RHIZOCTONIA SOLANI AS A COMPONENT IN THE BOTTOM ROT COMPLEX OF GLASSHOUSE LETTUCE

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RHIZOCTONIA SOLANI AS A COMPONENT IN THE BOTTOM ROT COMPLEX OF GLASSHOUSE LETTUCE

Proefschrift
ter verkrijging van de graad van
doctor in de landbouwwetenschappen,
op gezag van de rector magnificus,
dr. C.C. Oosterlee,
hoogleraar in de veeteeltwetenschap,
in het openbaar te verdedigen
op woensdag 14 september 1983
des namiddags te vier uur in de aula
van de Landbouwhogeschool te Wageningen.

ISN= 19213 4 - 03

This thesis is also published as Verslagen en Mededelingen
van de Plantenziektenkundige Dienst
te Wageningen nr. 160, 1983

STELLINGEN

I

Afwezigheid van directe nevenwerking van bestrijdingsmiddelen op nuttige organismen kan onder laboratoriumomstandigheden worden vastgesteld.

II

Gegevens over de inoculum-dichtheid van Rhizoctonia solani in de grond vóór de slateelt hebben ziektevoorspellende waarde.

Dit proefschrift

III

De beschikbaarheid van effectieve bestrijdingsmiddelen betekent niet dat de behoefte aan resistente rassen zou verminderen.

IV

De anastomose-conceptie verdient meer aandacht bij het onderzoek naar de bestrijding van en de veredeling tegen Rhizoctonia solani.

N.A. Anderson, 1982. Ann. Rev. Phytopathol. 20: 329 - 347.
Dit proefschrift

V

In het kader van de optimalisering van het gebruik van bestrijdingsmiddelen dient bij de toelating de mate van resistentie van de gewassen betrokken te worden.

VI

Bateman's brede conceptie van de "dynamic nature of plant disease" en zijn "multiple-component" hypothese over pathogenese en parasitisme kunnen zinvolle bijdragen vormen in de discussie over "gangbare" en "alternatieve" planteziektenkunde en gewasbescherming.

D.F. Bateman, 1978. In: Plant Disease, vol. III, edited by
J.G. Horsfall and E.B. Cowling, p. 53-83. Academic Press, New York.

VII

De omvang van het pakket eisen, dat door de overheid wordt gesteld met betrekking tot de risico's voor de volksgezondheid en het milieu in het kader van de toelating van biologische middelen, zoals schimmel-, bacterie- en viruspreparaten, staat de ontwikkeling van deze middelen in de weg.

VIII

De gedachte dat bij nieuwe teeltwijzen, zoals de steenwol- en voedingsfilmtechniek, ziekten en plagen verminderen is in zoverre onvolledig, dat deze teeltwijzen ook specifieke gewasbeschermingsproblemen met zich meebrengen.

IX

Het is af te keuren dat een sociale indicatie nog steeds geen criterium vormt voor formele arbeidsongeschiktheid.

X

Het Markermeer is meer waard dan de Markerwaard.

XI

Het moet worden afgewacht of de uit de gemeentelijke herindeling resulterende rigoreuze getalsmatige vermindering van de voormalige 11 steden en 30 grietenijen in Friesland, alleen maar reden geeft tot redactionele modernisering van het gezegde "op z'n elfendertigst", ofwel ook tot inhoudelijke aanpassing.

Proefschrift van T. Kooistra
Rhizoctonia solani as a component in the
bottom rot complex of glasshouse lettuce

Wageningen, 14 september 1983.

VOORWOORD

Bij het verschijnen van dit proefschrift wil ik graag allen bedanken die op enigerlei wijze aan de totstandkoming ervan hebben bijgedragen.

Prof. Dr. Ir. J. Dekker ben ik zeer dankbaar voor zijn bereidheid als promotor op te treden en voor de mogelijkheid een deel van het onderzoek over de relatie temperatuur en aantasting, op het Laboratorium voor Fytopathologie uit te voeren. Zijn suggesties en stimulerende begeleiding hebben grote invloed gehad op de inhoud en de vorm van dit proefschrift.

Mijn co-promotor en de initiatiefnemer van het onderzoek, Prof. Dr. A.F.H. Besemer, wil ik bedanken voor zijn belangstelling voor het onderzoek, zijn adviezen over fytofarmaceutische aspecten en voor het doornemen van het manuscript.

De directie van de Plantenziektenkundige Dienst ben ik zeer erkentelijk voor de geboden mogelijkheden het proefschrift te bewerken en uit te geven. Hierbij wil ik graag de collega's en medewerkers van de Binnen- en Buitendienst als ook de studenten betrekken, die hun bijdrage hebben geleverd aan de literatuurstudie, het experimentele werk, het veldonderzoek, de statistische verwerking, het typen, het tekenen van de grafieken, de vormgeving, de correctie en het drukken van het manuscript. Zonder hun medewerking was deze publicatie niet tot stand gekomen. Hartelijk dank.

Verder gaat mijn dank ook uit naar collega's buiten de Dienst en medewerkers van Voorlichtingsdiensten, Proefstations en Proeftuinen en naar tuinders voor hun medewerking en stimulerende contacten.

Veel dank ben ik verschuldigd aan Ing. G.M. Tichelaar, tot zijn pensionering verbonden aan het Instituut voor Plantenziektenkundig Onderzoek te Wageningen, voor de samenwerking gedurende een deel van het onderzoek en voor de waardevolle discussies over bodempathogenen en valkuilen bij grondonderzoek.

Dr. Ir. L. Bravenboer, Proefstation voor de Tuinbouw onder

Glas te Naaldwijk, ben ik erkentelijk voor het doornemen van een deel van het manuscript.

Ing. J. Kort, tot zijn pensionering verbonden aan de Plantenziektenkundige Dienst te Wageningen, komt een speciaal woord van dank toe voor het vertalen in het Engels.

Tenslotte wil ik Annie bedanken voor haar steun thuis tijdens de periode waarin ik aan dit proefschrift heb gewerkt.

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I. INTRODUCTION

I.1. THE CULTURE OF LETTUCE

I.1.a. *Economics*

In The Netherlands, butterhead lettuce are together with tomato and cucumber one of the three most important glasshouse crops. The acreage under lettuce is the largest (2700 ha in 1981), the production value is the third highest (Dfl. 237 million in 1981. Over 50 per cent of the culture is concentrated in the glasshouse district of Zuid-Holland. Other centres are in the provinces of Noord-Brabant, Limburg and Utrecht.

A great deal of the lettuce is grown in air-heated glasshouses, usually following the main crop tomato. Often two successive crops are cultivated during November-March. The most lettuce is grown during the winter and early spring: about 10 per cent in October-November, over 35 per cent in December-February and over 45 per cent in March-April.

More than 90 per cent of the glasshouse lettuce production is exported, 80 per cent to the German Federal Republic, about 10 per cent to the United Kingdom and the rest mainly to the Scandinavian countries.

The price of Dutch lettuce in Germany is dependant on the supply and on imports from France, where the yield greatly depends on the weather conditions. An example of the average price for 100 heads during three growing seasons is given in Table I.1.

The main part of the direct production costs (i.e. excluding labour costs) is plant material and, depending on the cropping period, heating. Costs for pesticides are only a few per cents of the direct costs. Ninety per cent of the labour costs are for planting, cutting and packing, ten per cent of these costs are for the remaining growing operations including crop protection.

The data for this paragraph are taken from: Proefstation voor Groenten- en Fruitteelt onder glas (1979), Landbouw Eco-

Table I.1.: Price per 100 heads in Dfl. in different production periods during three growing seasons.

Production period	Seasons		
	79/80	80/81	81/82
Oct./Nov.	22.80	42.50	34.40
Dec./Feb.	28.50	64.10	31.10
Mar./May	18.70	40.40	34.40
Average	22.50	49.00	33.50

nomisch Instituut (1981) and Rijksinstituut voor het Rassenonderzoek van cultuurgewassen (1982).

I.1.b. *Growing practices*

Lettuce is grown on different soil types, in which the pH and, in soils sensitive to drought, humus content should be not too low. Because lettuce is sensitive to salts, the soil is leached out by watering before cropping. Prior to planting the soil is dug or rotavated (to a depth of about 20 cm) and manured according to recommendations based on soil sampling analysis.

In autumn before the lettuce is planted, the soil is usually treated with methyl bromide to protect the tomato crop that follows lettuce, against nematodes and *Pyrenochaeta lycopersici*.

This treatment can not be done in spring before the tomato crop because the soil conditions are too wet and cold as a result of the heavy watering necessary for leaching out the bromide.

Propagation of seedlings takes place at specialized nurseries where pelleted seeds are sown in blocks, usually measuring 4 x 4 x 4 cm. Soil blocks of 5 x 5 x 5 cm with bigger plants are used in order to reduce the chance of drying out in warm periods or to shorten the cultivation period. The duration of propagation is 15-60 days, depending on the season. The plants in the soil blocks are planted in shallow holes; only in very early and very late crops blocks are then planted deeper to avoid drying out.

Table I.2.: Cropping periods.

Crop	Date of sowing	Date of planting	Date of harvesting
Early autumn	10 Aug. - 25 Aug.	20 Aug. - 5 Sept.	25 Sept.- 20 Oct.
Autumn	25 Aug. - 15 Sept.	5 Sept.- 1 Oct.	20 Oct. - 1 Jan.
Winter	15 Sept.- 25 Oct.	1 Oct. - 5 Dec.	1 Jan. - 1 Mar.
Spring	25 Oct. - 25 Feb.	5 Dec. - 22 Mar.	1 Mar. - 1 May
Late spring and summer	25 Feb. - 5 Aug.	22 Mar. - 20 Aug.	1 May - 15 Sept.

Depending on the time of harvest, five cropping periods can be distinghished (Table I.2.), each with specific growing requirements and cultivars.

Planting density in warm cropping periods is 16-19 plants per square meter, in colder periods 18-24 plants per square meter. The duration of culture is greatly dependent on the cropping period: three months for winter crops, one and a half month for early autunm and late spring crops. After harvest, the lettuce is cooled to + 2^{o}C in vacuum installations.

Lettuce is put up for auction in classes for weight and quality according EEC standards. Quality class I is intended mainly for export and realizes a much higher price than class II which is reserved for national consumption, class III, cooking lettuce, yields very few. A minimum weight of 12 kgs per 100 heads in the winter months or 13 kgs in other months is standard for class I. The following weight classes in kgs per 100 heads are distinghished 10-11, 12-13, 14-15.....23-24 and 25 upward.

The choice of cultivars is mainly determined by cropping period and susceptibility to fungal diseases and physiological disorders.

A very important factor in lettuce cultivation is the glasshouse climate. Processes of growth, development and head formation are determined by the temperature and light (duration and intensity) and especially by the ratio of these to each other. Particularly in winter crops under restricted

light and temperature conditions, it is difficult to combine satisfactory plant growth and adequate head formation.

During the night the temperature is maintained at 6-7°C by heating or ventilation; during the day at ± 12°C, rising to 18-20°C or higher during sunny periods. Additional carbon-dioxide (CO_2) is used to increase the growth rate and to broaden the leaves.

The watering regime can be very critical with regard to the appearance of fungal diseases and physiological disorders.

A complete summary of diseases, pests, disorders and weeds and their control is given in Consulentschappen voor plantenziektenbestrijding (1981). The main problems are the following. In propagation nurseries downy mildew *(Bremia lactucae)* and grey mould *(Botrytis cinerea)* may occur. *B. lactucae* is constantly forming new physiological races, which break down the crop resistance. Aphids are the most important insects, which not only cause damage by sucking but can also transmit virus and advance *B. cinerea.* Besides the above mentioned diseases and pests, in planted lettuce bottom rot is a problem, especially in maturing lettuce (see Chapter I.2.). Tipburn and glassiness are the most important of the physiological disorders.

The possibilities of chemical control of disease and pests late in the growing season are limited because of residual pollution. Lettuce is a thin-leaved vegetable with an unfavourable surface-contents ratio, that is eaten uncooked and often unwashed. Circumstances for break-down of residues are also unfavourable during December up to and including February, because the plant grows slowly and light is limited. Control in most instances is restricted to one single application soon after planting. A later application is often not practicable because of safety intervals or the very intensive, close planting, for which spraying equipment cannot be used, without damage to the plants.

In order to control residues in lettuce, the expected date of harvesting has to be reported in order to enable residue inspection for fifteen different chemicals, including bromide. In case the residue tolerance is in excess, an extra safety

interval is ordered or the sale even prohibited.

It should be emphasised that growing practices that avoid diseases and pests such as healthy propagation material and a favourable glasshouse climate which encourages regular growth are important factors in lettuce growing. See also Chapter I. 2.b.

The data for this paragraph has been taken from the above mentioned sources (Chapter I.1.a.) and from Bensink (1971), Consulentschappen voor planteziektenbestrijding (1981) and Van Holsteyn (1981).

I.2. BOTTOM ROT

I.2.a. *Pathogens*

Bottom rot is characterized by a soil-borne decay (in Dutch "aanslag" or "smet" = "smudge") of lettuce plants that are in head or nearly mature. The disease can make heads completely unmarketable due to extensive rotting, but even with small infections, extra trimming of the lower leaves makes cutting more laborious and can reduce lettuce quality class and head weight significantly.

Until 1963 in The Netherlands, *Botrytis cinerea*, *Sclerotinia sclerotiorum* and *S. minor* were considered to cause bottom rot. In 1963 Verhoeff and Thijssen isolated *Rhizoctonia solani* from plants showing black rot in the lower leaves. This symptom was first described in 1900 in the U.S.A. and in 1901 *R. solani* was recognized as the causal fungus (Townsend, 1934).

Kooistra et al. (1974) observed that "black rot" is often nonspecific; in many instances in which the practical diagnosis indicated *R. solani* as the causal fungus, heads appeared to be infested by *B. cinerea* and to lesser extend by *R. solani* and *Sclerotinia* spp. *Pythium* spp. were also often involved.

These observations were confirmed in a survey by Tichelaar (1976, 1977). From these investigations it became clear that bottom rot in most cases was caused by *B. cinerea*. The pathogens *Sclerotinia* spp. and *R. solani* were present to a lesser degree. In more than a quarter of all diseased heads more than one fungus was involved.

The observed occurrence of *Pythium spp.* as a component in the bottom rot complex led to taxonomic investigations by Blok and Van de Plaats - Niterink (1978). Besides to *P. sylvaticum*, a common species in Dutch soils, an unknown species was found, which the authors described as *P. uncinulatum nov. sp.* Some isolates were identified as *P. tracheiphilum*. From pathogenicity tests the authors concluded that *P. unicinulatum* and *P. tracheiphilum* might play a role in the bottom rot complex.

I.2.b. *Control*

Until 1978 the generally applied preventive control of the bottom rot fungi *Botrytis cinerea, Rhizoctonia solani* and *Sclerotinia spp.* in most instances, consisted of one single pre-planting soil surface application of quintozene in combination with a single dusting with thiram up till seven days after planting to control *B. cinerea*. A combined product of quintozene and dicloran or captan was also used. In cases where an attack by *Sclerotinia spp.* was expected, quintozene was incorporated into the upper soil layer. In all control schemes, fumigation using dicloran against *Botrytis* can be practised up till four weeks before harvest.

