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STUDIES ON *RHIZOCTONIA SOLANI* KÜHN, THE CAUSE OF THE BLACK SCURF DISEASE OF POTATO

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CHAPTER 1

GENERAL REMARKS ON THE *RHIZOCTONIA* DISEASE

1.1. INTRODUCTION

The black scurf or *Rhizoctonia disease* is one of the most common and widely spread diseases of the potato, *Solanum tuberosum* L. The disease occurs on the plants in any stage, and exhibits a number of interesting symptom characteristics which vary with climatic conditions, age of the host, and part of the plant attacked. The severity of the injury is highly dependent on environmental conditions.

The disease attracted wide attention among plant pathologists, and it has been investigated by many workers.

1.2. SUSCEPTS

The pathogen causing *Rhizoctonia* disease of potatoes has been reported on over 230 species of plants in 66 families. As the damage done in many cases consists of a damping off of seedlings, the disease is considered one of the most serious and troublesome maladies of seedlings.

It is known that the causal organism has an extremely wide host range, and comprises an indefinite number of races that differ culturally as well as pathogenically. There are, however, indications that certain races appear to be more specific for certain crops than others. Some experimental results for instance indicate that isolates from sclerotia on potato tubers seem to be less pathogenic to potatoes and sugar beet than isolates from potato stems or sugar beet.

Many cultivated crops other than the potato such as ornamentals and weeds are subject to this disease. One species of *Rhizoctonia* is reported even to be a parasite of other fungi (BUTLER and KING 1951).

1.3. NAMES OF THE DISEASE

The name '*Rhizoctonia*' is the most common in the literature and has much in favour to designate the disease. The disease was first named 'Schorf' or 'Grind', i.e. 'Scurf' or 'Scab'. Later, in the German literature the disease was called 'Pockenkrankheit' (= pox disease), and '*Rhizoctonia* Keimfäule' (= *Rhizoctonia* stem rot) (KÜHN 1858, SCHANDER and RICHTER 1924).

The disease has also been termed by other common names: viz. black scurf, little potatoes, dry stem, russet scab, black scab, lakschurft, scurf and *Rhizoctonia* rot.

1.4. HISTORY AND RANGE

Our knowledge of the *Rhizoctonia* disease of potatoes and other cultivated plants dates back to JULIUS KÜHN in Germany (1858). It appears that at that

time, the disease was quite common in Germany and probably in most of Europe.

In the United States of America, the disease was described by DUGGAR and STEWART in 1901. Since *Rhizoctonia* disease of beets, cotton, etc. had been recorded in the U.S.A. during 1890 to 1900, it seems probable that the disease was present but unrecognized in potatoes before 1900 (DUGGAR and STEWART 1901). In the Netherlands the disease has been reported by WESTERDIJK (1915) especially *R. crocorum* and *R. solani* appeared to be very abundant on potato in Holland. According to PELTIER (1916), the disease was first reported in England in 1904. In Australia, MCALPINE (1912) found *R. solani* very widely distributed on potato. The disease is found on potatoes in all countries that have pest control or reporting services.

The *Rhizoctonia* disease has undoubtedly been distributed throughout the potato growing areas of the world by means of the sclerotia present on the tubers. The pathogen has also been reported to occur in many areas as a natural inhabitant in non-cultivated soils.

1.5. IMPORTANCE OF THE DISEASE

Rhizoctonia disease of potatoes is regarded as one of the most serious diseases of potatoes because of its marked effect on tuber development and the presence of the black sclerotia on the tubers which presumably decreases the market value of the crop.

In many cases the sprouts are attacked and killed by the pathogen. This condition causes poor stands, single stemmed hills, and the production of 'little potatoes'. These effects are considered of great importance especially in seed potato growing areas.

1.6. NATURE OF LOSSES

Rhizoctonia disease occurs on all parts of the plant but three main parts of the plant are primarily attacked:

1. The sprouts.
2. The main stems which become girdled.
3. The tubers which may be injured directly by the occurrence of russeting or scab, by a cracking of the tuber, by the formation of pits at or near the lenticels, and by a wet rot of the tuber; or indirectly by the condition known as 'little potatoes' and by the presence of sclerotia on the tubers.

1.7. SYMPTOMATOLOGY

The disease has different symptom complexes and practically all parts of the potato plant show effects of the disease during some stage of their development.

In the same field there may be vigorous healthy-looking plants, others that are stunted, some that are weak and spindling and many others missing. The

affected plants usually have smaller leaves, abnormally upright, and maybe dark green. If such plants are pulled up, the underground portion of the stem shows a brown rotting of the cortex known as 'cankers', which may girdle the stem 2.5 cm or more below the soil surface.

Cankers may also occur on the stolons, separating the new tubers from the main stem, and thus preventing their further development. Such cankers interfere with the movement of food material to the roots and tubers and thus suppress their normal development.

On the tubers the disease is characterized by the presence of hard black or brownish-black bodies closely adhering to the skin. These sclerotia are entirely superficial, crust-like hyphal masses that range from minute to large irregular aggregates about 2.5 cm or more across. They are often referred to as 'the dirt that won't wash off' (Plate 1). If many of them are present, tubers are rendered unsightly and unmarketable. These dark brown or blackish sclerotia are the most characteristic and diagnostic signs of the disease.

1.8. ETIOLOGY

1.8.1. *General Survey of the literature*

As *Rhizoctonia* spp. are of great economic importance, their accurate identification is a matter of fundamental importance.

The first description of a *Rhizoctonia* disease was given by DUHAMEL (1728), who found it to cause a disease of saffron (*Crocus sativus*) in southern France. DUHAMEL, unaware of the fact that this disease was caused by an organism gave it the name tuberoides. DE CANDOLLE (1815), who discovered a similar disease on lucerne showed it was caused by a fungus which he named also *Rhizoctonia*. The genus includes also the species *R. crocorum* (Pers.) D.C. In fact the first valid description of the genus is that given in FRIES systema Mycologicum (1823).

In 1851, the TULASNE brothers classified all forms of *Rhizoctonia* as a single species *Rhizoctonia violaceae*, a classification which has been adopted by a number of authors.

The *Rhizoctonia* disease of potatoes was first described in 1858 by KÜHN, who gave the causal organism the name *Rhizoctonia solani* Kühn. In fact, KÜHN was acquainted only with the mycelial and sclerotial stages.

Since the time of KÜHN extensive studies have been made dealing with the nomenclature of this fungus. However, the nomenclature of this plant pathogen has had a controversial history. In the absence of any sporing stage the species was characterized by its hyphal morphology.

The perfect stage of *R. solani* was not known until PRILLIEUX and DELACROIX (1891) described a basidiomycetous fungus which they named *Hypochnus solani* Prill. et Delacr. However, they did not recognize it at that time as the perfect stage of the pathogen known to them as *Rhizoctonia solani* Kühn.

ROLFS (1903) working on the *Rhizoctonia* disease in Colorado observed the basidiomycetous stage constantly associated with this fungus on the stalks and

lower leaves of potato plants. BURT (1918) called it *Corticium vagum* B. and C. var. *solani* Burt.

In 1911 BOURDOT and GALZIN stated that the perfect stage should be classified in the genus *Corticium* rather than in *Hypochnus* as had been done by PRILLIEUX and DELACROIX. They therefore changed the name to *Corticium solani* (Prill. and Delacr.) BOURDOT and GALZIN. ROGERS (1943), classed the fungus under the new genus *Pellicularia*. Since that time the basidial stage is called *Pellicularia filamentosa* (Pat.), Rogers, whereas the mycelial stage is named *Rhizoctonia solani* Kühn.

Since the discovery of the perfect stage of *Rhizoctonia solani*, considerable research has been done to obtain this stage in pure culture. For this purpose several methods have been described. The study of the sexual stage in pure cultures has contributed much to clarify the existing confusion (CARPENTER 1949, EXNER and CHILTON 1944, FLENTJE 1956, FLENTJE et al. 1963, FLENTJE and STRETTON 1964, HAWN and VANTERPOOL 1953, KOTILA 1947, STRETTON et al. 1964, ULLSTRUP 1939, WARCUP and TALBOT 1962, and WHITNEY 1964).

Reviews on the nomenclature and taxonomy of *R. solani* have been given by DUGGAR (1915), FLENTJE (1956), KOTILA (1929, 1947), OLIVE (1957), PELTIER (1916), and ROGERS (1943).

The considerable confusion which later prevailed with regard to the nomenclature of the basidial stage of *Rhizoctonia* led to the thorough studies of DONK (1954, 1956, 1958). He made an extensive study on the taxonomy of the fungus, and amply discussed the literature pertaining to this subject. He concluded that the name *Pellicularia* is untenable for this group and created a new genus: *Thanatephorus* Donk.

DONK (1958) made clear that the species name is not *solani* because FRANK (1883) described at an earlier date a *Hypochnus* species, which he named *cucumeris*, and which is obviously identical with *Hypochnus solani* Prill. and Delacr. Therefore the name of the perfect stage of *R. solani* has to be *Thanatephorus cucumeris* (Frank) Donk.

FLENTJE et al. (1963) have recently reviewed the taxonomy of *R. solani* and confirmed that the analysis of DONK is the most satisfactory to date. WARCUP and TALBOT (1962) listed the following provisional synonymy:

Hypochnus cucumeris Frank; in Ber. dtsch. Bot. Ges. **1** (1883), p. 62.
Rhizoctonia solani Kühn, in Krankh. Kulturgew. 1858, p. 224.
Hypochnus solani Prill. and Delacr., in Bull. Soc. mycol. Fr. 7 (1891), p. 220.
Corticium solani (Prill. and Delacr.) Bourd. and Galz., in Bull. Soc. mycol. Fr. **27** (1911), p. 248.
Botrobasidium solani (Prill. and Delacr.) Donk, in Nederl. mycol. Ver. **18–20** (1931) p. 117; ROGERS Univ. Iowa. Stud. Nat. Hist. **17** (1935), p. 18.
Hypochnus filamentosa Patouillard sensu ROGERS.
Pellicularia filamentosa (Pat.) Rogers in Farlowia (1943), p. 113, fig. 11, Cunningham in Trans. Roy Soc. N.Z. **81** (1953), p. 328.
Ceratobasidium filamentosa (Pat.) Olive in Amer. J. Bot., **44** (1957), p. 429–435.

In the absence of the basidial stage, many investigators refer only to the hyphal

features for identification. However, many of these features do not provide definite proof for identification, for they are also common to the vegetative stage of other organisms. Therefore, it is important that one should attempt to produce the perfect stage of any *Rhizoctonia* being studied to avoid further confusion as far as possible. For the sake of convenience in this thesis the much used names of the imperfect stage *Rhizoctonia solani*, also *R. solani* or *Rhizoctonia* will be applied.

1.9. Scope of the present investigation

Since the sclerotia and the mycelium of the causal organism are generally prevalent in soils, whereas outbreaks of the disease occur only occasionally, it is likely that the development of the disease is highly dependent upon favourable environmental conditions.

It has already been reported that *Rhizoctonia* exists in natural soils in the form of several clones which may be distinguished morphologically and pathogenically, and which exhibit varying saprophytic and parasitic capabilities. It is the purpose of the present investigation to contribute to a better knowledge on the cultural characteristics of the different isolates obtained in the course of this investigation from sclerotia present on potato tubers. It was decided to investigate the effect of temperature, various media, and some antimicrobial agents on the growth rate of the fungus to gain more knowledge in this field. Pathogenicity tests were made in the hope of finding a relation between different types and degrees of virulence.

Moreover, adequate control measures of diseases caused by soil-inhabiting fungi such as *Rhizoctonia* cannot be developed unless the saprophytic phases of such primitive parasites have been studied adequately. Another point worked out in this paper was to develop a more reliable, simple and rapid method for detecting and isolating *Rhizoctonia* from soil. The present dissertation contains a description of this work.

In addition, knowledge regarding the saprophytic behaviour of the fungus in the soil, and its persistence under different conditions is of great importance. Because of the conflicting literature dealing with the saprophytic behaviour, it seemed advisable to investigate some aspects in this direction. In the course of these investigations, several isolates were obtained by the new method described. Since this method does not distinguish between pathogenic and non-pathogenic strains of *R. solani*, supplementary pathogenicity tests had to be made.

CHAPTER 2

PHYSIOLOGICAL STUDIES

2.1. Introduction

Since the time of Julius Kühn (1858) extensive literature has been published dealing with different physiological aspects of the fungus *Rhizoctonia*.

Many investigators have studied the variability of *R. solani* with respect to growth habits, physiology and pathogenicity. Duggar and Stewart (1901) were the first who studied *Rhizoctonia* as a cause of plant disease. The literature concerning cultural variation of *R. solani* prior to 1934 was reviewed adequately by Le Clerg (1934). Kernkamp et al. (1952) published a comprehensive review of investigations on physiologic specialization and parasitism of *R. solani* up to 1951. Since that time investigations on cultural variation of *R. solani* have included the following:

1. Studies of the type and range of cultural variations, 2. Attempts to determine whether certain cultural, morphological or physiological characteristics could be used for distinguishing species of *Rhizoctonia*, 3. Attempts to determine whether there is a correlation between cultural characteristics, sensitivity to chemicals, and origin of the isolate on the one hand and pathogenicity on the other hand.

Studies in this respect included isolates from different crop plants, and were made with tissue-culture, sclerotial, single basidiospore, and hyphal-tip isolates. From a survey of the literature concerning this subject, it is obvious that the species *Rhizoctonia solani* comprises an idefinite number of races or strains that differ in cultural characters as well as in pathogenicity.

It was decided, therefore, to investigate the influence of temperature, various media and some chemicals and antibiotics on the growth of sclerotial isolates of *Rhizoctonia*. As far as the author is aware no previous accounts on sclerotial isolates of the fungus have been published in the Netherlands. In addition, knowledge regarding the behaviour of *Rhizoctonia* may be of importance to develop control measures.

2.2. Materials and methods

2.2.1. *Source of isolates*

In order to study differences between isolates of the fungus, several hundred isolations were made from sclerotia present on tubers of several potato varieties, grown under different conditions in several localities in the Netherlands. In addition, a few isolates were obtained from seed potatoes grown in the Republic of Ireland.

According to their general morphological appearance on cherry agar slants the isolates could be divided into three groups:

1. With very little or no aerial mycelium.

2. With abundant aerial mycelium.
3. With intermediate growth habit.

In general, it was not difficult to distinguish between the three groups.

For comparison in physiological studies and for pathogenicity tests five isolates from each group were chosen. To warrant variation within each group, the selected isolates differed as to host variety, soil type and locality. The isolates included in this study are indicated by the following serial number, preceded by the prefix Sc. (= sclerotial).

Group I: 5, 8, 28, 34, 39
II: 1, 17, 18, 52, 55
III: 3, 13, 25, 33, 45.

The data concerning the host varieties, locality and soil type are listed in table 1.

TABLE 1. Source and locality of the 15 isolates of *R. solani* included in the present study. Isolates were obtained during the year 1961/1962.

Group	Isolates	Potato variety	Locality	Soil type
I	Sc. 5	Bintje	Middenmeer	clay
	Sc. 8	Voran	Rolde	sand
	Sc. 28	Bintje	Coevorden	clay
	Sc. 34	Sirtema	Coevorden	peat
	Sc. 39	Bintje	North East Polder	light clay
II	Sc. 1	Climax	Rolde	sand
	Sc. 17	Bintje	Schagen	sand
	Sc. 18	Surprise	Olst	clay
	Sc. 52	Meerlander	Kloosterburen	clay
	Sc. 55	Alpha	Bleiswijk	clay
III	Sc. 3	Bintje	Groningen	heavy clay
	Sc. 13	Surprise	Diepenveen	sand
	Sc. 25	Doré	Groningen	clay
	Sc. 33	Surprise	Coevorden	clay
	Sc. 45	Bintje	Haarlemmermeer	clay

2.2.2. *Isolation of the fungus from sclerotia*

Tubers with visible sclerotia were selected from different lots. The tubers were washed thoroughly in tap water to remove any adhering soil particles, then rinsed in sterilized water and left to dry for a few hours.

Small pieces of sclerotia were cultured on 2 % water agar in PETRI dishes and incubated at 24 °C until new mycelial growth developed. Transfers were made from the advanced edge of the colonies and maintained on cherry agar slants at 24 °C as stock cultures for further study. No contaminations occurred when the isolations were made in this way.

2.2.3. *Preparation of the inoculum*

A slightly modified FORSBERG method (1955) – the cotton thread method – has been used throughout this investigation to provide the inoculum. Coarse white cotton thread No. 8 was cut in pieces about 7 cm long, rinsed in distilled water and autoclaved for one hour. These pieces were then cut with sterile scissors into sections of about 2 mm long. About hundred sections were laid on the surface of the required medium in a PETRI dish.

The PETRI dishes were then inoculated in the centre with a block of agar with mycelium from the particular isolate. The PETRI dishes were incubated until the fungus had overrun the sections of cotton entirely. For growth studies one section of *Rhizoctonia* infested thread was transferred to the centre of every PETRI dish. This manner of preparing the inoculum usually obviates erratic behaviour of the fungus due to different size or character of inoculum.

2.3. INFLUENCE OF TEMPERATURE ON GROWTH RATE

2.3.1. *Review of the literature*

Rhizoctonia solani is distributed throughout the world and many investigators have shown that it comprises a vast number of cultural and pathological strains.[1] The ability to induce certain parasitic fungus diseases is conditioned on a specific range of soil temperature together with other factors.

Many observations and experiments have been made on the effect of soil temperature on the incidence and virulence of *Rhizoctonia solani*. From a survey of the literature, isolates of *Rhizoctonia* were shown to differ with regard to their response to temperature. From the numerous observations in the literature only the following were selected to illustrate the above conclusions.

RICHARDS (1921, 1923) was probably one of the first to call attention to the effect of soil temperature on *Rhizoctonia* infection of potatoes, peas, beans and cotton. He found that the optimum temperature for infection of these crops was 18°C.

JONES (1922) and RICHARDS (1921) found that *Rhizoctonia* was most virulent to potatoes at a soil temperature of 15°–21°C. On the other hand, ROLFS (1902, 1904) and PELTIER (1916) found that a high temperature (30°C) together with too much or too little moisture determined to a large degree the virulence of various strains of *Rhizoctonia*.

MATSUMOTO (1923) working with three isolates showed the optimum temperature to be about 24°C. MÜLLER (1924) studied different cultures of *Hypochnus solani* (= *Rhizoctonia solani*) and reported a temperature range of 4.5°C to 30.8°C, and an optimum of 24°C.

MONTEITH (1926, 1928) demonstrated that *Rhizoctonia* develops best at 28°C, when cultured in PETRI dishes. However, almost no growth was shown at 10°C or 16°C, and only a third as much at 34°C.

NEWTON and MAYERS (1935) working with a potato isolate found the mini-

[1] 'Strains' is used in this text for a group of isolates which exhibit the same characters as far as investigated.

mum temperature to be 6°C, the optimum 25°C, and the maximum 32°C. LE CLERG et al. (1942) tested 63 sclerotial isolates obtained from potato on potato-dextrose-agar (P D A) at 20°C, 25°C, and 30°C; they found an optimum at 25°C; whereas isolates from sugar beet showed optimum growth at 30°C. For studies made prior to 1951 see KERNKAMP et al. (1952).

BATEMAN and DIMOCK (1959) concluded from their survey of the literature that many isolates of *R. solani* fall into one of two major groups as far as optimum temperatures for growth are concerned. Isolates obtained from hosts favoured by high temperature grow best at 30°C and inflict greatest damage near this temperature. In contrast isolates obtained from hosts favoured by low temperature grow best at or below 25°C and inflict most damage on their hosts near or below 20°C. There are of course, some *Rhizoctonia* isolates that do not confirm entirely to the above generalization (KHAN, 1950, PERSON, 1944). RUSHDI and JEFFERS (1952) compared fifteen isolates of *Rhizoctonia solani* from various hosts and grew them under a variety of environmental conditions. They found that only one isolate made appreciable growth at 4°C. At 10°C all isolates except one from sugar beet leaves and another from soybean made moderate to good growth. At temperatures of 16°, 21°, 27° and 32°C all except one culture made moderate to good growth. Two isolates obtained from sugar beet and from *Euphorbia pulcherrima* made fair growth at 38°C while all other isolates made little or no growth.

