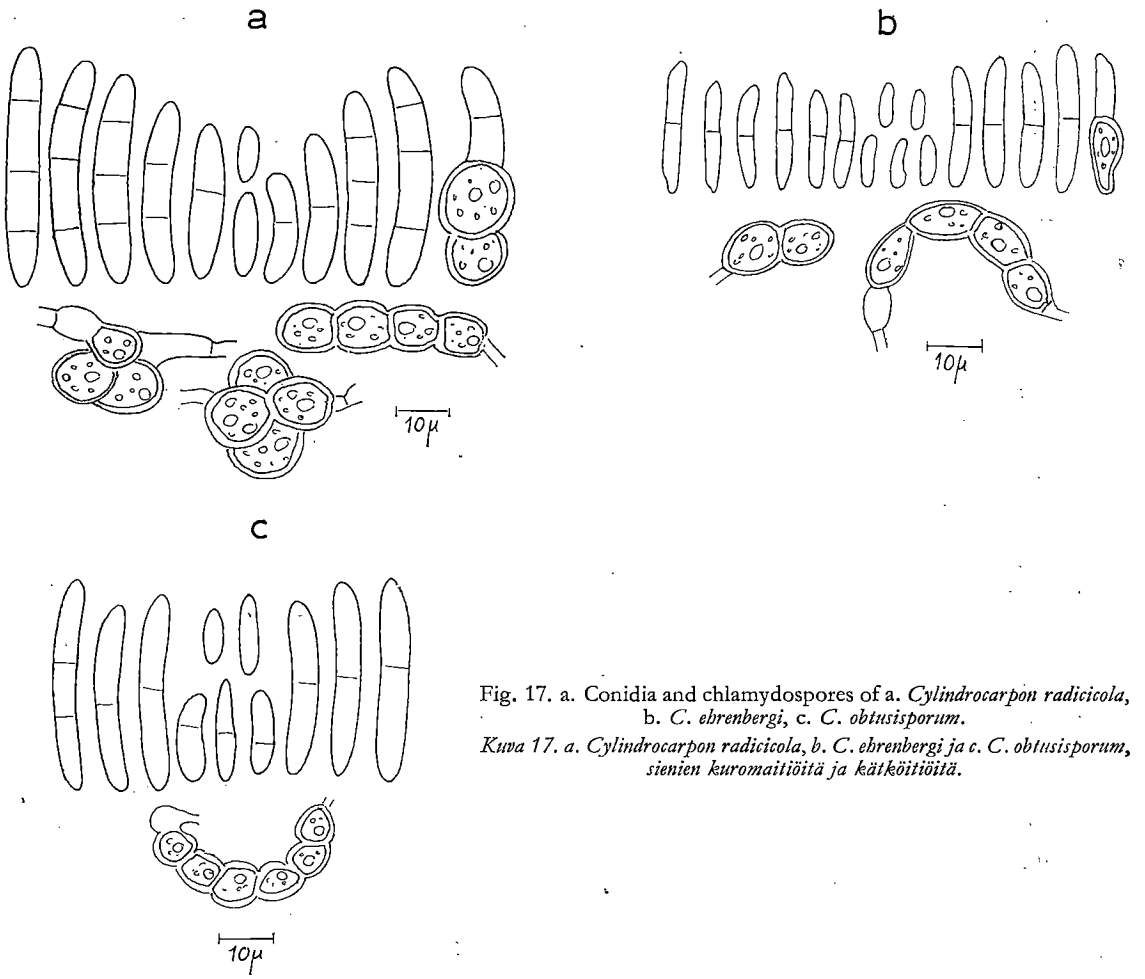


Fig. 17. a. Conidia and chlamydospores of a. *Cylindrocarpon radicicola*, b. *C. ehrenbergi*, c. *C. obtusisporum*.
Kuva 17. a. Cylindrocarpon radicicola, b. C. ehrenbergi ja c. C. obtusisporum, sienien kuromaitiöitä ja kätköitiöitä.

The mycelium of *C. ehrenbergi* in culture was white or slightly reddish brown in colour, cottony, and formed quite dense mats, which sometimes were light brown and resembled sclerotia. The conidia arose slowly, but in cultures 3—4 weeks old they were very abundant as a pale yellow mass in the sporodochia or pionnotes. The conidia were cylindrical, usually straight but occasionally slightly curved, and characteristically 1-septate. Their average size in μ was:

0-septate	3.3 × 9.6	(2.8—4.1 × 6.5—11.2)
1- »	3.7 × 22.0	(2.8—4.2 × 12.1—30.7)

There were few small-sized, chlamydospores, 6.6 × 8.4 (4.2—13.5) μ, usually occurring in chains and resembling bulbous swellings on the mycelium (Fig. 17).

This fungus comprised only 0.4 % of the isolates, and its pathogenicity to red clover seedlings was above average (Table 5, Fig. 18).

Cylindrocarpon obtusisporum (Cke. & Hark.) Wollenweber 1928

As in the case of the above-mentioned species, this fungus, is found in many different kinds of plants (Wollenweber 1928). Cormack (1937 a) and McDonald (1955) isolated it from e.g. alfalfa roots; the former author determined experimentally that it was only mildly or at most moderately pathogenic.

Rather abundant white or pale tan aerial mycelium was produced in culture; the hyphae were 2—3 μ in diameter. The stroma was light tan, becoming darker later. At first there were

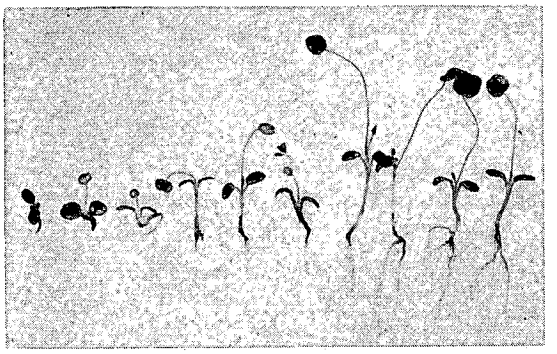


Fig. 18. Red clover seedlings injured by *Cylindrocarpon ehrenbergi.*

Kuva 18. Cylindrocarpon ehrenbergi -sienen vioittamia puna-apilan taimia.

few conidia in the mycelium; later they increased, notably in the sporodochia, which were cream coloured or yellowish white. The conidia were cylindrical, straight or curved, roundish at the end, 1-septate (0—3). Their average sizes (6 isolates) in μ were as follows:

0-septate	....	4.1 × 11.1 (3.0—5.0 × 5.6—17.0)
1- »	....	4.6 × 30.0 (2.8—6.0 × 14.0—38.1)
2- »	....	5.1 × 36.8 (3.7—6.0 × 22.8—40.9)
3- »	....	4.7 × 34.6 (4.2—5.0 × 30.0—40.9)

There were very few chlamydospores, and it was difficult to distinguish them from the bulbous swellings on the mycelium; their dimensions were 7 × 8 (5—10 × 5—12) μ (Fig. 17).

The fungus was found in only 0.2 % of the isolates and the pathogenicity was below average (Table 5).

Mycelia sterilia

Rhizoctonia solani Kühn 1858

The fungus is one of the most widely distributed soil pathogens and has a wide range of host plants. It is a complex of species, which varies greatly both morphologically and physiologically. Many different opinions have consequently been expressed concerning the taxonomic position of this fungus. At present its perfect stage is considered to be the *Basidiomycetes* fungus *Ceratobasidium filamentosum* (Pat.) Olive, syn. *Pellicularia filamentosa* (Pat.) Rogers (Olive 1957). Many research workers have demostrated that *R. solani* causes root rot in clovers and other legumes (cf. p. 26). In Finland the fungus has been found to be a common and injurious causal agent of damping off in vegetables and ornamentals (Linnasalmi 1952) as well as to cause decay of stored carrots (Mukula 1957).

In culture the fungus produced initially tan-coloured mycelium which later became darker; the hyphae, 3.7—10 μ in diameter, were characteristically branched at right angles near the septae and were distinctly constricted at the branching points. The mycelium also contained large cells 14—19 × 26—32 (39) μ in size, which were either cylindrical and round-ended or barrel-shaped and flat-ended; according to Saksena and Vaartaja (1960) they are chlamydospores. The mycelium produced small brown sclerotia, 1—3 mm in diameter, occurring mainly in clusters (Fig 19).

On the basis of the present material, *R. solani* is a common parasite in red clover roots (6.9 %) and since it was moderately pathogenic to seedlings, it is relatively injurious to clover.

Rhizoctonia crocorum (Pers.) De Candolle 1815

The fungus, whose perfect stage is *Helicobasidium purpureum* (Tul.) Pat., is a common root parasite and has many host plants (Wollenweber 1932). In the 19 th century it was already known in Europe as a destroyer of alfalfa roots under the name *R. violaceae* Tul. or *R. medicaginis* DC. or of clover roots under the name *R. violaceae* DC. f. *trifolii* (De Candolle 1815, Fuckel 1861; cf. also p. 26). In Finland the fungus was isolated from carrots by Mukula (1957).

The aerial mycelium of the fungus in culture was greyish violet, cottony, with hyphae 4—6.5 μ in diameter. The hyphae on the surface of the medium were 6.5—11 μ thick and developed sclerotia about 1 mm in size; these were initially pale grey, later becoming dark violet (Fig. 19). *R. crocorum* was encountered quite frequently in the root samples (3.5 %) and the pathogenicity was below average.

Rhizoctonia endophytica Saksena & Vaartaja 1960

The species was previously isolated only in Canada from young nursery plants of pine and spruce which were injured by damping off. It was also found to be pathogenic to seedlings of certain deciduous trees and vegetables (Saksena and Vaartaja 1960).