At the beginning of the seventies, the need for alternative compounds for quintozene arose, firstly as a result of problems concerning contaminations with hexachlorobenzene, later aggravated by residues of quintozene. In this connection research into fungicides to replace quintozene in lettuce were initiated by the Plant Protection Service (Kooistra et al., 1974).

At that time a new group of specific fungicides (dicarboximides) particularly effective against *Botrytis spp, Sclerotinia spp.* and *Rhizoctonia spp.* (Lacroix et al., 1974; Burgaud et al., 1975) became available. From 1974 till 1975 iprodione, procymidone and vinclozolin were tested for approval as substitutes for quintozene in controlling the bottom rot complex in lettuce.

In 1977 and 1978, iprodione (Rovral) and vinclozolin (Ronilan) respectively were registered for application in lettuce. Procymidone (Sumisclex) showed undesirable phytotoxici-

ty in this crop. Rovral is rather effective against *R. solani*, Ronilan against *Sclerotinia spp.* and *B. cinerea*.

In 1980 a combination product of dicloran and captan was registered for application prior to planting against *B. cinerea* and *Sclerotinia spp.* only.

Tolylfluanid, registrated in 1982 for the control of *B. cinerea* up till seven days after planting, has a limited application.

The availability of Rovral and Ronilan caused a great reduction in the use of quintozene. In 1983, the application of quintozene in lettuce will probably be prohibited in view of its persistance in the environment.

The above mentioned dicarboximides are applied in one single high volume soil spray within a week of planting, sometimes mixed with thiram, or followed by dusting with thiram and fumigation with dicloran up till four weeks before harvesting.

Steam sterilisation of the soil is recommended if a heavy Sclerotinia attack is expected.

Besides chemical control, cultivars with relatively little susceptibility to bottom rot can be grown. There are cultivars of which the lower leaves hardly touch the soil thus often escaping soil borne pathogens. The lower leaves of other cultivars turn yellow less quickly, thus offering resistance to *B. cinerea* for a longer period. The "upright" cultivars are at present only cultivated on a limited scale. The usual preventive chemical measures are also applied to these cultivars.

Cultural measures promoting an undisturbed plant growth minimize the chance of *B. cinerea* attack. In this connection a correct temperature-light ratio and a relative humidity which is not too high should be maintained. *B. cinerea* attack can further be avoided by not too close planting and moderate watering. The latter two measures apply to all other bottom rot fungi. Harvesting in good time can also reduce losses. In successive crops remnants of the previous crop should be carefully removed.

I.3. AIM AND OUTLINE OF THE PRESENT STUDY

In the previous paragraph (Chapter I.2.b.) the necessity of replacing quintozene as a fungicide against bottom rot was discussed. During the investigations into alternative compounds, a number of questions arose concerning the bottom rot complex, especially with regard to *R. solani*. Althougn this pathogen is one of the most extensively studied fungi in phytopathology (Parmeter, 1970; Domsch et al., 1980), a number of aspects of the biology and the host-parasite relationship in the specific situation of glasshouse lettuce growing in The Netherlands appeared to be underinvestigated with regard to the optimum control of *R. solani*.

The present study starts with the symptomatology and incidence of the fungi of the bottom rot complex and a survey of crop losses caused by these (Chapter II). The following five chapters are focused on *R. solani* as a component of the bottom rot complex. In Chapter III the pathogenicity and anastomosis groups are determined of *R. solani* isolates from glasshouse soil and lettuce. In view of the soil-borne character of *R. solani* attack, methods for the isolation of this fungus from soil are investigated (Chapter IV). By using a suitable bait method the spatial distribution and inoculum density of the soil in glasshouses is studied (Chapter V). The relations between the main factors and infection by *R. solani*, namely inoculum density, temperature and relative humudity are investigated in Chapters VI and VII. Investigations end with experiments on the chemical control of the bottom rot fungi *B. cinerea*, *R. solani* and *Sclerotinia spp.* (Chapter VIII). A general discussion of the results is given in Chapter IX.

II. BOTTOM ROT PATHOGENS: OCCURRENCE AND CROP LOSSES

II.1. INTRODUCTION

According to data from literature mentioned in Chapter I.2. it appeared that from lettuce heads with bottom rot obtained from glasshouses using current crop husbandry practices, one or more of the fungi *Botrytis cinerea, Pythium spp., Sclerotinia* spp. or *Rhizoctonia solani* could be isolated. The macroscopic indentification of the pathogens proved to be difficult, especially in an early stage of attack. This means that the insight into the incidence and severity of disease and the part played by the individual pathogens or combinations, is difficult. Also no information is available on potential crop losses due to bottom rot in the absence of control measures, or on the actual crop losses.

Experiments were set up in order to answer these questions. This chapter reports research on:

a. symptomatology of bottom rot fungi under field conditions,
b. evaluation of cherry agar as an isolating medium for indentification of bottom rot pathogens,
c. occurrence of the pathogens and resulting disease incidence and severity,
d. crop losses due to bottom rot fungi.

II.2. SYMPTOMS

In most instances bottom rot appears as a brownish-black discolouration of the lower leaves, followed by decay, sometimes with white mycelium. These a-specific symptoms may result from all bottom rot pathogens, mainly in the early stage of attack (Kooistra et al., 1974).

In the following lines the symptomatology of the fungi involved are described, based on many observations of attacked plants and heads, and checked by microscopic identification after plating of diseased tissue on agar.

Rhizoctonia solani ("Black rot")

The attack mostly starts only after the crop has covered the soil. This is connected to the hygrophylic character of the fungus (Chapter VII).

The first plant parts to become diseased are the leaf petioles and midrib portions which are in contact with the infested soil. The initial symptoms are rust-coloured, sharply defined lesions which expand and usually become darker ("black rot"). The infected plant parts in contact with the soil decay almost completely within a short time, becoming black leaf prints in which the main veins are visible as thin threads. These very thin remnants are difficult to remove from the ground.

Once it has started the decay spreads upwards and inwards, from leaf to leaf. Midribs retain their white-green colour the longest, sometimes revealing up to 2 cm long sunken brown lesions.

Another characteristic is that infected plants appear healthy because the outer leaves are mostly not attacked due to a too low relative humidity (Chapter VII). These leaves keep their green colour because the stem is not infested and also because superficially attacked midribs remain functioning.

A long lasting, very high relative humidity in glasshouses may cause an attack of the whole head. When this happens the plant turns into a slimy brown mass of disorganized tissue with the exception of the stem.

In some instances brown mycelium on the veins can be observed by the naked eye and dense mycelium on more decayed plants. Black, irregularly formed 1-10 mm sclerotia can be sporadicly found in affected heads or on the soil under plants that have been attacked.

Under favourable conditions, i.e. high temperature and high relative humidity (Chapter VII), the attack spreads very quickly (within a week).

Young plants in soil blocks may be attacked if the watering regime is incorrect resulting in drooping leaves to the

wet infested soil, after sprinkling.

The most specific symptoms of *R. solani* attack can be summarized as follows: Plants appear healthy but at harvest time the lowest and innnermost leaves have turned into black remnants with the exception of the midribs on which brown sunken lesions are found. From below, diseased heads look "fish boney". A cross-section of the stem does not show symptoms of attack.

Botrytis cinerea ("grey mould")

As a rule this fungus only attacks damaged, maturing or weakened plants. In lettuce crops attack has been observed on young, drooping plants which have been planted too late, on plants in which the growth had been interrupted during cultivation or on older plants with yellowing or damaged leaves.

The fungus penetrates into the stem base disturbing the vascular system. As a result, plants are wilting and the heads are not marketable. Attacked leaves or stems often show greyish-brown dispersing spores ("grey mould"). White mycelium may also become visible.

The disease mainly occurs in closely planted lettuce, commencing during the last period before harvest. Mesophyl and ribs of lower, old and already yellowing leaves turn brown and decay. Parts of leaves that are further up the stem may also become infested. Unmarketable heads can result when infested plant parts have been removed.

Specific to *B. cinerea* are spreading spores and, if the attack is heavier, the plants wilt as result of a disorganized stem base.

Sclerotinia sclerotiorum and Sclerotinia minor ("sclerotinia rot")

Symptoms in the beginning are very much similar to those caused by *B. cinerea*, namely infection of leaves that touch the soil and infection of the stem base. As with *R. solani* the attack may be restricted to the interior of the head. Edges of leaves keep their green colour for the time being.

In a more advanced stage the stem starts rotting, the plant

wilts and collapses. In this stage white mycelium and black sclerotia (5-10 mm in *S. sclerotiorum* and 1 mm in *S. minor*) can be found. The latter symptom is characteristic for "sclerotinia rot".

Pythium spp.

Attack is most common in mature plants of which lower leaves decay. No fungal spores can be seen by the naked eye. *Pythium* rot is slimier when compared to bottom rot caused by other pathogens. The disease shows more or less specific chocolate coloured veins and midribs, of which the tissues are completely decayed, and sometimes the disease is more advanced in ribs than in the surrounding mesophyl. According to the investigations of Blok and Van der Plaats - Niterink (1978) a number of *Pythium* species could be involved. It is not yet known if these species cause different symptoms.

II.3. EVALUATION OF CHERRY AGAR AS AN ISOLATION MEDIUM

In the foregoing section it appeared that often a-specific bottom rot symptoms occur. In order to be able to identify the pathogens, diseased plant parts need to be plated out. It is also known that diseased tissues can harbour several pathogens. For this reason it is possible for fast-growing fungi such as *B. cinerea* and *Pythium* spp. to overgrow certain *R. solani* strains which develop much more slowly on agar. The incidence of *R. solani* in a complex of fungi, on the heads could therefore be underestimated.

This difficulty can be overcome for the greater part by using a poor medium like cherry agar. In order to evaluate the usefulness of cherry agar for *R. solani* isolates in a complex of fungi, a selective medium for *R. solani* was prepared. Melted cherry agar of 50°C was used as the base medium to which equal parts of furalaxyl and dichloran were added in a range of concentrations. Furalaxyl has a known inhibiting effect on *Pythium* spp., dicloran on *Sclerotinia* spp. *R. solani* is relatively insensitive to these compounds. In a previous experiment it

Table II.1.: ED_{50} values in ppm a.i. furalaxyl plus dicloran in cherry agar for mycelium growth inhibition of bottom rot pathogens.

Pathogen	ED_{50} in ppm a.i. furalaxyl plus dicloran (1:1 mixture)
B. cinerea	6
P. irregulare	1.4
S. sclerotiorum	3.8
R. solani	> 128

was found that of the *Pythium* spp. which are found in lettuce (*P. irregulare, P. mamilatum, P. tracheiphilum, P. uncinulatum* and *P. ultimum*, see Blok and Van der Plaats - Niterink, 1978), *P. irregulare* was least affected by furalaxyl. This species was included in the experiment. The concentrations at which 50 per cent inhibition of the mycelium outgrowth of the bottom rot fungi occurred, was determined as described in Chapter IV.3.c. The results are shown in Table II.1.

It appeared that ED_{50} values of the mixed compounds for *R. solani* was > 128 ppm. The other fungi tested were inhibited at much lower concentrations. For the sake of the following evaluation experiment, the composition of the selective agar mixture was established on 20 ppm dichloran and furalaxyl respectively.

In order to examine whether underestimation of *R. solani* in attacked heads would occur by using cherry agar as an isolation medium, the incidence of bottom rot pathogens in 43 diseased heads was established by plating out infected tissue on selective medium as well as on cherry agar.

Per head two infected leaf- or midrib portions were cut into two equal parts. Each piece was again divided into halves (about 2 x 5 mm each). One half was plated on selective medium, the other on cherry agar. A hundred and seventy two tissue pieces from 43 heads under investigation were plated on each of the two media. The number of heads and the pathogens observed on each of the media is given in Table II.2.

Table II.2.: Number of 43 heads with specified pathogen(s), identified on selective agar (cherry agar + 20 ppm dicloran + 20 ppm furalaxyl) and on cherry agar.

Pathogen(s)	Number of heads	
	selective agar	cherry agar
Rhizoctonia solani (R)	33	0
Botrytis cinerea (B)	0	9
Pythium spp. (P)	0	1
B + R	0	30
P + R	0	2
B + P + R	0	1
No pathogen	10	0
Total R	33	33

From the results obtained with cherry agar it appeared that *R. solani* could be isolated from 33 out of 43 heads, namely in combination with *B. cinerea* (30 x), with *Pythium* spp. (2 x) and once with both these fungi. *Sclerotinia* spp. was not found on any of the heads. Using the selective medium, *R. solani* could be isolated from the same number (33) of heads.

These results indicate that the use of cherry agar for identification of fungi present in the same head will not lead to an underestimation of *R. solani* due to overgrowth by *B. cinerea* or *Pythium* spp. But it has to be mentioned that, using this acid medium, soft rot bacteria associated with advances stages of *R. solani* attack, as reported by Pieczarka and Lorbeer (1975), are not detected. However, according to these authors, bacteria are considered as secondary invaders after infection by primary fungal pathogens.

II.4. INCIDENCE OF PATHOGENS AND SEVERITY OF ATTACK

In previous investigations (Kooistra et al., 1974; Tichelaar, 1976,1977) the incidence of bottom rot causing pathogens was determined in small samples of diseased heads from

many crops treated with fungicides against bottom rot. The severity of attack was not involved in these investigations.

The results of the analysis of the incidence of bottom rot fungi and the severity of attack caused by these pathogens in plots without fungicides are reported in this section. Investigations covered eleven crops from five holdings, indicated as A, B, C, D and E.

Per crop 30 - 72 heads from 2 - 6 untreated plots (4.5 m^2) of fungicide trials were classified according to the severity of attack (0 = no attack, 1 = 1 - 2, 2 = 3 - 4, 3 = 5 - 6, 4 = > 6 leaves attacked respectively, 5 = not marketable) from which the average disease index (0 - 5) was calculated. From each head two infected parts were plated on cherry agar for identification of bottom rot fungi. Results are given in Tables II.3, 4 and 5.

Practically all heads on holding A (Table II.3.) were attacked by *B. cinerea*. The disease index amounted to 2.8.

All the heads on holding B (Table II.3.) were also diseased mostly by *B. cinerea* and *R. solani* or by a combination of the two. The disease index of the heads infected by the combination of the mentioned pathogens (i.e. 2.8) was somewhat higher than in case of the separate pathogens (i.e. 2.3). In this crop *Sclerotinia* spp. alone or in combinations occurred at a low rate.

On holding C (Table II.4) half of the crop was free from attack. The other half was slightly attacked by *B. cinerea* (disease index 1.1.). In the succeeding crop heads were particularly attacked by *Pythium* spp. and *B. cinerea* or combined (almost 90 per cent). Disease severity caused by *B. cinerea* was highest (disease index 2.6.) and by *Pythium* spp. lowest (disease index 2.1.). The combined attack took an intermediate position (disease index 2.4.).

In comparing the results in both crops it appeared that the disease attack in the spring crop is much lower and caused by only one pathogen. In the late crop there was more damage to all heads by one or more pathogens.

On holding D (Table II.4.) all heads were diseased in the spring crop, particularly by *B. cinerea* and *R. solani* or a com-

Table II.3.: Analysis of incidence (%) and severity (disease index) of pathogens causing bottom rot determined on heads from untreated plots in two glasshouses (A and B) at harvest. Disease index 0 = no attack, 5 = heads not marketable.