2.3.2. *Procedure of the experiments*

In this investigation, the influence of temperature on growth rate of the mycelium on cherry agar was studied by incubating the isolates in triplicate at 3.5, 10, 15, 20, 22, 24, 28, 30 and 50°C.

To study growth rate, measurements were made every 24 hours by recording the colony diameter in two directions at right angles. In about five days, at the most favourable temperature the fungus has overgrown the media in the PETRI dishes completely. Therefore, measurements made after an incubation period of 96 hours were used for comparing the growth rate of the 15 isolates at different temperatures.

2.3.3. *Results*

The linear growth of the colonies for each group of isolates grown at different temperatures is shown graphically in figures 1, 2, and 3.

The optimum temperature ranges between 22° and 24°C. No distinct differences were found between the isolates of the different groups, although the group I isolates might have a somewhat lower temperature optimum. The minimum temperature for growth was between 3.5 and 10°C. At 3.5°C one isolate (Sc. 55) grew poorly, whereas the maximum temperature for all isolates is between 30° and 35°C. The growth rate near the optimum temperature varies between the isolates, but there is no correlation between growth habit and growth rate.

The results show that neither optimum temperature nor growth rate are

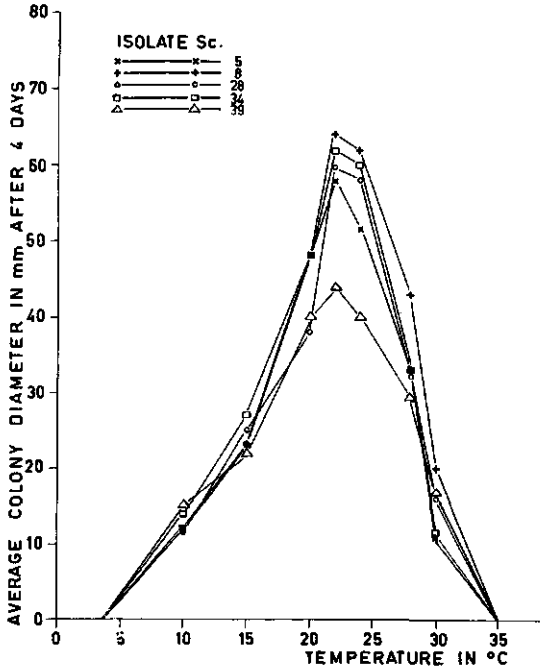

FIG. 1. The average growth on cherry agar of five sclerotial isolates of *R. solani* (group I) after 4 days.

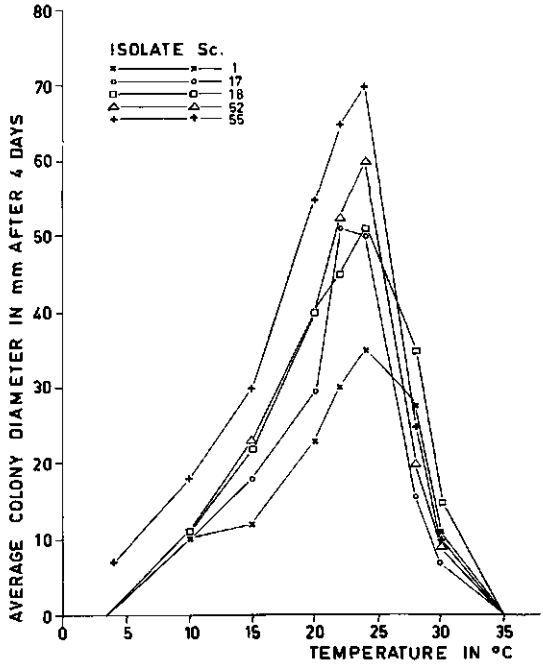

FIG. 2. The average growth on cherry agar of five sclerotial isolates of *R. solani* (group II) after 4 days.

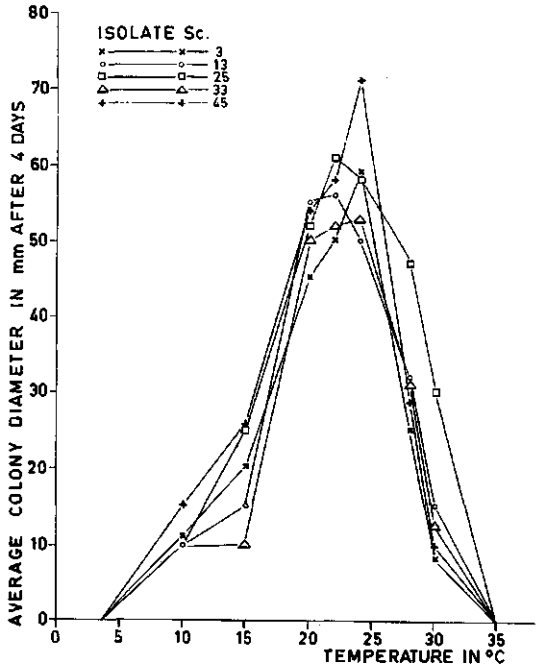

FIG. 3. The average growth on cherry agar of five sclerotial isolates of *R. solani* (group III) after 4 days.

significantly correlated with growth habit. Therefore, these characteristics can not be used in conjunction with other growth characters in classifying an isolate.

It is to be noted that the various optimum temperatures reported for growth of *R. solani* and for disease development on its various hosts further indicate its heterogenicity.

2.4. INFLUENCE OF DIFFERENT MEDIA ON GROWTH RATE OF THE FUNGUS AT TWO DIFFERENT TEMPERATURES

2.4.1. *Procedure*

In the present investigation the growth rate for the same 15 isolates was studied on 3 different media at 24° and 28°C. The media used were:

1. Cherry agar[1]
2. Potato-dextrose-agar (PDA)
3. Czapek-Dox agar-Modified (Difco)

The inoculum was prepared in the same manner as previously described, and five replicates were used for each medium.

[1] The cherry agar medium is prepared as follows: 100 g cherry fruits, 12.5 g agar-agar The fruits are boiled in 500 ml water for 1.5 hour, filtered through glasswool, the volume brought to 500 ml, and heated in a waterbath for 1.5 hour for sterilization.
N.B. Never sterilize in an autoclave, as the agar-agar will not solidify.

2.4.2. *Results*

Table 2 shows the average diameter of the colonies for each medium and each temperature after 72 and 96 hours of incubation. As regards media, at both temperatures PDA generally gives the highest, and cherry agar the lowest growth rate.

LE CLERG (1941) and CHEN (1943) working with different media reported that different isolates of *R. solani* respond differently to various nutrients. RUSHDI and JEFFERS (1952) studied fifteen isolates of *R. solani* grown on 6 standard media and incubated under a wide range of temperature. Growth was good on all media, but Czapek-dox gave best results.

MAIER (1959) obtained 245 cultures of *R. solani* from diseased cotton seedlings. These isolates were grouped into 10 strains on the bases of their cultural variation and on their ability to parasitize cotton seedlings. He reported limited cultural variation among the 10 strains when they were grown on carrot agar, potato-dextrose-agar (PDA) and on nitrate-dextrose-agar media.

From the results of the previous temperature study and from these data, it is concluded that *R. solani* isolates exhibit a great degree of variation, however, the effects of temperature and media are interrelated since the effect of the temperature is expressed differently on different media.

2.5. EFFECT OF SOME ANTIMICROBIAL AGENTS ON GROWTH RATE OF *R. SOLANI* IN RELATION TO VARIATION IN SENSITIVITY

2.5.1. *Review of the literature*

To study whether the isolates used in this study differed in their tolerance to chemicals and whether the degree of tolerance could be used as a means of differentiation between isolates some experiments were carried out.

In the literature there are considerable data about the sensitivity and tolerance of *Rhizoctonia solani* to chemical substances.

PAPAVIZAS and DAVEY (1959) studied the effect of combinations of three antibiotics: aureomycin hydrochloride + albamycin sodium salt + chloromycetin; neomycin sulfate + streptomycin sulfate + terramycin hydrochloride. They also used two other antimicrobial agents: oxgall and sodium propionate. They showed that the first combination allowed rapid growth of the fungus, whereas the other combination and the two antimicrobial agents suppressed growth considerably. No difference in tolerance between the four strains tested could be established.

REAVILL (1954) investigated the influence of certain chloronitrobenzenes (TCNB and PCNB) on germination, growth and sporulation of a number of fungi grown on agar media in PETRI dishes. Three strains of *R. solani* recovered from potato and from seedlings of cabbage and lettuce were included in his study. From the data presented, it was shown that growth of *R. solani* was equally retarded by TCNB and PCNB, and that the three isolates behaved in a similar way.

LE CLERG (1939) and KHAN (1950) reported different degrees of tolerance to

TABLE 2. The growth of colonies, in millimeters of fifteen isolates of *Rhizoctonia solani* after 72 and 96 hours on 3 different media kept at 24 and 28 °C temperature (M = maximum growth on PETRI dish).

Isolates of *Rhizoctonia*	Media, temperature, and hours of incubation											
	24 °C						28 °C					
	Cherry agar		PDA		Cz-Dox (M)		Cherry agar		PDA		Cz-Dox (M)	
	72 hr	96 hr	72 hr	96 hr	72 hr	96 hr	72 hr	96 hr	72 hr	96 hr	72 hr	96 hr
Sc. 5	38	55	56	M	48	M	19	33	32	56	35	70
Sc. 8	35	62	50	M	52	M	24	43	57	M	55	M
Sc. 28	32	58	52	80	17	22	19	32	42	67	27	42
Sc. 34	43	60	70	M	70	M	14	33	30	51	35	70
Sc. 39	30	40	56	M	53	M	13	29	34	63	32	58
Sc. 1	22	35	34	53	23	35	12	28	36	57	15	29
Sc. 17	25	50	58	M	48	M	10	15	40	70	25	49
Sc. 18	42	51	58	M	51	70	13	35	43	77	15	26
Sc. 52	44	60	49	78	32	50	11	20	24	42	12	25
Sc. 55	46	70	50	75	50	M	13	25	26	41	27	47
Sc. 3	36	59	55	M	44	72	12	25	21	30	20	40
Sc. 13	35	50	80	M	60	M	15	32	53	M	20	35
Sc. 25	39	58	72	M	53	M	29	43	55	M	30	62
Sc. 33	31	53	53	M	50	M	17	31	38	65	33	62
Sc. 45	46	71	58	M	52	82	14	29	42	67	34	60

certain chemicals among races of *R. solani*. CHEN (1943) studied the growth of several races of *R. solani* and their variants on media containing a great number of toxic chemicals. CHEN concluded that three parent strains and their variants respond differently to various chemicals. MAIER (1962), in his tests with 12 *Rhizoctonia* isolates found considerable differences in their tolerance to 12 fungitoxicants, although Dexon, PCNB and thiram were effective against most of the isolates. In the tests of SHATLA and SINCLAIR (1963), 36 hyphal-tip isolates of *R. solani* varied greatly in their tolerance to PCNB when applied at a wide range of concentrations. This subject has been recently reviewed by VAARTAJA (1964).

From the literature, there appears some evidence that adaptation to increased concentrations of chemicals is a common feature to *Rhizoctonia solani*. SINCLAIR (1960) was the first to report that isolates of *R. solani* obtained from diseased cotton seedlings varied in their ability to grow on agar containing different concentrations of soil fungicides: PCNB, captan, dichlone, and nabam. The experimental data from field, greenhouse, and laboratory studies presented by him showed that some isolates of *R. solani* seem to be less affected than others by the same test chemical or combination of chemicals. Other workers (EL SAID 1963, SHATLA and SINCLAIR 1963, and THOMAS 1962) demonstrated that adaptation to certain fungicides takes place in *Rhizoctonia solani*.

2.5.2. *Materials and methods*

Three isolates Sc. 8, Sc. 55 and Sc. 3 each representing one group were included in this study. Two antibiotics, terramycin hydrochloride and streptomycin sulfate each at 100 ppm were used. Desiccated ox-bile (= oxgall) was applied at concentrations of 1, 2.5, and 5 g/l, and boric acid at concentrations of 1 and 3 g/l respectively.

Four different media were included in these experiments viz: a. cherry agar, b. potato-dextrose agar (PDA), c. peptone agar, and d. Czapeck-Dox agar (Modified). The inoculum of *R. solani* was prepared in the same manner as previously reported. Five replicates were used for each treatment. The PETRI dishes were incubated at 24°C and colony diameters were measured every 24 hours.

2.5.3. *Results*

Table 3 shows the results of the experiments on linear growth of the three isolates. The data represent the average diameters recorded at the end of 3 days incubation, measurements showed very little variation between replicates.

The growth was not affected by incorporation of terramycin or streptomycin in any of the four media. The growth rate was reduced considerably by incorporating 1 g/l oxgall or 1 g/l boric acid to the media with the exception of oxgall in PDA or Cz-Dox. Apparently in these cases the effect of oxgall has been neutralized by the medium.

Boric acid in a concentration of 3 g/l inhibited the growth of the three isolates completely. However, isolate Sc. 55 showed slight growth on cherry agar. The

TABLE 3. Effect of two antibiotics and some toxic substances on linear growth of three isolates of *R. solani* on four media. Average growth in mm after 72 hours incubation at 24 °C.

Treatment	Medium and isolate of *R. solani*											
	Cherry agar			PDA			Peptone agar			Cz–Dox (M)		
	8	55	3	8	55	3	8	55	3	8	55	3
Check / no agents	40	58	42	61	30	54	25	26	25	74	45	69
Streptomycin 100 ppm	40	47	42	51	33	40	27	23	26	72	49	63
Terramycin 100 ppm	45	50	45	55	40	45	23	24	21	71	43	54
Terramycin 100 ppm + oxgall 1 g/l	12	9	13	47	35	38	9	0	15	43	25	40
Oxgall 1 g/l	10	9	12	42	34	39	11	trace	9	41	25	41
Oxgall 2½ g/l	0	0	trace	24	16	19	trace	0	trace	19	10	18
Oxgall 5 g/l	0	0	0	16	16	15	0	0	0	13	9	16
Terramycin 100 ppm + boric acid 1 g/l	33	27	24	22	26	32	12	11	15	10	10	11
Boric acid 1 g/l	26	23	24	18	28	35	10	8	16	10	8	13
Boric acid 3 g/l	0	10	trace	0	0	trace	0	0	0	0	0	0

graded series of concentrations of oxgall showed slight differences between isolates. On peptone agar, isolate Sc. 55 seems to be more sensitive. On the other hand the same isolate appears to be a little bit more tolerant to boric acid (3 g/l) on cherry agar. As in the foregoing experiment, only slight differences in sensitivity between strains have been noted, it seems likely that toxic materials cannot be used to differentiate between different strains of *Rhizoctonia*. It should be mentioned, however, that only few materials have been included in this study. Therefore more extensive investigations have to be done before differences in sensitivity can be used for separating different isolates of *R. solani*. Increased knowledge about fungicide tolerance in a pathogen as variable as *R. solani* could lead to the establishment of better control measures.

CHAPTER 3

PATHOGENICITY OF ISOLATES

3.1. Review of the literature

Since the work of DUGGAR and STEWART (1901) several investigators have noted that cultures of the fungus *Rhizoctonia solani* isolated from different localities, different species of host plants, or even from various parts of the diseased plant, may vary greatly in morphology, physiology and pathogenicity. The damage done to potatoes and other crops by the cosmopolitan soil inhabitant *R. solani* has resulted in a more thorough study of pathogenicity. Attempts have been made to correlate cultural characteristics with pathogenicity but with varying success.

PELTIER (1916) who was the first to study thoroughly the parasitism of *R. solani* found no outstanding differences in the pathogenicity of a number of isolates. LE CLERG (1934) concluded from his experiments that isolates vary greatly in pathogenicity.

Since 1934 numerous papers have been published dealing with various phases in the parasitism of cultures of *R. solani* and several theses have been devoted to studies on this subject. KERNKAMP et al. (1952) summarized all the literature pertaining to the subject up to 1951. Therefore only some examples illustrating the conclusions will be mentioned here.

LE CLERG (1934) studied the parasitism of *R. solani* on several crop plants. He found that sugar beet isolates were pathogenic as a group to potatoes. On the other hand he isolated strains of *R. solani* from potato sprouts that were virulent on sugar beet roots. SANFORD'S results (1938) were in general agreement with those of LE CLERG. He obtained parasitic races from sclerotia, stem lesions, and basidiospores on potatoes. From 20 to 25 percent of the sclerotial isolates were weakly pathogenic to non-pathogenic on potato stems, whereas 100 percent of the stem isolates were pathogenic. Basidiospore isolates gave rise to a large proportion of virulent cultures. In 1939 LE CLERG (1939) attempted to distinguish races of *Rhizoctonia* on the basis of parasitism on several crops. Since the results obtained were very variable, he concluded that races could not be accurately distinguished by this method.

Working with three cultural types of *Rhizoctonia*, HOUSTON (1945) claimed, that there are indications of a correlation between cultural types and pathogenicity. STOREY (1941) stated that some strains have a restricted host range while others are not so specific. DE ZEEUW (1940) studied 65 isolates from potato tubers, potato stems, flax, peanuts, sweetclover, delphinium, muskmelon, and sugar beet. He demonstrated that there were many differences among the isolates, the most important of which was the differential parasitism of isolates from potato varieties.

Since the year 1951 approximately 20 papers have been published regarding various factors affecting the pathogenicity of *R. solani*. Some of the general

conclusions will be mentioned here. The most recently published data on the relative pathogenicity on different hosts appear to be those of BARKER and WALKER (1962). They studied the relationship between pectolytic and cellulolytic enzyme activity and the pathogenicity of *R. solani*. From their experiments they concluded that a high correlation was found between polygalacturonase activity in vitro of various isolates and their pathogenicity. The same was found for the relationship between cellulase activity and pathogenicity. They also found that most sclerotial isolates from potato were not very pathogenic to any host tested, whereas those from bean were most virulent on bean and potato.

SHEPHARD and WOOD (1963) investigated the effect of environment and nutrition of pathogen and host. They found that environmental factors were rarely of more than secondary importance in determining whether or not an isolate would attack a particular host plant; pathogenicity depends mainly on intrinsic properties of the host and isolate.

Working with 38 isolates of *Pellicularia filamentosa* (Pat.) Rogers (= *R. solani*) obtained from different host plants, AKAI et al. (1960) found that non-pathogenic isolates showed poor mycelial growth, whereas pathogenic ones showed rapid growth of mycelium. When they tested the different isolates for pathogenicity on cucumber seedlings, they found that isolates obtained from diseased cucumber seedlings generally were more infective to cucumber seedlings.

RICHTER and SCHNEIDER (1951) investigating the parasitism of *Rhizoctonia* on potato found that the fungus was most injurious to the stolons and stems, but the roots were not susceptible. VAN EMDEN (1958) found from his experiments in vitro on rooted sprouts that they were always attacked through the stems or leaves while the roots remained healthy. He also found that light and temperature are important factors in determining the infection with *Rhizoctonia* on potato.

The influence of the origin of the isolates of *Rhizoctonia* on its pathogenicity was the object of study by TOLBA and MOUBASHER (1955). They tested several *Rhizoctonia* strains obtained from different host plants for pathogenicity. From the results they obtained, they concluded that there was no specificity for the *Rhizoctonia* isolates in connection with their respective hosts.