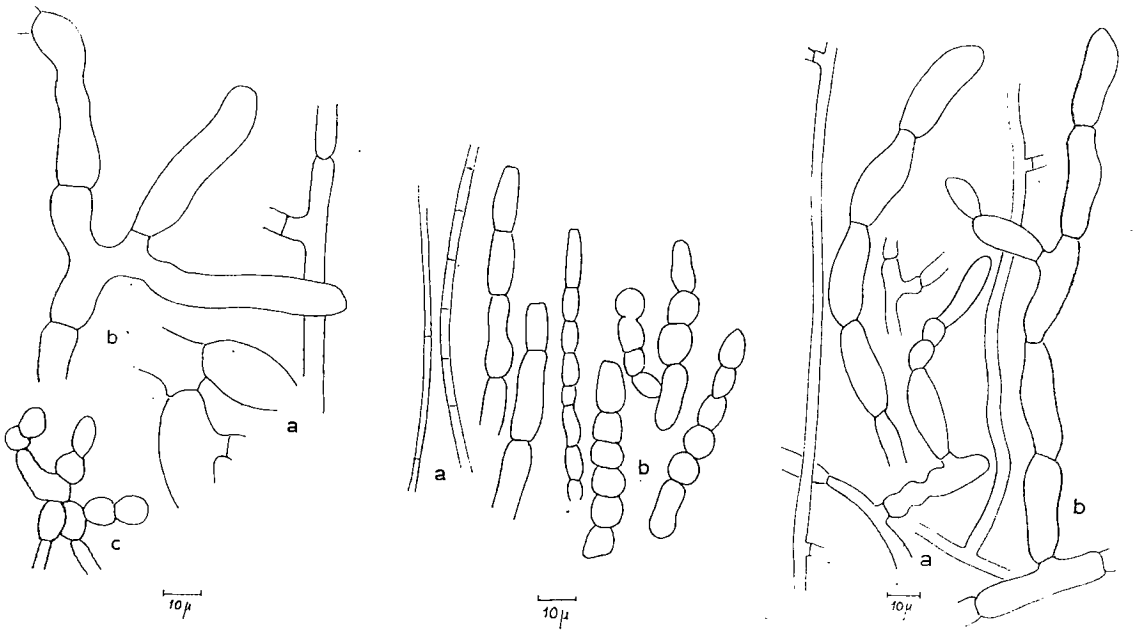


Fig. 19. *Rhizoctonia* species: A. *R. solani*, B. *R. crocorum* C. *R. endophytica*, a. vegetative hyphae, b. chlamydospores, c. sclerotial mycelium.

Kuva 19. Rhizoctonia-lajit: A. R. solani, B. R. crocorum C. R. endophytica, a. sienirihmoja, b. kätköitiöitä, c. pahkarihmastoa.

The fungus grew quite rapidly in culture, and its hyaline surface mycelium formed scattered small, white, cottony clumps. Later the colony was cream yellow or with a tinge of brown. The vegetative hyphae were hyaline, later becoming slightly tan, and had a diameter of 3.7—4.7 μ. They formed branches at nearly right angles and were constricted at the branching points. The branches anastomosed quite profusely. Chlamydospores occurred either singly or usually in loose clusters (Fig. 19); when young they were in the shape of elongated, round-ended cylinders, later becoming barrel-shaped, 8.3—13.0 × 14.9—25 (30) μ.

Rhizoctonia endophytica was found in 4.1 % of the root samples. Its pathogenicity was below average to red clover seedlings (Table 5).

Pathogenicity of the fungi

The discovery of micro-organisms in the roots of red clover plants diseased with root rot does not necessarily prove that these organisms were the primary cause of the disease, despite the fact that they were isolated as aseptically as possible from the margin of healthy and diseased tissue, as was done in the present studies. It is possible that such fungi promoted secondarily the development of the disease which was caused by some other agent. The primary factor could be some other micro-organism or animal or a certain growth factor which weakened the host plant.

In order to demonstrate the pathogenicity of the fungi isolated from red clover roots, a large number of trials were carried out under controlled conditions both with red clover seedlings and with older plants.

Table 4. Pathogenicity tests with seedlings
Taulukko 4. Patogeenisuuskokeet siementaimilla

Fungus — *Sieni*	Test series I — *Koesarja I*				Test series II — *Koesarja II*			
	No. of isolates *Isol. lukum.*	No. of tests *Kok. lukum.*	Av. % of diseased and dead seedlings *Sair. ja kuolleita* %	Significance χ^2 isolates *Merkitsevyys*	No. of isolates *Isol. lukum.*	No. of tests *Kok. lukum.*	Av. % of diseased and dead seedlings *Sair. ja kuolleita* %	Significance χ^2 isolates *Merkitsevyys*
Chaetomium spp.	—	—	—	—	2	3	35.7	69.17***
Marasmius graminum	2	2	41.6	15.42***	4	6	60.7	37.09***
Phoma medicaginis v. *pinodella*	2	2	20.0	—	6	6	65.8	147.08***
Pestalotia truncata	—	—	—	—	1	3	80.0	—
Alternaria tenuis	1	1	6.7	—	3	4	30.2	8.50*
Fusarium poae	3	5	19.0	11.26***	7	8	28.6	32.41***
» *arthrosporioides*	23	26	52.0	142.58***	11	18	43.8	294.41***
» *avenaceum*	29	35	58.7	256.58***	18	30	55.9	545.98***
» *acuminatum*	29	48	54.0	171.15***	23	26	57.2	637.54***
» *culmorum*	6	6	42.3	2.83	4	4	58.3	69.38***
» *graminearum*	—	—	—	—	1	3	81.3	—
» *sambucinum*	7	8	13.3	9.81°	8	9	41.0	316.10***
» *oxysporum*	17	19	15.3	45.17**	15	21	32.6	181.71***
» » v. *redolens*	2	2	15.0	0.14	5	5	48.9	43.88***
» *solani*	1	2	16.7	—	8	13	46.7	437.95***
Cylindrocarpon radicicola	10	10	22.3	47.60***	12	17	49.0	465.73***
» *ehrenbergi*	—	—	—	—	3	8	63.8	149.81***
» *obtusisporum*	1	1	80.0	—	4	5	30.5	6.50 (*)
Rhizoctonia solani	—	—	—	—	6	12	36.3	68.29***
» *endophytica*	2	2	26.7	12.28***	3	3	25.1	9.23**
» *crocorum*	—	—	—	—	3	4	42.0	13.52**
Total — *Yhteensä*	135	169	—	—	147	208	—	—
Control	—	26	1.0	—	—	19	8.9	—

Pathogenicity to seedlings

Two series of laboratory trials were performed; in the first, 10 newly germinated seeds were sown in each tray, while in the second 50 seeds were sown. There were four replicates in each series. The number of trials in the 10-seed series was 26 and in the 50-seed series 19, and they comprised a total of 377 fungal inocula from 238 different isolates. The pathogenicity of *Fusarium* and *Cylindrocarpon* was thoroughly studied, since these were the two most commonly isolated fungi obtained from diseased clover plants (Table 3).

As regards the most frequently occurring fungal species in the roots, a sufficient number of trials were conducted with as many isolates as proved necessary to obtain a clear picture of the degree of pathogenicity of the fungus concerned (Tables 4 and 5). Definite conclusions cannot be drawn about the pathogenicity of all the isolated fungal species, since some of them were tested in one or only a few trials. Accordingly they do not all appear in Tables 4 and 5.

A wide variation in pathogenicity was observed between the various species and also in the different isolates of the same species (Table 4). The differences between the isolates of most of the species were very large and significant, as can be seen from the χ^2 values, a fact which reflects the great variations occurring within these species.

Three fifths of the isolates killed at least 50 % of the seedlings, while one third caused losses of 26—50 % (Table 5). The most pathogenic species to seedlings were *Fusarium graminearum* and *Pestalotia truncata*, both of which killed on an average ca. 80 % of the plants. *Cylindrocarpon ehrenbergi* was also highly pathogenic, likewise *Pythium debaryanum* and *Sclerotinia trifoliorum* which were only in a preliminary test. High or above average was the pathogenicity of *Fusarium avenaceum*, *F. acuminatum* and *Marasmius graminum*. In the other tested fungi the order of pathogenicity was: *Fusarium arthrosporioides*, *Phoma medicaginis* var. *pinodella*, *Rhizoctonia solani*, *Cylindrocarpon obtusisporum*, *Rhizoctonia crocorum*, *Fusarium culmorum*,

Table 5. Percentage of fungal isolates in different pathogenicity classes in trials with clover seedlings
Taulukko 5. Sieni-isolaattien lukumäärän jakaantuminen patogeenisuusluokkiin prosentteina siementaimikokeissa

Pathogenicity classes (diseased and dead plants):
Patogeenisuusluokat (sairaita ja kuolleita kasveja):
I = 100—76 %, II = 75—51 %, III = 50—26 %, IV = 25—0 %

Fungus — *Sieni*	No. of tests *Kok. lukum.*	No. of isolates *Isol. lukum.*	Pathogenicity class *Patogeenisuusluokka*			
			I	II	III	IV
Chaetomium spp.	2	3	—	50	—	50
Marasmius graminum	4	8	—	67	16	17
Phoma medicaginis v. *pinodella*	7	8	25	25	38	12
Pestalotia truncata	1	3	100	—	—	—
Alternaria tenuis	3	5	—	—	75	25
Fusarium poae	9	13	10	10	50	30
» *arthrosporioides*	26	44	18	32	32	18
» *avenaceum*	38	65	26	30	38	6
» *acuminatum*	47	74	23	36	30	11
» *culmorum*	8	10	—	40	50	10
» *graminearum*	1	3	100	—	—	—
» *sambucinum*	13	17	7	20	13	60
» *oxysporum*	22	40	—	9	46	45
» » v. *redolens*	6	7	—	43	29	28
» *solani*	8	15	22	11	22	45
Cylindrocarpon radicicola	22	27	9	18	46	27
» *ehrenbergi*	3	8	67	—	33	—
» *obtusisporum*	4	6	20	—	80	—
Rhizoctonia solani	6	12	—	50	17	33
» *endophytica*	5	5	—	20	40	40
» *crocorum*	3	4	—	33	67	—
Total — *Yhteensä*	238	377	—	—	—	—
Average — *Keskimäärin*	—	—	15	27	36	22

Alternaria tenuis, *Fusarium oxysporum* var. *redolens*, *F. solani*, *Cylindrocarpon radicicola*, *Fusarium poae*, *Chaetomium cochliodes*, *Rhizoctonia endophytica*, *Fusarium sambucinum*, *F. oxysporum*, *Chaetomium aureum*.

All the fungal species tested were capable of causing both pre-emergence killing and post-emergence damping off, regardless of whether they were actual root inhabiting fungi or soil inhabiting fungi, the latter of which GARRET (1960) called primitive parasites.

The artificial infection tests produced symptoms similar to those found in the diseased root samples taken from the field (cf. p. 16). Weakly pathogenic fungi, which were unable to kill seedlings, caused deformations or other mild injuries in the roots.