Glasshouse	A		B	
Cropping period	winter		late autumn	
Number of heads examinated	30		60	
Pathogen(s)	heads %	disease index (0-5)	heads %	disease index (0-5)
No pathogen	0		0	
Botrytis cinerea (B)	93	2.8	32	2.3
Pythium spp. (P)				
Rhizoctonia solani (R)			17	2.3
Sclerotinia spp. (S)			5	1.7
B + P	7	1.5	7	1.8
B + R			27	2.8
B + S			10	2.8
P + S			2	2.0
B + P + R			2	2.0

bination of both (almost 90 per cent). Disease severity was equally high for both pathogens (disease index 2.2-2.3). Also in the second, late spring crop, *B. cinerea* and *R. solani* were the dominant pathogens; *B. cinerea* scored a disease index of 2.3 and *R. solani* alone or in combination with *B. cinerea* 3.2 and 3.3 respectively. In this crop *Pythium* spp. were rare.

A comparison of these successive crops on this holding shows in both crops a hundred per cent attack by *R. solani* or *B. cinerea* or a combination of both. In the second culture, cropped in a warmer period, the disease severity caused by *R. solani* was much higher than in the first crop.

On holding E (Table II.5.) five crops were analysed during two successive years. In the first crop (79-1), all heads were attacked: 47 per cent by *B. cinerea* and 53 per cent by *B. cinerea* plus *Pythium* spp. or by *Pythium* spp. alone. The disease se-

Table II.4.: Analysis of incidence (%) and severity (disease index) of pathogens causing bottom rot in two successive crops in two glasshouses (C and D), determined on heads from untreated plots at harvest. Disease index: 0 = no attack, 5 = heads not marketable.

Glasshouse	C				D			
Cropping period	spring		late spring		spring		late spring	
Number of heads examinated	36		60		58		42	
Pathogens	heads %	disease index (0-5)	heads %	disease index (0-5)	heads %	disease index (0-5)	heads %	disease index (0-5)
No pathogen	50	0	0		0		0	
Botrytis cinerea (B)	47	1.1	30	2.6	37	2.2	41	2.3
Pythium spp. (P)			40	2.1	7	1.5	10	1.8
Rhizoctonia solani (R)			2	1.0	32	2.3	29	3.2
Sclerotinia spp. (S)			7	1.8				
B + P	3	2.0	17	2.4			2	1.0
B + R					19	2.2	17	3.3
B + S			2	3.0				
P + R			2	3.0	2	1.0	2	3.0
B + P + R					4	3.0		

Table II.5.: Analysis of incidence (%) and severity (disease index) of pathogens causing bottom rot in successive crops during a period of two years, determined on heads from untreated plots at harvest. Disease index: 0 = no attack, 5 = heads not marketable.

Glasshouse	E									
Cropping period	winter		spring		autumn		early spring		spring	
Crop No.	79-1		79-2		80-1		80-2		80-3	
Number of heads examinated	36		36		60		72		72	
Pathogen(s)	heads %	disease index (0-5)	heads %	disease index (0-5)	heads %	disease index (0-5)	heads %	disease index (0-5)	heads %	disease index (0-5)
No pathogen	0		3	0	52	0	54	0	17	0
Botrytis cinerea (B)	47	2.3	64	1.5			11	2.4	1	3
Pythium spp. (P)	11	1.3			45	1.4	20	1.1	40	1.7
Rhizoctonia solani (R)			11	4.5			3	2.0	33	4.7
Sclerotinia spp. (S)			3	2.0						
B + P	42	1.1	3	2.0	3	2.5	11	1.3		
B + R			14	2.2						
P + R							1	1.0	8	2.5

verity for *B. cinerea* was highest (disease index 2.3), for *Pythium* spp. and the combination with *B. cinerea* somewhat lower (disease index 1.3 and 1.1 respectively). In the following spring crop (79-2), beside *B. cinerea* (64 per cent), *R. solani* alone or in combination with *B. cinerea* was found. The disease index for the attack by *R. solani* was 1.5, the index for *R. solani* alone was much higher (disease index 4.5). In combination with *B. cinerea* the attack was intermediate (disease index 2.2). In comparison to the first crop the higher incidence and disease severity as a result of *R. solani* is striking.

In 1980, fifty per cent of the autumn crop (80-1) was not diseased. The other half was attacked by *Pythium* spp. at a low rate (disease index 1.4). In the second early spring (80-2) again half of the heads were again attacked, namely by *Pythium* spp. and *B. cinerea* alone or in combination. Disease severity caused by *B. cinerea* was somewhat higher than by *Pythium* spp. (disease index 2.4 and 1,1 respectively). In the case of a combined attack the disease index was intermediate: 1.3. In the following spring crop (80-3) more than 80 per cent of the heads were diseased, mainly due to *Pythium* spp. (40 per cent) and *R. solani* (33 per cent) and 8 per cent due to combination of the two. The disease severity caused by *R. solani* was much higher than by *Pythium* spp.: disease index 4.7 and 1.7 respectively. The disease index for the combination was again intermediate: 2.5.

In comparing crops on holding E in 1979 and 1980 it appears that the attack was heavier in 1979, mainly due to *B. cinerea*, whereas *Pythium* spp. and *R. solani* were less prevalent. In 1981 *Pythium* spp. and *R. solani* were the main pathogens. In both years the disease severity in *R. solani* was highest, especially in the crops cultivated during warm periods.

The observations of eleven crops at five holdings give rise to the following conclusions. Eighty to a hundred per cent of the heads of most crops were attacked; in some instances half of the crop was free from disease. Most crops were attacked by two or more pathogens. About 30 per cent of the heads were infected by more than one fungus. Two crops were attacked by

B. cinerea alone and one crop by *Pythium* spp. alone.

B. cinerea was found on most holdings, followed by *Pythium* spp., *R. solani* and *Sclerotinia* spp. This conforms the reports of Kooistra et al. (1974) and Tichelaar (1976,1977).

The severity of disease per crop showed a great variation. In most instances *B. cinerea* caused the highest disease index; in crops grown during warm periods *R. solani* took over this position. The severity of the disease caused by *Pythium* spp. was usually the lowest. It was observed that all pathogens could cause unmarketable heads (disease index 5). However, except for *R. solani* in some cases, the average disease index due to fungi that cause bottom rot, seldom exceeds value 3, which amounts to 5 - 6 diseased leaves per head.

B. cinerea is prevalent in winter crops, the other bottom rot pathogens more often appear in warmer cropping periods. This is in agreement with the findings of Kooistra et al. (1974) and Tichelaar (1976, 1977). There is little correlation between the successive crops and the attack. However, when *R. solani* is found in the preceding crop, incidence as well as severity will be greater in the following (warmer) cropping period.

II.5. CROP LOSSES

From 1978 - 1980 field trials were carried out for the approval of the fungicides against bottom rot. The disease and yield data from 28 glasshouse trials were analysed. In a number of holdings *R. solani* and *Sclerotinia* spp. were known to occur in the soil.

The potential yield loss was based on the difference in the trial concerned of the yield data of the untreated object and treated objects with lowest disease index (attainable yield). The actual loss was estimated from the difference between the theoretical and the attainable yield.

The trials were carried out in six replicates and the plots measured 3 x 1.5 m . At harvest forty plants were examined from the centre of each plot. The following yield components

were determined: average number of marketable heads from 40 plants, average weight of marketable heads, percentage of marketable heads of export quality class and the disease index (0 - 5; 0 = no attack, 5 = not marketable, see Chapter II.4). The occurrence of the main pathogens in the different glasshouses was determined visually. In relevant cases differences in yield results were checked by analysis of variance. The yield differences of the mean of all glasshouses were proved with the t-test of means (paired observations) according to Wallis and Roberts (1965).

The results (Table II.6.) show that as an average in treated plots 39.7 (99.1 per cent) marketable heads were harvested, i.e. almost the maximum of 40 heads. The range was narrow: 38 - 40 heads. In the untreated plots the average number of marketable heads was significantly lower; 36.8 (92.1 per cent). Also the range was wide, namely 20 - 40 heads. Therefore, the potential loss of marketable heads is 7.0 per cent, with a range of 0 - 50 per cent.

The average weight of marketable heads in the untreated plots did not differ significantly from the average weight of heads in the treated plots in most glasshouses. If the results from all the glasshouses are considered, the average difference of almost 10 g (equal to 1 kg per 100 heads) is just significant. In the glasshouses where the lettuce weighed less than 25 kgs per 100 heads and there was a significant difference weight between treated and untreated plots (glasshouses 3, 8 and 11), the cash return from the untreated crop would be less because the lettuce would be in a lower weight class (see Chapter I.2.).

The average percentage of heads of export quality class in the treated plots is high, 90.7 (36.7 - 100). In the untreated plots this percentage is significantly lower: 83.2 (32.2 - 100) Therefore the potential loss in export quality class is on an average 7.5 per cent. On some of the holdings this loss is much greater, up till 30 per cent (glasshouse 3).

In most of the glasshouses the difference in disease index between treated and untreated plots is highly significant. The

Table II.6.: Bottom rot and yield data for crop loss analysis from 28 glasshouses. Figures are based on treated and untreated plots of 40 plants, 6 replicates.

Glass-house	Main pathogen(s) 1)	Average number of marketable heads per plot of 40 plants			Average weight (g) of marketable heads			Percentage marketable heads in export quality class			Disease index (0-5) 2)		
		untr.	treat.	sign. 3)	untr.	treat.	sign.	untr.	treat.	sign.	untr.	treat.	sign.
1	B	39.2	40.-		164.9	164.6	NS	97.0	95.8		0.46	0.04	xx
2	B, (R)	35.3	40.-	xx	204.2	232.1	NS	86.8	91.3		2.31	0.56	xx
3	R, P	36.0	39.5	xx	232.4	260.3	x	39.8	70.9		3.13	1.10	xx
4	B, P	32.5	39.8	x	172.8	166.5	NS	66.2	77.4		3.07	0.81	xx
5	B, (R)	34.7	39.7	NS	300.5	263.4	NS	77.9	97.1		2.17	0.97	xx
6	S, B, (R)	34.7	37.3	NS	304.9	295.1	NS	75.0	74.1		2.49	1.77	x
7	S, B, (R)	20.0	38.0	xx	291.5	320.6	NS	73.3	90.4		3.79	1.16	xx
8	B	35.5	39.7	xx	155.8	185.1	xx	61.0	87.0		2.43	0.85	xx
9	B	40,-	40.-	NS	292.5	288.8	NS	100.-	100.-		0.66	0.23	xx
10	B (R)	33.8	39.8	xx	188.2	195.2	NS	85.7	94.6		1.82	0.33	xx
11	B	32.7	39.0	xx	199.5	224.8	xx	91.3	96.6		2.58	0.69	xx
12	S, B, P, R	39.5	39.7		325.9	357.8	x	77.2	96.2		2.11	0.81	xx
13	B, P	39.3	40.-		190.7	195.8	NS	32.2	36.7		1.1	0.55	xx
14	B, S	39.7	40.-		367.2	368.3	NS	89.9	98.8		3.43	1.20	xx
15	R, P, B	39.7	39.8		184.7	184.9	NS	97.5	98.3		2.20	1.35	NS

Table II.6.: (continued). Bottom rot and yield data for crop loss analysis from 28 glasshouses. Figures are based on treated and untreated plots of 40 plants, 6 replicates.

Glass-house	Main pathogen(s) 1)	Average number of marketable heads per plot of 40 plants			Average weight (g) of marketable heads			Percentage marketable heads in export quality class			Disease index (0-5) 2)		
		untr.	treat.	sign. 3)	untr.	treat.	sign.	untr.	treat.	sign.	untr.	treat.	sign.
16	S, B, (R)	34.2	39.3	x	270.7	318.2	xx	74.6	94.5		2.98	1.43	xx
17	B, (R)	39.0	39.7		332.9	330.9	NS	93.6	94.1		1.00	0.68	NS
18	B, (R)	39.8	40.-		261.9	270.4	NS	100.-	100.-		0.33	0.18	NS
19	B, (P)	38.8	39.8		236.5	251.0	NS	68.2	88.7		2.15	0.48	xx
20	B, (P)	40.-	40.-		281.3	282.9	NS	99.6	100.-		1.15	0.63	x
21	B	39.5	40.-		273.0	271.3	NS	97.5	97.5		0.48	0.13	NS
22	B	31.7	39.2	NS	136.1	143.8	NS	92.1	98.7		1.88	0.58	NS
23	B, (P)	39.0	39.8		245.9	258.8	NS	97.4	99.2		1.18	0.65	x
24	(B)	40.-	40.-		220.8	220.2	NS	99.2	97.5		0.01	0.0	
25	B	39.3	40.-		136.9	137.5		92.4	92.1		1.43	1.02	NS
26	B	38.3	40.-		190.9	195.4	NS	69.1	75.0		1.31	0.73	x
27	B	39.7	40.-		260.7	262.3	NS	94.5	96.7		0.14	0.06	NS
28	B, P	39.7	40.-		205.7	215.8		99.2	99.6		1.67	0.70	xx
Aver.		36.8	39.7	xx	236.8	245.1	x	83.2	90.7	xx	1.77	0.70	xx

1) B = B. cinerea, P = Pythium spp., R = R. solani, S = Sclerotinia spp., ( ) = sporadic incidence

2) 0 = no attack, 5 = unmarketable

3) x = $P \leq 0.05$, xx = $P \leq 0.01$, NS = not significant

disease index in the treated plots is 0.70 as an average with a range of 0 - 1.77. The attack in the untreated plots is significantly higher, 1.77 and the range is 0.01 - 3.79.

Economically the heads of export quality class determine the yield of the lettuce crop (Chapter I.1.b.). It appears from the above discussed data that for the treated plots the average percentage of marketable heads of export quality class amounts 99.1 x 90.7 = 89.9 per cent. This has to be considered as the attainable yield and is relatively set at 100. For the untreated plots the data are 92.1 x 83.1 = 76.6 per cent heads (relatively 83.2). From these figures the potential loss due to bottom rot appears to be 100-83.2 = 16.8 per cent heads of export quality class. In fact the financial loss is a little less because marketable heads of other than export quality, realize, a relatively low, price. The calculated potential loss of 16.8 per cent is an average value. On individual holdings (e.g. glasshouse 7), the potential loss can be more than 50 per cent.

The actual loss due to bottom rot, i.e. on treated plots, is much lower. In the foregoing it has been shown that treated fields yielded 89.9 per cent heads of export quality. If 100 per cent is considered to be the theoretical yield, the actual loss would be 10.1 per cent heads. However, in fact this figure is lower, because other factors, than bottom rot, that also affect quality have to be considered (see Chapter I.1.b.), as was the case in glasshouse 13. In this crop the attack of bottom rot fungi was low (disease index 0.55, i.e. 0.8 diseased leaf per head), but only 36.7 per cent of the heads were classified in the export class. A more realistic estimate of the average actual yield loss would be approximately 5 per cent of the heads, because in the majority of the glasshouses, over 95 per cent of the yield was classified as export quality (Table II.6.). In addition, costs for chemical control and the removal of diseased leaves have to be considered as actual losses.

II.6. SUMMARY

From observations on diseased plants and heads from many commercial glasshouses, bottom rot symptoms were described, caused by *B. cinerea, Pythium* spp., *R. solani* and *Sclerotinia* spp. Only in certain, usually advances stages of attack are symptoms specific for the pathogen involved. In most cases the leaves infected are a-specific decayed and the causal fungi have to be identified microscopally after plating of attacked tissue. Cherry agar appeared to be a suitable medium on which to isolate pathogens from plant material with a complex of fungi.