MAIER (1962) studied the sensitivity of 12 *Rhizoctonia solani* isolates for various soil chemicals. He also tested 12 isolates for their pathogenicity on cotton. He stated that there was no correlation between pathogenicity to cotton and sensitivity to chemicals. SHATLA and SINCLAIR (1963) on the other hand, demonstrated that pathogenicity of *R. solani* isolates and tolerance to pentachloronitrobenzene (PCNB) were correlated to some extent under greenhouse conditions.

Other investigators (DANIELS 1963, MAIER 1959, MAIER and STAFFELDT 1960, PITT 1964, SHATLA and SINCLAIR 1963) studied the parasitism of *Rhizoctonia solani* on a wide range of hosts. Their results showed great variability in pathogenic activity, which ranged from non-pathogenic to highly-pathogenic. DANIELS (1963), for example, investigating the parasitic activities of some isolates of

Corticium solani, claimed that the degree of pathogenicity to different host plants may arise together with a complete range from no effect to lethal attack on any of the hosts studied. MAIER (1959) investigated the pathogenic variability of 245 *Rhizoctonia* isolates on cotton seedlings. The isolates were grouped into 10 strains or races on the basis of their cultural variation and on their ability to parasitize cotton seedlings. He found limited cultural variation among the 10 strains, but the pathogenicity ranged from non-pathogenic to highly-pathogenic.

FLENTJE and SAKSENA (1957) studying the pathogenic and cultural characters of sixty-eight isolates of *Pellicularia filamentosa* and twelve isolates of *P. praticola* obtained from various hosts, came to the conclusion that there was no correlation between cultural characters and pathogenicity.

Very recently HADLEY and HARVAIS (1964) developed a rapid method for testing the pathogenicity of *R. solani* to potato. They used detached potato leaflets and placed them over the growing cultures of the fungus in PETRI dishes. After incubation, the leaflets were examined for the infection of *R. solani* strains manifested by the browning and discolouration of the leaf area. They showed that isolates from potato gave a high infection while strains isolated as pathogens of other hosts showed very low or no infection.

The present studies were undertaken with the object of finding a possible relation between culture types and degree of injury on the potato plants.

3.2. MATERIALS AND METHODS

The same 15 isolates used in the previous experiments were studied in relation to pathogenicity. All inoculations were carried out on the potato variety Bintje. The tubers were carefully selected for uniformity in size and absence of visible sclerotia. Moreover, the tubers were treated by desinfecting them in a solution containing 0.6 % 'Aretan-nieuw' for half an hour in order to kill any viable sclerotia if present. After drying, the tubers were stored in cold storage at 4°C until needed. Before planting, the tubers were transferred from the cold storage to a higher temperature for the development of the sprouts. When the tubers were well sprouted, all sprouts were removed except one apical sprout for each tuber.

The inoculum used was prepared by growing cultures of the isolates in one-liter ERLENMYER flasks containing steamed soil with a moisture content of 25 %. Each flask was inoculated by an agar disk from a culture of the isolate concerned, and then incubated for one month at 24°C. The flasks were shaken after two weeks in order to mix the fungus evenly through the medium. After one month, the inoculum was mixed with natural un-sterilized sandy soil which had been tested and appeared to be free from *Rhizoctonia*. Inoculum was added in the proportion of 1:9 (w/w) inoculum:soil. Soil and inoculum were mixed thoroughly. The soil prepared in this way was distributed in clean sterilized clay pots 20 cm in diameter by 19 cm deep. Two days after filling the pots, five '*Rhizoctonia* free' seed potatoes were planted in each pot, at a depth of 12 cm. Five pots were used for every isolate, and the pots were arranged in a rando-

mized design, kept in a greenhouse maintained at an average temperature of 18 °C. The pots were kept for two months and the moisture holding capacity (M H C) was adjusted to approximately 40 % by regular watering and weighing.

At the end of the growing period, observations were made for the surviving plants only, including the length of sprout, the massing of sclerotia on the stem (see fig. 1) or on the new tubers, and the total weight of the new tubers for each replicate.

To record the degree of infection on stems and stolons, four arbitrary classes of infection types were distinguished.

class 0 – no lesions present on sprouts (no infection)
class 1 – light infection
class 2 – moderate infection
class 3 – heavy infection, often completely girdling the stem.

3.3. Results

Table 4. The degree of pathogenicity of 15 isolates of *Rhizoctonia solani* on the potato variety. Bintje. Greenhouse experiment, finished after 2 months.

Group No.	No. of isolate	Average-height of 25 shoots (cm)	Total weight of tubers produced[1] by 25 plants	Degree of injury [2]	Sclerotial production [3]
I	Sc. 5	26	790	1.4	+++
	Sc. 8	23	730	1.4	++
	Sc. 28	27	850	1.0	+
	Sc. 34	28	780	1.5	+++
	Sc. 39	30	770	1.4	+++
II	Sc. 1	32	950	0	0
	Sc. 17	34	960	0	0
	Sc. 18	30	900	1.1	+
	Sc. 52	28	800	2.0	+
	Sc. 55	32	950	1.1	0
III	Sc. 3	27	930	1.3	++
	Sc. 13	27	950	1.0	++
	Sc. 25	22	950	1.4	++
	Sc. 33	26	980	1.6	++
	Sc. 45	24	960	1.4	+
None (control)		40	1010	0	0

[1] Analysis of variance shows that differences in tuber weights between group I and group II and III are highly significant; whereas there is no significant difference between group II and group III.

[2] The average degree of injury for the 25 isolates is based on a scale of 0 (no disease) to 3 (completely girdling).

[3] The symbols used denote degree of sclerotial production as follows: 0 no sclerotia produced, + light contamination, ++ moderate contamination, +++ heavy contamination with sclerotia.

The results of these trials are presented in table 4. Most of the isolates tested gave light to moderate infection on the sprouts. None of the isolates caused heavy infection with complete girdling of the plants. This is in agreement with results obtained by other investigators, who stated that sclerotial isolates from potato are only weakly pathogenic on that and other hosts. (BARKER and WALKER 1962, LE CLERG 1941, SANFORD 1938).

Isolates Sc. 1 and Sc. 17 did not cause infection on stems or stolons, and subsequently no sclerotia appeared on the new tubers. Isolate Sc. 55 which likewise formed no sclerotia, caused only light infection on the stems. For the rest of the isolates the production of sclerotia on new tubers varies from light to heavy. It is important to note that three of the isolates of group II which formed abundant aerial mycelium, showed no sclerotia, whereas the two remaining caused only a light contamination. These results agree with the previous work of HOUSTON (1945).

In general, no correlation between type of isolate and pathogenicity for the potato could be established. It is evident from several studies that definite conclusions with regard to pathogenic ability of a group of isolates can only be made after testing a fairly large number of isolates under different environmental conditions.

CHAPTER 4

A RAPID METHOD FOR THE ISOLATION OF *RHIZOCTONIA SOLANI* KÜHN FROM SOIL AND SOME EXPERIMENTS USING THIS METHOD

4.1. Introduction

The study of the fungal flora of the soil has reached a stage where broad surveys can provide new information to the soil microbiologists. While it is known that fungi may exist in soil both as active mycelium and as dormant structure, such as spores and other resting bodies like sclerotia, a major problem in the study of soil fungi has been to discover which fungi in soil are present as mycelium.

To obtain such information, in recent years considerable progress has been made towards the development of new methods and selective media for the isolation and identification of soil fungi.

Rhizoctonia is easily identified by the morphologic characters of the hyphae. This and the economic importance of the diseases incited by *Rhizoctonia* may be the reason that many investigations have been directed towards the development of new methods for the isolation and detection of this fungus.

Three commonly used methods to isolate *Rhizoctonia* from soil are:

1. Direct isolation from soil particles, from fragments of plant debris, and from superficial runner hyphae occurring on the root surfaces of plants.
2. Indirect isolation by using:
   a. special devices introduced into the soil to be investigated such as; plate-profile technique, immersion tubes, soil microbiological sampling tubes (SMST) and several other devices.
   b. susceptible host plants grown in the test soil, and subsequent isolation of the fungus from the infected plant.
3. Indirect isolation through substrate colonization (trapping or baiting methods). This is carried out by introducing into the infected soil:
   a. different substrates of plant material such as corn, buckwheat, oats, several others to the soil and to be colonized by the fungus.
   b. baits of cellulose film.

4.2. Review of the literature

Many papers dealing with the above mentioned methods have appeared. These papers will be reviewed, also a brief description of the methods will follow.

4.2.1. *Direct isolation*

Warcup (1955) designed a hyphal isolation method for the isolation of fungi occurring in soil as sterile mycelium. Essentially, the method depends on the

observation that when a soil suspension is prepared, many of the fungal hyphae remain with the heavier soil particles of the residue. The fungal hyphae are removed from the residue, placed in a dish, covered with melted agar and incubated at room temperature. He thus succeeded in isolating and identifying the hyphae of *Rhizoctonia* originating from residual soil particles.

BOOSALIS and SCHAREN (1959) were able to recover colonies of *R. solani* from plant debris particles screened from soil naturally infested with this fungus. To this end they devised a special plating method. Nondisinfected plant debris particles were cultured on acidified water agar to which streptomycin sulfate was added. Colonies of *R. solani* from plant debris particles were discerned in 4–98 hours.

DANIELS (1963) was able to obtain pure cultures of *R. solani* derived from superficial runner hyphae harboured on the root surfaces of host plants. The hyphae were pulled off with forceps from the roots and incubated on nutrient agar.

4.2.2. *Indirect isolation by using special devices*

The ROSSI-CHOLODNY slide technique, first devised by the Italian microbiologist G. ROSSI (1928) and later modified by the Russian microbiologist N. CHOLODNY (1930) provided an extremely simple and elegant technique for studying the soil microflora. They obtained a 'contact picture' of the soil microflora by pressing a clean glass microscope slide against an exposed face of soil. *R. solani* was among the group of fungi recovered by this method.

The immersion tube method designed by CHESTERS (1940, 1948) for the purpose of isolating actively growing mycelium from soil, has been particularly successful in isolating *R. solani*. The method consists of a glass tube with spirally arranged invaginated capillaries filled with a nutrient agar. A soil core is removed in the field and an immersion tube inserted in its place, for 7–14 days. After that time fungi are isolated by plating out portions of soil core removed from the tube.

MUELLER and DURRELL (1957) have described a modification of CHESTERS' tube technique. Application of plastic centrifuge tubes bored with holes, instead of the breakable glass tubes proved to be more convenient and less contamination occurred. THORNTON (1952) described a method which he called screened immersion plate method, as a development of the ROSSI-CHOLODNY slide method. He employed glass microscope slides coated with a thin layer of water agar carried in a 'perspex' box provided with a lid containing 10 spaced holes for entrance of fungal growth. After burying them in soil for a suitable period, colonies of fungi could be recovered and identified. From a comparative study THORNTON (1956) found that mycelium of *R. solani* was predominant on screened plates while it was not recorded on any occasion using soil plates. Also with the ROSSI-CHOLODNY technique he observed the occurrence of *Rhizoctonia* mycelium as a constant feature. More recently MARTINSON and BAKER (1962) using basically the technique applied by MUELLER and DURRELL (1957) in which so-called soil microbiological sampling tubes (SMST) are used, modified

certain procedures in the method. They tried different media and found that *R. solani* was easily recovered from soil when using potato-dextrose agar (PDA) or RICHARDS solution with agar. They also reported that *R. solani* was isolated more frequently when exudates from radish seeds were added to the test tubes.

WOOD and WILCOXSON (1960) described another screening immersion plate, in which they used plastic plates, to isolate different genera of fungi. They found that *Rhizoctonia* was frequently isolated.

ANDERSON and HUBER (1962) developed their plate-profile technique for the isolation of the fungus *Rhizoctonia* from soil.

4.2.3. *Indirect isolation by parts of living plants*

Among the numerous methods used for the isolation of *Rhizoctonia* an older method – the infected host method – was described to isolate *R. solani* from field soil. PELTIER (1916) was probably among the first to isolate *R. solani* from field soil by using plant material. In his method, he selected small patches of ground over the field to be examined. Every space was cleared except for a small living plant. After watering the soil thoroughly, he managed to maintain the soil moisture by covering the plants. After a period of five days, the plant parts were examined for the presence of *Rhizoctonia*, and pure cultures were obtained.

FLENTJE and SAKSENA (1957), after planting different seed crops in the field succeeded in obtaining several isolates of *R. solani* by plating infected seedlings on soil-extract agar.

4.2.4. *Indirect isolation through colonization of substrate*

Colonization of special substrates or baits has been used as a method to study the presence of certain pathogens in soil not only qualitatively but also quantitatively. Recently, a trapping method was used as a means to evaluate soil fungicides applied to the soil as a control measure against *Rhizoctonia* and other soil borne fungi. Basically these methods are a compromise between the selective plating method and the host plant infection test, their success depends on the saprophytic activity of the fungus. Substrates for soil fungi may be defined as 'living or dead', virgin or partially decomposed plant or animal tissues lying in or upon the soil, or soluble products diffusing therefrom (GARRETT 1956). The 'bait' consists of a substrate that is more likely to be colonized by species in the desired group than by any others.

The various ways that plant material has been used as baits or traps to isolate microorganisms from soil have been reviewed by DURBIN (1961). MESSIAEN (1957) was the first to explore the ability of *R.solani* to colonize on corn seed kept in soil. KENDRICK and JACKSON (1958) investigated the factors likely to favour isolation of the fungus. From their experiments they concluded that by selecting appropriate combinations of moisture, temperature, and incubation period, recovery of the fungus was promoted.

PAPAVIZAS and DAVEY (1959) developed a colonization method for the iso-

lation of *R. solani* from soil by employing as a bait, standardized pieces of mature stems of buckwheat (*Fagopyrum exculentum* MOENCH). Small pieces of the material were mixed with the soil to be investigated, after a suitable exposure in the soil for 3–4 days, the pieces were recovered by hand, treated in a special way and placed on an agar medium containing antibiotic and on which *R. solani* grows rapidly. Recently (1962) they tested several other substrates to be colonized by *Rhizoctonia*, and they came to the conclusion that stem segments of buckwheat, cotton and lima bean were rather satisfactory, whereas those of corn, oats and soybean were not. The method gives useful data on the prevalence of *Rhizoctonia* in soil.

PITT (1964) who slightly modified the method of PAPAVIZAS and DAVEY (1959) indicated that 90 % of naturally infected straws of wheat yielded *R. solani*. LIVINGSTON et al. (1962) by using the buckwheat stem segments as a substrate were able to assay the inoculum levels of *Rhizoctonia* in potato fields. They also used this method to evaluate terraclor as a soil treatment to control *Rhizoctonia* disease of potato. By using sterile bean stem sections THAYER and WEHLBERG (1963) were able to evaluate the populations of *Rhizoctonia* and *Phythium* in soil fungicide tests.

DANIELS (1963) using square pieces of cellulose film as baits, was able to obtain pure cultures of *R. solani* by introducing the baits to the test field. After an incubation period of 12 days, the baits were removed from the soil, washed free from soil particles and transferred to nutrient agar in PETRI dishes on which *R. solani* grew out from the baits.

Although the methods reported above may be used with success for the detection and isolation of *R. solani* from soil, they are still laborious and need a great amount of time and effort. Moreover, in some instances with the plating-method techniques the fungus *Rhizoctonia* is rarely recovered, whereas it has been occasionally detected by other techniques (BISBY et al. 1935, CHESTERS 1948, KAUFMAN et al. 1963, MUELLER and DURRELL 1957 and THORNTON 1956).

DAVEY and PAPAVIZAS (1962) compared four methods used for the isolation of *Rhizoctonia* from soil to show the merits and advantages of each method when applied under nearly identical conditions. The four methods they compared were: a. debris particle method, b. immersion tube method, c. buckwheat colonization method and d. infected host method. They concluded that each method had certain distinct advantages and characteristics which would suggest its use under particular circumstances.

They found that the infected host method was the best when the range of clones of the fungus pathogenic to certain plants should be studied. The immersion tube method recovered a limited number of clones of the fungus. By using the debris particle method along with the buckwheat colonization method, complete information could be achieved, since the debris particle method reflected the status of the quiescent *Rhizoctonia* in soil whereas the buckwheat colonization method provided information on the active *Rhizoctonia*.

While it is now possible to isolate *Rhizoctonia* from the soil in situ by introducing various organic substrates which can be colonized by the fungus,

several experiments have shown that cellulolytic ability is a normal characteristic of *R. solani* whatever its natural habitat. It is evident from the results of the investigations mentioned above that *R. solani* can persist in soil both as mycelium and as sclerotia. It can make free and independent growth in natural soil (BLAIR 1943).

TRIBE (1960) through his elegant use of buried cellulose film as a substrate for the study of microbial successions observed that *R. solani* was the dominant fungal colonizer of cellulose test pieces.

GARRETT (1962) from his experiments showed that *R. solani* grew well on filter paper, he concluded that the fungus can utilize forms of cellulose as carbon source in pure culture and in unsterile soil.

DANIELS (1963) demonstrated the cellulolytic ability of *R. solani* by using processed and native forms of cellulosic substrates under different conditions in pure culture and in unsterile soil.

EL ZARKA (1963) deviced a method in which small dried stem pieces of jews' mallow (*Corchorus olitorius* L.) were put on stainless steel insect pins and introduced in the test soil. The method takes advantage of the vigorous competitive saprophytic ability of this particular pathogen in colonizing the jews' mallow. A detailed account of this method is given in the following pages.

4.3. DESCRIPTION OF THE METHOD

4.3.1. *General*

Requirements for a substrate to be used for isolation are:

1. The substrate has to be suitable as a nutrient source.
2. The substrate has to be easily recovered.
3. The substrate has to withstand various manipulations after recovery by keeping its coherence and its firmness.

From experiments which included material from mature plants, the dry mature stem of jews' mallow = Nalta jute (*Corchorus olitorius* L.) appeared to be the best to meet the above mentioned requirements.

The chief constituents of the dried stem of jews' mallow are: lignin 18.5 %, pentosan 15.8 %, alpha-cellulose 29 %, total carbon 48 % and total nitrogen 0.52 %. The carbon/nitrogen ratio of the dried jews' mallow is 92. DAVEY and PAPAVIZAS (1963) studied the effect of C/N balance on the saprophytic activity of *Rhizoctonia*, they indicated that the C/N ratio may be more important than the absolute amount of C or N added to the soil in producing an effect on *Rhizoctonia*.

Dried stem pieces of the jews' mallow plant were obtained as follows. The plants were grown at 24–25 °C. Just before flowering the leaves were stripped off and the stems were harvested and dried. The stems were then cut into small sections 8 to 10 mm long. These stem sections were used without any previous treatment as a substrate in the following manner. A stainless steel insect pin about 4 cm long (No. 3 pin) was inserted perpendicularly through the piece of stem (Plate 2). The pins to which the stem pieces were attached, were placed in

the soil to be investigated nearly to their full length with the head-end down in order to keep the pieces attached to them. Except when otherwise noted, the pins were recovered after 24 hours from the soil. This period is called the substrate incubation period. Experiments were carried out to determine the optimum incubation period and to study what happens when the substrate incubation period is prolonged beyond the optimum period.

The stem pieces were then washed thoroughly in tap water to remove any adhering soil particles, surface sterilized in 1 % sodium hypochlorite solution for 1–2 minutes and rinsed several times with sterile water. Finally they were soaked for two hours in a fresh sterile solution of 100 ppm terramycin hydrochloride at room temperature in order to suppress bacterial growth.

After removal from the solution the pieces were placed on sterile paper towels and air dried. The stem segments were then plated in PETRI dishes containing 12 ml of solidified agar medium. Ten pieces were plated in one dish with a total of 100 pieces for each treatment. Several media were tested during this investigation for plating out the pieces: e.g. cherry agar, potato-dextrose agar, prune agar, water agar, Czapek-dox agar (M), and peptone agar. All media proved to be suitable for growth of the fungus. For serial work cherry agar proved to have advantages over the other media, and therefore was used throughout the work. Experiments with regard to the influence of the medium are described under 4.3.4.