A high relative humidity in the microclimate of the clover stand promoted the growth of root rot fungi and enhanced their virulence. On the other hand, abundant moisture in the soil retarded the growth of most of the fungi, especially of the *Fusarium* species, and reduced their aggressiveness (cf. p. 50).

Pathogenicity to young plants

Seventeen trials were carried out in the greenhouse with red clover plants 2—10 months old which were grown especially for this purpose. The *Fusarium* fungi were most extensively tested, but in addition there were isolates of *Cylindrocarpon*, *Phoma*, *Pestalotia*, *Marasmius*, *Rhizoctonia* and LTB. In many of the trials the pathogenicity of the fungi was determined concurrently in both injured and healthy roots. Studies were also made on the mechanism of the infection process, notably the route of fungal invasion and the factors predisposing infection, which will be described later (p. 42). The trials with older plants involved difficulties, since most root rot fungi are relatively weak pathogens and consequently the

Table 6. Inoculation trial with 3-month old red clover plants
Taulukko 6. Inokulointikoe 3 kuukauden ikäisillä puna-apilan taimilla

A cut was made at the neck of the root and a piece of agar containing mycelium was placed on the cut. Duration of trial 87 days.
Juuren niskaan tehtyyn haavaan pantiin sienen rihmastoa. Koeaika 87 vrk.

Fungal species and isolate *Sienilaji ja isolaatti*		Condition of roots — *Juurien kunto* 5—0			
		Tammisto		Tepa	
		intact *haavoittamaton*	cut *haavoitettu*	intact *haavoittamaton*	cut *haavoitettu*
Fusarium poae	62205—9	4.5	3.0	4.5	3.0
» *arthrosporioides*	6091—5	4.3	3.0	3.8	3.3
» *avenaceum*	62196—9	3.8	3.5	3.8	3.5
» *acuminatum*	6038—1	2.3	2.3	3.8	1.5
» »	612—13	0.8	0.5	1.3	0.0
» *culmorum*	6089—1	2.5	2.5	3.8	2.3
» *graminearum*	62119—9	0.0	0.0	2.3	0.0
» *sambucinum*	6091—8	3.5	4.3	4.3	4.0
» *oxysporum*	6232—8	3.8	3.5	3.5	2.0
» » v. *redolens*	622—3	4.0	4.0	4.0	2.5
» *solani*	6131—5	3.0	3.0	4.3	3.0
Control		5.0	5.0	5.0	5.0
F-value — *F-arvo*		11.34***	10.97***	7.98***	7.02***
LSD		1.4	1.3	1.1	1.7
Significance of cutting — *Haavoittamisen merkitsevyys*		0.3 < P < 0.2		P < 0.001	

death of old plants takes place very slowly, much more slowly than with seedlings. Even in prolonged trials, complete destruction of the clover plants was rare. Many of the fungal species were gradually able to penetrate into even undamaged roots (Table 6). The severity of infection, however, was essentially dependent on the inoculum potential (GARRET 1960). For example, in a certain trial with 9-month old clover plants, where the inoculum consisted of a liberal conidial-mycelial suspension prepared from 7 fungal isolates *(Fusarium arthrosporioides, F. avenaceum, F. acuminatum, F. graminearum, F. oxysporum, Cylindrocarpon radicicola, Rhizoctonia solani)* the roots, which initially were healthy, became severely diseased within as short a period as three weeks.

	No. of roots in condition classes						Average condition % of roots
	5	4	3	2	1	0	
Control	6	2	0	0	0	0	95
Inoculation	0	1	2	3	0	2	40

The fungi attacked wounded roots very readily, and destruction of the plant took place rapidly and completely (Table 7). Consequently, root rot fungi are most often facultative wound parasites of older clover plants.

The order of pathogenicity of the fungi to post-seedling plants was about the same as to seedlings.

Table 7. Infection trial with 3-month old red clover plants in the greenhouse
Taulukko 7. Infektiokoe 3 kuukauden ikäisillä puna-apilan taimilla kasvihuoneessa

Inoculum placed in a cut at the neck of the root; duration of trial 40 days.
Juuren niskaan tehtyyn haavaan sienen rihmastoa. Koeaika 40 vrk.

Treatment — *Käsittely* Fungus and isolate — *Sieni ja isolaatti*		Condition of roots *Juurien kunto* 5—0	
		Tammisto	Tepa
Control I		4.8	4.8
» II, cut — *haavoitettu*		4.6	4.5
Marasmius graminum	62140—8	1.9	2.5
LTB	218	3.2	2.9
Phoma medicaginis v. *pinodella*	6254—6	3.6	2.7
Pestalotia truncata	62232—8	2.3	3.1
Fusarium avenaceum	6150—1	2.4	1.7
» *graminearum*	62119—9	1.4	1.7
» *oxysporum* v. *redolens*	638—9	1.5	1.7
Cylindrocarpon ehrenbergi	62118—6	1.8	1.3
F-value — *F-arvo*		19.80***	37.66***
LSD		0.8	0.6

Factors favouring infection of clover plants

Routes of invasion

The chief natural sites of invasion of root rot organisms are the openings in the plant epidermis. This fact became clearly evident in the studies carried out by the author on the mechanism of infection using peas and beans. These plants were chosen for the experiments because they grow more rapidly than clover and are thus better suited for such investigations. In conformity with the trials of CORMACK (1937 a, b) and HAWN (1959) with alfalfa as well as with those of CHRISTOU and SNYDER (1962) with beans, it was found that at least the *Fusarium* fungi readily invade seedlings through the stomata of the hypocotyl (Table 8, Fig. 20). According to the trials of CORMACK (op. cit.), *Cylindrocarpon ehrenbergi* was also able to enter the roots of alfalfa through its lenticels.

In the case of clover seedlings, infection usually took place via the above-mentioned natural openings, although sometimes by means of other suitable organs of the plant. For instance, the root hairs and the young epidermal cells which produce them are not cutinized, and are easily penetrated by the fungal hyphae. At least the hyphae of certain *Fusarium* species readily penetrate the young epidermal cells of the root tips of red clover. From these epidermal cells, the fungal hyphae grow through the cortical tissue into the xylem, from where the invasion progresses mainly along the tracheids. The mycelium grows both inter- and intracellularly. Since the root hairs originate in the pericycle of the stele, the fungus readily advances along this tissue into the phloem of the primary root. The vascular wilt fungi, which include most species of *Fusarium*, invade in particular, the vascular tissues.

In the case of older clover plants, the conditions favouring infection are different from those of seedlings, since the root epidermis is more difficult to penetrate. In such cases, environmental factors have an important bearing on the process of infection. According to experiments

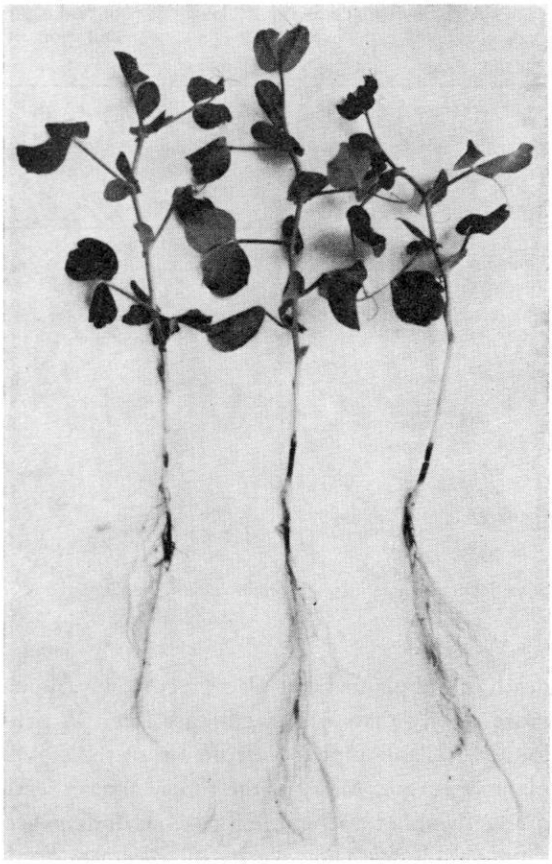


Fig. 20. Pea seedlings inoculated with *Fusarium avenaceum;* the fungus was located at two levels in the soil.
Kuva 20. Fusarium avenaceum -sienellä inokuloituja herneen taimia; sieni oli sijoitettu kasvualustalle kahteen tasoon.

Table 8. Inoculation trial with pea seedlings in the greenhouse
Taulukko 8. Inokulointikoe herneellä kasvihuoneessa

Treatment — *Käsittely* Fungus and isolate — *Sieni ja isolaatti*		Condition of roots *Juurien kunto* 5—0
Control		5.0
Marasmius graminum	6046—k	4.1
Phoma medicaginis v. *pinodella*	6254—6	4.6
Pestalotia truncata	62232—8	4.1
Fusarium avenaceum	6150—1	3.1
» *acuminatum*	6091—7	3.2
Cylindrocarpon ehrenbergi	62118—6	4.4
Rhizoctonia crocorum	6256—7	4.4
F-value — *F-arvo*		32.80***
LSD		0.3