Analysis of pathogens from heads from untreated plots in eleven crops, showed that *B. cinerea* was found in almost every crop. The incidence of *Pythium* spp., *Sclerotinia* spp. and *R. solani* was much lower. Most crops were attacked by two or more pathogens. About 30 per cent of the heads were infected by more than one fungus.

Disease severity of heads was highest when attacked by *R. solani* especially in warm cropping periods, least by *Pythium* spp.

The incidence of *Pythium* spp., *Sclerotinia* spp. and *R. solani* was higher in warm cropping periods, *B. cinerea* was more common in winter crops.

The average potential loss on 28 holdings due to bottom rot, determined on plots without fungicide treatment was estimated at about 17 per cent heads of the export quality class. In one glasshouse a loss of more than 50 per cent was found.

The actual loss, i.e. on treated plots, was estimated to be about 5 per cent heads. The other losses are costs of control and extra costs of harvesting (i.e. cutting diseased leaves).

III. CHARACTERISTICS OF RHIZOCTONIA SOLANI

III.1. INTRODUCTION

In the previous chapter the parasitic occurrence of *Rhizoctonia solani* in lettuce was studied, showing that this fungus is a relatively important parasite in this crop.

With regard to parasitic survival the question of susceptibility of crops planted before and after lettuce may arise. The host plant range is known to be very wide (Baker, K.F., 1970). In The Netherlands the most important glasshouse crops tomato and cucumber are known to be attacked by *R. solani* (Consulentschappen voor planteziektenbestrijding, 1981).

The aim of the first part of the present study was the investigation of the susceptibility of 17 glasshouse plants to *R. solani*, artificially inoculated in soil.

According to current concepts, the species *R. solani* consists of four hyphal anastomosis groups, designated AG-1, AG-2, AG-3 and AG-4 (Parmeter et al., 1969). From Japan two additional groups have been described (Ogoshi, 1976; Kuninaga et al., 1979). According to Sherwood (1969), each anastomosis group is characterized by distinctive morphologic, pathologic and physiologic norms with some overlapping of isolates in different groups. Anderson (1982) reviewed the genetical and pathological research from 1965, focused on the AG-concept. Recent research has pointed out that one crop, e.g. sugar beet can be attacked by strains belonging to at least two anastomosis groups (Herr and Roberts, 1980) which mainly attack leaves and roots respectively.

The second part of the present study was undertaken to determine the AG-group of isolates in soils and attacked lettuce heads from glasshouses in different regions of The Netherlands.

III.2. MATERIALS AND METHODS

Host range experiments

The susceptibility to *R. solani* isolates from soil (R 200-00-79 and R 201-04-79) of the glasshouse plants listed in Table III.1. was determined. From each crop 1-2 weeks old seedlings and 4-6 weeks old plants were transfered into plastic containers filled with artificially infested pot soil (one well-dispersed cherry agar plate colonized by *R. solani* in 500 g of soil). Each container held 3-5 plants and there were three containers per object. Containers filled with non infested soil served as a control. In order to obtain about 100 per cent relative humidity, containers were placed at room temperature in day-light under plastic cover. Plants were inspected and the development of the disease was assessed daily; the following categories of susceptibility were used: +++ (very susceptible, plant dead after 2-4 days), ++ (susceptible, plant dead after 5-7 days), and + (moderately susceptible, plant dead after 8-11 days).

Determination of AG-groups

The tester-strains used, A, D, F and C (after Richter and Schneider, 1953) were supplied by Dr. R. Schneider, Biologische Bundesanstalt für Land- und Forstwirtschaft, Berlin-Dahlem, BRD. These groups correspond with the AG-groups 1, 2, 3 and 4 respectively of Parmeter et al., (1969).

Thirty five glasshouse isolates were tested, 11 isolates originated from soil from two holdings; the remaining 24 had been isolated from attacked lettuce on 13 holdings. Cultures were maintained on cherry agar.

To observe anastomosis, sterile glas slides were covered with a thin water-agar layer. Two disks (8 mm in diameter) were cut from the edge of fungal colonies and placed 2-3 cm apart on the coated slides. The slides were placed in petridishes and incubated for 24-48 hours at 15° or 22°C. The area of hyphal contact was examined at 100 x and 400 x magnifications. In some cases hyphes were stained with 0.5 per cent

aniline blue in lactophenol. Isolates were placed in the same anastomosis group as the tester strain, if hyphal contact with cytoplasmic fusion was observed (Parmeter et al., 1969).

III.3. HOST RANGE

The observations showed that the hypocotyle and the cotyledons were particularly attacked and from there the parasite spread to the upper stalk and leaves. None of the 17 tested crops were immume (Table III.1.). No differences were found in infectivity between the isolates used.

The susceptibility of seedlings and older plants was practically the same under the experimental conditions. Most crops were very susceptible. Gherkin, sweet pepper and tomato appeared to be susceptible; beans and cucumber were moderately susceptible.

Chemical control is advised for 7 of the 17 crops tested (Consulentschappen voor planteziektenbestrijding, 1981).

III.4. ANASTOMOSIS GROUPS

From the results obtained (Table III.2.) it appears that all four European *R. solani* anastomosis groups were found in 35 isolates obtained from soil or lettuce heads on 13 holdings. Two isolates from soil and lettuce from one holding did not fuse with one of the tester strains.

The soil isolates, collected from only two holdings, belonged mainly to AG-4. Two isolates from one of these holdings fused with tester strain AG-3. It is generally known that representatives in this group occur almost exclusively on *Solanaceae*, especially potato. Potatoes had been grown in the past on the holding concerned.

It is striking that AG-1 was found in lettuce heads from most of the holdings. AG-2 and AG-4 were found on only a few holdings. In lettuce from glasshouses 9 and 13, two groups

Table III.1.: Susceptibility of seedlings and plants of glasshouse crops in artificially inoculated soil with two *Rhizoctonia solani* isolates. The susceptibility classes are based on the number of days after which the plants died. Three replicaties, each consisting of 3-5 plants. The last column gives the possible control advice.

Glasshouse crop	Complete attack after ... days				Susceptibility class[3]	Chemical control advice[4]
	seedlings[1]		plants[2]			
	R 200-00-79	R 201-04-79	R 200-00-79	R 201-04-79		
aubergines (Solanum melongena)	3- 4	2- 4	3 - 4	3 - 4	+++	no
beans (Phaseolus vulgaris)	8-11	8-11	-	-	+	no
black radish (Raphanus sativus var. niger)	3- 4	3- 4	3 - 4	3 - 4	+++	yes
cauliflower (Brassica oleracea ver. botrytis)	3- 4	3- 4	3 - 4	3 - 4	+++	no
celery (Apium graveolens)	3- 4	3- 4	3 - 4	3 - 4	+++	no
chicory (Cichorium intybus)	3- 4	3- 4	3 - 4	3 - 4	+++	no
chili peppers (Capsicum annuum)	3- 4	3- 4	3 - 4	3 - 4	+++	no
cucumber (Cucumis sativus)	9-10	9-11	-	-	+	yes
endive (Cichorium endiva)	2- 3	2- 3	3 - 4	3 - 4	+++	yes
gherkin (Cucumis sativus)	5- 7	5- 7	-	-	++	yes
kohlrabi (Brassica oleracea var. caula-rapa)	3- 4	3- 4	3 - 4	3 - 4	+++	no
lettuce (Lactuca sativa)	2- 4	2- 4	3 - 4	3 - 4	+++	yes
parsley (Petroselinum crispum)	2- 3	2- 3	3 - 4	3 - 4	+++	no
radish (Raphanus sativus)	4- 5	4- 5	4 - 5	4 - 5	+++	yes
spinach (Spinacia oleracea)	2- 3	2- 3	3 - 4	3 - 4	+++	no
sweet pepper (Capsicum annuum)	4- 6	4- 6	4 - 7	4 - 7	++	no
tomato (Lycopersicon esculentum)	4- 7	4- 7	4 - 9	4 - 9	++	yes

1) 1-2 weeks old

2) 4-7 weeks old

3) +++: very susceptible
++ : susceptible
+ : moderate susceptible

4) Consulentschappen voor planteziektenbestrijding (1981)

Table III.2.: Determination of anastomosis group of *Rhizoctonia solani* isolates obtained from soil and lettuce of different glasshouses.

Glasshouse	Isolate	Source	Anastomosis group
1	R 200-00-79	soil	AG-4
	-01-		AG-4
	-03-		?
	-04-		AG-4
	R 408-02-79	lettuce	?
2	R 201-01-79	soil	AG-3
	-02-		AG-3
	-03-		AG-4
	-04-		AG-4
	-05-		AG-4
	-07-		AG-4
	-09-		AG-4
	R 409-00-79	lettuce	AG-1
	-01-		AG-1
	-03-		AG-1
3	R 401-00-76	lettuce	AG-4
	-01-		AG-4
4	R 402-01-76	lettuce	AG-4
5	R 404-00-76	lettuce	AG-1
6	R 405-00-76	lettuce	AG-2
7	R 406-00-77	lettuce	AG-1
8	R 407-00-77	lettuce	AG-1
9	R 410-00-79	lettuce	AG-1
	-01-		AG-2
10	R 411-00-79	lettuce	AG-4
11	R 412-00-79	lettuce	AG-1
12	R 413-00-79	lettuce	AG-1
13	R 416-02-79	lettuce	AG-1
	-04-		AG-2
	-07-		AG-2
	-08-		AG-2
	-10-		AG-2
	-11-		AG-2
	-12-		AG-2
	-15-		AG-2

were observed, e.g. AG-1 and AG-2.

All three isolates from lettuce in glasshouse 2 belonged to AG-1, whereas in the soil AG-3 and AG-4 were isolated.

III.5. DISCUSSION

All seventeen glasshouse crops tested appeared to be more or less susceptible to *R. solani* which is in accordance with the very wide host range of the species (Baker, K.F., 1970). It is, therefore, assumed that the presence of host plants may contribute to the survival of *R. solani* in glasshouse soils (Papavizas, 1970).

Older plants were as susceptible as seedlings. This seems contradictory to the generally accepted idea that old plants are relatively resistant. This idea is largely based on the fact that older plants have moved into an environment that is unfavourable for the fungus (Baker, K.F., 1970). In the experiments the relative humidity was high in plots with both seedlings and older plants and, therefore, favourable for fungus development. Both stages of development of the test plants were equally susceptible.

From Table III.1. it also appears that, on some of the crops tested, *R. solani* does not occur to such an extent in practice that control measures are recommended. In many cases the fungus will cause no problems because cultural measures i.e. dryness or the use of "sterile soil blocks" for planting seedlings, inhibit *R. solani* development. Moreover it can be assumed that the inoculum density in part of glasshouse soils is low as a result of frequent soil desinfection with methyl bromide (Chapter VIII.4.e.).

However, it cannot be excluded that changing cultural measures, for instance energy saving glasshouse isolation, resulting in higher relative humidity, will stimulate *R. solani* to attack a number of potentially susceptible crops. Moreover, in the future the application of methyl bromide (Chapter VIII. 4.e.) will be further restricted and it looks as though sub-

stitute compounds will control *R. solani* less effectively.

From the determination of anastomosis groups in 35 isolates it appeared that AG-1, AG-2 and AG-4 were found in attacked lettuce. AG-3 is known to occur exclusively in *Solanaceae* (Ogoshi, 1976).

Although the number of isolates and holdings tested was too limited to draw general conclusions it was striking that AG-1 was found on most holdings. The combination of AG-1 and AG-2 occurred at a number of them.

In other crops like sugar beet (Herr and Roberts, 1980) it was found that different anastomosis groups caused different disease syndromes (for instance damping off, root rot and foliage blight). This aspect has not been included in the experiments.

But during the evaluation of disease attack in lettuce heads it often appeared that in heavily diseased heads sometimes only mesophyll was destroyed whereas in other equally diseased heads, the midrib was also infested, causing cancers. The differences may be due to differences in pathogenicity between strains linked to the anastomosis groups, as was the case in the forementioned experiments with beet by Herr and Roberts.

From a practical point of view the question may arise whether anastomosis groups from lettuce differ in sensitivity to fungicides. A provisional experiment in vitro showed that all tested groups were equally sensitive to iprodione.

According to Anderson (1982) the principle of the anastomosis group concept i.e. each group is a seperate, more of less independent unit, is an important factor to make progress in devising methods for disease control and in obtaining disease resistance.

III.6. SUMMARY

In artificially infested soil the susceptibility of 17 glasshouse crops in the seedlings and older plant stage to two *R. solani* isolates were tested. It appeared that all crops proved to be susceptible, though to a different extent.

The determination of anastomosis groups in 35 isolates from soil or lettuce from 13 holdings indicated the presence of three of four known groups (AG-1, AG-2 and AG-4, of which AG-1 was predominant) in diseased heads. On some holdings two anastomosis groups were present. AG-3 ("potato group") was found in soil at only one holding.

Practical consequences of the findings concerning host plant susceptibility and anastomosis groups are discussed.

IV. ISOLATION OF RHIZOCTONIA SOLANI FROM THE SOIL

IV.1. INTRODUCTION

The soil-borne fungus *Rhizoctonia solani* is generally considered a facultative parasite. Saprophytic occurrence and behaviour in the soil are factors of major importance for the parasitic appearance of the fungus. *R. solani* may be present in the soil saprophytically in colonized debris, as sclerotia, as hyphae or as basidiospores (Baker and Martinson, 1970). Relatively large fungal particles dominate in most soils (Boosalis and Scharen, 1959; Weinhold, 1977; Clark et al., 1978).

In the past thirty years many methods for detecting *R. solani* in soil have been developed. Modified after Bouhot (1979), three categories of methods can now be distinguished for estimating: inoculum density, competitive saprophytic ability and inoculum potential respectively.

The first category includes the most recently developed methods. The number of *R. solani* propagules per unit of weight of soil is determined by direct plating of soil on selective agar (Ko and Hora, 1971; Henis et al., 1978, Doornik, 1981[b]) or by microscopic observation of sieved soil suspended in water or agar (Ui et al., 1976; Weinhold, 1977; Clark et al., 1978; Roberts and Herr, 1979). Using this method saprophytic and parasitic strains cannot be distinguished without time consuming isolation and testing, and no clear information is obtained on the inoculum capacity. However, an important feature of these methods is the quantitative aspect, namely data on the actual number of propagules in the soil. From a historical point of view it is noteworthy that this, youngest, category of quantitative methods was developed only after an appropriate selective medium for *R. solani* became available recently (Ko and Hora, 1971). It then became clear that the fungus occurs at comparatively very low densities in natural soils (Baker and Cook, 1974). For this reason generally employed soil-dilution-techniques were not satisfactory in the case of

R. solani, as other micro-organisms mostly occurring at high densities. Hence, the sieving methods mentioned above that have since then been developed are largely based on the opposite principle: concentration of the population by sieving of the soil.