From observations with the naked eye it appeared that already after six hours of incubation *R. solani* started to develop from the colonized pieces. For serial work, an incubation period of 16–18 hours proved to be more suitable throughout the experiments and unless otherwise indicated, this period did not exceed 18 hours because fast growing fungi including *Rhizoctonia* might overrun the adjoining pieces of substrate, interfering in this way with the identification of *Rhizoctonia* or with the quantitative evaluation of the experiment (Plate 3).

Observations with the naked eye and under the microscope made at regular intervals to determine the best time for these observations are described under 4.3.5.

The soil to be tested was partially air dried and passed through a sieve. The soil was mixed thoroughly, divided into equal portions and placed in sterilized containers. The water content was adjusted to approximately 50 % of the moisture holding capacity (MHC), and the containers were kept at 18–19 °C in the greenhouse. Each treatment consisted of ten containers arranged according to a randomized design. Usually, when artificially inoculated soils were included in the experiments, the inoculum soil mixtures were kept for two days before introducing the jews' mallow pieces in order to allow the fungus to spread throughout the inoculated soils.

In the following four experiments a sandy soil which proved to be free from *Rhizoctonia* was used. A ratio of 10:90 inoculum: soil was applied, and isolate Sc. 55 was used to provide the inoculum for all four experiments.

These experiments were carried out with the following objectives in mind:

1. To find out the time required for the substrate to be colonized by the fungus.

2. To investigate the frequency of recovery of the substrate when incubated for prolonged periods.
3. To study the influence of media on the frequency of isolation of the fungus from the colonized substrate and,
4. To know the time needed for *Rhizoctonia* to develop out of the colonized pieces.

Results of such investigations are reported in the following:

4.3.2. *The time required for the jews'mallow to become colonized by R. solani from an artificially inoculated soil*

It has been shown previously that an incubation period of the substrate in the soil for 24 hours is sufficient to provide an almost 100 % colonization. To study how fast *Rhizoctonia* can establish itself on the inserted substrate, an experiment with incubation periods of 6, 12, 18 and 24 hours was set up.

Isolate Sc. 55 and the ratio 10:90 inoculum: soil dilution were used. Four sterilized clay flats were used with one hundred pieces in each. After the recovery of the pieces they were treated as previously described and plated on cherry agar.

TABLE 5. Percent isolation of *R. solani* from jews' mallow stems kept for 6, 12 and 24 hours respectively in artificially inoculated sandy soil. Isolate Sc. 55

Inoculum: soil ratio	Substrate incubation period in hours			
	6 hr	12 hr	18 hr	24 hr
	Percent isolation of *R. solani*			
10:90	0	32	82	100

The results of this experiment are set in table 5.

These data revealed that twelve hours incubation for the substrate is sufficient for *Rhizoctonia* to settle on the substrate in such a way that it can be recovered. With increasing incubation periods, the number of recoveries increased progressively. With the 10:90 ratio it reaches 100 % after an incubation period of 24 hours.

4.3.3. *The frequency of recovery when the substrate is incubated for prolonged periods*

The present work was attempted to investigate the effect of length of incubation of jews' mallow substrate in soil on the percent isolation of *R. solani*. Isolate Sc. 55 was used to provide the artificial inoculation, and the inoculum was obtained the same way as previously described. The inoculum-soil mixture was divided among six clean sterilized square clay flats, 25 × 25 cm with 12.5 cm deep. After the two days of incubation of the infested soil, 150 pieces of jews' mallow stem segments were introduced to every flat with a total number of 900 pieces for the dilution 10:90. The pieces were then recovered from the ino-

culum soil mixture after 1, 2, 4, 10, 20, 30, 45, 60 and 90 days, treated in the same way and plated out on cherry agar medium. For each incubation period one hundred pieces of jews' mallow were recovered.

TABLE 6. Percent isolation of *R. solani* from jews' mallow kept for 1, 2, 4, 10, 20, 30, 45, 60 and 90 days respectively in artificially inoculated sandy soil. Isolate Sc. 55.

Inoculum: soil ratio	Substrate incubation period in days								
	1	2	4	10	20	30	45	60	90
	Percent isolation of *R. solani*								
10:90	100	100	100	45	38	35	12	8	4

The results given in table 6 show that stem pieces incubated for 2 or 4 days yielded the highest percentage of *R. solani*. After 4 days the number of isolations decreases rather abruptly between 4 and 10 days, and from there on gradually until after 90 days *Rhizoctonia* is isolated only to a very low percentage.

The decrease in the number of isolations from the 4th day on is important from an ecological point of view. Evidently *R. solani* is an early and vigorous colonizer of suitable organic tissues, but it is rapidly succeeded or antagonized and masked by other species.

After 90 days incubation, the substrate jews' mallow still contained viable *Rhizoctonia*. The incubated pieces, however, deteriorated partially, but still retained their rigidity and firmness.

4.3.4. *Influence of various media on the frequency of isolating R. solani from the colonized substrate*

In this study the influence of six different media was investigated with respect to:

1. Total number of *Rhizoctonia* colonies recovered from the colonized substrate.
2. Number of substrate pieces which apparently yielded only pure growth of *Rhizoctonia*.

The same six media which were previously mentioned under section 4.3.1. were used for incubation of the substrate. For each medium 100 pieces of the substrate were distributed over ten PETRI dishes as described before. The same isolate Sc. 55 was used in this study, and the dilution 10:90 inoculum:soil was included, and the mixture was distributed into sterilized flats kept at 18 °C in the greenhouse. From preliminary experiments when three media were used, it had been shown that with the above mentioned ratio usually 100 % colonization was attained.

Four flats were used, with a total of 600 pieces of substrate. After a period of two days of incubation, the pieces were recovered and treated in the same way as described before, then plated on the different media at 24–25 °C for 18 hours; and then examined under the microscope. Counts were made of the number of pieces yielding *R. solani* on each medium, and of the number of pieces showing only growth of *Rhizoctonia* (Table 7).

TABLE 7. Number of colonies of *R. solani* secured from jews' mallow pieces after incubation on various media for 18 hours. Substrate incubation for two days. Isolate Sc. 55.

Media	Number of pieces colonized by *R. solani*	Number of pieces yielding only growth of *Rhizoctonia*
Cherry agar	100	83
PDA	100	58
Prune agar	99	63
Water agar	100	76
Czapek-Dox agar	100	59
Peptone agar	99	61

From the data presented in table 7 almost 100 % of the pieces developed *Rhizoctonia*, irrespective of the media used. In some instances, other fungi were observed growing from the colonized pieces. These fungi, however, did not interfere with the growth of *R. solani*. Consequently, identification of *R. solani* was possible in all instances.

It is significant to mention that a relatively high percentage of pieces showed apparently only growth of *Rhizoctonia*. This indicates that the jew's mallow substrate is a very favourable selective substrate for the isolation of *Rhizoctonia*.

Cherry agar, and water agar gave the highest percentage (about 80 %) of pure growth of the fungus, as can be seen from the second column of Table 7. The other media gave a lower percentage (about 60 %).

In spite of the treatment of the pieces by the antibiotic terramycin (100 ppm) to suppress the growth of bacteria, in some cases still some colonies of bacteria developed. Cherry agar and water agar again proved to be superior to the other media. Since the fungus on cherry agar medium develops a more vigorous growth than on water agar, and since it is relatively easy to prepare, these facts were considered very desirable points. Unless otherwise indicated, cherry agar was used for the isolation in the subsequent experiments.

4.3.5. *Observations on the time needed for R. solani to develop out of the colonized pieces*

This investigation was initiated to gain further information about the time required for *R. solani* to develop its initial growth from colonized pieces of jews' mallow. For this purpose, four media were selected for plating the pieces: a. cherry agar, b. PDA, c. prune agar, and d. water agar. The experiments were set up with the following objectives:

1. In the first one observations were recorded with the naked eye every six hours until 24 hours from the time the pieces were plated on the media. It appeared as it has been mentioned before that after six hours of incubation, growth of *Rhizoctonia* could be detected in 100 % of the colonized pieces. This was the case irrespective of the medium used.
2. The purpose of the second experiment was to determine how fast *Rhizoctonia* can develop out of the pieces. To this end hourly observations were made under the microscope.

Already after one hour of incubation, few pieces showed a trace of hyphal growth; after two hours of incubation on cherry agar, PDA, prune agar and water agar, a percentage of 96, 98, 100 and 88 was recorded respectively. From the foregoing observations, it is evident that *R. solani* develops very fast from colonized pieces of jews' mallow substrate and it is obvious that a period of six hours of incubation is ample to recognize growth of the fungus with the naked eye.

4.4. Application of the method in the isolation of *Rhizoctonia* from soil

4.4.1. *Introduction*

From the data obtained in the previous experiments, it has been shown that the dried stem pieces of the jews' mallow substrate proved to be very favourable plant material to be colonized by *R. solani*. The method described proved to be quick and simple to apply, it also enabled comparisons between soil mixtures even at low dilution of the inoculum.

Papavizas and Davey (1961) by using the buckwheat as a substrate, investigated the saprophytic activity of 5 isolates of *Rhizoctonia* mixed with unsterilized sandy loam soil at the rate of 1 % (w/w), and kept them for various incubation periods ranging from 14 days to 196 days. From the data they presented, they found that the rate of decrease of colonized pieces varied among the isolates. They also reported that after 196 days of incubation the loss in activity ranged from 24 %–91 % for the different isolates.

The technique previously described by the author (4.3.) provides a valuable tool for the isolation of *R. solani* from soil. Because of the flexibility and relative simplicity of the method, it was decided that further studies had to be carried out by using this method to gain information concerning the distribution of the soil-inhabitant *Rhizoctonia solani* under a wide range of artificially inoculated soils, and naturally infested soils.

For this reason a number of experiments was carried out.

4.4.2. *The effect of varying the inoculum: soil ratio and the length of the substrate incubation period*

For this experiment, mainly the same procedure as described in the previous experiment (4.3.2.) was followed, except that three dilutions of the inoculum-soil: 1:99, 0.3:99.7, and 0.01:99.99 were included. Results of the dilution 10:90 previously obtained were also included for comparisons (Table 8).

Table 8 illustrates the results of the number of colonized pieces for each dilution and after every incubation period.

From the data presented in this table, it appears that *Rhizoctonia* has been recovered from colonized pieces in artificially inoculated soil tested at the 4 different dilutions. Already after 12 hours of incubation, a rather high percentage of isolations can be achieved, even when the inoculum: soil ratio is very low (0.01 % of inoculum).

TABLE 8. Percent of *R. solani* from jews' mallow stems kept for 6, 12, 18 and 24 hours in artificially inoculated sandy soil. Isolate Sc. 55.

Inoculum: soil ratio	Substrate incubation period in hours			
	6	12	18	24
	Percent isolation of *R. solani*			
10:90	0	32	82	100
1:99	0	49	66	78
0.3:99.7	0	33	60	70
0.01:99.99	0	36	48	52

With increasing the substrate incubation period, the number of recoveries increased progressively. However, with lower inoculum ratios, lower percentages have been recorded.

In a second experiment the pieces were recovered after 1, 2, 4, 10, 20, 30, 45, 60 and 90 days. For each incubation period and for each dilution, one hundred pieces of jews' mallow were used. In such an experiment lasting for prolonged periods, the soils in the flats were maintained at 50 % (MHC) throughout by daily weighing and watering.

TABLE 9. Percent isolation of *R. solani* from jews' mallow stems kept for 1, 2, 4, 10, 20, 30, 45, 60 and 90 days in artificially inoculated sandy soil. Isolate Sc. 55.

Inoculum: soil ratio	Substrate incubation period in days								
	1	2	4	10	20	30	45	60	90
	Percent isolation of *R. solani*								
10:90	100	100	100	45	38	35	12	8	4
1:99	78	98	86	28	22	21	9	7	3
0.3:99.7	70	91	88	21	14	16	6	4	2
0.01:99.99	52	83	83	28	19	19	5	3	2

Table 9 represents the number of colonized pieces for the three dilutions and after every incubation period. It also includes the results for the dilution 10:90 for comparison.

The results given in table 9 show that stem pieces incubated for 2 or 4 days in the three inoculum soil mixtures tested yielded the highest percentage of *R. solani* and those for 10, 20, 30, 45, 60 and 90 days the lowest. After 4 days, the number of isolations decreases rather abruptly between the 4 and 10 days series and from there on gradually until after 90 days (Fig. 4).

For lower inoculum ratios, the trend is the same, however, the number of isolations is lower.

In a third experiment two additional isolates: Sc. 8 and Sc. 3 were included in the study besides Sc. 55. The inoculum for the three isolates was obtained in the same way as previously described. The following inoculum: soil dilution mixtures were selected for the test; 50:50, 10:90; 5:95; 1:99; 0.3:99.7 and

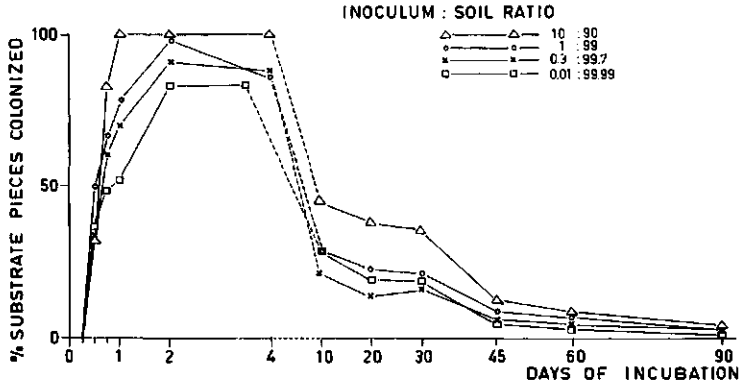

FIG. 4. Effect of length of jews'mallow incubation in the soil on the percentage of colonization. Isolate Sc. 55, and four inoculum: soil dilutions.

0.01:99.99. The incubation periods for the jews' mallow substrate were 1, 2, 4 and 10 days. The experiment was set up in the same way as previously described and also for the treatment of the substrate after recovery.

TABLE 10. Percent isolation of *R. solani* from soil artificially inoculated with 3 isolates tested at varying inoculum: soil dilutions and after varying substrate incubation periods.

Inoculum: soil ratio	Isolate Sc. 8				Isolate Sc. 55				Isolate Sc. 3			
	Substrate incubation period in days											
	1	2	4	10	1	2	4	10	1	2	4	10
	Percent isolation of *R. solani*											
50:50	100	100	100	85	100	100	100	80	95	100	98	75
10:90	90	98	91	32	90	100	100	43	70	82	85	40
5:95	80	85	80	25	85	100	100	43	45	75	70	30
1:99	40	44	30	16	80	90	92	35	36	70	66	16
0.3:99.7	24	30	26	4	70	86	90	22	8	30	26	10
0.01:99.99	6	13	12	4	50	80	75	30	10	18	8	12

Results on percentage of isolation of *R. solani* as affected by the length of incubation of jews'mallow stem pieces in the soil for the three isolates are recorded in table 10. From these data it is obvious that the number of colonized pieces varies from one isolate to another. However, in all cases of inoculum:soil dilutions tested with the three isolates, stem pieces incubated for 2 or 4 days yielded the highest percentage of *R. solani* (Fig. 5).

4.4.3. *The effect of keeping the inoculum: soil mixtures for varying periods before introducing the jews' mallow stems*

In all previous experiments the inoculum: soil mixtures were kept for two days before introducing the jews' mallow segments. In this experiment the time during

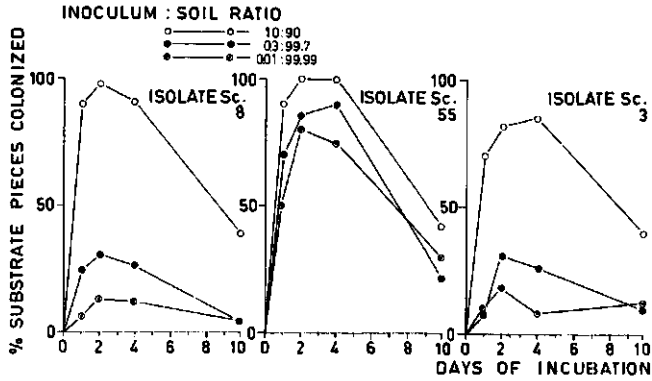

FIG. 5.
Effect of length of jews' mallow incubation in the soil on the percentage of colonization. Isolates Sc. 8, Sc. 55, and Sc. 3. Three inoculum: soil dilutions.

which the inoculum: soil mixture was kept, varied from 2 to 180 days. Isolate Sc. 8 has been used to provide the inoculum. The inoculum was increased in the same way as previously described, and it was used after one month of incubation.

The inoculum was added to the natural unsterilized sandy soil (w/w), by diluting it with increasing proportions of the unsterilized soil. The inoculum: soil mixtures were mixed thoroughly in separate piles, and care was taken when mixing the inoculum with the soil to avoid contamination from outside sources.

The following range of inoculum:soil dilution mixtures were tested 100:0 (inoculum control, 50:50, 5:95, 1:99, 0.5:99.5, 0.3:99.7, 0.1:99.9, 0.03:99.97, 0.01:99.99 and 0:100 (soil control). The inoculum: soil mixtures were distributed equally among sterilized 10 cm clay pots, adjusted at approximately 50% (MHC) which was maintained throughout the experiments. The pots were then kept on benches in the greenhouse at 18–19 °C.

Periods for incubating the inoculum: soil mixtures were as follows: 2, 4, 10, 30, 60, 90, 120, 150 and 180 days before application of the jews' mallow stem pieces. In all series of inoculum: soil mixture the pieces were incubated for 24 hours and after that treated in the usual way. Ten pots were used for every period of keeping, and for every inoculum:soil mixture with a total of 90 pots for every dilution. Ten pieces of the dry mature jews' mallow substrate were inserted into every pot.

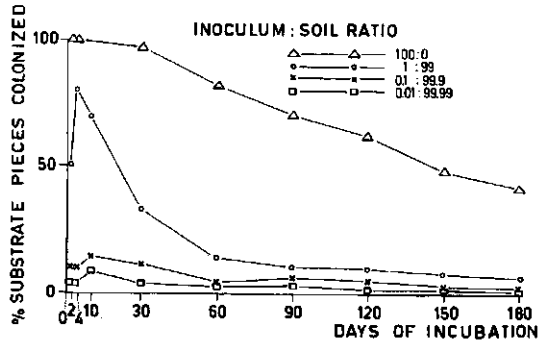

FIG. 6. Effect of length of inoculum incubation in soil before introducing the jews' mallow substrate on the percentage colonization. Isolate Sc. 8, and four inoculum: soil dilutions.

TABLE 11. The effect of keeping the inoculum: soil mixtures for varying periods before introducing the Jews' mallow stems. Isolate Sc. 8.

Inoculum: soil ratio	Period in days of keeping the inoculum: soil mixtures before introducing the jew's mallow substrate								
	2	4	10	30	60	90	120	150	180
	Percent isolation of *R. solani*								
100:0 (inoculum control)	100	100	100	97	82	70	62	48	42
50:50	100	100	96	90	64	52	43	23	20
5:95	90	91	76	67	63	43	45	24	23
1:99	51	80	69	33	14	10	10	7	6
0.5:99.5	27	26	14	16	2	7	6	1	3
0.3:99.7	30	30	22	17	13	14	5	1	3
0.1:99.9	10	10	15	12	4	6	5	2	2
0.03:99.97	6	6	6	7	3	4	2	1	1
0.01:99.99	4	4	9	4	3	3	1	1	1
0:100 (soil control)	0	0	0	0	0	0	0	0	0

Table 11 presents the number of pieces of the substrate colonized by *Rhizoctonia* in every dilution and after every period of keeping.