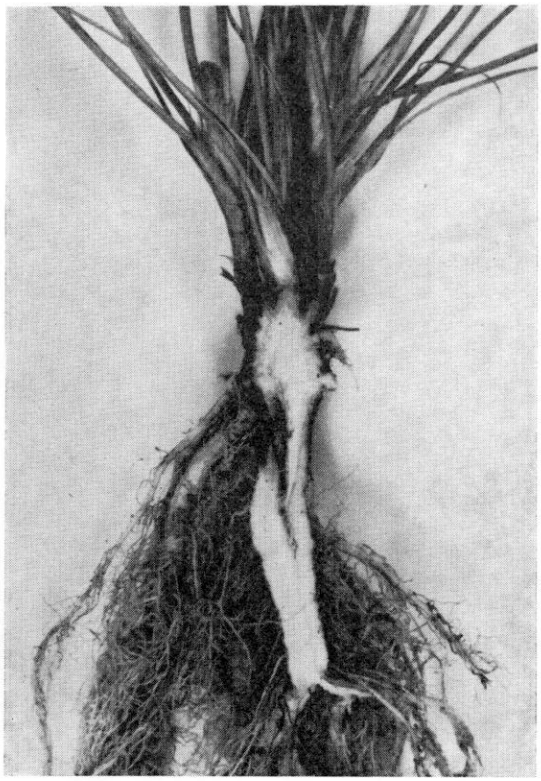
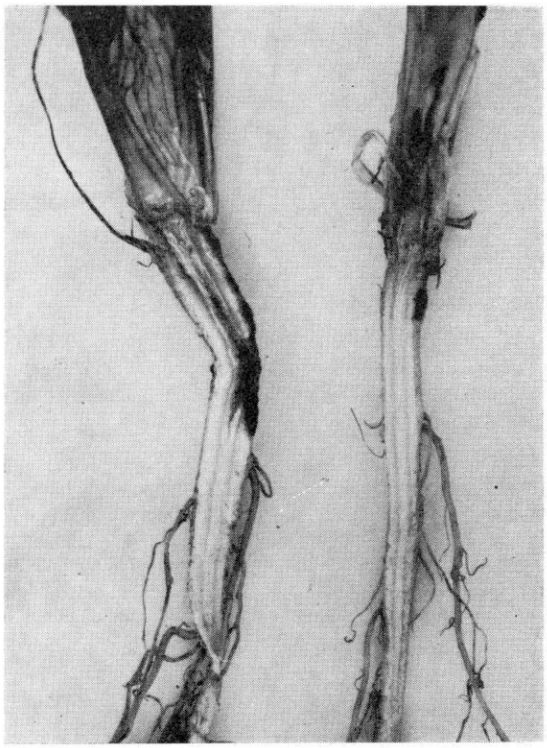


Fig. 21. Nine-month old clover seedling from pathogenicity test with *Fusarium avenaceum* after 26 days from infection, root wounded by puncturing; longitudinal section.

Kuva 21. 9 kuukauden ikäisenä pistämällä haavoitettu ja Fusarium avenaceum -sienellä infektoitu puna-apilan taimi halkaistuna 26 vrk:n kuluttua infektoinnista.



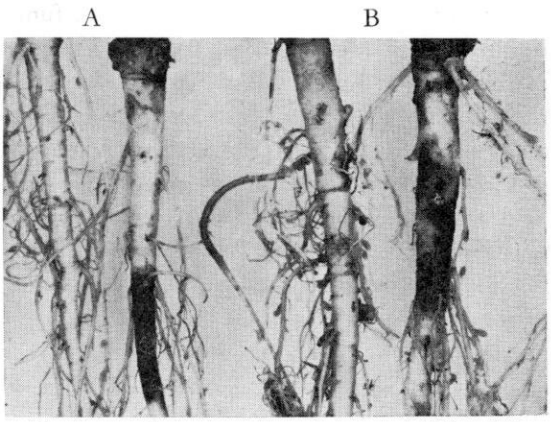
a b

Fig. 22. Red clover seedlings 2½ months old, wounded by puncturing; a = infected with *Cylindrocarpon radicicola*, b = not infected.

Kuva 22. Pistämällä haavoitettuja 2½ kuukauden ikäisiä puna-apilan taimia; a. infektoitu Cylindrocarpon radicicola -sienellä, b. infektoimaton.

performed by the author (cf. p. 48), it was found that at least certain species of *Fusarium* were able to invade the healthy roots of even mature clover plants, evidently chiefly through the natural openings in the root epidermis. It is possible that part of the fungal invasion may take place directly through the young parts, such as the root tips. This is suggested by the fact that in many cases destruction of the roots began at the tips.

It was clearly demonstrated in the experiments that wounds in the roots of red clover increased the success of fungal infection. A puncture or cut made at the neck of the tap root was sufficient to ensure the immediate entrance of the parasite (Figs. 21, 22). Wounds made lower in the tap root led to the same result (Fig. 23). As



A B

a b c d

Fig. 23. Red clover seedlings infected with *Fusarium arthrosporioides* (A) and *F. avenaceum* (B); a and c = intact roots, b and d = roots wounded by puncturing.

Kuva 23. A. Fusarium arthrosporioides ja B. F. avenaceum -sienellä infektoituja puna-apilan taimia, a ja c haavoittamattomia, b ja d haavoitettu pistämällä.

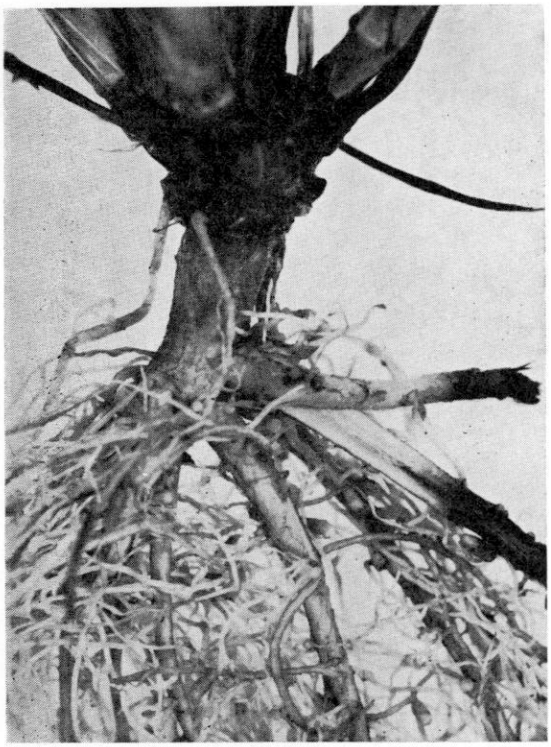


Fig. 24. Red clover seedling showing severed branch root infected with *Fusarium avenaceum*.

Kuva 24. Puna-apilan katkaistu juuren haara on infektoitu Fusarium avenaceum -sienellä.

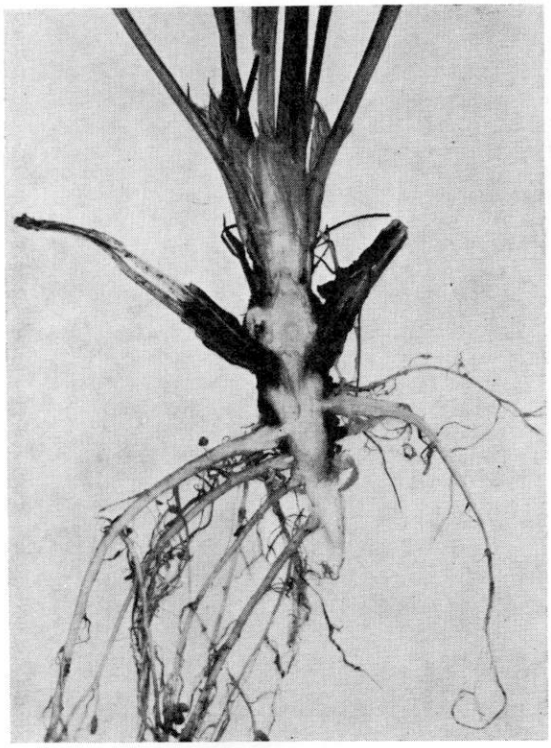


Fig. 25. Severed shoots of red clover infected with *Fusarium avenaceum*.

Kuva 25. Fusarium avenaceum -sienellä infektoituja puna- apilan katkaistuja varsia.

evidence of the importance of fungi in causing root rot, it can be mentioned that mechanical injury to the root without the presence of fungi resulted merely in a scar being formed at the site of the wound (Fig. 22 b). When the secondary roots are broken in one way or another, the fungi easily enter the plant and pass to the primary root (Fig. 24). Under the conditions prevailing on the field, this route of infection is extremely important for initiating root rot, since there are many possibilities for mechanical injury and breaking of the roots.

After clipping, the severed ends of the clover stems — especially on seed fields — provide good sites of invasion for various micro-organisms. They penetrate into the stem and advance as far as the parenchymous tissues of the crown of the plant (Fig. 25). The fungi easily destroy this tissue with its large, thin-walled parenchymous cells extending for a short distance into the tap root. The first symptom of root rot, discolouration of the tissue, is usually seen in this part of the plant (Fig. 1, B 9).

Although the trials showed that root rot fungi could invade completely uninjured clover roots, as was also observed by e.g. GARREN (1955), FEZER (1961) and CHI et al. (1964), it was obvious that wounding of the roots in one way or another greatly facilitated infection of the plant. Wounding of the roots is considered by many investigators to be the principal factor for the initiation of root rot infection (KILPATRICK et al. 1954 a, FULTON and HANSON 1960, BOLLOW and DIERCKS 1960).

From their initial invasion site, the hyphae of root rot fungi proceed both upwards and downwards along the vascular tissue (Fig. 26). The first result of this is a brown discolouration followed later by clogging of the tissue (Fig. 27) and a gradual withering of the plant. In its initial

stages, root rot is a typical tracheomycosis. The immediate cause of withering induced by root rot has been studied in a number of investigations in the case of tomato (e.g. GOTHOSKAR et al. 1955, PIERSON et al. 1955). On the other hand relatively few studies have been made with clover, but the infection mechanism appears to be similar to that in tomatoes (WOODBURY et al. 1962, CHI and HANSON 1964).

It was earlier believed that *Fusarium* and certain other root rot fungi produced toxins, which caused darkening of the tissue and subsequent withering of the plant (GÄUMANN et al. 1950). However, recent studies have shown that the main reason for the withering is clogging of the vascular tissues, partly with mycelia but apparently chiefly with the decomposition products from the vessel walls caused by the biochemical action of the fungus (SCHEFFER and WALKER 1953, PIERSON et al. 1955).

Some species of *Fusarium* have been found to produce a pectinase enzyme which decomposes the pectin-containing vascular tissue of the plant (GOTHOSKAR et al. 1955, PIERSON et al. 1955, CHI and HANSON 1960). Thus the fungi pass into the surrounding tissues, where under favourable conditions they may sporulate (cf. CHI and HANSON 1964). A direct relationship has been observed between the virulence of *Fusarium oxysporum* isolates and their capacity to produce pectinase (PAQUIN and COULOMBE 1962). Vascular wilts are systemic diseases caused by certain soil fungi (GARRET 1960). They spread very rapidly and finally penetrate into nearly all the tissues of the plant. On the other hand, it has been found that fungal infection stimulates the host plant to produce a counter-agent, phytoalexine, which checks the advance of the fungus if the plant possesses at least some degree of resistance (CRUICKSHANK 1963).