The second category of methods are mostly the older techniques. Here, competitive saprophytic ability is determined by burying selective agar, impregnated baits or plants parts in the soil, after which the colonization by *R. solani* is assessed. The older methods have been reviewed by Sinclair (1970), the more recent ones by Geijpens (1974) and Bouhot (1979). Surveys were made by Clark et al., (1978) and Camporota (1980). The results obtained are relative ones: the inoculum activity is expressed as colonized proportion of buried baits. Generally employed dilution end-point-quantification techniques (Bouhot, 1979) have great disadvantages when assessing *R. solani* (Menzies, 1963). Bait methods are intended for investigating aspects such as the saprophytic phase of *R. solani*, changes of the inoculum density, multiplication and survival in the absence of the host (Bouhot, 1979). Also a close correlation appears to exist between the inoculum density measured with bait methods and the resulting disease (Sneh et al., 1966; Geijpens, 1974 and Papavizas et al., 1975).

The third category of methods measure the inoculum potential using susceptible host plants of which the disease is assessed. A summary of these techniques is given by Sneh et al. (1966), Sinclair (1970) and Camporota (1980). It appears that most authors conceive the inoculum potential in a broad sense: "The result of the action of the environment, the vigor of the pathogen to establish an infection, the susceptibility of the host and the amount of inoculum present" (Baker, 1978). It is clear that experimental results obtained with these methods are essentially dependent on the chosen conditions. This concept of inoculum potential sensu Baker (1978) is discussed by Vanderplank (1975) and Meijer (1979). The employment of mathematical transformations of inoculum density data for interpretational purposes as suggested by Baker (1971) and Baker

and Drury (1981) is criticized by Vanderplank (1975), Grogan et al. (1980) and Leonard (1980). An attempt to standardize this type of methods in order to increase the predictive value for disease incidence was made by Bouhot (1979). He postulated a number of experimental conditions by which in fact the maximum potential of the inoculum sensu Vanderplank (1975) is determined. Mitchell (1979) defined this as follows: the capacity of a pathogen population to infest a population of fully susceptible host plants under optimum conditions for infection. Based on the above mentioned standardization, Camporota (1980, 1982) recently developed methods for characterizing *R. solani* populations causing damping-off.

Our aim was to have firstly at our disposal a reliable isolation technique in order to study the relationship between inoculum density and disease in commercial glasshouses and secondly the selection of appropriate fields for testing candidate fungicides against *R. solani* in lettuce. In this connection the paper disk bait method of Herr (1973) was modified and further developed. The inoculum density data obtained with this method are relative. In addition a new quantitative soil sieve method was introduced in order to be able to estimate the actual number of *R. solani* propagules.

IV.2. BAIT METHOD USING PAPER DISKS

IV.2.a. *Introduction*

The paper disk bait method after Herr (1973) is based on the competitive saprophytic ability of *R. solani* propagules to colonize paper disks, soaked in a selective medium, in soil. The method has been developed for in situ research in fields.

Paper disks, 6 mm in diameter, were placed over holes in aluminium plates, which were inserted vertically into the soil without disturbing the growing plants. The plates were respectively incubated, recovered, plated on selective agar and examined for *R. solani*.

Tichelaar (personal communication) modified this field method for the examination of soil samples in the laboratory. Paper

disks are not mounted in aluminium plates but placed in petri-dishes and covered with soil. The procedure is principally the same as the original method by Herr (1973). We adopted Tichelaar's modifications for the greater part, a few details being changed. Disks were not placed on the bottom of petri-dishes but inserted vertically into dishes which had been half-filled with soil. No antibiotica were added to the water agar for plating of paper disks.

The description of the method is as follows: petri-dishes, 9 cm in diameter, are filled with 50 g of well-mixed soil samples and eight 6 mm paper disks soaked in selective medium (according to Herr, 1973) are inserted vertically just under the soil surface. Dishes are incubated at 25°C for a fixed number of days. Disks are then removed by tweezers and freed of excess soil by running tap water. The disks are then dried on filter paper for approximately half an hour, transferred onto 9 cm dishes containing 2 per cent water agar (eight disks per dish) and incubated at 25°C for 24 hours. The edges of the disks are examined microscopically (x 100) for *R. solani* outgrowth based on the morphological characteristics of the mycelium according Parmeter and Whitney (1970); in questionable cases the possession of multinucleate cells in actively growing hyphae is determined (Commonwealth Mycological Institute, 1968). Numbers of colonized baits are expressed as a percentage or transformed to corrected number of colonizations per 100 paper disks according to the concept of multiple infection (Gregory, 1948; Vanderplank, 1975). In standard analysis, five dishes each holding eight disks per soil sample are used and incubated in soil for 48 hours. Based on the assumed at random distribution of the propagules (because of homogenization of the sampled soil) the lowest detectable infestation level with a probability of 95 per cent, is 8 per cent colonized paper disks using the above mentioned sample size (n = 40 disks).

IV.2.b. *Length of incubation period in the soil*

A rapid routine method requires the shortest possible incubation period. The effect of the length of the incubation period on the results using different baits has been demonstrated by Papavizas and Davey (1962), Martinson (1963), Elzarka (1965) and Sneh et al. (1966). Depending on the kind of baits, the authors obtained the best results with an incubation period of 2-4 days. Herr (1973) in his paper disk method, however, found 5-7 days optimum, hypothetically referring to some inhibition of *R. solani* as a consequence of using selective inhibitors. In our opinion another explanation for this comparatively long incubation times lies in the possibility of secondary colonization from neighbouring disks because of the very short distance between baits in Herr's set-up. When compared to primary colonization data, the yield is greater, and later due to the necessary time for secondary colonization to develop.

In view of this an incubation time experiment was carried out in which secondary colonization was excluded. Small plastic cylinders (1.35 cm in diameter, 1 cm high) were filled with approximately 1.8 g of natural infested sandy soil, containing one paper disk. Cylinders were incubated in petridishes at 25°C and periodically inspected for the presence of *R. solani*. At the end of each incubation period fourty paper disks were recovered. (Table IV.1.).

Results show that a high percentage of colonized baits is

Table IV.1.: Corrected number of colonizations per 100 disks after 1-14 days of incubation in naturally infested sandy soil (n = 40 disks).

Incubation (days)	Corrected number of colonizations (per 100 paper disks)
1	20
2	22
3	29
4	14
7	11
14	11

found after only one day. After 2-3 days, the yield is somewhat higher but thereafter decreased abruptly. This trend confirms the finding of the authors quoted. The optimum incubation period appears to be 2-3 days. The distinct difference in optimum incubation period between our modified technique and the original method by Herr (1973) will be further discussed in the next paragraph.

In order to save time, the possibility of a two days' incubation period was investigated. Soil from eleven glasshouses was analysed by the standard method at different incubation periods (1, 2 or 3 days). The results are given in Table IV.2.

The figures show that on an average in the sandy soils the yield after a 3 days' incubation period is not significantly higher than after 2 days. In clay soils the yield tends to be somewhat higher after 3 days. More data are needed to confirm this difference. Therefore, in routine research the chance of underestimating to some extent the inoculum density after two days of incubation will probably be smaller in sandy soils and

Table IV.2.: Corrected number of colonizations per 100 disks, after 1, 2 and 3 days incubation in natural infested soils (n = 40 disks).

Sample		Days of incubation		
		1	2	3
Sandy soil	1	22	29	25
	2	42	82	82
	3	5	20	43
	4	9	11	11
	5	6	48	48
	6	1	20	16
	7	5	20	27
	8	5	17	16
	total	95	247	268
Clay soil	1	0	14	24
	2	2	3	8
	3	5	11	22
	total	7	28	54

perhaps somewhat greater in clay soils. For most research an incubation period of two days is justified.

IV.2.c. *Secondary colonization*

With respect to the paper disk method, the term "secondary colonization" is used when a propagule is growing from one colonized paper disk to a neighbouring disk, which will lead to an over estimation of the inoculum density of *R. solani* in the soil sample.

Several authors reported a relatively fast growth of *R. solani* in soil from the food base, especially in sterilized soils (Papavizas, 1970; Doornik, 1980). We checked, by using eight baits per dish and an incubation period of two days (2 cm distance between baits) the possibility of secondary colonization. In sterilized soil, infested disks were placed vertically at 2 cm distance from non-colonized paper disks. No secondary colonization was observed after one day of incubation at 25°C. After two days, colonization by aerial mycelium of the control disks was completed, indicating a growth of more than 1 cm per day. The growth of mycelium in soil is slower. In a similar experiment, infested disks were covered with sterilized soil. After two days of incubation 55 per cent of the control disks were colonized.

The significance of the food base (Papavizas, 1970), i.e. paper disks impregnated with the selective medium, was illustrated by the result of an experiment with mycelium as inoculum source instead of colonized disks. Non-colonized control disks were again placed at 2 cm distance from the mycelium. After an incubation period of four days none of the control disks were colonized.

In view of this, there is little chance that secondary colonization will occur in our experiments (2 cm distance between baits, two days incubation period). Primary colonization requires namely 1-3 days (see Table IV.2.). In sterile soil a further 1-2 days are required before the mycelium reachs neighbouring disks. In natural soils this will take even longer. A two day incubation period is too short and a 2 cm distance be-

tween paper disks is too wide for secondary colonization to occur.

In Herr's technique (1973), the distance between disks was only 0.75 cm, making secondary, and possibly tertiary, colonization possible. In this case, the optimum incubation time becomes longer and the inoculum density is overestimated. The actual incubation time for primary colonization is shorter than the value (5 - 7 days) found by Herr. In our experiment this was 2 - 3 days which is in accordance with data in literature for other substrates (see Chapter IV.2.b.).

IV.2.d. *Pathogenicity of isolates*

In the paper disk method, saprophytically active inoculum is assessed. The question is to what extent this inoculum proves to be pathogenic for lettuce. In the literature on competitive saprophytic ability a close correlation between colonization and pathogenic activity of the fungus is reported (see Chapter IV.1.). Thus Papavizas et al. (1975) used sterilized table beet seed as a bait and tested different *R. solani* isolates for pathogenicity on seedlings in four crops including lettuce. All isolates were pathogenic for lettuce. Of the remaining isolate and host plant combinations, 5 per cent did not cause disease syptoms.

In order to study this aspect of the paper disk method, we investigated 62 isolates from four glasshouse soils. Results (Table IV.3.) show pathogenicity for lettuce of all isolates

Table IV.3.: Pathogenicity for lettuce of *Rhizoctonia solani* isolates obtained from soil using the paper disk bait method.

Soil	Number of examined isolates	Number of pathogenic isolates
1 clay soil	27	27
2 sandy soil	30	30
3 sandy soil	3	3
4 sandy soil	2	2

tested. Great differences occurred in the degree of pathogenicity with regard to the incubation period and infection rate as described by Papavizas et al. (1975).

Pathogenicity for lettuce of all isolates may be connected with the frequency of lettuce cropping (1 - 2 crops per year) in the glasshouses concerned. It can be concluded that, using the paper disk bait method, the determined relative inoculum density in soil from lettuce glasshouses refers to *R. solani* isolates which are pathogenic for lettuce.

IV.3. SIEVING METHOD, USING SELECTIVE MEDIUM

IV.3.a. *Introduction*

In the literature, methods for estimating the actual number of *R. solani* propagules in field soils (see Chapter IV.1.), are based on one of the following principles. In one method, the soil is sieved, which means that greater quantities of soil can be used (Weinhold, 1977). A disadvantage is the following time-consuming microscopical examination. A second method is that the soil is directly placed on a selective agar, demanding little microscopic assessment (Ko and Hora, 1971). But this means that only very small quantities of soil can be used. In view of these facts, a combination of both methods was developed which has the advantages of both methods and the disadvantages are eliminated.

IV.3.b. *Description of the method*

Soil samples in a number of replicates of 50-100 g of soil are washed over a 0.40 mm sieve. The soil retained is drained off by filter paper at the bottom of the sieve up to just under water-holding capacity and then divided into 50 aliquots. Five to ten aliquots are placed in petri-dishes with modified (see Chapter IV.3.c.) selective medium according to Ko and Hora (1971) and incubated at 25°C for 24-48 hours. Thereafter the edge of clumps are examined microscopically (x 100) for outgrowth of *R. solani* (see Chapter IV.2.a.). The percentage of colonized clumps is determined, corrected for multiple colo-

nization (see Chapter IV.2.a.) and the inoculum density i.e. the number of living propagules per 100 g of soil is calculated.

IV.3.c. *Improvement of the selective medium*

Ko and Hora's selective medium (1971) includes fenaminosulf, a light unstable compound used to inhibit contaminiation by *Oomycetes*. This medium has to be stored and incubated in the dark. In order to avoid this type of storage, furalaxyl, a recently developed fungicide with high activity against *Oomycetes*, was tested as a substitute.

A number of isolates of *Pythium species* and *R. solani* from soil and lettuce were tested for sensitivity to furalaxyl. Six mm diameter cherry-agar disks with mycelium of these fungi were placed in dishes with Ko and Hora's selective medium without fenaminosulf but with furalaxyl in concentrations ranging from 0-256 ppm. For *Pythium spp.*, the minimum effective dose, which would inhibit the growth of mycelium was determined. The inhibition of mycelium growth of *R. solani* at the different dosages was compared with growth in the untreated check. The concentration, at which 50 per cent inhibition is obtained, was estimated by plotting the data on logarithmic probability paper.

From the results (Table IV.4.) it appears that the growth of most *Pythium* species is inhibited completely at 32 ppm furalaxyl. For the common species *P. irregulare* and *P. sylvaticum* ♂, however, the minimum effective dosis is 128 and 256 ppm respectively. Table IV.5 indicates an ED_{50} value of furalaxyl for *R. solani* of 180 ppm.

On account of these results, the concentration of furalaxyl in Ko and Hora's selective medium was standardized at 180 ppm. Although some retardation of growth of *R. solani* on the agar can be expected, interference by contaminating *Pythium spp.* is avoided.

IV.3.d. *Occurrence of R. solani in different sieve fractions*

In the various sieving methods for determining the actual number of propagules in soil (see Chapter IV.1.), different sieve diameters have been employed. Roberts and Herr (1979)

Table IV.4.: Minimum concentrations of furalaxyl (ppm) in the selective medium for inhibition of mycelium growth of *Pythium* species.

Pythium species	Minimum effective concentration of furalaxyl in ppm.
P. ultimum	4
P. oligandrum	8
P. paroecandrum	8
P. tracheiphilum	8
P. intermedium	16
P. gracile	16
P. sylvaticum ♀	32
P. mamilatum	64
P. irregulare	128
P. sylvaticum ♂	256

used 0.25 mm, Uit et al. (1976) 0.3 mm, Weinhold (1977) 0.35 mm and Clark et al. (1978) 0.425 mm. Loss of *R. solani* propagules rarely occurred.

The wider the openings in the sieve the more effective the procedure is. Therefore the possibilities for soil from lettuce greenhouses of a 0.40 mm sieve were investigated. Three soil samples with a rather high inoculum density were washed over sieves of different meshes.

Table IV.5.: Inhibition of *Rhizoctonia solani* mycelium on selective agar with different concentrations of furalaxyl.

Concentration of furalaxyl (ppm)	Mycelium growth inhibition (percentage)
0	0
32	7
64	13
128	38
256	60
ED_{50}: 180 ppm	

From the first sample, 300 g of soil was divided into six aliquots. Three sub-samples were washed over a 0.40 mm sieve, the other three over a 0.25 mm sieve. Fractions were then divided into 50 soil clumps and transferred to Ko and Hora's modified medium (1971) and inspected for *R. solani* outgrowth. It was assumed that no propagules would pass through a 0.25 mm sieve. Should the number of propagules in both fractions prove to be equally high, then no propagules had passed through the 0.40 mm sieve.