The results of this experiment indicated that the number of colonized pieces of the jews' mallow substrate increased when the amount of inoculum was increased (Fig. 6). The substrate proved to be satisfactory for isolating and detecting the fungus from the soil even at a relatively low inoculum level. From the data it is furthermore evident that *Rhizoctonia* can be detected and isolated through colonization after a rather long keeping period of the inoculum:soil mixtures, even at a low inoculum: soil ratio (0.01 %).

However, the number of the colonized pieces decreased after prolonged storing of the inoculum:soil mixture. Evidently this is due to a decrease of the saprophytic activity.

CHAPTER 5

ISOLATION OF *RHIZOCTONIA SOLANI* FROM NATURALLY INFESTED SOILS

5.1. Introduction

The occurrence of certain outbreaks of *R. solani* disease in the field, together with the ability of *R. solani* to exist in soil and its extensive host range, suggests that fields may contain numerous types of the fungus which are morphologically and pathogenically distinct.

The lack of suitable techniques appears to be the limiting factor in attempts to gain information concerning inoculum potential of the fungus in various soils, and the ecological relationships of root disease fungi. The introduction of certain substrates to the soil has made it possible to facilitate the isolation of *R. solani* from soil. From the foregoing experiments, we now have convincing evidence that *R. solani* can readily colonize the dried plant tissue jews' mallow substrate as a competitive saprophyte.

The method previously described has proved useful and reliable in the isolation of the root pathogen *R. solani* from soil, thus, it seemed possible that by using this technique comparisons could be made between different soil types naturally infested with *Rhizoctonia* and cropped with various crops in order to gain information about the distribution of *Rhizoctonia* and its inoculum level.

5.2. Samples for investigation

Different areas in the Netherlands were chosen to collect soil samples to be tested. These samples were collected from different soils known to be infested with *Rhizoctonia*, and from soils where its presence was not expected. The origin of each sample, the soil type and the previous crops of each field are shown in table 12.

The soil samples were collected at random spots from each field and taken from the 2.5–12.5 cm layer. Two 20 kg samples were collected from each field and kept in plastic bags until needed. The bags were stored in a cool place, and normally the storage period did not exceed a week.

The samples were partially air-dried, sieved through a 5 mm sieve, mixed thoroughly; subsamples were taken for assay. For every soil sample ten sterilized 10 cm pots were used, and in every pot ten segments of the jews' mallow substrate were introduced. The pots were kept in the greenhouse at 18°–19°C, and the moisture content was adjusted to approximately 50 % of their moisture holding capacity (MHC).

In this investigation the jews' mallow substrate was incubated for two periods viz: one and two days. At the end of the incubation period, the substrate pieces were recovered, treated in the same manner, and plated on cherry agar to determine the percentage of colonization by *Rhizoctonia*. Evidently every

TABLE 12. Origin of samples, soil type, and previous crops.

Sample No.	Place of origin	Province	Soil type	Previous crops
1	Rolde	Drenthe	sandy loam	1960 summer wheat 1961 meadow grass 1962 meadow grass
2	Groningen	Groningen	clay	1960 oats 1961 summer wheat 1962 potato
3	Bennekom	Gelderland	sand	1960 oats 1961 rye 1962 potato
4	Wageningen	Gelderland	sandy loam	1960 French bean 1961 witloof chicory 1962 potato
5	Koewacht	Zeeland	clay	1960 sugar beet 1961 barley 1962 potato
6	Koewacht	Zeeland	clay	1960 potato 1961 peas 1962 barley
7	Koewacht	Zeeland	clay	1960 barley 1961 flax 1962 barley
8	Steenbergen	Noord Brabant	clay	1960 sugar beet 1961 clover 1962 potato
9	Steenbergen	Noord Brabant	light clay	1960 potato 1961 barley + clover 1962 flax
10	Steenbergen	Noord Brabant	clay	1960 peas 1961 sugar beet 1962 potato
11	Kruisland	Noord Brabant	clay	1960 winter wheat 1961 peas 1962 potato
12	Steenbergen	Noord Brabant	light clay	1960 potato 1961 sugar beet 1962 peas
13	Steenbergen	Noord Brabant	light clay	1960 winter wheat 1961 peas 1962 potato
14	Kruisland	Noord Brabant	clay	1960 potato 1961 meadow grass 1962 sugar beet
15	Kruisland	Noord Brabant	clay	1960 peas 1961 sugar beet 1962 potato
16	Espel	Noord Oost Polder	light clay	1961 wheat 1962 meadow grass 1963 potato
17	Espel	Noord Oost Polder	light clay	1961 sugar beet 1962 barley 1963 potato

TABLE 12. continued.

Sample No.	Place of origin	Province	Soil type	Previous crops
18	Espel	Noord Oost Polder	light clay	1961 sugar beet 1962 wheat 1963 potato
19	Wageningen	Gelderland	sand	1961 raspberry 1962 raspberry 1963 raspberry
20	Dronten	Oostelijk Flevoland	light clay	1962 meadow grass 1963 meadow grass 1964 potato
21	Rutten	Noord Oost Polder	light clay	1962 fodder beet 1963 summer wheat 1964 potato
22	Biddinghuizen	Oostelijk Flevoland	clay	1962 common reed 1963 common reed 1964 common reed
23	Dronten	Oostelijk Flevoland	clay	1962 common reed 1963 common reed 1964 common reed
24	Haarlemmermeer	Noord Holland	clay	1962 meadow grass 1963 meadow grass 1964 potato

colonized piece provides a site for isolation of the fungus. The percentage of pieces colonized by *R. solani* for each sample and after the two different periods of incubation of the substrate are presented in table 13. Hyphal-tip isolations were made from colonized pieces of jews' mallow to cherry agar slants and kept for further study. Comparative cultural studies of the stock cultures were made on cherry agar after 3 weeks of incubation at 24°C to determine the number of different types for each soil sample on the basis of their morphological appearance on this particular medium.

5.3. RESULTS

It can be seen in table 13 that the soils tested differed considerably in their natural infestation with *Rhizoctonia*. The foregoing survey demonstrates that the immediate previous crops of the 24 soils, played an important role in determining the frequency of isolation of the fungus.

The potato plant is one of the numerous hosts of the phytopathogenic fungus *R. solani*. With a few exceptions, the soils cropped with potato showed a relatively high percentage of *Rhizoctonia*. Soils No. 2, 13, 15, 17, 20 and 21, however, indicated a low percentage of colonized pieces which could be attributed either to the preceding crop (Table 12) or to the time of collecting the sample. It is evident from the data collected, that frequency of isolation of *R. solani* was influenced by the incidence of disease on potato during the growing season.

LIVINGSTON et al. (1962) investigated the inoculum levels of *R. solani* in

TABLE 13. The relative frequency of isolation of *R. solani* from 24 naturally infested soils after 1 and 2 days incubation.

Sample No.	Percent jews'mallow pieces colonized		Number of types for each soil sample [1]
	1 day incubation	2 days incubation	
1	1	1	1
2	0	3	3
3	16	14	3
4	13	16	7
5	10	11	1
6	22	37	2
7	30	34	2
8	13	29	3
9	5	4	2
10	12	13	3
11	6	15	2
12	6	4	3
13	3	9	1
14	1	1	1
15	3	6	2
16	14	30	2
17	3	5	1
18	18	25	1
19	0	0	0
20	1	2	1
21	2	10	3
22	3	10	4
23	3	5	2
24	6	13	3

[1] Distinguished morphologically on cherry agar slants after 3 weeks of incubation at 24°C.

potato fields. Using the special plate technique and the buckwheat stem-section method, they found that a definite correlation existed between field soil inoculum level and incidence of disease on potato during the growing season.

A significantly higher isolation frequency (37 %–34 %) was obtained from soils No. 6 and No. 7 respectively after two days incubation for the substrate and cropped with barley. The soil samples were collected for assay after crop harvest, and abundant straw litter was scattered. Since *Rhizoctonia* is considered a pioneer colonizer of fresh organic matter lying on or in the soil, the fungus may have been stimulated by the presence of barley straw.

ANDERSON and HUBER (1962) studied the isolation frequency of *Rhizoctonia* from rhizospheres under several cropping sequences and obtained a high isolation frequency (36 %) from the barley rhizosphere.

It is of interest to note that soil sample No. 19 which was cropped with raspberry for several years proved to be free from *Rhizoctonia*, and soil No. 1 which was planted with meadow grass for two successive years was almost free.

It is perhaps significant to state that soils No. 22 and 23 which were newly reclaimed from the sea showed the presence of *Rhizoctonia*. After reclamation

and before cultivation of the first crop, these soils were seeded with common reed (*Phragmites communis*) to combat the development of other weeds. The occurrence of the pathogen *Rhizoctonia* in non-cultivated soils has been reported by several investigators. (MENZIES 1952, PRATT 1918, and THORNTON 1956). It indicates that the soil may be infested before the first crop is planted. Therefore the sclerotia carried on contaminated seed potatoes are not necessarily the only source of inoculum.

From the different soil samples, several morphologically distinct clones were obtained (Table 13). Since differentiation has been made only on cherry agar, the number of types for each soil sample, therefore, may be regarded as conservative. Isolates may look more or less alike on a medium, but completely different on another medium. It is evident from table 13 that the population of *Rhizoctonia* in soil occurs as distinct types which vary in morphological appearance and virulence.

According to our experiments, the soil with the highest percentage of colonization do not necessarily yield the highest number of distinct types. This is not in agreement with PAPAVIZAS and DAVEY (1962), who reported from their experiments on 15 naturally infested soils, that generally the soils with the highest percentage of colonization gave the highest number of distinct types (= clones which exhibited distinct morphological differences on PDA according to PAPAVIZAS and DAVEY).

From the foregoing results it is concluded that the frequency of isolation of *Rhizoctonia* depends on the immediate previous crop, soil type or region as combined factors. It can be assumed that other factors play an important role in determining the frequency of isolation, viz: time of collecting the soil sample, content of organic material and the types of *Rhizoctonia* which exist in the soil.

During the course of this investigation, several species of other fungi were observed growing from the colonized substrate together with *R. solani*. However, none of these fungi interfered noticeably with the growth of *R. solani*. Among the genera developing on the medium in association with *R. solani* were: *Fusarium* spp., *Sclerotium bataticola*, *Cephalosporium* spp., *Trichoderma* spp., *Alternaria* spp., *Helminthosporium* spp., and many other fungi.

The method used is quick and simple to apply, it enables the assay of various soils, and may also prove useful in studying the effects of various soil treatments on soil borne plant pathogens. The technique devised has proved useful not only for detecting and isolating *Rhizoctonia*, but also for revealing the presence of a wide range of root-invading or root-surface fungi, thus the method is widely applicable to gain valuable information concerning the ecology of soil fungi.

Finally, for a general survey of *Rhizoctonia* spp. in soil, and for determining the range of types of the fungus, the jews' mallow colonization method appears to be quite satisfactory. However, the method recovers mostly the active clones in the soil. It is obvious that the number of colonized jews' mallow segments more or less indicates the degree of infestation of soil. It may be expected that the higher the percentage of colonized pieces, the more heavily the soils are infested by the fungus. However, it must be kept in mind that no close corre-

lation can be expected because of saprophytic ability – which may differ between types – also influences the results of the above mentioned experiments.

In the experiments described in this chapter no information was obtained concerning the frequency of occurrence of pathogenic types. This problem will be treated in chapter 7.

CHAPTER 6

PERSISTENCE OF *RHIZOCTONIA SOLANI* IN THE SOIL AS MEASURED BY DIFFERENT PLANTS AND THROUGH COLONIZATION OF JEWS' MALLOW SUBSTRATE

6.1. Introduction and literature review

Many studies have been devoted to the saprophytic life of soilborne organisms associated with root-rot diseases of cultivated plants. The question of how long a particular plant pathogen can survive in soil is very important, but extremely difficult to answer precisely.

The group of fungi brought together in the genus *Rhizoctonia* are widely distributed in the soil, but yet *Rhizoctonia* diseases are not correspondingly prevalent. Since we are dealing in the case of *R. solani* with a pathogen which is very variable as regards all characteristics including pathogenicity, and therefore can be considered a rather complex biological system, it is certain that the disappearance of the pathogen from soil will seldom be abrupt. We can expect a reduction to a low level of survival that may not be detectable by the current methods, but is still sufficient to cause disease under favourable conditions.

Evidently, the major problem in gaining such information is the lack of reliable methods for a direct assay of the pathogen population. Most of the information in the literature on the length of time that plant pathogenic organisms can survive in soil is based on the occurrence of the disease on a host plant. Recently, in many other studies portions of plant tissues have been utilized to demonstrate the presence of pathogens in samples of soils. There is no question that some strains consistently disappear from soils within a few months, whereas others seem to persist indefinitely.

Each organism in the soil is in a state of fluctuating equilibrium with others, regulated by the physical, chemical and biological conditions of the soil. Variation of a factor of the soil condition may change the balance and affect development of a plant pathogen.

Baker (1959) attributed the change to three factors:

1. Stimulation of antagonists in the microflora, consequently the activity of the pathogen is reduced.
2. Suppression or elimination of antagonists and then increase of pathogen activity.
3. Essential destruction of soil microflora, so that the first organism to return luxuriates without competition.

6.1.1. *Overwintering of Rhizoctonia*

The most common way in which the root-infecting fungi survive for long periods in soil is through the development of resting spores or sclerotia (Garrett 1956). Many root-infecting fungi produce sclerotia which serve as efficient survival structures in soil.

Overwintering in the soil is the common feature for *Rhizoctonia,* and since this fungus does not form spores commonly, it is apparent that sclerotia are important in the survival and dissemination of the pathogen. In the early investigations about the existence of the fungus, it was not definitely known whether the fungus exists from one growing season to the next as mycelium, sclerotia or both (MÜLLER 1924, PELTIER 1916, PRATT 1918). As a result of various studies it has now been established that *Rhizoctonia* may exist as mycelium and/or sclerotia.

THORNTON (1956) observed that *Rhizoctonia* occurred in a mycelial state in natural grassland soils, and constituted 49% of his isolates on screened-immersion plates. WARCUP (1955, 1957, 1959) found that *Basidiomycetes, Rhizoctonia* spp., and sterile fungi, especially those with dark-coloured hyphae, formed the main component (70%) of the population of viable hyphae in a field soil survey.

BOOSALIS and SCHAREN (1959) using their assay procedure based on recovery of plant debris fragments, demonstrated that *Rhizoctonia solani* pathogenic to sugar beet persisted in soil in the form of sclerotia on the surface of plant debris particles, and in thick-walled hyphae within such particles. Moreover, they reported that *Rhizoctonia* was detected from soil samples of a sugar beet field 7 months after the crop was harvested.

DANIELS (1963) from her investigation on a field soil where an extensive population of *Rhizoctonia* was known to exist on the roots of a variety of weeds, found only a small amount of the fungus away from plant roots.

Since the sclerotia under favourable conditions germinate to produce new vegetative mycelium, and provide potential foci for dissemination of the organism, factors influencing the production and survival of sclerotia have been studied by several investigators. It became apparent then that sclerotia may be directly or indirectly implicated in the control of diseases caused by this organism. However, the occurrence of the pathogen in non-cultivated soils and the wide range of suscepts of the virulent strains indicates that the soil may be the more important source of inoculum.

Several investigators studied the factors influencing the formation of sclerotia by *Rhizoctonia solani and the survival of such sclerotia* (BRAUN 1930, BRITON-JONES 1925, ELMER 1942, HERZOG and WARTENBERG 1958, HOUSTON 1945, MATSUMOTO 1921–1923, SANFORD 1947–1956, TSIANG 1947, TYNER and SANFORD 1935).

For a complete understanding of the problems concerning persistence a further review of the literature will follow.

6.1.2. *Review of the literature*

Rhizoctonia solani is known to be capable of prolonged saprophytic life in the soil (GARRETT 1950). Other evidence indicates that certain isolates of *R. solani* may depend for survival to some extent upon a parasitic phase. Moreover, BLAIR (1943) and GARRETT (1962) indicated that the fungus is capable of indefinitely prolonged saprophytic life in soils, devoid either of living plant roots or of

recently dead plant material. It, therefore, may presumably be living on residual cellulose in plant remains.

There is much support for the view of BLAIR and GARRETT, by the wide distribution of this pathogen in cultivated fields. Moreover its occurrence in non-cultivated land has been reported from time to time. The evidence obtained by the author in the preceding studies (Chapter 6) is also in entire agreement with its ubiquity.

Several investigators showed that *R. solani* can be suppressed in field soils, by incorporating various agents in the soil. The agents used were: wheatstraw (BLAIR 1943), corn-meal combined with *Trichoderma* (DE BOER 1962), wheat oats or corn-residues (KOMMEDAHL and YOUNG 1956), and maize-meal, dextrose, or sodium nitrate (SANFORD 1947).

DAVEY and PAPAVIZAS (1960) tested four dry, mature amendments alone and in combination with additional nitrogen for the control of *Rhizoctonia solani* and the effect on the competitive saprophytic activity in soil. They reported that the suppression of *R. solani* in soil by mature organic amendments and supplementary nitrogen appears to be related to the general microbial activity in soil, partially related to the C/N ratio of the amendments, but not related to the soil reaction.

HERZOG (1961) demonstrated from his experiments that *R. solani* was retarded in its development by antibiosis in the soil. HERZOG (1961), on the other hand, showed that the growth of *R. solani* may also be stimulated by the action of some substances released by the roots of higher plants. This should result in an increase of the fungus resistance to antibionts or protect it from its own antibionts.

ELMER (1942) found that *Rhizoctonia* from potatoes could persist from year to year as sclerotia and mycelium in the soils of eastern Kansas, but if temperatures during the growing season were too high for sclerotial production and survival of soil-borne mycelium the disease declined in the absence of a susceptible crop immediately succeeding the potatoes.

SANFORD (1952) showed from his experiments that *R. solani* disappeared from heavily infested soils in less than 4 months, when associated with wheat, oats, barley, or maize when replanted every 21 days. When soils were cropped in the greenhouse with susceptible crops i.e. potatoes, beans, or peas, the pathogen can persist a prolonged period up to 8 months. From these results, SANFORD concluded that *R. solani* depends entirely on parasitic nutrition for its persistence in the soil. CHRISTOU (1962) and DANIELS (1963) indicated that a parasitic phase might be important in the persistence of certain strains of *R. solani*.

HERZOG (1961) studied the effect of the rhizosphere of different crop plants on the persistence of *R. solani* in soil. He found that the pathogen was less stimulated in the rhizosphere of *Gramineae*, and more in that of beet and legume crops, whereas it reached its maximum in the rhizosphere of tomato and potato.

ANDERSON and HUBER (1962) on the other hand obtained a high isolation frequency (36 %) from the barley rhizosphere, whereas the isolation frequency from sweet clover, corn, wheat, and sugar beet rhizospheres were less and approximately the same (15–25 %).

FLENTJE and SAKSENA (1957) conducted a field trial in Australia in which they obtained an isolate of *R. solani* from Cungena soil. They inoculated it at a high rate into a field at Waite, Australia, in the presence of a susceptible host. They reported that the organism failed to survive from one season to the next. From field trials carried out by BOYLE (1956), it has been shown that the pathogen *R. solani* causing root rot of peanut disappeared from the soil between crops if the crop refuse was screened from the soil between plantings, but remained active if stems and leaves of peanut, cotton, soybean, or corn were incorporated.

It is now well established that *R. solani* can colonize dead plant tissues as competitive saprophyte, and may exist in soil as distinct clones. In fact, this evidence has been demonstrated by PAPAVIZAS and DAVEY (1959), and EL ZARKA (1963), when they used stem sections of plant tissues as a substrate to trap *Rhizoctonia* from soil. It should be noted that there is a wide variation within the species *R. solani* both as regards pathogenicity and saprophytic growth habits. Undoubtedly, some clones of the fungus have greater ability to survive saprophytically in soil than others, and this feature is not necessarily correlated with a specific parasitic ability.