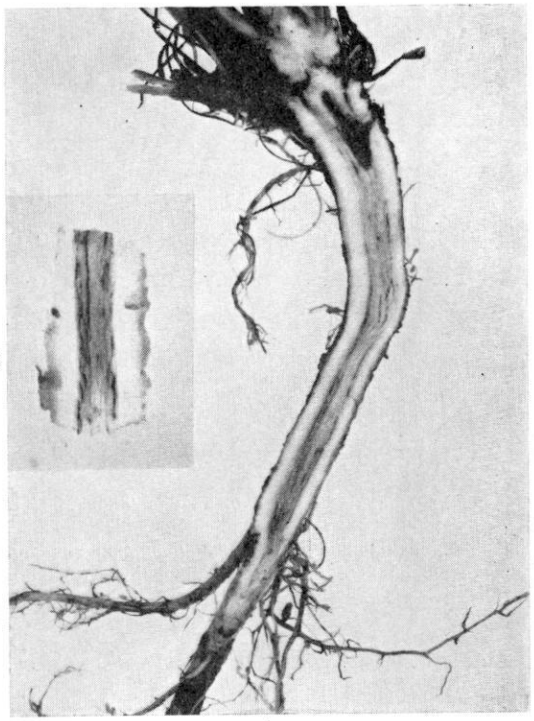


Fig. 26. A. Longitudinal section of clover root showing the invasion of the root rot fungus along the vascular tissues of the tap root after having entered a secondary root; B. Vascular tissue of red clover root invaded and discoloured by fungal mycelium.

Kuva 26. A. Päästyään sisään valtaamastaan juuren haarasta sienet etenevät johtosolukkoa myöten pääjuuressa; B. Sienirihmojen valtaamaa ja ruskettamaa puna-apilan juuren johtosolukkoa.

Factors causing mechanical injuries to roots

Many investigators have pointed out the significance of certain soil-inhabiting insects in causing mechanical injury to roots of clover and other legumes and thus favouring their invasion

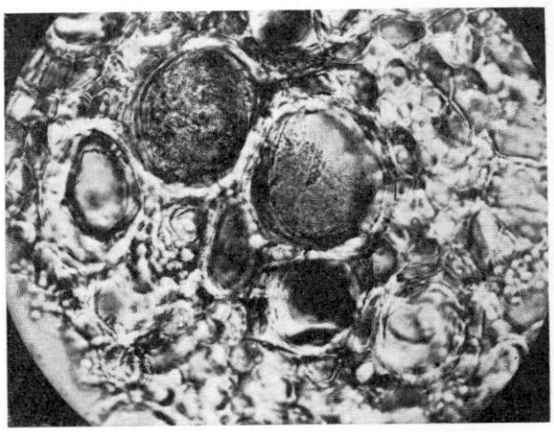


Fig. 27. Cross section of red clover root, showing tracheids clogged by *Fusarium acuminatum*. 350 ×

Kuva 27. Fusarium acuminatum -sienen tukkeamia putkiloita puna-apilan juuressa.

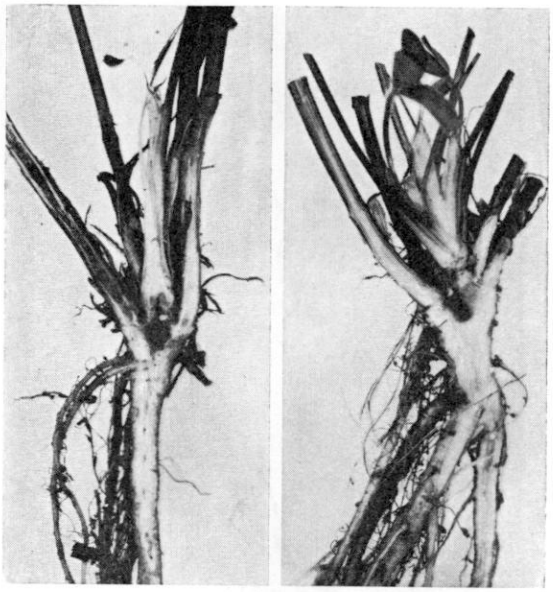


Fig. 28. Larvae of *Apion* sp. in the basal part of the stem of red clover plants.
Kuva 28. Apion sp. -toukkia puna-apilan varren tyvessä.

by root rot organisms. *Sitona* species are considered to be especially important by e.g. KILPATRICK and DUNN (1958), GRAHAM and NEWTON (1960) and LEACH et al. (1963), while BOLLOW and DIERCKS (1960) believe that species of *Apion* are the most important in this respect. KILPATRICK (1961) demonstrated that larvae of *Sitona flavescens* and *S. hispidulus* feed on the root nodules of clover and also transmit fungi, such as *Fusarium oxysporum*, *F. solani* and *F. roseum*. Other initiators of root rot have been found to be *Hylastinus obscurus* Marsh. (GRAHAM and NEWTON 1959) and *Calomycterus setarius* Roelofs (NEWTON and GRAHAM 1963). The importance of insects in promoting the initiation of root rot, without mentioning individual species, has been emphasized by e.g. KREITLOW et al. (1953), KILPATRICK et al. (1954 a) and FULTON and HANSON (1960).

Nematodes have been found to aid the entrance of root rot fungi as well as to transport fungi (e.g. MCGUIRE et al. 1958, BAXTER and GIBSON 1959 and POWELL 1963).

In Finland, as in other countries, clover is parasitized by species of *Apion* and *Sitona*. Of the 11 species of *Sitona* encountered in this country, larvae of at least the following species commonly inhabiting clover were found by MARKKULA (1958, 1959 a) to damage the roots of red clover: *S. hispidulus* Fabr., *S. flavescens*, Marsh., *S. sulcifrons* Thunb, *S. decipiens* Lindb. *S. lineatus* L. and *S. puncticollis* Steph. No data is available on the extent of their damage to clover in Finland.

Apion virens Herbst, which in its larval stage lives in the stems of red clover, as well as *A. seniculus* Kirby, parasitizing the stems of alsike clover, are relatively common in southern and central Finland (MARKKULA 1959 b).

Larvae of *A. virens* were discovered in many red clover samples, especially in those collected from seed fields. They appeared to penetrate downward through the stem into the upper part of the root, thus providing an easy route of invasion for root rot fungi (Fig. 28). However, according to STEIN (1965), *A. virens* larvae do not penetrate to the root but instead return upward through the stem. Certain studies have shown that larvae of *A. seniculus* bore tunnels in the crown of red clover plants (BOLLOW and DIERCKS 1960).

The fact that only a few insects of *Apion* and *Sitona* were found in the root rot samples (cf. Table 2) does not mean that these insects are of minor importance in initiating and promoting root rot. It is very possible that some of the larvae could have escaped from the samples during collection and handling. According to numerous observations made by the author on the field, it is evident that such insects are considerably more significant in promoting root rot than would appear from the above-mentioned analysis results.

A very important group of factors responsible for the initiation of root rot in Finland consists of abiotic winter damage (YLIMÄKI 1962 b). Root injury taking place during the winter season, particularly in autumn and spring, is caused especially by soil disruption due to alternate freezing and thawing of the ground, which damages and breaks the roots. Similar damage is

caused by pronounced drying of the soil (cf. KREITLOW et al. 1953, KILPATRICK et al. 1954 a). The author has observed this during dry summers in Finland, particularly on clay and silt soils. Livestock grazing on fields may also cause serious damage to the crown of clover plants. In some winters a long-lasting cover of ice or water on the surface of the field may weaken the plants and make them susceptible to fungal invasion.

In addition to actual injury, clover plants may also become subject to fungal attack if they are physiologically weakened. GRAHAM and NEWTON (1959) observed that *Hylastinus obscurus* depleted the carbohydrate reserves of clover roots. CHI et al. (1964) expressed the opinion that the effect of root injury in promoting disease is due principally to its weakening influence on the plant rather than the provision of a route of invasion for the fungi. Many environmental factors affecting the growth of plants as well as other diseases, such as virus infections, have been found to increase the extent and severity of root rot (WATSON and GUTHRIE 1946, OSHIMA and KERNKAMPF 1957).

The influence of environmental factors on infection

Since the fungi causing root rot of clover are common in the soil and many of them can live for long periods in a large number of different host plants — some are able to survive saprophytically without host plants — it is evident that clover plants are constantly menaced by the possibility of infection. As to when and how severely the fungi succeed in invading the plants in each particular instance depends to a great extent on the prevailing conditions. On the one hand, infection is dependent on the natural susceptibility or resistance of the plant to disease, and on the other, on the favourability of the environmental conditions to fungal attack. Since there may be a large number of different factors simultaneously influencing infection, it is extremely difficult — often quite impossible — to distinguish them under field conditions. In many cases it is difficult even to decide which factor has been the most important.

Owing to the multiplicity of root rot fungi and the many diverse factors affecting them, the damage caused by them may occur in very different conditions throughout the entire growing season, from early spring to late autumn. Mostly, however, root rot is observed in early spring soon after the melting of the snow (cf. KILPATRICK et al. 1954 a, FEZER 1961). This is partly due to the fact that in this early stage the diseased, stunted clover plants can more easily be distinguished from the healthy plants, but also partly due to the relatively larger numbers of infected plants in the ley in the springtime. The majority of such diseased plants die in the following growing season. As stated previously, there is a close correlation between the extent of winter injuries and the severity of root rot under the conditions prevailing in Finland. In addition, the clover plants are in a weak condition in spring after their winter dormant period, and are consequently more susceptible to root rot.

Weakening of clover

The weakened state of clover plants in the spring may be a result of soil and nutritional factors, climatic conditions, or various biotic factors. One of the most vital factors affecting the winter hardiness of clover plants is their supply of food reserves, in particular carbohydrates (GREATHOUSE and STUART 1936, VIRTANEN and NURMIA 1936, KASPAROVA and PROSKURNIKOVA 1944, SMITH 1950). In northern regions, such as Finland, the plant food reserves become greatly depleted during the course of the long winter, and as a result the plants are susceptible to cold in the following spring and are generally in a weak condition. Similarly, they are liable to attacks by fungal parasites.

Clipping leys many times and to a short stubble, as well as grazing, in the latter part of the summer and especially in the autumn, retard and prevent an accumulation of carbohydrate reserves thus weakening the winter hardiness of clover plants (cf. POHJAKALLIO 1939, KASPAROVA and PROSKURNIKOVA 1944, TEITTINEN 1959). The growth of clover after flowering and seed pro-

Table 9. Effect of number of clippings and application of fertilizers on condition of red clover roots

Taulukko 9. Niittokertojen lukumäärän ja lannoituksen vaikutus juurien kuntoon

At the start of the trial the plants were 7½ months old. Basal fertilizer equal in all treatments. Duration of trial 74 days.