The other two soil samples were divided into two sub-samples of 50 g each and both were washed over a 0.40 mm and a 0.25 mm sieve in succession. Fractions on both sieves, ⩾ 0.40 mm and < 0.40 - > 0.25 mm respectively, were divided into 50 clumps and transferred to selective medium plates. Here, it was expected that no *R. solani* would be found in the finest fraction.

The results are summarized in Table IV.6.
The data from the experiment with the first soil sample show no significant difference in the number of collected propagules using 0.25 or 0.40 mm sieves. In soil samples 2 and 3, a single propagule was found in the finest fraction. In this connection there are indications that during the washing procedure coarse soil particles (obviously with one propagule) have splashed onto the fine sieve. It may be concluded that the great majority of *R. solani* propagules are retained by a 0.40 mm sieve. This is in agreement with the literature cited.

Table IV.6.: Occurrence of *Rhizoctonia solani* propagules in different sieve fractions of three soils. Mean number of soil clumps with *Rhizoctonia solani* (n = 50).

Soil	Fractions (mm)			
	> 0.25	> 0.40	> 0.25 - < 0.40	> 0.40
1[1)]	12.0	11.7		
2[2)]			0.5	17.5
2[2)]			0.5	21.0

1) 3 replicates 2) 2 replicates

IV.3.e. *Size of sieved samples*

In order to increase the capacity of the method, it is necessary to know whether the use of greater quantities of soil as Weinhold (1977) indicated (50 g soil per sample) influences the inoculum density results. In this connection it should be taken into account that, by dividing sieved soil samples into 50 clumps, part of the clumps may contain more than one propagule (multiple colonization). During growth on agar plates this will not be noticed, therefore the number of propagules in the sample may be underestimated. This may occur more frequently when inoculum densities are higher and the size of samples is bigger. Assuming that propagules are dispersed at random by mixing the soil, the multiple colonization transformation (see Chapter IV.2.a.) offers possibilities for correction.

In determining the effect of the quantity of sieved soil and the usefulness of multiple colonization correction, three soil samples with a low, intermediate and high inoculum density were examinated. From each sample 50, 100 and 200 g of soil was washed in two replicates, divided into 50 clumps and plated. Clumps from samples of more than 200 g soil could not be handled. Results are shown in Table IV.7.

Table IV.7.: Occurrence of *Rhizoctonia solani* in clumps of different amounts of sieved soil from samples of different degree of infestation from which inoculum densities were calculated (propagules/100 g soil) without and with using correction for multiple colonization.

Soil sample	Amount of sieved soil (g)	Clumps (n=50) with *R. solani*		Inoc. density (prop./100g)	
		mean number	%	not corr.	corr.
1	50	1	2	2	2
	100	2.5	5	3	3
	200	5	10	3	3
2	50	7	14	14	15
	100	15	30	15	18
	200	21	42	11	14
3.	50	14.5	29	29	34
	100	20.5	41	21	26
	200	37.5	75	19	35

Results show that in the case of sample 1 the three different amounts of soil produce an equal value for inoculum density. At this low density level multiple colonization is not expected. It appears that for sample 2 a lower inoculum density value is obtained with 200 g than with smaller amounts of soil. The difference is eliminated by correction for multiple colonization. The effect of the amount of sieved soil proved to be strong in sample 3 at a high density level. Without correction, the estimation of the inoculum density in 100 g and 200 g of soil proved to be lower than in the case of 50 g. After correction for multiple colonization a good conformity was obtained. To conclude, different amounts of sieved soil reach an equal value for inoculum density provided that these are corrected for multiple colonization. The capacity of the new sieving method, with regard to the amount of soil handled, has been increased fourfold by the possibility of sieving 200 g instead of 50 g of soil when compared to Weinhold's method (1977).

IV.3.f. *Efficacy of soil sieving*

The efficacy of sieving soil over a 0.40 mm sieve is dependent on the granular composition of the soil and the character of the organic material present. In sandy soils the fraction >0.40 mm in generally greater than in clay soils. Soils in glasshouses for lettuce cultivation often have a fairly high humus content.

This aspect was investigated with three sandy soils and a clay soil. From each sample, 2 x 100 g of soil were washed over a 0.40 mm sieve. Dry weight was established in the non-sieved soil and in the >0.40 mm fraction. From dry-weight values the percentage >0.40 mm were calculated. The difference with respect to 100 per cent was considered the efficacy percentage of sieving. The results obtained are given in Table IV. 8.

The figures show that about 90 per cent of the soil passes the sieve. This efficacy indicates an increase in the capacity by a factor 9 as compared to Ko and Hora's method (1971) where

Table IV.8.: Efficacy of sieving at four soils.

Soil	Fraction > 0.40 mm	Efficacy of sieving
1 (sand)	11%	89%
2 (sand)	12%	88%
3 (sand)	10%	90%
4 (clay)	9%	91%

non-sieved soil is placed on a selective medium.

IV.3.g. *Pathogenicity of isolates*

As for the paper disk bait method (Chapter IV.2.d.), pathogenicity for lettuce of 54 isolates obtained with the sieve method was tested. Isolates from two sandy soils and one clay soil were used (Table IV.9.).

All isolates proved pathogenic for lettuce, however to different degrees with regard to the incubation period and infection rate. This agrees with the results obtained by Weinhold (1977), who found with his sieving method that all the isolates from soil of cotton fields were pathogenic for cotton.

IV.4. COMPARISON OF METHODS

IV.4.a. *Introduction*

In Chapter IV.1. the practical advantages of the applica-

Table IV.9.: Pathogenicity for lettuce of isolates obtained by the sieving method.

Soil	Number of	
	examined isolates	pathogenic isolates
1 sandy soil	20	20
2 sandy soil	25	25
3 clay soil	9	9

tion of the paper disk method for estimating the inoculum density has been mentioned. The evaluation of the results obtained requires data on the effectiviness of the bait method.

In the literature, a number of experiments comparing different isolation methods have been described. Sneh et al. (1966) compared percentages by *R. solani* colonized units from different baits at progressive degrees of infestation of the soil. For all substrates, a very close correlation (r = 0.96) with the concentration of the inoculum in the soil was found. The highest percentages of colonized baits were obtained with bean segments. These investigations demonstrate that, by using bait methods, a good (relative) estimation of the inoculum density can be obtained.

This also applies to the method in which clumps of soil are plated directly onto the selective medium after Ko and Hora (1971). Henis et al. (1978) found a high correlation between the percentage of colonized clumps and artificially introduced degrees of inoculum densities.

Weinhold (1977) compared the quantitative results, which he obtained with his sieving method examinating 26 natural infested soils, with the data obtained by using the bean segment bait method. A good correlation (r = 0.90) was found. Weinhold concluded: "The high correlation between the inoculum density obtained by stem colonization and the population obtained by the screening assay strongly supports the conclusion that both procedures accurately determine *R. solani* levels in soil".

Based on these literature data, a good correlation between results from our two methods is expected. Because our sieving method is a combination of the Weinhold (1977) and the Ko and Hora (1971) procedures, our paper disk bait method is in agreement with the bait methods applied by Sneh et al. (1966).

The aim of the experiments described in this paragraph were to test the accuracy of both methods and to quantify the relative data on inoculum density from the paper disk method, by comparing these with the actual numbers of propagules obtained with the sieving method.

The experiments require a broad range of inoculum densities,

therefore, samples were taken from a glasshouse in which great differences in level of the infestation were known to occur.

IV.4.b. *Correlation between the results of the sieving and the paper disk methods*

From a lettuce glasshouse on sandy soil, twelve soil samples were taken and treated according to the two isolation methods. The percentages of colonized soil clumps obtained by the sieving method were corrected for multiple colonization and from these the numbers of propagules per 100 g of soil were calculated. Similarly percentages of colonized baits obtained by the paper disk method were corrected for multiple colonization and given as numbers of colonizations per 100 paper disks. Based on these figures, the regression of the paper disk method results on the sieving method data were calculated (Figure IV.1.).

It appears that a rather broad range of inoculum densities were involved and that a very good correlation exists between results from both methods ($r = 0.99$). The regression line passes through the origin. These findings confirm our expectation and support the conclusion that the inoculum density is accurately estimated by both methods.

IV.4.c. *Quantification of the results obtained by the paper disk method*

The interpretation of inoculum density results obtained by the paper disk bait method requires quantification of the percentages of colonized disks to numbers of propagules per 100 g of soil. The sieving method provides the actual number of propagules of living *R. solani* per unit of soil.

In Chapter IV.4.b. a good correlation between results of the both methods has been demonstrated. Therefore, the number of living propagules per 100 g of soil ($\hat{X}$) can be calculated from data (Y) obtained by the paper disk method, using the regression equation, $\hat{X} = 0.26\ Y$, based on the data of the previous paragraph (Figure IV.1.).

The application of this figure indicates the actual value of the threshold level of the paper disk method. Using the

standard procedure of the method (n = 40 paper disks) the threshold of detection was determined on 8 per cent colonized disks (Chapter IV.2.a.). Quantification of this figure with the above mentioned regression equation gives the value of 2 propagules of *R. solani* per 100 g of soil. Soil samples examined by the standard procedure, that showed none colonized baits, may, therefore, contain 0 - 2 propagules per 100 g of soil.

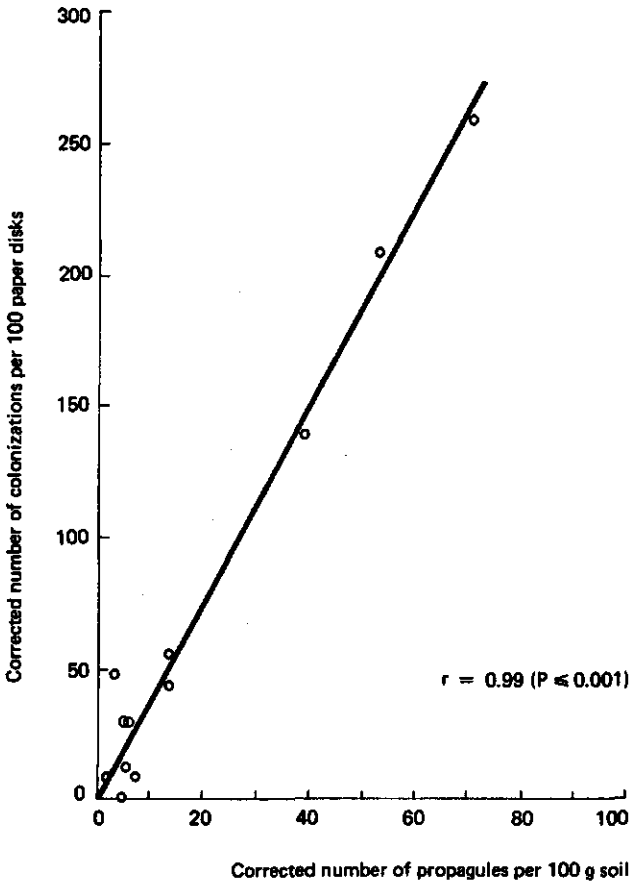

Figure IV.1.: The relationship between the corrected number of propagules per 100 g soil (calculated from the data obtained by the sieving method) and the corrected number of colonizations per 100 paper disks (calculated from the data obtained by the paper disk method).

IV.5. DISCUSSION

The modified paper disk bait method proved to be an accurate and relatively fast and simple way of estimating the levels of *R. solani* in soils.

The paper disks should be incubated in the soil for only two days. Under these conditions and using a two cm distance between disks, no secondary colonization occurred and as a consequence, also no overestimation of the inoculum density took place. In our opinion the possibility of the disturbing appearance of secondary colonization occurring with some baiting methods has received too little attention in the literature concerned (Chapter IV.1.). It has been pointed out that the optimal incubation period published by Herr (1973) was too high probably due to the occurrence of secondary colonization. This also applies to the recently published results of Camporota (1981), which he obtained by adapting Herr's method.

Ecologically, *R. solani* colonized suitable organic material very quickly and abundantly (Garret, 1956), but obviously it is readily succeeded, antagonized or masked by other species (Papavizas and Davey, 1959; Sneh et al., 1966). The rather abrupt decrease of percentage colonized baits at longer incubation times in our experiments confirms this.

As distinct from natural baits which are used in other methods, the composition of the impregnated paper disks is standardized. Another advantage may be the selectivity of the medium used in the paper disk method. Examination for *R. solani* rarely was disturbed by *R. solani*-like fungi. Jager (1979), using segments of *Juncus effusus* as a bait in potato field soils, frequently came across *Rhizoctonia*-like fungi which hindered the examination.

The use of the paper disk method, as it measures the competitive saprophytic ability of the *R. solani* population, was determined also by the generally accepted correlation of results of baiting methods with disease (Sneh et al., 1966; Geijpens, 1974; Papavizas et al., 1975). Chapter VI goes further into this aspect.

A possible restriction of the paper disk method and of bait methods in general is an underestimation of the inoculum density occurring at hundred per cent colonized substrate units (Roberts and Herr, 1979). The chance is small that such high levels of *R. solani* in mixed soil samples from lettuce glasshouses will be found (Chapter V). Another possibility of underestimating the population density is the appearance of multiple colonization at higher density levels. However, from the experimental data about the correlation with the results of the sieving method, the suitability of the correction for multiple colonization according to Gregory (1948) can be derived. This agrees with the generally accepted use of this correction (Vanderplank, 1975).

A good correlation between results of the paper disk method and the sieving method has been demonstrated. It could be concluded that the inoculum density is accurately estimated by both methods. A regression formula was introduced for the quantification of the relative inoculum density data from the paper disk method into actual numbers of *R. solani* propagules per 100 g of soil. It should be borne in mind that these experiences are based on soil samples from one glasshouse. Should more glasshouse soils involved, the observed correlation coefficient ($r = 0.99$) perhaps would be less high and the regression coefficient (0.26) have another value, because of the possible differences in competitive saprophytic ability of *R. solani* field populations or differences in the conductivity of soils for *R. solani*. However, differences between soils with the same crop are on average obviously small as Weinhold (1977) found when he did similar experiments of twenty-six cotton soils. He calculated a correlation coefficient of $r = 0.90$ for the data from two inoculum density determining procedures. The results obtained by Camporota (1981) with a modified paper disk method indicate a similar competitive saprophytic ability of *R. solani* populations from four soils.

In contrast to other quantitative assays the described new

sieving technique is time-saving because it combines the advantages of two principles. By sieving samples, about 90 per cent of the soil is discarded, while microscopic observation is limited to the well-defined edges of clumps of soil plated on selective agar. Apart from cutting down on the amount of microscopic examination necessary, the capacity of the new quantitative sieving method is four and nine times greater than the original assays of Weinhold (1977) and Ko and Hora (1971) respectively.

The accuracy of the method was demonstrated in the experiment on the relationship between the inoculum density data in glasshouse soil, as determined by the sieving technique, and the paper disk method.

The occurrence of the multiple colonization of soil clumps has proved to be corrected adaquately according the method of Gregory (1948).

By the sieving method it is also possible to estimate the vigour of *R. solani* propagules in soil as reflected by the number and length of hyphae emerging from each clump (Henis et al., 1978).