The early findings of WARCUP (1959) when he observed the hyphae of *Rhizoctonia* in field soil, led TRIBE (1960) and GARRETT (1962) to conclude that *Rhizoctonia* can utilize cellulose film. In further studies on the saprophytic activity of *R. solani*, DANIELS (1963) demonstrated in her experiments the saprophytic ability of the fungus by growing it on processed and native forms of cellulose substrates.

SANFORD (1952), however, indicated that in the absence of vegetation, a heavy soil infestation of *R. solani* in the late autumn practically had disappeared by the following June. Evidently SANFORD used potato planted as a host to measure the persistence of *Rhizoctonia* during his experiments.

According to WINTER (1951), *R. solani* was never found growing in bare soil without a nutrient base. When the nutrient base was exhausted the fungus ceased growth and even by adding organic nutrients to the soil, the mycelium could not be made to grow on again. If, however, the fungus came upon living roots, it surrounded them and grew vigourously in all directions from the nutrient base. WINTER used the inoculation plate method throughout his investigations to isolate the fungus.

BOSCH (1948) observed that the rate of spread of *R. solani* depends upon the density of susceptible hosts. Obviously, *R. solani* cannot extract the substances necessary for its growth from the soil alone, but depends upon substances produced by the living root, although perhaps only supplementary ones.

This was confirmed to a certain extent by BOYLE (1956) who failed to isolate *R. solani* from bare soil to which he added soya-bean hay, even though the organic material was interspersed with it. He, too, came to the conclusion that the spread of the fungus in soil depends on the availability of organic substances. The root systems of grown out peanut plants served as a substrate to detect the presence of *Rhizoctonia* and to measure the incidence of the disease on plants.

Working with isolates of *R. solani*, the causal fungus of sharp eyespot disease of cereals, PITT (1964) indicated that isolates were incapable of prolonged saprophytic survival in bare soil even following an initially high infestation. The pathogen quickly died out in artificially infested soil and showed only limited survival in naturally infected cereal straw buried in soil. This evidence, however, indicates that certain isolates of *R. solani* may depend for survival to some extent upon a parasitic phase. On the other hand, in earlier studies, PELTIER (1916) demonstrated, that *Rhizoctonia* survived in a previously infested soil and on plant parts during the winter season.

The formation of sclerotia in nature is rather common on many hosts. Undoubtedly the sclerotia of *R. solani* play an important role in the survival of the pathogen in nature, and several interesting papers have explored the role of sclerotia in survival of *Rhizoctonia*.

From his investigations, PELTIER (1916) reported that sclerotia and mycelium of *Rhizoctonia* can persist under adverse conditions for 3 years, even for longer, when soil cultures were kept under laboratory conditions.

GADD and BERTUS (1928) showed that sclerotia of *R. solani* could survive for 6 years in the laboratory, and PALO (1926), working with the pathogen from rice found that sclerotia survived burying in soil for 6 months. Working with isolates of *R. solani* from potatoes, HERZOG and WARTENBERG (1958) demonstrated from their experiments that sclerotia in soil remained viable for 5 years. BOOSALIS and SCHAREN (1959) were able to detect viable sclerotia of *Rhizoctonia* causing damping-off and seedling blight of sugar beet, 7 months after harvesting the crop.

PITT (1964) thoroughly investigated the survival of sclerotia formed by the strain *R. solani* responsible for sharp eyespot disease of wheat. His results showed that buried sclerotia of *R. solani* in the soil survived for a period of 6 months when left undisturbed in the laboratory.

The sequence of persistence of *Rhizoctonia* in soil is a matter of conjecture. However, the occurrence of heterokaryosis in *Rhizoctonia solani* Kühn has been suggested by several workers (HAWN and VANTERPOOL 1953, KOTILA 1929, MÜLLER 1924, SAKSENA 1961, SANFORD and SKOROPAD 1955, WHITNEY and PARMETER 1963). BUXTON (1960) from his studies suggested that heterokaryosis can be a possible means of survival in soil-borne parasitic fungi. By forming heterokaryonts, fungi can adjust their genetic capabilities for colonization of many different kinds of substrate.

6.2. MATERIALS AND METHODS

From the study of the literature pertaining to the persistence of *Rhizoctonia solani*, apparently there is some controversy on this subject. Because of these conflicting reports, a large portion of the present studies was devoted to the investigation of the persistence of the fungus to increase our knowledge in this field. Studies have been undertaken with soil planted to various hosts and soil without vegetation. The first series of experiments were conducted in the

greenhouse because plants could be grown throughout the year. To study the persistence under more natural conditions the second series was conducted outdoors.

The persistence of *R. solani* was measured by the following two methods: a comparative disease rating on the stems of crop plants and the degree of colonization of the jews' mallow substrate. In the greenhouse series, two experiments were carried out. The aim of the first experiment was to compare the effect of various crop plants on the persistence of *R. solani*. In the second experiment, which was a repetition of the first one, persistence was determined by both methods.

Five different crops representing different families and known to be host or non-host were selected: potato var. Bintje, pea var. Big Ben, winter rye var. Petkuser, Egyptian clover (*Trifolium alexandrinum* L.), and winter wheat var. Michigan amber. Three cultures were included in this study each representing one group of isolates namely Sc. 8, Sc. 55 and Sc. 3. For the study of persistence during the winter season, only Sc. 8 was used to provide the inoculum.

The soil used was unsterilized sandy soil brought to the laboratory in its natural state. It was found to be free from *R. solani*, and the soil sample was sieved; in this way, all organic matter except traces of short segments or fine roots, were removed. The isolates were grown as previously described. The inoculum – of one month old cultures – was added to the natural unsterilized soil at a ratio of 1:9 inoculum:soil. The artificially inoculated soil was apportioned among the containers as required.

For the potato, the soil was put in 20 cm clay sterilized pots, and for the rest of the hosts and in the winter experiment 10 cm pots were used. The soil moisture in the pots was adjusted to approximately 80 % of their moisture holding capacity. The experiments were carried out in a temperature control chamber at 18°–19°C.

The seeds of the five hosts were surface disinfected prior to planting. The crops were planted at the rate of 20 seed potatoes/pot, 40 seeds for peas, and 80 seeds for the rest of the crops without adding any fertilizer. All treatments were set up in quadruplicate and a randomized block design was used throughout. The plants of every crop were removed after 25 days and successive replantings for each crop were made after a few days. After the fifth replanting, potato seeds were planted in all pots for all crops and these were also pulled after 25 days.

After each replanting the plants of every crop were carefully examined and rated for disease according to a numerical scale of 0 (no disease) to 3 (plants completely girdled). Also the percentage of emergence and the average height of the plants were determined.

For testing the degree of colonization a total of one hundred pieces of the jews' mallow substrate was inserted to the pots of each crop. Directly, after pulling the crop, the pieces were introduced, incubated for 24 hours, and then recovered, treated in the same manner as previously indicated and then plated on cherry agar medium.

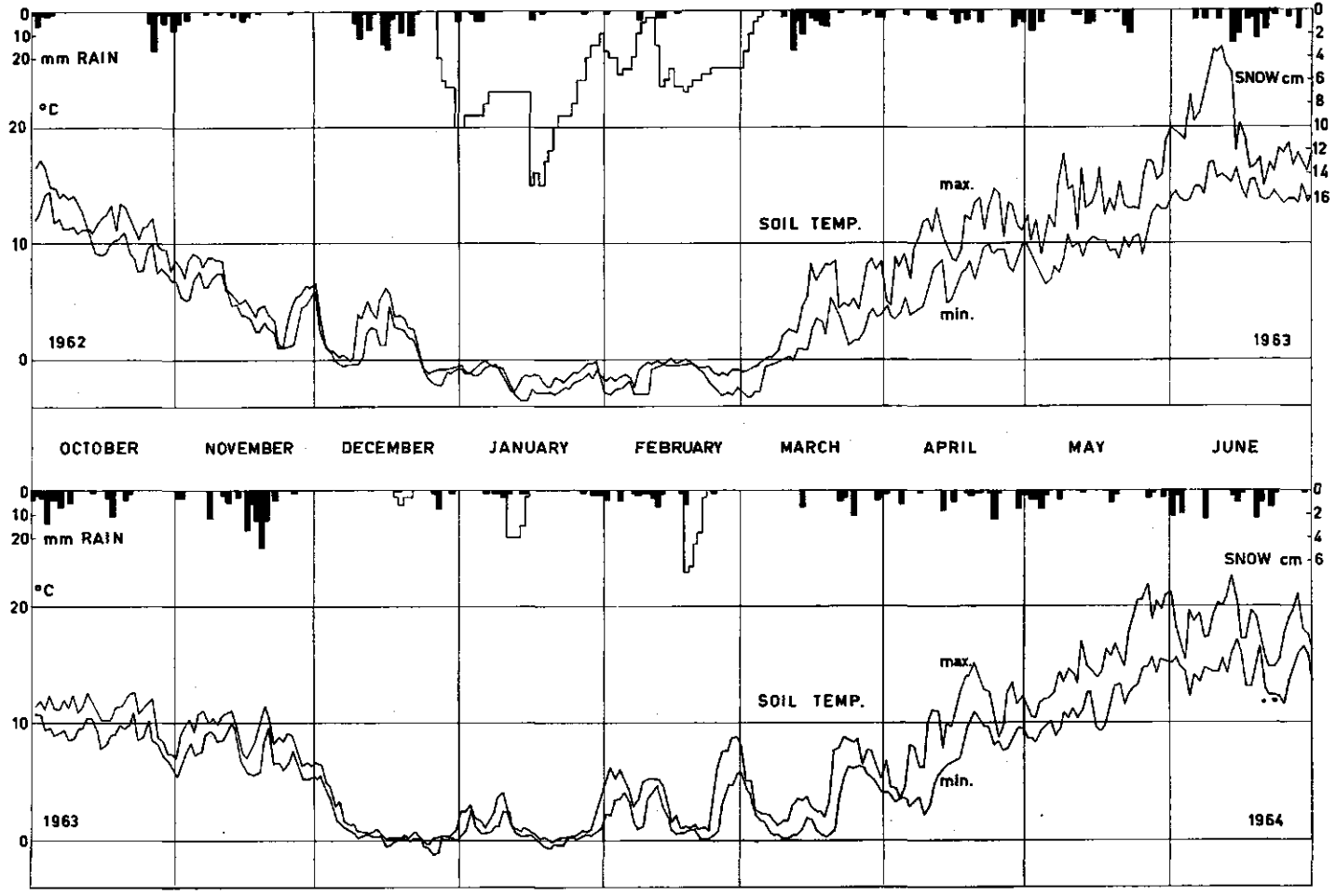

FIG. 7. Seasonal course of some weather factors 1962–1964.
Soil temperature : Temperature at 5 cm below soil surface under short grass in heavy clay soil.
Daily maximum and minimum as recorded by a soil thermograph.
Rain : Rainfall in mm between 09.00 h and 09.00 h of the foregoing day (black histogram)
Snow : Height of snow cover in cm at 09.00 h (open histogram).
(Data obtained from the Institute of Phytopathological Research (I.P.O.) by courtesy of Mr. van Rheenen).

For the outdoor experiment, the pots were tested for percentage isolation of *Rhizoctonia* by colonization of jews' mallow substrate, after a period of two days incubation in the greenhouse. Always 100 % vigorously colonized pieces of the substrate were secured from the pots. Directly after this test, at the beginning of October, the pots were transferred to the field, kept in a trench with the upper edge of the pots 10 cm below the soil surface and covered with polyethylene sheets until needed for test, to prevent quick drying of the soil.

Forty pots were used, and on November 1st, five pots were taken to the laboratory where 20 pieces of the jews' mallow substrate were introduced into each pot. The segments were incubated for 24 hours, after which they were recovered and treated in the same way as previously described. The percent colonization of pieces with *Rhizoctonia* was recorded. The same procedure was followed every month. The last pots were tested at the beginning of June. The percentage of jews' mallow stem pieces colonization was considered as a numerical expression of the persistence of *Rhizoctonia* in soil.

During the experimental period the soil temperature 5 cm below soil surface was recorded and the average monthly degrees were computed. Rainfall and snow were also recorded. This experiment was done twice in successive seasons namely 1962/1963 and 1963/1964 (Fig. 7).

The data obtained are presented in tables 14, 15, 16, 17 and 18.

6.3. Results

Although there is some inconsistency in the experimental results obtained, part of this lack of consistency might be attributed to differences in environmental conditions between pots during the experiments. Still some interesting conclusions may be drawn from the tables.

Considering the potato, it is obvious that the infection index remains more or

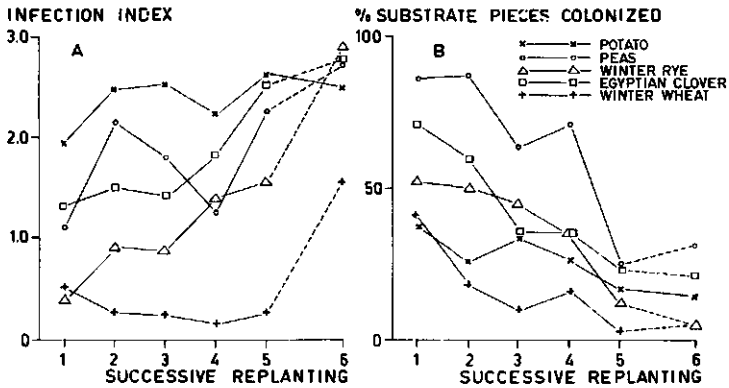

FIG. 8. The effect of successive replantings of 5 different crop plants on the persistence of *R. solani* in inoculated sandy soil. The sixth planting was potato in all series.
A. Persistence measured by infection index.
B. Persistence measured by colonization of jews' mallow after each replanting.

TABLE 14. Percentage of seedling emergence of 5 different crop plants and average height of plants when grown in unsterilized sandy soil inoculated with *R. solani* (1:9 ratio). First experiment.

Sequence	Isolate number	Potato		Pea		Rye		Egyptian clover		Wheat	
		Emergence in %	Height in cm	Emergence in %	Height in cm	Emergence in %	Heigth in cm	Emergence in %	Height in cm	Emergence in %	Height in cm
	Sc. 8	90	21	100	16	100	27	45	6	100	28
1	Sc. 55	100	19	100	14	100	27	45	6	100	30
	Sc. 3	80	11	100	14	100	26	33.7	6	100	28
	Sc. 8	35	22	95	16	99	27	70	5	100	31
2	Sc. 55	100	14	100	15	99	28	51	6	98.7	31
	Sc. 3	45	10	100	15	97	27	45	5	98.7	30
	Sc. 8	60	21	77.5	14	93	27	57.5	6	97	30
3	Sc. 55	60	20	70	15	97	26	45	7	98.7	32
	Sc. 3	40	22	92.5	15	100	27	53.7	7	100	31
	Sc. 8	80	12	97.5	14	95	19	66	6	100	24
4	Sc. 55	90	18	85	17	99	23	60	7	97.5	27
	Sc. 3	75	20	97.5	17	98	20	77.5	7	98.7	25
	Sc. 8	100	18	85	15	95	18	85	7	98.7	20
5	Sc. 55	85	19	80	18	100	21	90	6	97.5	24
	Sc. 3	100	19	95	16	100	19	73.7	7	97.5	23
					Potato planted in all series						
	Sc. 8	90	17	100	19	95	15	100	17	90	16
6	Sc. 55	100	29	100	28	100	21	100	19	100	19
	Sc. 3	100	25	100	23	95	21	100	20	95	18

TABLE 15. Persistence of *R. solani* in a sandy soil as affected by different crops planted in soil inoculated with the pathogen (1:9 ratio). First experiment.

Sequence	Isolate number	infection index of crop				
		Potato	Pea	Rye	Egyptian clover	Wheat
	Sc. 8	1.75	1.48	1.00	1.20	0.00
1	Sc. 55	1.30	1.05	0.50	1.70	0.00
	Sc. 3	1.05	0.67	1.00	1.50	0.00
	Sc. 8	2.45	2.13	1.10	1.46	0.00
2	Sc. 55	1.25	1.30	0.79	1.72	0.02
	Sc. 3	2.70	1.37	1.25	1.59	0.00
	Sc. 8	2.45	1.99	1.20	1.11	0.60
3	Sc. 55	1.35	0.93	0.89	0.69	0.02
	Sc. 3	2.60	0.87	0.60	0.61	0.00
	Sc. 8	2.00	1.77	1.77	2.40	0.60
4	Sc. 55	1.65	0.96	0.96	0.70	0.02
	Sc. 3	2.00	0.90	0.90	0.98	0.60
	Sc. 8	2.20	1.86	1.44	2.59	0.62
5	Sc. 55	1.70	1.55	1.10	1.73	0.48
	Sc. 3	1.95	1.54	1.40	1.92	0.40
		Potato planted in all series				
	Sc. 8	2.60	2.35	2.25	2.35	1.40
6	Sc. 55	1.60	0.65	0.75	0.50	0.00
	Sc. 3	1.45	2.20	1.75	2.25	2.45

less constant from the first to the sixth crop in succession, whereas the colonization experiment clearly indicates a decrease of the colonization percentage. This demonstrates that even the lowest colonization percentage obtained (14 %) is still high enough to give a high infection index (Fig. 8).

After cultivation of the other crops, the infection index remains constant or shows a tendency to increase. An increase is for instance apparent in the second experiment for pea, rye and Egyptian clover, but not for wheat. In no case the infection index decreases, in sharp contrast with the colonization percentage which – as was the case for potato – decreases very clearly for all crops. The lowest infection index is found after wheat. It must be concluded therefore that wheat is not a favourable host for the isolates used in this study. However, it is remarkable that after 5 wheat replantings the infection index on potato is not appreciably lower than after the other crops. The colonization percentage after wheat decreases in the same manner as after the other crops, however, the colonization level is apparently lower than with the other crops. Especially when wheat is compared with pea and Egyptian clover the difference is striking. Potato and rye have an intermediate rank.

When the effects of the three isolates are compared, one might conclude that Sc. 8 has the highest infection index. It may be considered therefore, the

TABLE 16. Percentage of seedling emergence of 5 different crop plants and average height of plants when grown in unsterilized sandy soil inoculated with *Rhizoctonia* (1:9 ratio). Isolate Sc. 8. Second experiment.

Sequence	Potato		Pea		Rye		Egyptian clover		Wheat	
	Emergence in %	Height in cm	Emergence in %	Height in cm	Emergence in %	Height in cm	Emergence in %	Height in cm	Emergence in %	Height in cm
1	92	25.0	98	16.7	96	27.8	92	7.9	99	30.9
2	100	26.6	100	15.4	100	27.6	90	7.9	100	24.6
3	98	25.0	86	14.0	96	20.0	88	5.5	98	23.0
4	84	17.7	74	12.5	100	20.5	73	6.8	100	24.5
5	36	19.5	78	14.3	96	20.3	79	8.6	100	20.8
					Potato planted in all series					
6	56	18.7	40	17.0	64	17.4	36	20.0	64	24.0

TABLE 17. Persistence of *R. solani* in an inoculated sandy soil as indicated by 5 different crop plants and by the number of colonized pieces of jews'mallow substrate. Isolate Sc. 8.

Sequence	Potato		Pea		Rye		Egyptian clover		Wheat	
	Infection index	Colonization in %	Infection index	Colonization in %	Infection index	Colonization in %	Infection index	Colonization in %	Infection index	Colonization in %
1	1.92	37	1.10	86	0.39	52	1.32	71	0.53	41
2	2.48	25	2.14	87	0.93	50	1.50	60	0.28	18
3	2.56	33	1.80	64	0.89	45	1.42	36	0.25	10
4	2.24	26	1.26	71	1.40	35	1.83	35	0.16	16
5	2.64	16	2.26	24	1.58	12	2.25	23	0.39	3
					Potato planted in all series					
6	2.52	14	2.72	31	2.90	5	2.76	21	1.60	5

most virulent strain. Sc. 55 and Sc. 3 have a lower infection index, the pathogenicity of Sc. 55 being on the average a little lower than Sc. 3.