Taimet kokeen alkaessa 7½ kk:n ikäisiä. Kaikissa koejäsenissä sama peruslannoitus. Koeaika 74 vrk.

Treatment *Käsittely*	Condition of roots *Juurien kunto* 5—0
11 clippings — *niittoa*	1.2
» — » + nitrogen — *typpi*	1.0
» — » + boron — *boori* ...	1.2
1 clipping — *niitto*	2.7
» — » + nitrogen — *typpi* ..	2.5
» — » + boron — *boori*	3.3
F-value — *F-arvo*	11.15***
LSD	0.9

duction has been found to take place almost exclusively at the expense of the carbohydrate reserves (SMITH 1950), accordingly seed fields of clover are particularly susceptible to root rot.

The effect of repeated clippings in weakening clover plants and increasing root rot was observed in greenhouse experiments carried out by the author, of which two examples are presented in Tables 9 and 10. Clippings made every second week resulted in increased susceptibility to clover rot, and this effect was not mitigated by nitrogen application nor by boron treatment (Table 9). Clipping to a short stubble increased the sensitivity of clover plants to clover root rot more than an augmentation in the number of clippings (Table 10). Similarly, FEZER (1961) observed that as the number of clippings increased, the vigour of red clover declined and the extent of root rot increased. He also noted that root rot was more prevalent under short-day than under long-day conditions and attributed this to the difference in amount of accumulated carbohydrate reserves. During short days, photosynthetic activity is less intense than during long days, and consequently smaller carbohydrate reserves accumulate in the roots. The effect of day-length may also be the opposite: a long day may stimulate flowering of clover, which in turn weakens the disease resistance of the plant (UMAERUS and ÅKERBERG 1963). The effect of certain insects in weakening clover with a resultant decrease in carbohydrate accumulation in the roots has been referred to earlier (p. 47).

Table 10. Effect of clipping on condition of red clover roots

Taulukko 10. Niittotavan vaikutus juurien kuntoon

At the start of the trial the plants were 210 days old. Clipping performed 3 times. Duration of trial 109 days.

Taimet olivat kokeen alkaessa 210 vrk:n ikäisiä. Niittokertoja 3. Koeaika 109 vrk.

Treatment *Käsittely*	Condition of roots *Juurien kunto* 5—0
No fungus — *ei sientä*, normal stubble — *norm. sänki*	4.7
No fungus — *ei sientä*, short stubble — *lyhyt sänki*	3.3
Fusarium spp. — *ei sientä*, normal stubble — *norm. sänki*	3.0
Fusarium spp. — *ei sientä*, short stubble — *lyhyt sänki*	1.3
F-value — *F-arvo*	17.16***
LSD	1.0

Effect of nutrients and soil charasteristics

Since, as explained above, a deficiency of food reserves in clover plants in late winter and spring increases their susceptibility to root rot, it could be assumed that prevention of such a deficiency would check the occurrence of root rot. According to the literature, experiments have been carried out to determine this aspect with regard to the principal plant nutrients and the trace elements. It has been found that phosphorus and potassium are vitally important for clovers. For example, KOROBEINIKOVA (1956 b) demonstrated that applications of superphosphate to the soil decreased *Fusarium* infection of clover plants, while CHI and HANSON (1961) proved by means of pot trials and histological studies that especially potassium makes the tissues of clover resistant to root rot. On the other hand, in many cases it has been found that a suitable equilibrium of nutrients provided by multiple fertilizers is

more important than the absolute quantities of the nutrients (KOROBEINIKOVA 1956 b, TVERSKOI et al. 1950, CHI and HANSON 1961, FEZER 1961).

Mature clover plants infected with root rot have symptoms resembling those occurring in e.g. cauliflower suffering from boron deficiency. Several pot trials were accordingly carried out to test the effect of boron on red clover plants of different ages. Different amounts of borax or boric acid were applied, either in the irrigation water or sprayed on the leaves, and various timing schedules were tested. It was found that boron did not prevent or retard the occurrence of root rot in a single case. On the other hand, excessive doses of boron, which produced yellowish and brownish discolouration of the edges of the leaflets, also caused a hollowing of the crown of the plant with subsequent browning. No micro-organisms were found in samples taken from such affected parts, indicating that the browning of the tissues was probably due exclusively to the high rate of boron used.

Since the optimum soil pH range for clover is around 6—7, plants growing in soil with this pH are more resistant to fungal attacks than plants in more acidic soil (TVERSKOI et al. 1950, CHI and HANSON 1959). PARKINSON (1961) observed that the occurrence of *Fusarium* and *Cylindrocarpon* fungi is greatly dependent on the acidity of the soil: in acidic conditions *Fusarium* species dominate, while in alkaline conditions *Cylindrocarpon* is predominant, although the pH of the soil alone does not govern the relative amounts of these fungi.

The quantity of organic matter in the soil affects in many ways for example the growth and persistence of *Fusarium* fungi. SEQUEIRA (1962) observed that the addition of organic matter to soil caused a decrease in the amounts of *F. oxysporum* f. *cubense*, which he attributed mainly to the stimulation of chlamydospore germination, the lytic action of soil bacteria on the mycelium and thin-walled spores, and to the cessation in the formation of new chlamydospores.

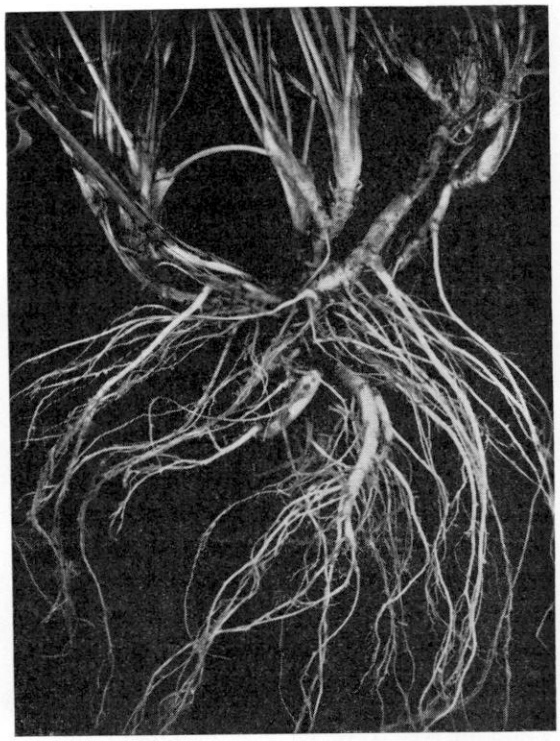


Fig. 29. Red clover plant whose tap root has been destroyed by root rot but which has produced abundant secondary roots from its crown and shoots.

Kuva 29. Juurilahon tuhottua pääjuuren puna-apila on kasvattanut juuren niskasta ja varsistakin runsaasti versojuuria.

Effect of temperature and moisture

Owing to the large number of the different fungi causing root rot, their temperature and moisture requirements vary a great deal. Consequently, they may produce injury to clover plants under very variable conditions. In tests the optimum temperature for the growth of most root rot fungi (e.g. *Fusarium* and *Cylindrocarpon*) as well as for the infection caused by them was relatively high, in the range of 20—30°C. Nevertheless, infection occurred even at relatively low temperatures, although in these cases the process of decay and ultimate death of the plant was considerably slower, since new secondary roots were produced in the crown to replace the destroyed tap root (Fig. 29). The effect of temperature on root rot is evidently chiefly indirect, affecting primarily the growth

and development of the fungi and host plant rather than the initiation of infection and its progress.

Moisture appears to be more important than temperature in initiating root rot. It has been found that abundant soil moisture increases the sensitivity of clover seedlings to damping-off caused by *Pythium*, while older plants do not respond in this way to soil moisture (CHI and HANSON 1962).

Trials were carried out in the greenhouse on the effect of moisture on root rot caused by *Fusarium* species in 3½-month old red clover seedlings. During the first two months, for instance an extremely pathogenic isolate of *F. graminearum* caused equal destruction under both wet and dry conditions, however, during the following three months the plants growing in dry soil suffered more damage. This can be seen in the following tabulation, in which normal moisture was maintained by giving the plants daily 47 ml water/pot, while the wet treatment was 84 ml/pot and the dry treatment 9 ml/pot.

	Condition of roots 5—0 June 17	October 6
Normal moisture	5.0	5.0
» » + fungus	2.6	2.2
Wet + »	1.7	1.8
Dry + »	1.7	1.0

Similar instances have been observed many times by the author in the field. Clover plants which are damaged by root rot in the spring but are still living, readily die later in the summer during periods of drought. This is understandable, since once the tap root is destroyed, the new secondary roots produced in the crown have no access to water (cf. Fig. 29).

Fusarium fungi thus seem to thrive in relatively dry conditions and to be more active than in wet conditions. This appears to be due mainly to the aerobic nature of the *Fusarium* species (STOVER 1953). However, another aspect related to this is the fact that bacteria which compete for oxygen and nutrients in the soil and which are partly antagonistic, thrive better under relatively dry conditions (GARRET 1960).

DISCUSSION

Taxonomy of *Fusarium* species

In recent decades determinations of the *Fusarium* species made throughout the world have generally employed either the system of WOLLENWEBER and REINKING (1935) or that of SNYDER and HANSEN (1945). Recently, however, use has been made of new modified systems (e.g. RAILLO 1950, GORDON 1952, 1960) based on that of the former research workers.

While studying the *Fusarium* material in the clover root samples, it was observed that when grown in culture, certain species in particular (e.g. *F. arthrosporioides*, *F. avenaceum*, *F. acuminatum*, *F. sambucinum*) showed distinct variations within the species which were both temporary, environmentally-caused as well as permanent divergences, possibly due to mutations (cf. MILLER 1946, CORMACK 1951). In the pathogenicity tests, there were similarly morphological differences between the isolates as well as large variations in their degree of pathogenicity, phenomena which were also observed by all the above authorities on *Fusarium* taxonomy. Thus it is extremely difficult to distinguish between different species of *Fusarium* in systems such as that of WOLLENWEBER and REINKING (1935), in which there are many species and only slight differences between them. In the opposite case, in the system of SNYDER and HANSEN (1945) the internal variations within the few species are very great, making it difficult to distinguish the species from one another. Since the author believes that Wollenweber's system modified by GORDON (op. cit.) is the most practical and suitable for the present purpose, it has been followed in this study.