Isolates obtained by our sieving method were pathogenic for lettuce; Weinhold (1977) in using his sieving method also found all examinated *R. solani* isolates from cotton fields pathogenic for cotton.

From the experiments with different sieving-diameters it appeared that also in lettuce glasshouses *R. solani* is practically restricted to coarse fractions (> 0.40 mm), corresponding literature data with regard to *R. solani* in other crops (see Chapter IV.3.d.).

IV.6. SUMMARY

A relatively fast and simple bait technique for detection of *R. solani* in soil has been improved and standardized, using paper disks impregnated with selective compounds. After two days incubation in soil and one day on water agar the disks are microscopally examined for the presence of *R. solani*; rel-

atively few disturbing fungi appear. Underestimation of the relative inoculum density due to multiple colonization of the disks could be removed by correction the percentage of colonized disks using the formula on transformation for multiple infection (Gregory, 1948). All isolates tested from four glasshouses obtained with this method, were pathogenic for letttuce.

A new quantitative sieving method has been developed for estimating the actual number of active propagules of *R. solani* in soil by plating clumps of the soil fraction > 0.40 mm on an improved selective medium. After incubation of the soil the number of clumps with outgrowth of *R. solani* is determined by microscopic examination. Multiple colonization has been adaequately corrected by Gregory's (1948) formule. The sieving method is based on the combination of principles of two exsisting techniques whereby the advantages of these assays are maintained and the disadventages eliminated. Apart from reducing the time for microscopic examination the capacity of the new method is four and nine times greater than the original techniques, respectively. All isolates tested from three glasshouses obtained with the sieving method are pathogenic for lettuce.

It has been concluded from the good correlation between the results of the paper disk method and sieving method, that inoculum density of *R. solani* is estimated accurately by both methods.

For quantification of the relative inoculum density data obtained with the paper disk method a regression formula has been calculated.

V. OCCURRENCE OF RHIZOCTONIA SOLANI IN THE SOIL

V.I. INTRODUCTION

In the preceding chapters several times attention has been focused on the soil-borne character of the attack on lettuce by *Rhizoctonia solani*. Because of this property, knowledge of the occurrence and the behaviour of the fungus in the soil is essential to understand the appearance of the disease in glasshouses.

The little information available in literature on these aspects of the fungus in mainly confined to field crops such as potatoes, sugar beets, snap beans (Papavizas, 1970). However, the author is not aware of publications relevant to glasshouse crops, with their specific cultural measures (e.g. soil disinfestation). This lack of knowledge is particularly felt with regard to the control aspect of *R. solani* in lettuce.

In Chapter IV, it has been shown that the paper disk method is a suitable procedure for measuring *R. solani* in soil. Using this technique, the following aspects are investigated in lettuce glasshouses: the spatial pattern of *R. solani* propagules in the soil, the changes of inoculum density during successive lettuce crops, the occurrence of the fungus in the soil of a number of commercial glasshouses and its relationship to glasshouse characteristics.

V.2. SPATIAL DISTRIBUTION

V.2.a. *General*

In view of field sampling procedures for the determination of inoculum densities and for disease dynamics studies, information on the spatial pattern of *R. solani* propagules in the soil is desirable.

Experimental evidence concerning the spatial distribution of soil-borne pathogens in scarce. Campbell and Pennypacker (1980), in reviewing the literature, reported the following

demonstrated or suggested spatial distributions: tetrahedal or regular (for modelling purposes), uniform, random, uneven and non-uniform (clustered).

In lettuce glasshouses a pattern of scattered patches of diseased plants has often been observed both by growers and the author. This indicates a horizontally clustered pattern of *R. solani* population in the soil. According to Dijkstra (personal communication), the negative binomial frequency distribution function has been particularly applicable in describing data involving clustering of organisms in general. The parameters of this distribution are: p = probability of paper disk colonization by *R. solani* and k = relative index to clustering. The "k" parameter gives an indication of the degree of clustering present. Small values of "k" i.e. approaching zero, indicate extreme clustering, as "k" approaches infinity, clustering decreases and random distribution is defined. General information about the application in phytopathology of the negative binomial distribution function is given by Campbell and Pennypacker (1980).

The statistical analysis of the experimental data are based on the publications of Anscombe (1950), Bliss and Fisher (1953) and Taylor (1961).

The procedure was as follows. On rectangular plots (see V. 2.b.) every 25 cm, corresponding to the distance between the lettuce plants, soil samples of 9 cm diameter and 1.5 cm depth were transferred to petri-dishes of the same size and provided with 10 paper disks each. After incubation the number of colonized disks and from that the corrected number of colonizations per 10 disks for each sample was determined.

From these figures a frequency table of the numbers of soil samples with specified numbers of colonizations per 10 disks was composed. The mean number of colonizations per 10 disks and the variance of the soil samples were also calculated.

From these data the variance-to-mean ratio was calculated and the statistical equality to unity was tested. A variance-to-mean ratio not significantly different from unity indicates a distribution of *R. solani* propagules conforming to a Poisson

serie and, therefore, represents an even or purely random dispersion of sampled individuals. A variance-to-mean ratio significantly greater than unity could indicate a clustering of propagules.

To describe the clustering of the *R. solani* propagules in the horizontal layer of the soil the above mentioned frequency data were further analysed for goodness of fit for the negative binomial distribution and the "k" parameter was determined to indicate the degree of clustering present.

In order to inquire into the vertical dispersion of propagules, plots (see Chapter V.2.c.) were sampled with an auger (22.5 cm high, 6 cm diameter). The soil sample obtained was divided into 2.5 cm slices of soil. Each slice was transferred onto a petri-dish with 8 paper disks. After incubation the percentages of colonized disks were determined and corrected for multiple colonization. With these data the linear regression on the depth of the slice of soil was calculated.

V.2.b. *Horizontal distribution*

This was investigated in two glasshouses, A and B, on sandy soil. In A, a healthy tomato crop was ready for harvest. This crop was planted after *R. solani* diseased lettuce. The size of the rectangular trial plot was 50 x 175 cm. This area was sampled every 25 cm in 2 rows of 175 cm long and 25 cm apart. The results of the 24 samples are given in Figure V.1.

Inoculum densities show great differences between sampling sites only 25 cm apart. Some positions have 8 or 9 colonized disks out of 10 in contrast to border positions with none.

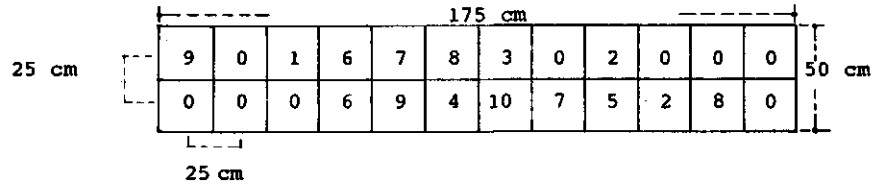

Figure V.1.: Pattern of inoculum density in a section of glasshouse A. Map of the number of colonized paper disks per soil sample with 10 disks, taken at 25 x 25 cm distance.

Table V.1.: Glasshouse A. Analysis for pattern of inoculum density. Frequencies of corrected number of colonizations per 10 disks of 23 soil samples and the goodness of fit of expected frequencies for negative binomial distribution.

Corrected number of colonizations per soil sample [1]	Frequency[2]	Expected frequency for negative binomial
0	9	9.0
1	1	2.9
2	2	1.8
3	1	1.3
4	0	1.1
5	1	0.9
6	1	0.7
7	0	0.6
8	0	0.5
9	2	0.5
10	6	3.7

Mean corrected number of colonizations: 5.8
Variance: 56.26
Variance-to-mean ratio: 9.71
"k" value: 0.34
Goodness of fit for negative binomial distribution:
chi-square value 3.52. Critical value 6.64,
$P < 0.01$, 2 degrees of freedom

1) Numbers obtained from the data of figure VI.1. by correction for multiple colonization of disks, 10 paper disks per soil sample.
2) Number of soil samples having specified number of colonizations per 10 disks, 23 soil samples.

In order to answer the question whether propagules were dispersed at random or clustered, data on inoculum density were analysed further statistically. The result of the position in which 10 disks were colonized (100 per cent) has not been used in the calculations.

From the data in Table V.1. it appears that the variance-to-mean ratio of the number of colonizations per 10 disks is significantly greater than 1. This indicates a departure from a pure random distribution of the inoculum density. This was confirmed by the fitting of the observed frequency data for colonizations by the negative binomial distribution, with a relatively low "k" value.

Table V.2.: Glasshouse B. Analysis for pattern of inoculum density. Frequencies of corrected number of colonizations per 10 disks of 140 soil samples and the goodness of fit of expected frequencies for negative binomial distribution.

Corrected number of colonizations per soil sample[1)]	Frequency[2)]	Expected frequency for negative binomial
0	76	76.0
1	37	33.8
2	15	15.8
3	6	7.5
4	0	3.6
5	2	1.7
6	3	0.8
7	0	0.4
8	0	0.2
9	1	0.2

Mean corrected number of colonizations: 0.87
Variance: 2.04
Variance-to-mean ratio: 2.34
"k" value: 0.91
Goodness of fit for negative binomial distribution: chi-square value: 0.76. Critical value 9.21, $P < 0.01$, 3 degrees of freedom

1) Numbers obtained by correction for multiple colonization of disks, 10 paper disks per soil sample.

2) Number of soil samples having specified number of colonizations per 10 disks, 140 soil samples.

The conclusion may be that the results obtained indicate a clustering of the *R. solani* propagules in the soil of the examined area. This is visible in Fig. V.1.: positions with high inoculum densities are concentrated in the centre of the plot.

In the second glasshouse B, a relatively large area was sampled one week after planting lettuce. In the preceding crop about 50 per cent of the lettuce plants were diseased by *R. solani*. In two 18 meters rows (mutual distance 25 cm) samples were taken at 25 cm from each other (140 soil samples).

In Table V.2. the frequencies of the corrected inoculum densities data are shown and the analysis of their spatial distribution over the plot are given.

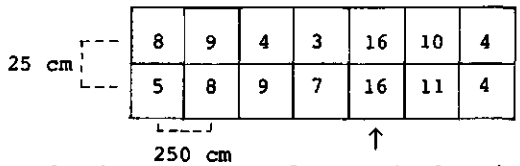

Figure V.2.: Glasshouse B. Map of summarized number of colonized disks per 10 soil samples (2.5 m), 10 paper disks each.

Analogous to results in the first glasshouse, data from the second glasshouse indicate clustering of *R. solani* propagules in the soil, whereby the dispersion of the inoculum density can be very well described by the negative binomial distribution. The parameter "k" in this case is also relatively low, which indicates to a high degree of clustering. This is illustrated in Figure V.2., in which inoculum density data per 2.5 meter (10 successive samples) has been summed up for each row.

It appears that there is a distinct local concentration of high inoculum densities (see arrow).

Summarizing it can be concluded that *R. solani* propagules in both glasshouses (during the preceeding crop and at the start of lettuce cultivation, respectively) were not distributed at random but clustered. With respect to density, data could be characterized well by the negative binomial frequency distribution.

V.2.c. *Vertical distribution*

This was investigated in two glasshouses, C and D, on sandy and clay soil respectively. In glasshouse C, at the time that the late spring lettuce was ready for harvest, 9 loci were sampled up to a depth of 30 cm. Results are shown graphically in Figure V.3.

A negative linear regression appears to exist between inoculum density and sampling depth. Extrapolation of the regression line results in the intercept with the X-axis between the 11th and 12th slice of soil (27.5 - 30.0 cm), below which, therefore, the chance of finding *R. solani* is poor. In this glasshouse the fungus could therefore be found throughout the whole of the plough layer.

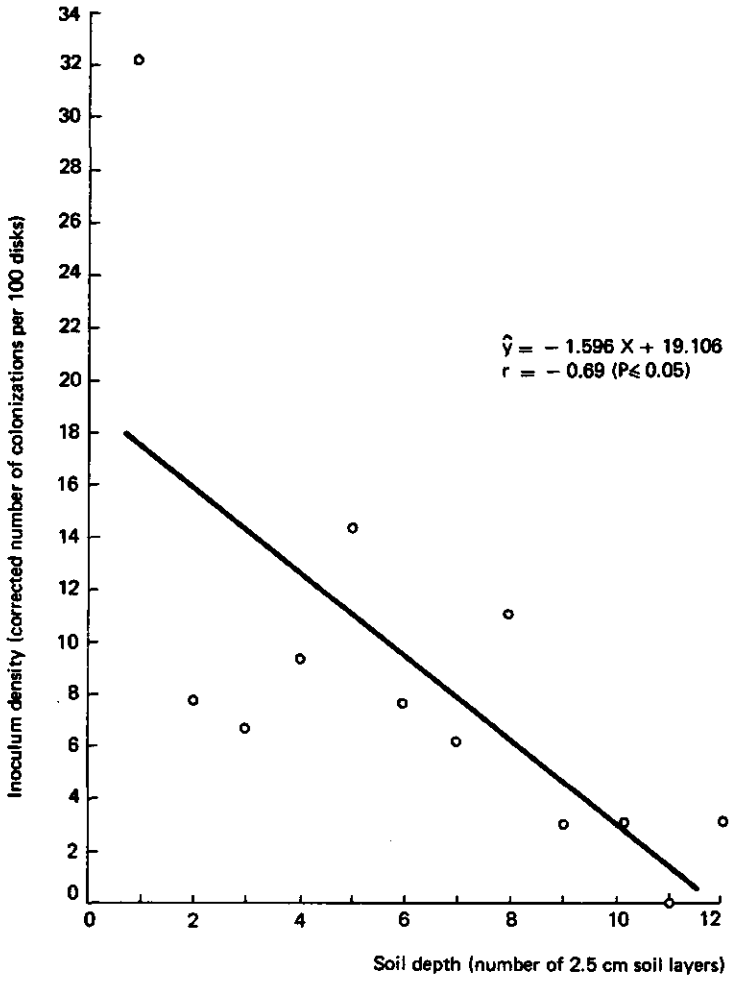

Figure V.3.: Glasshouse C. Relationship between 2.5 cm soil layers and inoculum density (number of colonizations per 100 paper disks) at the time of harvest of lettuce in a sandy soil glasshouse. Density data represent the average of 9 soil samples.

In the glasshouse D on clay soil, 12 samples were taken to depth of 15 cm at the time of harvest of tomatoes. Results are given in Figure V.4.