There is no clear indication that one of the isolates might be more or less adapted to one of the hosts tested. The only conclusion that perhaps might be drawn is that Sc. 55 is influenced by the successive replanting of pea, rye, Egyptian clover, and wheat in such a way that its virulence to potato decreases. In the case of potato after five replantings of wheat it apparently even lost its pathogenicity.

With respect to pathogenicity judged by emergence and height of plants, no clear picture could be obtained. In some cases emergence was lower than normal. This is not necessarily attributed to *Rhizoctonia* attack, but might be related partly also to unfavourable soil conditions. The height of the plants is not any more a good measure to judge for the disease severity. A correlation with infection index could not be established.

TABLE 18. Persistence of *R. solani* under field conditions in artificially inoculated bare sandy soil (1:9 ratio). Isolate Sc. 8.

Month	Percent isolation of *Rhizoctonia*	
	1962/1963	1963/1964
October	100	100
November	100	100
December	72	80
January	80	80
February	69	76
March	25	35
April	11	15
May	15	16
June	10	15

The results of the experiments regarding the persistence of *Rhizoctonia* under field conditions are presented in table 18.

The persistence was measured by the rate of colonization of jews' mallow substrate. The results clearly indicate that *Rhizoctonia* can persist for a long period, and can easily stand extreme weather conditions in winter. This is in agreement with other results reported from literature and also of experiments previously described in chapter 4. The winter of 1962/63 was exceptionally cold in the Netherlands as can be seen from figure 7.

Our results are in disagreement with those of SANFORD (1952) who stated that in the absence of potatoes, a heavy soil infestation with *Rhizoctonia* disappeared over winter.

The aspect of heterokaryosis might usefully be considered in the study of the many varied micro-environments that *Rhizoctonia* and other fungi encounter in a complex habitat such as the soil, thus, offer much scope for experiment.

CHAPTER 7

PATHOGENICITY OF *RHIZOCTONIA* SAPROPHYTICALLY EXISTING IN SOIL AND ISOLATED BY COLONIZATION OF JEWS' MALLOW SUBSTRATE

7.1. INTRODUCTION

Since *Rhizoctonia* is known to exist in many, if not most soils, a wide variety of isolates of *Rhizoctonia solani* KÜHN has been obtained during the last fifty years. Several investigators deviced different techniques for detection and isolation of *Rhizoctonia* from soil.

Rhizoctonia solani is known to be extremely variable in both host range and virulence. In fact, this is probably a general feature of soil-inhabitant fungal pathogens. Only rarely a pathogen will be distinctive in appearance, and not associated with non-pathogenic strains. Concerning *R. solani*, it has been shown that there are many non-pathogenic strains in the soil that are morphologically indistinguishable from pathogenic strains.

The methods described so far for detecting *Rhizoctonia* from soil with exception of the methods using living plants, do not distinguish between pathogenic and non-pathogenic strains of *R. solani*. The usual way to overcome this difficulty at present is to perform pathogenicity tests on randomly selected isolates recovered by the particular method.

FLENTJE and SAKSENA (1957) studied the occurrence, distribution and pathogenicity of a wide range of *Rhizoctonia* strains obtained from various infected plants. They came to the conclusion that the species of what they name *Pellicularia filamentosa* = *Rhizoctonia* consists of a wide range of pathogenic strains, varying from specific to one host family to a wide range of families, yet other strains appear to be non-parasitic.

PAPAVIZAS and DAVEY (1962) tested twenty selected clones recovered by their buckwheat colonization method, and representing 10 different naturally infected soils. Their investigation led to the conclusion that the population of *Rhizoctonia* in various soils is composed of a number of morphologically and pathogenically distinct clones.

Later DAVEY and PAPAVIZAS (1962) in a comparative study of four methods for isolation of *Rhizoctonia*, obtained a number of clones of the fungus. The range of pathogenicity of these clones was determined on five species of plants. They observed a wide range in pathogenicity. Clones obtained by the debris particle, buckwheat colonization, and immersion tube methods, ranged from apparently complete saprophytes to strong pathogens. All clones obtained with the infected host method possessed pathogenic capabilities.

The present work was undertaken to determine whether saprophytically existing types which were previously obtained by the jews' mallow colonization method (Table 13) have any pathogenic potentialities.

7.2. Materials and methods

Forty isolates of *Rhizoctonia* selected at random from isolates originating from 24 soils, were included in this study. These isolates were derived from various soils from widely separated geographic areas in the Netherlands (Table 12). Each isolate was preceded by the letter R (= *Rhizoctonia*) to differentiate it from the sclerotial isolates.

Inocula were prepared as previously described (Chapter 3). The inoculum was added to non-sterilized sandy soil believed to be free from *Rhizoctonia* contamination at the rate of 1:9 inoculum:soil on the oven-dry weight basis of the soil. The inoculum was mixed thoroughly with the soil and the infested soil was placed in suitable containers.

Twenty-five surface disinfected seeds each of cabbage, radish and lettuce, 10 seeds of broad beans, and 5 seeds of potatoes were planted in each pot containing the infested soil and the non-infested control soil. Four replicate containers of each host were used with each isolate of *Rhizoctonia* tested. The pots were kept in a greenhouse, and the soil was maintained at about 40 % of its moisture holding capacity which had previously been found by Papavizas and Davey (1961) to be near optimum for both growth of plants and activity of *Rhizoctonia*.

Percentage stand of all crops except potato was determined 14 days after planting. At the end of a 3 weeks growing period all surviving plants were pulled out to record the degree of infection according to an arbitrary scale ranging from 0 (no infection) to 3 (plants completely girdled); plants completely killed before emergence were placed in class 4.

7.3. Results

The results of the inoculum experiments of the 40 isolates of *Rhizoctonia* confirmed the expected wide range of pathogenicity on the 5 hosts tested (Table 19). The following designations will be used.

a. infection index 0.1–0.9 pathogenicity low
b. infection index 1.0–1.9 pathogenicity moderate
c. infection index 2.0–2.9 pathogenicity high
d. infection index 3.0–4.0 pathogenicity very high.

With regard to potato, the isolates ranged from non-pathogenic to highly pathogenic (highest infection index 2.8). On the other crops all isolates showed more or less severe infection, indicating – with only a few exceptions – a moderate to very high pathogenicity under the conditions of the experiments.

On potato only 4 isolates showed high pathogenicity (infection index > 2.0), whereas 12 isolates were non-pathogenic or showed only very slight infection (infection index 0.0–0.4). When the average infection index on the other hosts was determined separately for the 4 isolates most pathogenic, for the 12 isolates non-pathogenic on potato and for the 24 remaining isolates, it is apparent that

TABLE 19. Pathogenicity of 40 isolates of *Rhizoctonia* obtained by the jews'mallow substrate from 24 soils as determined by percentage of emergence and infection indices of 5 different crop plants.

Isolate number	Emergence in %					Infection index				
	Cabbage	Radish	Lettuce	Bean	Potato	Cabbage	Radish	Lettuce	Bean	Potato
R 1	77	89	77	88	100	1.11	2.45	1.36	3.31	1.0
R 2	67	92	71	100	100	1.54	1.85	2.00	2.66	1.5
R 4	59	83	49	78	100	1.70	2.05	2.20	3.03	2.2
R 5	46	83	61	70	100	2.18	2.01	1.83	3.20	2.7
R 7	56	82	73	82	100	2.58	2.26	1.56	3.12	2.7
R 8	72	92	69	95	100	1.61	1.43	1.86	3.01	2.8
R 11	58	82	63	90	100	1.76	1.39	1.80	3.08	0.4
R 12	57	86	65	42	100	2.00	1.78	1.79	3.74	0.2
R 13	64	75	73	15	100	1.86	2.23	1.06	3.73	0.4
R 14	64	86	50	98	100	1.82	1.23	2.24	2.90	1.6
R 15	59	83	9	90	100	2.67	1.85	3.80	2.66	1.6
R 16	69	93	73	88	100	1.84	0.57	1.12	2.63	1.3
R 17	86	92	83	95	100	1.20	0.48	0.72	2.48	1.0
R 18	65	93	55	80	100	2.22	0.97	2.50	2.73	1.6
R 19	0	0	68	80	100	4.00	4.00	1.28	2.76	1.0
R 20	21	10	86	85	100	3.28	3.65	0.92	2.66	1.5
R 21	93	93	73	90	100	0.95	1.48	0.88	2.84	0.8
R 23	54	95	36	88	100	2.92	2.65	3.20	3.37	0.4
R 24	52	40	8	85	100	3.64	2.41	3.95	3.29	0
R 25	72	78	66	45	100	1.67	1.94	2.93	3.73	0

TABLE 19. continued

Isolate number	Emergence in %					Infection index				
	Cabbage	Radish	Lettuce	Bean	Potato	Cabbage	Radish	Lettuce	Bean	Potato
R 27	0	0	84	85	100	4.00	4.00	0.12	2.83	0
R 28	68	82	69	80	100	2.20	3.21	1.20	3.12	1.0
R 29	63	77	51	85	100	1.88	1.85	1.79	2.64	0
R 30	67	76	61	85	100	1.48	2.65	1.75	3.04	1.0
R 31	58	82	65	78	100	1.73	2.59	1.44	3.04	0
R 33	67	81	55	62	100	1.48	1.36	1.80	3.20	0
R 34	80	78	58	90	100	2.56	3.06	2.88	2.87	1.0
R 36	66	85	61	68	100	1.94	1.72	2.04	2.90	0
R 37	86	79	65	88	100	3.02	2.97	2.38	3.05	0
R 39	68	71	67	75	100	2.17	2.64	2.42	3.21	1.4
R 40	68	82	46	58	100	2.25	1.78	2.68	3.12	1.4
R 41	6	0	67	85	100	3.92	3.99	2.28	2.94	1.8
R 42	7	2	57	72	100	3.73	3.93	3.45	3.01	1.9
R 44	79	66	22	75	100	1.79	2.45	3.91	3.00	1.3
R 45	14	2	61	75	100	4.00	4.00	2.75	3.52	1.5
R 47	91	83	54	88	100	1.31	3.26	2.77	3.04	1.5
R 48	75	20	69	72	100	1.97	3.86	2.59	2.94	1.6
R 49	63	67	66	60	100	1.68	3.18	2.40	3.32	1.4
R 51	75	60	50	92	100	2.11	2.82	3.51	2.84	0.8
R 53	58	61	74	68	100	2.63	3.04	2.65	3.20	1.6
None control	100	100	100	100	100	0	0	0	0	0

there exists no correlation between pathogenicity or lack of pathogenicity on potato and that on the other 4 hosts (Table 20).

TABLE 20. Grouping of the 40 isolates according to their pathogenicity on the different crops. The data are compiled from table 19.

Number of isolates	Highly pathogenic on	Infection index	Average infection index				
			Cabbage	Radish	Lettuce	Beans	Potato
8	Cabbage	≧ 3.0	3.7	3.6	2.2	3.0	1.0
32		< 3.0	1.9	2.1	2.2	3.0	1.1
12	Radish	≧ 3.0	2.9	3.6	2.1	2.6	1.3
28		< 3.0	2.0	1.9	2.1	3.0	1.0
6	Lettuce	≧ 3.0	2.8	2.7	3.6	3.0	1.0
34		< 3.0	2.2	2.4	1.9	3.0	1.1
24	Beans	≧ 3.0	2.2	2.5	2.3	3.2	1.1
16		< 3.0	2.4	2.4	1.9	2.8	1.0
4	Potato	≧ 2.0	2.0	1.9	1.9	3.1	2.6
24		0.5–1.9	2.3	2.7	2.2	2.9	1.2
12	[1]	< 0.5	2.3	2.0	2.0	3.2	0.1
40	–	–	2.2	2.4	2.1	3.0	1.1

[1] Isolates non-pathogenic on potato.

As regards beans, pathogenicity was high to very high and no great variation occurred between isolates. The infection indices varied between 2.5 and 3.7. When isolates were divided into two groups, one with a pathogenicity as high as or higher than 3.0, and the other with a pathogenicity lower than 3.0, no correlation could be demonstrated between pathogenicity on beans and that on the other hosts.

With respect to lettuce, 6 isolates were very highly pathogenic on this crop (index 3.0 or higher). As can be seen from table 20, on the average no correlation exists between pathogenicity on lettuce and that on the other hosts. However, two isolates when considered separately, were an exception. R 42 combined a very high phatogenicity on lettuce with a very hig pathogenicity on cabbage and radish, and R 24 was very highly pathogenic on lettuce and on cabbage.

The results of the inoculation experiments on cabbage and radish can be considered together, because there is apparently a rather close correlation between the pathogenicity on both hosts. Out of the 40 isolates, 14 isolates were very highly pathogenic on one or both hosts. From these, 6 isolates showed very high pathogenicity on both hosts, 2 only on cabbage and a high pathogenicity on radish and 6 only on radish and a high or moderate pathogenicity on cabbage. There is no correlation between the pathogenicity on radish and/or cabbage on the one side and that on the other hosts on the other side. As already mentioned under lettuce strains R 24 and R 42 were an exception.

From the foregoing results, it might be concluded, that there are indications for a certain specialization of the isolates on different hosts. With the restriction

that the specialization is not absolute, one can distinguish between a cruciferous strain, a lettuce strain, and a potato strain. This would be in agreement with the results previously obtained by FLENTJE and SAKSENA (1957) and with some other investigators. It must be kept in mind, however, that the strains obtained by FLENTJE and SAKSENA showed much more specialization. Differences in experimental results may be also attributed to differences in the conditions under which the experiments were carried out.

It must be noted that, whereas 12 isolates were non-pathogenic on potato or nearly so, all isolates were more or less pathogenic on the other 4 hosts tested, with the exception of R 27 which was only very slightly pathogenic on lettuce.

SUMMARY

The black scurf disease of potato incited by *Rhizoctonia solani* KÜHN [= *Thanatephorus cucumeris* (FRANK), DONK], which is practically present in all potato growing areas, is considered one of the most important diseases of the potato.

In chapter 1, some general aspects of the disease are discussed, in particular the nomenclature of the causal organism. Special attention has been paid to the historical accounts and the most important literature was reviewed, with the conclusion that the pathogen should be named *Thanatephorus cucumeris* (FRANK) DONK for the basidial stage. For convenience sake, however, the names *Rhizoctonia* or *R. solani* which are generally applied in the literature are used in this work.

In chapter 2, some physiological aspects were investigated, with respect to the effect of temperature, of various media and of some antimicrobial agents on the growth rate of the fungus.

Several hundreds of isolates from sclerotia on potato were obtained, 15 isolates being selected from them for further studies (Table 1). The selection was made on the basis of cultural characters. The isolates were separated into three culture types designated as group I, II and III based upon the following cultural character: I. Very little or no aerial mycelium, II. Abundant aerial mycelium and III. Intermediate growth habit. The isolates showed an optimum temperature between 22° and 24°C; the minimum was between 3.5° and 10°C, and the maximum was 30° (Fig. 1, 2, 3). Neither optimum temperature nor growth rate were correlated with growth habit. The influence of 3 different media, viz. cherry agar, potato-dextrose agar and Czapek-dox agar (Modified) was studied at 24° and 28°C (Table 2). At both temperatures PDA generally gave the highest, and cherry agar the lowest growth rate.

Antibiotics and other antimicrobial agents have been used for differentiation between strains, and their use in isolating soilborne phytopathogenic fungi has been suggested by several workers. In the present investigation, two broad spectrum antibiotics (terramycin hydrochloride, and streptomycin sulfate); and two other antimicrobial agents (oxgall, and boric acid) were incorporated in four different media. Neither terramycin nor streptomycin suppressed the growth of *Rhizoctonia* when incorporated with the four media. The linear growth of the three isolates tested was reduced by oxgall and boric acid at 1 g/l on peptone agar, and cherry agar, but not on PDA or Cz.-dox (M) agar (Table 3). It appears that differentiation between strains of *R. solani* cannot be based on the sensitivity obtained by the toxic materials used.

In chapter 3, the 15 isolates were tested for their pathogenicity on potato. The results (Table 4) were similar to those obtained by other investigators. They show that the majority of the isolates produced light to moderate infection, and no heavy infection occurred. No correlation between type of isolate and pathogenicity could be demonstrated.

Chapter 4 deals with the method, used throughout these studies for the iso-

lation of *R. solani* from soil and with different experiments concerning its applicability. A comprehensive review of literature about the different methods previously described forms the first part of this chapter. The method itself which proved to be rapid, simple and reliable, can be described as follows. The dried stem pieces of the jews' mallow plant were used as a substrate for colonization by *Rhizoctonia*. The stems were cut into small sections, 8 to 10 mm long, and a stainless steel insect pin was inserted perpendicularly through each piece and then placed in the test soil nearly to full length with the head-end down. After a certain period of exposure in the soil, the pins were recovered and the stem pieces were treated in a special way and plated on cherry agar medium for identification of the fungus which grows from the colonized plant tissue.

From experiments, it appeared that *R. solani* can already be isolated from jews' mallow stems after a substrate incubation period of 12 hours. After 24 hours substrate incubation it can be recovered from all pieces (Table 5). Likewise, a high percentage of *Rhizoctonia* can be obtained after 2 and 4 days of substrate incubation (Table 6). After a prolonged period of incubation, however the percentages of *Rhizoctonia* recovered from the colonized pieces showed a continuous decrease. After 90 days it was isolated only to a very low percentage (Table 6).

For isolation of *Rhizoctonia* from the colonized substrate, cherry agar and water agar proved to be the most suitable medium as can be seen from table 7. After one hour some growth developed, and after two hours already from a high percentage of pieces, growth of *R. solani* could be observed. For serial work 16–18 hours incubation of the stem pieces on the medium proved to be the most convenient.

To test whether the jews' mallow stem method gives also good results at low and very low inoculum:soil ratios, an experiment was carried out with different inoculum:soil mixtures. As can be seen from tables 8 and 9 and figure 4, the percent isolation of *R. solani* decreases with decreasing inoculum:soil ratios, but even at a ratio of 0.01:99.99, the fungus could be recovered from the stem pieces for 83 % after substrate incubation periods of 2 and 4 days. From table 10 and fig. 5, it may be concluded that isolate Sc. 55 has a greater competitive saprophytic ability than isolates Sc. 3 and Sc. 8. At low and very low inoculum: soil ratios, the percentage recovery of isolate Sc. 55 was much higher than that of the two other isolates. An experiment was set up to investigate the effect of a prolonged period of inoculum:soil mixtures before introducing the jews' mallow stem pieces. Normally the mixtures were kept for 2 days before inserting the substrate. The results given in table 11 and fig. 6 indicate that *R. solani* decreased continuously. However, at the lowest inoculum:soil ratios even after 180 days, *Rhizoctonia* could be recovered.

In chapter 5, trials were performed to isolate *R. solani* from naturally infested soils from widely separated areas in the Netherlands, in an attempt to compare the inoculum levels in various fields (Table 12). As can be seen from table 13 the frequency of isolation of *Rhizoctonia* – apparently *R. solani* in all cases – from 24 naturally infested soils varies greatly. Only one soil sample did not give any

colony, whereas 5 samples gave a percentage between 25 and 37. The other samples were in between. From a comparison of tables 12 and 13, it is clear that the frequency of isolation is not related in a simple way with crop rotation, the immediate previous crop, soil type or region. It must be assumed that frequency of isolation depends on the combined effect of the above-mentioned factors, in addition to other factors as time of soil sampling, content of organic material in the soil, and the types of *Rhizoctonia* existing in the soil.

From the naturally infested soils tested, several morphologically distinct types could be recovered. Forty-nine types could be distinguished on cherry agar medium. Soils with the highest percentage of colonization are not necessarily the soils which contain the highest number of morphological types as can be seen from table 13. This is not in agreement with PAPAVIZAS and DAVEY (1962).