Since most of the species in Gordon's system can be considered as combinations of several of the species occurring in the scheme of Wollen-

weber and Reinking, and since this system has been used only as from 1952 (in Finland MUKULA 1957), it has been considered desirable to describe briefly all the *Fusarium* species isolated in the present study according to Gordon's system.

Marasmius graminum and the LTB fungus

In terms of its morphological characters and its production of HCN, *M. graminum* resembles the LTB fungus occurring in Canada and the U.S.A. (p. 22). However, their response to temperature is different, since the mycelial growth of LTB declines sharply at temperatures higher than 15—17°C, whereas *M. graminum* grows well even at 30°C (Fig. 9). The temperature had no appreciable effect on the pathogenicity of this fungus in the trials.

It was especially noticeable that the two *M. graminum* isolates 6 221—2 and 62 140—8, which produced abundant rhizomorpha, were similar to one another but differed from the other isolates as regards their temperature and pH requirements as well as their HCN production (cf. p. 23). Neither of these two types resembled LTB in these respects.

Pathogenicity of root rot fungi

Root rot is a disease complex which can be incited by many fungal species either singly or in combination. In the present trials the pathogenicity of the fungi was tested mainly with individual species and using disinfected growth medium. Consequently, the conditions were completely different from those on the field, where the infection is affected by the microbes in the soil as well as by many environmental factors. Thus the results of the pathogenicity tests and the trials dealing with the mechanism of infection cannot as such be compared with the conditions existing in nature. In the present experiments, which were simplified and carried out under conditions as similar as possible, the differences in pathogenicity of most of the fungi were extremely large, owing to variations within the fungal species themselves (see. p. 39). This demonstrates that pathogenicity trials must be performed with many isolates and using a large number of parallel tests before even an approximate picture can be obtained about the relative pathogenicity between the different species.

Should the pathogenicity tests be carried out with single-spore or with wild cultures? From the purely mycological standpoint, the former would be most logical. Since, however, root rot is a disease complex, it would be more in accordance with nature to use »wild» cultures (cf. GORDON 1952). The author performed several comparative experiments with seedlings using both wild and single-spore cultures of the same *Fusarium* isolates. Since no significant differences were noticed between these two methods, it was decided to use single-spore cultures in the pathogenicity tests.

Clover rot and root rot

Clover rot has hitherto been regarded as the most common and damaging infective disease of field legumes in Finland (POHJAKALLIO 1939, YLIMÄKI 1956). Under conditions favourable for the causal fungus, clover rot may indeed reach epidemic proportions and inflict heavy, occasionally total, damage especially in young clover leys. In regions with a thick snow cover and a long period of snow-covered ground, repeated and serious losses due to clover rot often occur. This disease is thus a case of winter injury.

In contrast, root rot is quite different in character. Its injuries develop throughout the entire growing season and they are much more difficult to observe. Occasionally the damping-off form of this disease, which may cause severe damage to seedlings, is completely overlooked in newly established leys. Similarly, the actual form of root rot causes a slow decline of clover in older leys which is hardly noticed. It is difficult to distinguish the diseased clover plants except in the spring when growth has just begun. Since clover plants infected with root rot may linger on for years, the yield of the stand steadily decreases despite the relatively large number of plants growing (YLIMÄKI 1962 a, 1966).

The present investigation has demonstrated that under the conditions in Finland, root rot is a very common and injurious disease. In comparing the present material with unpublished data on the occurrence of clover rot, the author has come to the conclusion that, from the overall point of view, root rot is considerably more damaging than clover rot. Injuries caused by root rot have previously been attributed to either clover rot or to abiotic factors.

Control of root rot

When clover is cultivated for many years on the same place, the fungi causing root rot continually multiply in the soil. Moreover, as certain of the more important root rot fungi, among others the *Fusarium* species, are polyphagous and can parasitize e.g. cereals, they have good possibilities to persist and multiply in arable soils. It has been shown that the *Fusarium* species occurring in cereals used as nurse crop for clover are able to incite root rot in clover; (CORMACK 1937 b, KOROBEINIKOVA 1956 a, SHATILOV 1963); oats is the nurse crop which is most resistant to these fungi (KHRENOVA 1961, CHAMBERS 1962).

Since most root rot fungi are polyphagous and many of them can also live saprophytically in the soil, the chances of controlling them by means of crop rotation, which has otherwise been successfully used in the control of certain specialized soil fungi, seem rather remote.

The breeding of clover resistant to root rot, although as yet in the early stages of research, appears to have some potentialities for becoming a feasible means of controlling this disease MENDE 1954, CHI 1959, HENSON 1962).

SUMMARY

Studies on the condition of red clover roots were made by collecting 8 838 roots on 429 leys in different parts of Finland. Examination of the material showed that 10.8 % of the samples were completely healthy. Root rot was found to be common in roots from all parts of the country, although it was less injurious in the north. More diseased roots were encountered in samples taken from old leys than from young ones, even though they were numerous also in first- and second-year leys.

Root rot occurs both as damping-off of seedlings and as a relatively slow decaying of the roots of older clover plants. For this reason, and also because various micro-organisms cause the disease, the symptoms of root rot vary greatly in kind and degree. Besides directly killing clover plants, root rot also causes yield losses by decreasing the productive capacity of diseased plants.

Root rot is a more serious and complicated problem on leys than clover rot, since it is found every year and in all localities, in contrast to clover rot, which occurs only in certain years and localities.

A total of 1 441 isolates of micro-organisms were made from the root samples, and 32 fungal species were determined. Of these, 82 % were of the group *Fungi imperfecti*, and the most prevalent species were: *Cylindrocarpon radicicola* Wr., *Fusarium acuminatum* (Ell. & Ev.) Wr., *F. arthrosporioides* Sherb., *F. avenaceum* (Fr.) Sacc., and *Rhizoctonia* spp. The following species had not previously been described in Finland: *Phoma medicaginis* var. *pinodella* (L. K. Jones) Boer., *Pestalotia truncata* Lev., *Stemphylium botryosum* Wallr., *Cylindrocarpon ehrenbergi* Wr., *C. obtusisporum* (Cke. & Hark.) Wr., *Rhizoctonia endophytica* Saks. & Vaart., *Chaetomium aureum* Chivers and *Chaetomium cochliodes* Palliser.

The *Basidiomycetes* fungus *Marasmius graminum* (Lib.) Fr. isolated from 10 root samples resembles in its morphological characteristics and its production of HCN the LTB fungus occurring in Canada and the U.S.A.

Greenhouse tests showed that most of the fungi isolated from the diseased roots were pathogenic to red clover seedlings. The pathogenicity varied widely between the fungal spe-

cies and also between different isolates of the same species. The most pathogenic species to seedlings were *Fusarium graminearum* Schwabe, *Pestalotia truncata* Lév., *Cylindrocarpon ehrenbergi* Wr., *Pythium debaryanum* Hesse and *Sclerotinia trifoliorum* Erikss. Above average in pathogenicity were: *Fusarium avenaceum* (Fr.) Sacc., *F. acuminatum* (Ell. & Ev.) Wr., and *Marasmius graminum* (Lib.) Fr. Moderately pathogenic species were: *Fusarium arthrosporioides* Sherb., *Phoma medicaginis* v. *pinodella* L. K. Jones) Boer. and *Rhizoctonia solani* Kühn. Below average was the pathogenicity of *Cylindrocarpon obtusisporum* (Cke. & Hark.) Wr., *Rhizoctonia crocorum* (Pers.) DC., *Fusarium culmorum* (W. G. Sm.) Sacc., *Alternaria tenuis* Neerg., *Fusarium oxysporum* v. *redolens* (Wr.) Gordon, *Fusarium solani* (Mart.) App. & Wr. emend. Sn. & Hans., *Cylindrocarpon radicicola* Wr., *Fusarium poae* (Pk.) Wr., *Chaetomium cochliodes* Pall., *Rhizoctonia endophytica* Saks. & Vaart., *Fusarium sambucinum* Fuckel, *Fusarium oxysporum* Schl. emend. Sn. & Hans. and *Chaetomium aureum* Chiv.

The fungi were found to enter seedlings chiefly through natural openings in the plant epidermis, such as stomata in the hypocotyl, but also through other suitable organs, notably the youngest epidermal tissues of the roots.

Many of the fungal species tested were gradually able to penetrate even uninjured roots of mature clover plants, though they invaded wounded roots far more effectively. A large number of the root rot fungi were found to be typical wound parasites. Certain fungi are relatively weak pathogens and are hardly able alone to cause root rot, however, they can accelerate the decay initiated by other factors or organisms.

In its early stages in mature plants, root rot is a typical vascular disease. When the vascular tissue is destroyed, the fungi invade other tissues and cause their decay.

The sites of fungal entrance are wounds in the tap root as well as the severed ends of the secondary roots and other injuries in them. A very favourable site for fungal invasion, notably for the *Fusarium* species, is the cut end of the stem, and the clover plants may easily become infected immediately after clipping.

Under the climatic conditions in Finland there is a close correlation between winter injury to clover and root rot. Mechanical damage to clover roots during the winter is caused mainly by soil frost disruption due to abrupt fluctuations in temperature, which may break or otherwise injure the roots. In some winters, clover plants are excessively weakened by remaining under a cover of water or ice and are consequently more liable to fungal attack. At the end of the long winter period, when the food reserves of the plants are depleted they are anyhow in a weak condition. Root rot is not, however, primarily a winter disease, since most of the actual damage occurs during the growing season.

Although larvae of the insects *Apion virens* Herbst and *Sitona* spp. are known to cause mechanical injuries to clover roots, under the conditions in this country they appear not to be as important as in some countries in promoting the initiation of root rot.

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SELOSTUS

Juurilaho puna-apilan hävittäjänä Suomen niittonurmissa

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Puna-apilan juurien terveydentilan tutkimiseksi kerättiin eri tahoilta maata 188 kunnasta 429 niittonurmelta lähes 9 000 puna-apilan juurta (taul. 1). Näytteistä oli 80 % peräisin ensimmäisen ja toisen vuoden nurmista ja 90 % kivennäismailta. Aineisto edusti verrattain hyvin maamme eri osia ja niiden nurmia antaen siten melko selvän kuvan juurilahon levinneisyydestä. (kuva 2).