To study the persistence of *R. solani* in soil two series of experiments were carried out, which are described in chapter 6. In both series unsterilized natural soil, inoculated with *R. solani* was used. In the first series which was carried out in the greenhouse 5 different crops including potato were grown, each crop five times in succession. In the first experiment the degree of attack (infection index) was used as a measure for the degree of infestation of the soil. In the second experiment the degree of attack as well as the colonization percentage of the jews' mallow substrate after the crop had been lifted were used (Tables 14, 15, 16 and 17, and Fig. 8). Results indicate that-irrespective of the crop – the infection index remained more or less constant or showed tendency to increase. Wheat showed the lowest infection index throughout. After the last replanting potatoes were planted in all cases. It increased the infection index still more. In contrast with the infection index, the percentage of colonization which was initially rather high decreased after the fifth successive plantings to a low or rather low level.

These results make probable that the degree of attack (infection index) is a poor standard for the inoculum level in the soil. The experiments also demonstrate that even at a low colonization percentage, a relatively high infection index was obtained. In the second series conducted during two seasons outdoors under field conditions from October to June, the soil was not planted. The colonization percentage, which was initially 100 %, dropped to 10 and 15 respectively. One of the winters was extremely cold. From these experiments, it can be concluded that *R. solani* can easily stand adverse winter conditions and persists in the soil for at least nine months (Table 18 and Fig. 7).

In chapter 7, forty selected isolates representing 24 soils were tested for pathogenicity on five different host plants including potato. The results show that a wide range of infection indexes was obtained (Table 19). The isolates with a high pathogenicity on potato were not exceptionally pathogenic on the other host plants tested. It indicates that there is no correlation between a high pathogenicity on potato and on other hosts. The same is true – with a few exceptions – for isolates with a high pathogenicity on lettuce and for those with a high pathogenicity on cabbage and lettuce (Table 20). It indicates that one can distinguish between a potato, a lettuce and a cruciferous strain, which is in agreement with the results obtained by FLENTJE and SAKSENA (1957) and others.

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SAMENVATTING

Aardappelschurft veroorzaakt door *Rhizoctonia solani* KÜHN [= *Thanatephorus cucumeris* (FRANK), DONK], die praktisch overal waar aardappelen verbouwd worden voorkomt, wordt als een van de belangrijkste ziekten van de aardappel beschouwd.

In hoofdstuk 1 worden enkele algemene aspecten van de ziekte besproken, in het bijzonder de nomenclatuur van de schimmel, die de ziekte veroorzaakt. Bijzondere aandacht werd besteed aan de historie van deze ziekte, en een overzicht werd gegeven van de belangrijkste literatuur, met de conclusie, dat het pathogeen *Thanatephorus cucumeris* (FRANK) DONK genoemd moet worden voor wat betreft het perfecte stadium. Voor het gemak is evenwel de in de literatuur gangbare naam *Rhizoctonia* of *R. solani* gebruikt in dit werk.

In hoofdstuk 2 werden enkele physiologische aspecten onderzocht met betrekking tot het effect van de temperatuur, van verschillende media en van enkele antimicrobiële stoffen op de groei van de schimmel.

Uit sclerotiën op aardappelknollen werden enkele honderden isolaties verkregen, waarvan er 15 werden uitgezocht voor verder onderzoek (Tabel 1). De selectie werd gemaakt op basis van cultuureigenschappen. De isolaties werden onderverdeeld in 3 cultuur-types, aangeduid als groep I, II en III, gebaseerd op de volgende eigenschap: I. Zeer weinig of geen luchtmycelium, II. Overvloedig luchtmycelium en III. Tussenvorm tussen groep I en groep II. De optimum temperatuur van de isolaties lag tussen 22 en 24 °C, de minimum temperatuur tussen 3,5 en 10 °C en de maximum temperatuur was 30 °C (Fig. 1, 2, 3).

De optimum temperatuur noch de mate van groei waren gecorreleerd met cultuur-type. De invloed van 3 verschillende media, n.l. kersenagar, aardappel dextrose agar en Czapek-dox agar (gewijzigd) werden bestudeerd bij 24 en 28 °C (Tabel 2). Bij beide temperaturen gaf aardappelglucose agar betere groei dan kersenagar.

Antibiotica en andere antimicrobiële stoffen zijn gebruikt om stammen van elkaar te onderscheiden, en de mogelijkheid ze toe te passen bij isolatie van phytopathogene bodemschimmels is door verschillende onderzoekers geopperd. In dit onderzoek zijn twee breed spectrum antibiotica (terramycine hydrochloride en streptomycine sulfaat) en twee andere antimicrobiële stoffen (osse-gal en boorzuur) geïncorporeerd in vier verschillende media. Terramycine noch streptomycine onderdrukten de groei van *Rhizoctonia* na incorporatie in de vier media. De lineaire groei van de drie getoetste isolaties werd gereduceerd door osse-gal en boorzuur, 1 g/l in pepton agar en kersenager, maar niet in aardappelglucose agar of Czapek-dox agar (Tabel 3). Differentiatie tussen stammen van *R. solani* op basis van hun gevoeligheid voor de gebezigde toxische stoffen is blijkbaar niet mogelijk.

In hoofdstuk 3 werden de 15 isolaties getoetst op pathogeniteit voor de aardappel. De resultaten (Tabel 4) waren gelijk aan die verkregen door andere onderzoekers. De meerderheid der isolaties bleken lichte tot matige infectie teweeg te

brengen, en zware infectie trad niet op. Er kon geen correlatie aangetoond worden tussen cultuurtype en pathogeniteit.

Hoofdstuk 4 behandelt de methode, die in dit onderzoek gebruikt is voor de isolatie van *R. solani* uit grond, en de verschillende proeven betreffende de bruikbaarheid ervan. Een summier overzicht van de literatuur betreffende de verschillende vroeger beschreven methoden vormt het eerste deel van dit hoofdstuk. De methode zelf, die snel, eenvoudig en betrouwbaar bleek, kan als volgt beschreven worden. Stukjes gedroogde stengel van de 'jews' mallow' plant werden gebruikt als substraat voor kolonisatie door *Rhizoctonia*. De stengels werden in smalle delen gesneden, 8 tot 10 mm lang, en een insectenspeld van roestvrij staal werd loodrecht door elk stukje geprikt en daarna in de te onderzoeken grond geplaatst, bijna over de volle lengte, met het kop-einde naar beneden. Na een bepaalde tijd werden de spelden weer uit de grond gehaald en de stengelstukjes op een bepaalde manier behandeld en uitgelegd op kersenagar ter identificatie van de schimmel, die uit het gekoloniseerde planteweefsel groeit.

Uit proeven bleek, dat *R. solani* reeds uit 'jews' mallow' stengels geïsoleerd kan worden na 12 uur incubatie van het substraat. Na 24 uur incubatie van het substraat kan het uit alle stukjes geïsoleerd worden (Tabel 5). Evenzo kan een hoog percentage *Rhizoctonia* verkregen worden na 2 en 4 dagen incubatie van het substraat (Tabel 6). Na een langere incubatieperiode, evenwel, toonde het percentage *Rhizoctonia*, dat uit de gekoloniseerde stukjes geïsoleerd kan worden een geleidelijke daling. Na 90 dagen werd slechts een zeer laag percentage geïsoleerd (Tabel 6).

Voor isolatie van *Rhizoctonia* uit gekoloniseerd substraat, bleken kersenagar en wateragar de meest geschikte media, zoals blijkt uit tabel 7. Na één uur trad reeds enige groei op, en na twee uur kon op een groot percentage stukjes groei van *R. solani* waargenomen worden. Voor seriewerk bleek 16–18 uur incubatie van de stengelstukjes het meest geschikt.

Om na te gaan of de methode met 'jews' mallow' stengels ook goede resultaten geeft bij lage en zeer lage inoculum-grond verhoudingen, werd een proef uitgevoerd met verschillende inoculum-grondmengsels. Zoals blijkt uit de tabellen 8 en 9 en uit figuur 4, neemt het percentage *Rhizoctonia* isolaties af met afnemende inoculum-grond verhoudingen, maar zelfs bij een verhouding van 0.01:99.99 kon de schimmel uit 83 % der stengelstukjes geïsoleerd worden na incubatieperioden van het substraat van 2 en 4 dagen. Uit tabel 10 en figuur 5 kan geconcludeerd worden dat isolatie Sc. 55 een groter competitief saprophytisch vermogen heeft dan isolaties Sc. 3 en Sc. 8. Bij lage en zeer lage inoculum-grond verhoudingen was het percentage geslaagde isolaties van Sc. 55 veel hoger dan dat van de beide andere stammen. Normaal werden de inoculum-grond mengsels twee dagen bewaard voordat het substraat er in aangebracht werd. Wanneer de duur van deze periode verlengd werd, nam *R. solani* geleidelijk af (Tabel 11, Fig. 6). Evenwel kon bij de laagste inoculum-grond verhoudingen zelfs na 180 dagen nog *Rhizoctonia* geïsoleerd worden.

In hoofdstuk 5 werden proeven uitgevoerd om *R. solani* uit natuurlijk besmette grond te isoleren, afkomstig van uiteenliggende plaatsen in Nederland, ten-

einde de inoculum niveaus in verschillende percelen te vergelijken (Tabel 12). Zoals blijkt uit tabel 13, schommelt de frequentie van isolatie van *Rhizoctonia* – klaarblijkelijk in alle gevallen *R. solani* – uit 24 natuurlijk besmette gronden aanzienlijk. Slechts één grondmonster gaf geen enkele kolonie, terwijl 5 monsters een percentage gaven tussen 25 en 37. De andere monsters lagen hier tussenin. Uit vergelijking van tabel 12 met tabel 13 is het duidelijk dat de frequentie van isolatie niet op een eenvoudige wijze samenhangt met vruchtwisseling, gewas van het voorafgaande jaar, bodemtype of streek. Aangenomen moet worden dat de frequentie van isolatie afhangt van het gezamenlijk effect van de bovengenoemde factoren, zoals tijdstip van monsterneming, gehalte aan organisch materiaal in de grond, en de types van *Rhizoctonia*, die in de bodem voorkomen.

Uit de natuurlijk besmette grond konden verschillende morphologisch van elkaar te onderscheiden types geïsoleerd worden. Op kersenagar waren negen en veertig types te onderscheiden. Grond met het hoogste percentage kolonisatie hoeft niet noodzakelijkerwijze ook het grootste aantal morphologische types te bevatten, zoals blijkt uit tabel 13. Dit is niet in overeenstemming met de resultaten verkregen door PAPAVIZAS en DAVEY (1962).

Om het overblijven van *R. solani* in grond te bestuderen werden twee series experimenten uitgevoerd, die worden beschreven in hoofdstuk 6. In beide series werd niet gesteriliseerde, natuurlijke grond gebruikt, die geïnoculeerd werd met *R. solani*. In de eerste serie, die in de kas uitgevoerd werd, werden vijf verschillende gewassen opgekweekt, inbegrepen aardappelen, en elk gewas vijf maal achter elkaar. In de eerste proef werd de mate van aantasting (ziekte-index) gebruikt als een maat voor besmetting van de grond. In de tweede proef werden zowel de mate van aantasting als het percentage kolonisatie van de 'jews'mallow' stukjes na verwijderen der planten gebruikt (Tabellen 14, 15, 16 en 17, Fig. 8). De resultaten wijzen erop dat – onafhankelijk van het gewas – de ziekte-index min of meer constant bleef of een tendens tot toeneming vertoonde. Tarwe gaf in het algemeen de laagste ziekte-index. Nadat de laatste planten verwijderd waren, werden in alle gevallen aardappelen gepoot. Dit deed de ziekte-index nog meer toenemen. In tegenstelling tot de ziekte-index, nam het percentage kolonisatie, dat aanvankelijk nogal hoog lag, na de vijf opeenvolgende gewassen af tot een laag of nogal laag niveau. Deze resultaten maken het waarschijnlijk dat de mate van aantasting (ziekte-index) onvoldoende informatie geeft omtrent het inoculum niveau in de grond. De proeven tonen ook aan dat zelfs bij een laag kolonisatie percentage een betrekkelijk hoge ziekte-index werd verkregen.

In de tweede serie, gedurende twee seizoenen uitgevoerd buiten de kas onder veld-omstandigheden van oktober tot juni, bleef de grond braak. Het kolonisatiepercentage, dat aanvankelijk 100 % bedroeg, viel terug tot 10, respectievelijk 15 %. Een der beide winters was bijzonder koud. Uit deze proeven kon geconcludeerd worden dat *R. solani* strenge winter omstandigheden gemakkelijk kan doorstaan, en gedurende ten minste 9 maanden in de grond kan overblijven (Tabel 18, Fig. 7).

In hoofdstuk 7 werden 40 geselecteerde isolaties uit 24 gronden getoetst op pathogeniteit voor vijf verschillende waardplanten, de aardappel inbegrepen.

Uit de resultaten blijkt een grote fluctuatie van de ziekte-index (Tabel 19). De isolaties met een grote pathogeniteit voor aardappel waren niet bijzonder pathogeen voor de andere onderzochte waardplanten. Dit duidt erop dat er geen correlatie is tussen hoge pathogeniteit voor aardappel en voor andere waardplanten.

Hetzelfde geldt – op een paar uitzonderingen na – voor de isolaties met een hoge pathogeniteit voor sla en voor die met een hoge pathogeniteit voor kool en sla (Tabel 20). Het wijst er verder op dat onderscheid gemaakt kan worden tussen een aardappel-, een sla- en cruciferenstam, wat in overeenstemming is met de resultaten, verkregen door FLENTJE en SAKSENA (1957) en andere onderzoekers.

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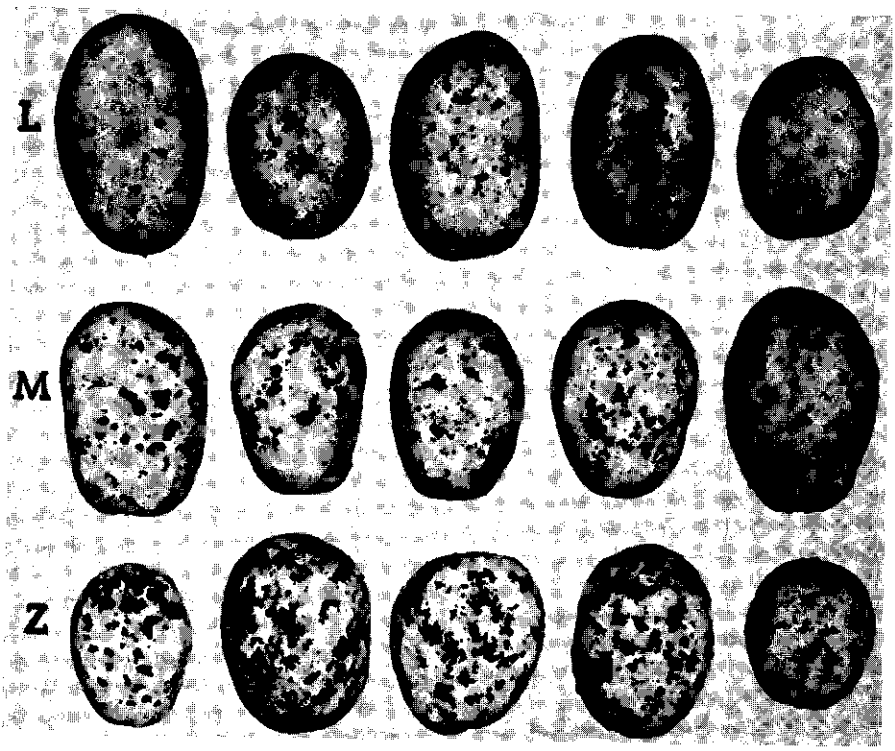

PLATE 1. Formation of sclerotia of *R. solani* on potato tubers.
L. light contamination
M. moderate contamination
Z. heavy contamination.

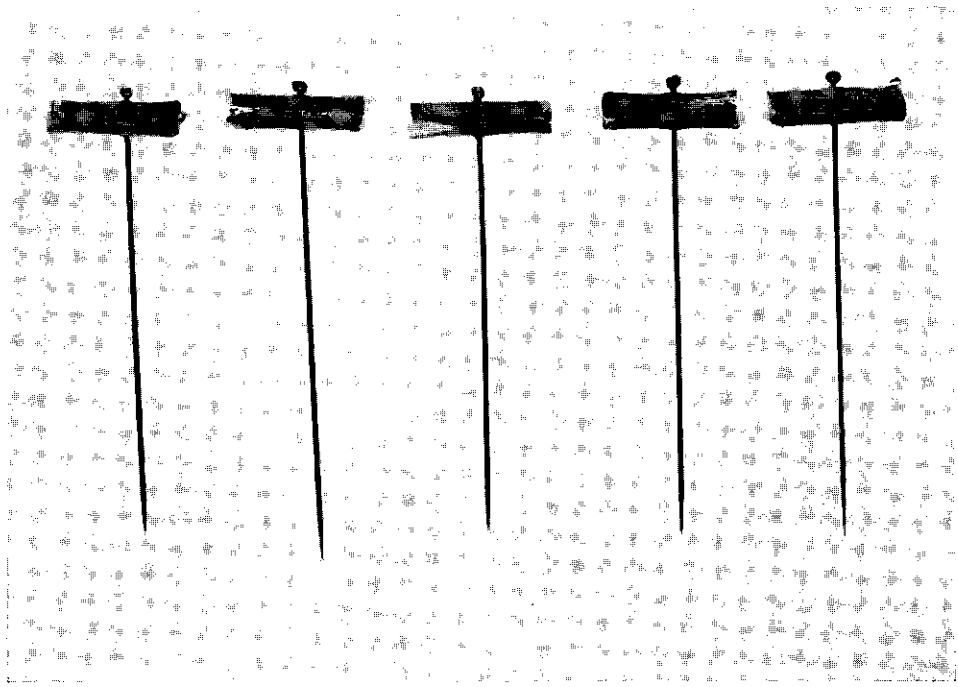

PLATE 2. Mature jews'mallow stem segments attached to stainless steel insect pins ready for use.

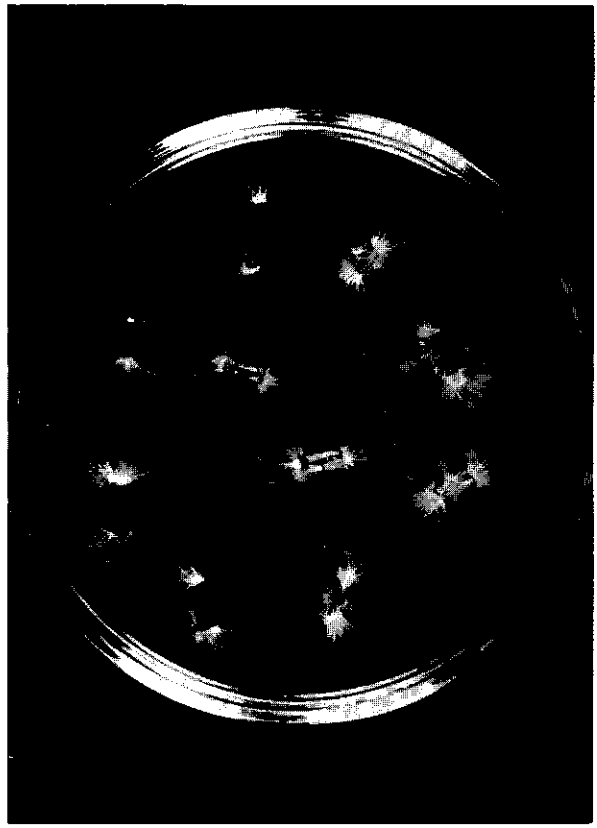

PLATE 3. Colonized stem segments of jews'mallow by *R. solani* showing growth of the fungus after 18 hours incubation on cherry agar medium.