Aineiston juurista oli täysin terveitä vain vajaat 11 %. Sairaita juuria oli vähemmän Pohjois-Suomesta kuin muualta peräisin olleissa näytteissä. Sairaissa juurissa todettiin kaikkialla esiintyvän juurilahoa, apilan juuria jo taimiasteelta alkaen tuhoavaa tautikompleksia. Taimiasteella tautia aiheuttavat pieneliöt joko tappavat idun heti alkuunsa ennen sen maan pinnalle tuloa tai tuhoavat vasta maan pinnalle jo ehtineen taimen (kuva 18). Varttuneissa apiloissa juurilaho saattaa ilmetä taudin aiheuttajista, isäntäkasveista ja ympäristöoloista riippuen vaihtelevin oirein. Juuriston lahoaminen voi olla joko yleistä tai vain johonkin sen osaan paikallistunutta. Lievissä tapauksissa tauti voi rajoittua vain juurien kuoriosaan muodostuviin laikkuihin tai syöpymiin, mutta yhtä yleistä on juurien johtosolukon vioittuminen ja lopulta sen tuhoutuminen kokonaan (kuvat 1 ja 3). Pieneliöiden tukkiessa johtosolukkoja ja tuhotessa ne vihdoin täysin apiloiden aineenvaihdunta häiriintyy. Sen seurauksena kasvit vähitellen näivettyvät. Juurilaho aiheuttaa satotappioita sekä alentamalla apiloiden tuotantokykyä että tappamalla niitä.

Vaikka juurien lahoamista tapahtuu kaikkina vuodenaikoina, havaitaan tuhot selvimmin keväällä, jolloin apilat ovat talven heikentäminä erityisen alttiita taudinaiheuttajille.

Juurinäytteistä otettiin 1 441 pieneliöeristettä, joista määritettiin 32 useimmin esiintynyttä sienilajia (taul. 3 ja kuva 11). Niistä kuului 82 % *Fungiim perfekti*-ryhmään, ja yleisimmät lajit olivat *Cylindrocarpon radicicola* Wr., *Fusarium acuminatum* (Ell. & Ev.) Wr., *Fusarium arthrosporioides* Sherb., *Fusarium avenaceum* (Fr.) Sacc. ja *Rhizoctonia* -lajit. Ensi kerran Suomessa kuvattuja olivat *Phoma medicaginis* var. *pinodella* (L. K. Jones) Boer., *Pestalotia truncata* Lév., *Stemphylium botryosum* Wallr., *Cylindrocarpon ehrenbergi* Wr., *Cylindrocarpon obtusisporum* (Cke. & Hark.) Wr., *Rhizoctonia endophytica* Saks. & Vaart., *Chaetomium aureum* Chivers and *Chaetomium cochliodes* Pall. Kymmenestä juurinäytteestä tavattiin kantasieni *Marasmius graminum* (Lib.) Fr., joka sekä morfologisilta että myös eräiltä fysiologisilta ominaisuuksiltaan muistuttaa jossain määrin Kanadassa ja U.S.A:ssa talvituhosienenä tunnettua LTB-sientä.

Suurin osa eristetyistä sienistä oli varsin patogeenisia puna-apilan siementaimille. Sienilajien ja myös niiden isolaattien patogeenisuus vaihteli erittäin paljon (taul. 4 ja 5). Patogeenisimmat lajit olivat *Fusarium graminearum* Schwabe, *Pestalotia truncata* Lév., *Cylindrocarpon ehrenbergi* Wr., *Pythium debaryanum* Hesse ja *Sclerotinia trifoliorum* Erikss. Hyvin patogeenisia olivat myös *Fusarium avenaceum* (Fr.) Sacc., *Fusarium acuminatum* (Ell. & Ev.) Wr. ja *Marasmius graminum* (Lib.) Fr. Kohtalaisen patogeenisia olivat *Fusarium arthrosporioides* Sherb., *Phoma medicaginis* v. *pinodella* (L. K. Jones) Boer and *Rhizoctonia solani* Kühn. Keskinkertaista heikompia patogeenisuudeltaan olivat *Cylindrocarpon obtusisporum* (Cke. & Hark.) Wr., *Cylindrocarpon radicicola* Wr., *Rhizoctonia crocorum* (Pers.) DC., *Rhizoctonia endophytica* Saks. & Vaart., *Fusarium culmorum* (W. G. Sm.) Sacc., *Fusarium oxysporum* Schl. emend. Sn. & Hans., *Fusarium oxysporum* var. *redolens* (Wr.) Gordon, *Fusarium solani* (Mart.) App. & Wr. emend. Sn. & Hans., *Fusarium poae* (Pk.) Wr., *Fusarium sambucinum* Fuckel, *Alternaria tenuis* Neerg., *Chaetomium cochliodes* Pall. ja *Chaetomium aureum* Chiv.

Sienien todettiin tunkeutuvan nuoriin siementaimiin pääasiassa päällysketon luonnollisista aukoista, kuten alkeisvarren ilmaraoista, mutta myös juurien nuorimpien solukkojen kautta (taul. 8, kuva 20). Monet kokeillut sienilajit kykenivät vähitellen tunkeutumaan varttuneidenkin apiloiden vioittumattomiin juuriin, mutta haavoitettuihin juuriin ne iskeytyivät paljon tehokkaammin. Useat juurilahoa aiheuttavat sienet todettiin tyypillisiksi haavaloisiksi. Eräät heikot patogeenit tuskin pystyivät itsenäisesti aiheuttamaan juurilahoa, mutta jouduttivat kuitenkin muiden syiden alulle panemaa lahoamista. Juurilaho on alkuvaiheessaan tyypillinen johtojännetauti (kuvat 26 ja 27), mutta johtosolukon tuhouduttua sienet tunkeutuvat ympärilläkin oleviin solukkoihin ja lahottavat ne.

Pääjuuressa olevien haavojen ohella myös pääjuuren haarojen katkeamat tai muut haavoittumat voivat olla sienien infektioteinä (kuva 24). Erittäin edullisia infektioteitä olivat ainakin *Fusarium*-sienille katkaistujen varsien tyngät, joten saastunnalle on heti apilan niiton jälkeen hyvin suuret mahdollisuudet (kuva 25).

Maamme ilmasto-oloissa on apilan talvivaurioilla ja juurilaholla kiinteä syy-yhteys. Nurmissa aiheuttaa apilalle juuristovioituksia talven aikana ennen kaikkea rouste, joka katkoo juuria ja murtaa niihin haavoja. Pintaveden ja jääpeitteen alla apila saattaa joinakin talvina heikentyä ja tulla normaalia alttiimmaksi sienien hyökkäyksille. Keväällä talvilevon päättyessä apilat ovat ravintovarojen huvettua vähiin jo muutenkin heikentyneessä tilassa. Juurilaho ei kuitenkaan ole varsinainen talvituho, sillä lahoamista tapahtuu koko kasvukauden ajan.

Vaikka apilan juurissa tavataan hyönteistoukkien (*Apion virens* Herbst ja *Sitona* -lajit) aiheuttamia vioituksia, ei niillä liene meillä juurilahon tien avaajina yhtä suurta merkitystä kuin eräissä muissa maissa.

Juurilaho on niittonurmissamme suurempi ja vaikeampi tautiongelma kuin apilamätä, koska juurilahoa esiintyy joka vuosi kaikkialla, kun taas apilamätää tavataan vain paikka paikoin joinakin vuosina.

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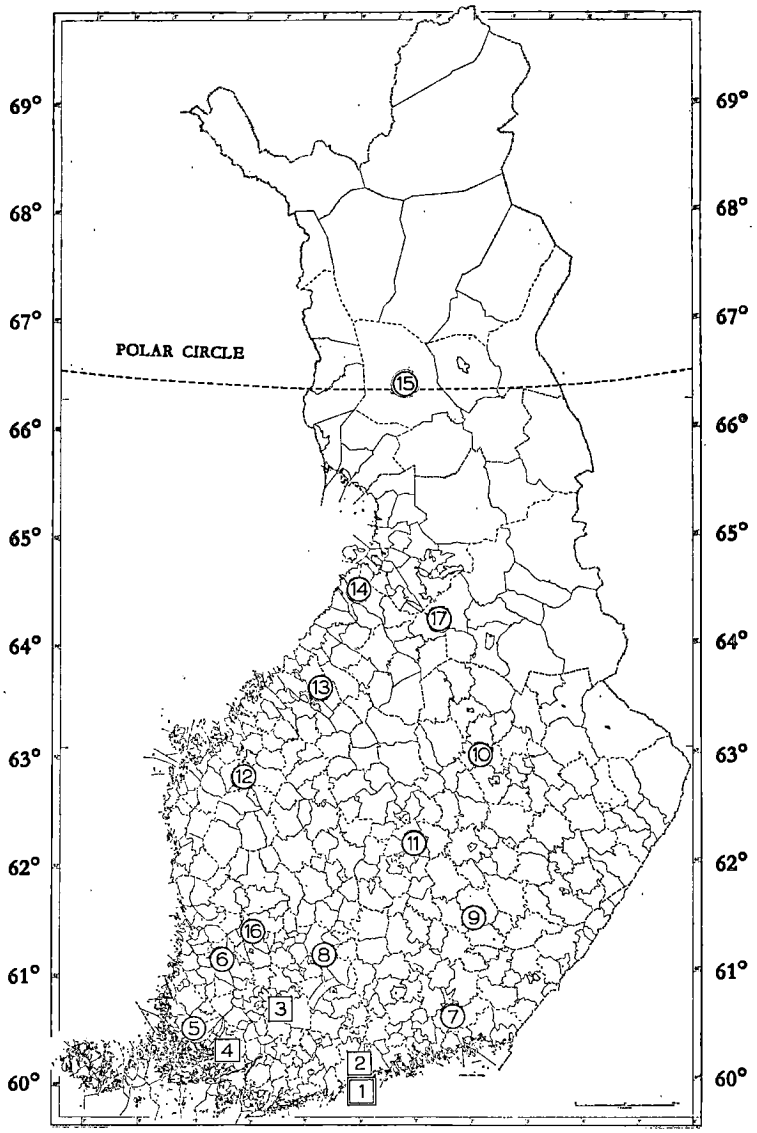

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