As in glasshouse C there is a rather high negative correlation between inoculum density and depth of sampling. Extrapolation of the regression line results in the intercept with

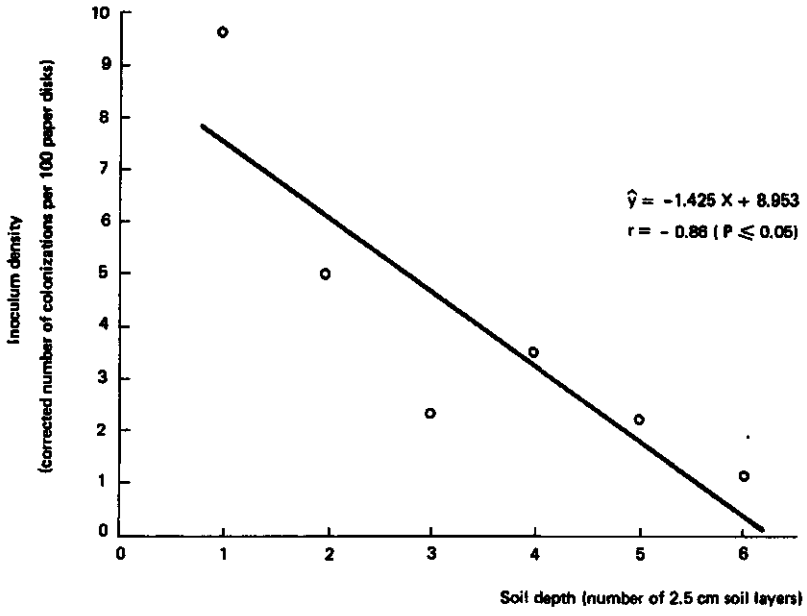

Figure V.4. Glasshouse D. Relationship between 2.5 cm soil layers and inoculum density (number of colonizations per 100 disks) at the time of harvest of tomatoes in a clay soil glasshouse. Density data points represent the average of 12 samples.

the X-axis just outside the sixth slice of soil (15 cm). Below this depth the chance of finding *R. solani* propagules is poor.

V.3. CHANGES IN INOCULUM DENSITTY

V.3.a. *General*

The purpose of this study was to collect data on the changes of the inoculum density of two successive lettuce crops in three glasshouses, in order to obtain in particular data on the relation to soil temperature.

The inoculum density of the soil between lettuce plants was determined periodically in two glasshouses (A and B) on sandy soil and one glasshouse (C) on clay soil. In each glasshouse six plots, measuring 1 x 1 meter, were sampled during the cultures 3 - 6 times by taking 12 cores at 5 cm depth with an au-

ger, 4 cm diameter. The 12 sub-samples were mixed making one composite sample per square meter. These samples were analysed with the standard procedure of the paper disk method (see IV. 2.a.). Plots were not treated with fungicides. In glasshouse B the inoculum density of the soil of the preceding tomato crop and the next cultivations of the soil were also determined.

The soil temperature at a depth of 3 cm was measured daily or weekly, as nearly was possible at the same time of day.

At time of harvest the percentages of *R. solani* diseased lettuce heads from the plots were determined.

V.3.b. *Results in glasshouses*

Glasshouse A on clayey sand, was kept dry during the early spring crop (11-2-1979/18-4-1979) and very dry during the late spring crop (30-4-1979/6-6-1979). Results of the changes of the inoculum density and soil temperatures during both crops are given in Figure V.5.

During the first spring crop the inoculum density appeared to remain constant on a relatively low level (1 - 3 colonizations per 100 disks). Soil temperatures remained low during the early growing period of this crop (4 - 7°C) but nearer harvest time the temperature was higher (10 - 12°C). At time of harvest none of the 36 lettuce heads examined, was attacked by *R. solani*.

During the second, short-term late spring crop the soil was sampled three times. The inoculum density remained relatively low (2 - 8 colonizations per 100 paper disks) but it tended to increase during the growing period. Soil temperature (16 - 20° C) was higher than in the first crop. A slight attack by *R. solani* (3 per cent of 60 lettuce heads) was observed.

In comparing population levels in both crops, there were no significant differences, although the soil temperature during the second crop was considerably higher. It seems that no correlation exists between soil temperature and inoculum density under these glasshouse conditions

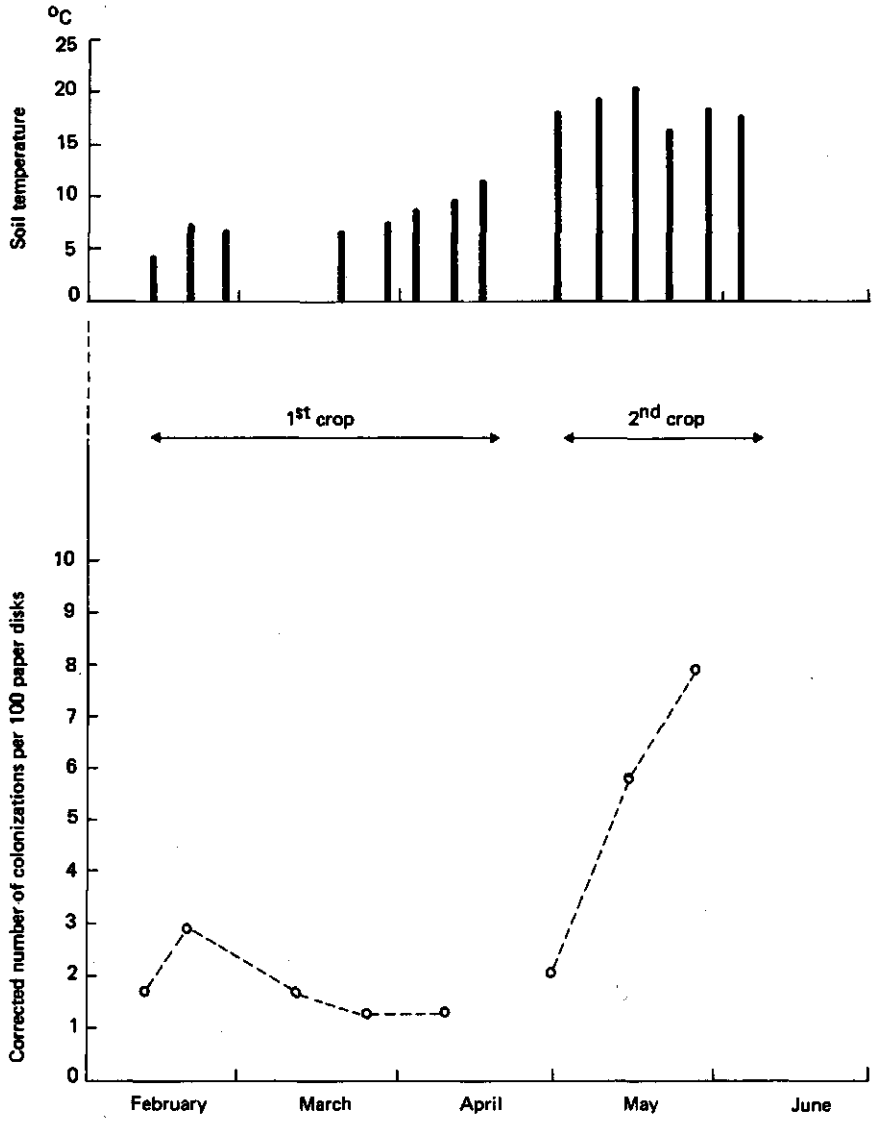

Figure V.5.: Glasshouse A. Inoculum density (corrected number of colonizations per 100 paper disks) and soil temperature (° C at 3 cm below soil level at 8 a.m.) during two successive lettuce crops. Density data points represent averages of six replications (plots).

In glasshouse B, on rather humid sandy soil, the early spring crop was from 13-1-1979 to 9-4-1979, the late spring crop from 14-4-1979 to 28-5-1979.

In this glasshouse supplementary soil samples were taken at the end of the preceding tomato crop (9-10-1978), after soil drainage (12-12-1978) and close before (29-12-1978) and after (30-12-1978) rotary tillage.

Observed data from this glasshouse are shown in Figure V.6.

Results show a high inoculum density at the end of the summer tomato crop (55 colonizations per 100 paper disks). After harvest (December) in the unheated glasshouse, the level of infestation of the soil was significantly lower (23 colonizations). Drainage and tillage had no distinct effect on the inoculum density.

During the first crop the population level (9 - 13 colonizations per 100 disks) was maintained. In the beginning, the temperature was below 10°C. In the later period it rose to 14° C. Twelve per cent of the 60 investigated lettuce heads had been attacked at harvest.

During the second crop the inoculum density increased considerably on the last two sampling dates: 156 and 116 colonizations per 100 disks respectively. Soil temperature fluctuated around 20°C. Attack by *R. solani* was severe: of the 36 lettuce heads examinated 55 per cent were infected.

In conclusion it can be stated that the inoculum density was high in the preceding tomato crop. Thereafter it decreased to a level that remained constant during the soil treatments and the following lettuce crop. A strong increase occurred at the end of the second crop. During this period the temperature was relatively high. Results obtained from this glasshouse indicate a positive relationship between inoculum density and temperature.

In glasshouse C on clay soil, the winter crop was from 6-11-1978 to 12-2-1979 and spring crop from 24-2-1979 to 17-4-1979. Changes of inoculum densities and soil temperature during both crops are shown in Figure V.7.

During the first cropping period, with the exception of sampling dates 3-1-1979 and 17-1-1979, no significant differences in inoculum densities were found. There is no explanations for the two extreme values on these dates. The score was low

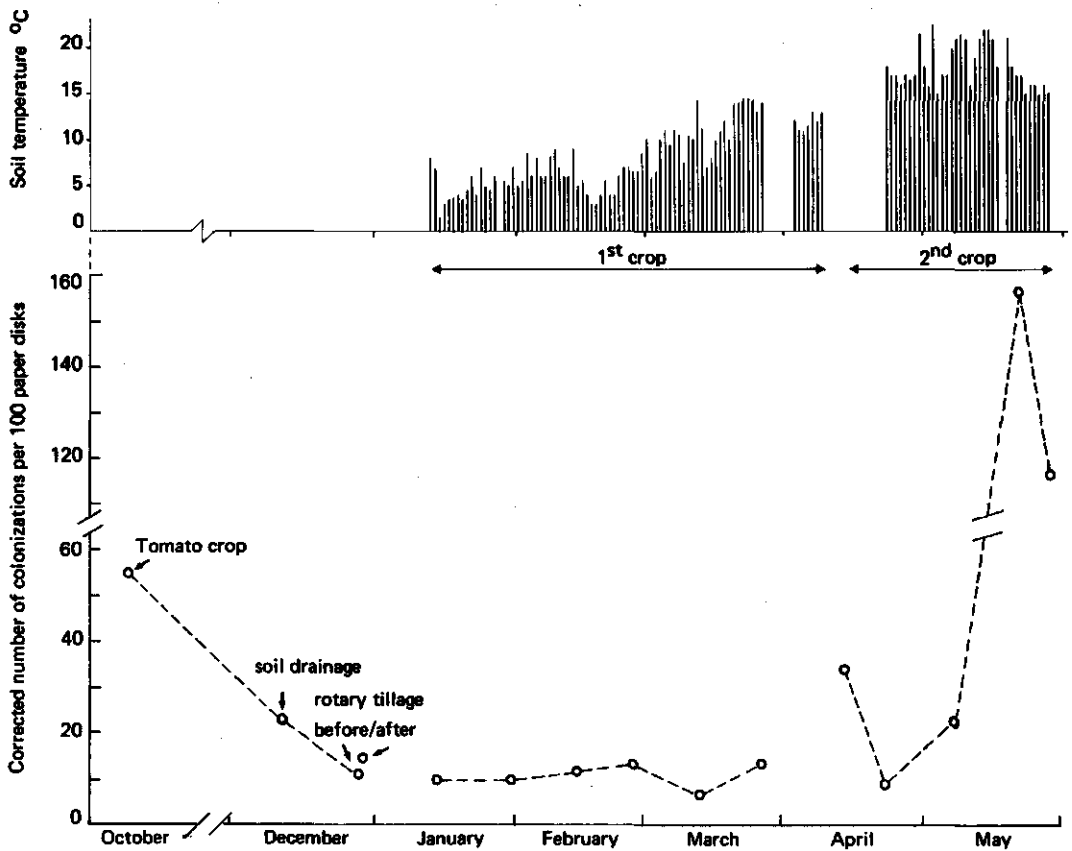

Figure V.6.: Glasshouse B. Inoculum density (corrected number of colonizations per 100 paper disks) and soil temperature (°C at 3 cm below soil level at 1 p.m.) during two successive lettuce crops, and the inoculum density of the previous crop tomato (October), after soil drainage, before and after rotary tillage (December). Density data points represent averages of six replications (plots).

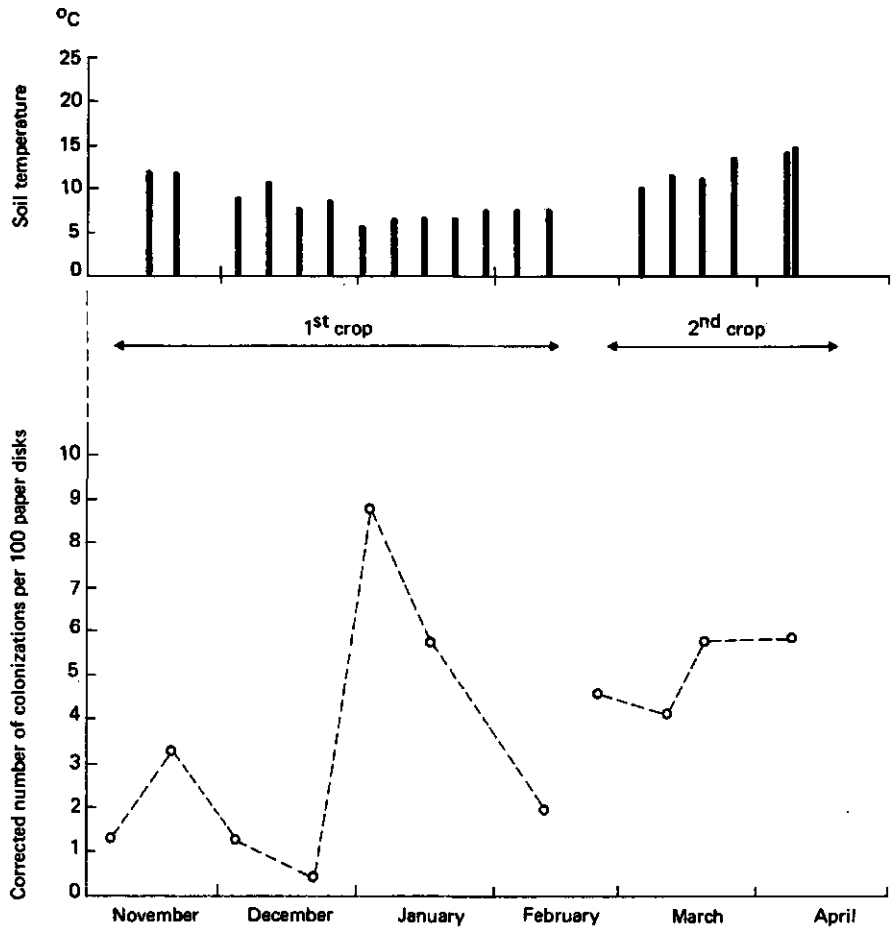

Figure V.7.: Glasshouse C. Inoculum density (corrected number of colonizations per 100 paper disks) and soil temperature (°C at 3 cm below soil level at 9 a.m.) during two successive lettuce crops. Density data points represent averages of six replications (plots).

on the other dates (0.5 - 3.5 colonizations per 100 disks). At the beginning, soil temperature was just over 10°C but fell during the last two months of the growing period to 7 - 8°C. At harvest time, none of 36 lettuce heads examinated was found infected by *R. solani*.

Inoculum densities during the second crop did not differ significantly. The levels remained low (4.5 - 6 colonizations per 100 disks). Soil temperature increased up to 10 - 14°C. At

harvest, 9 out of 36 lettuce heads were attacked by *R. solani* (25 per cent).

In summarizing, it can be concluded that the inoculum density remained low during both crops.

V.4. OCCURRENCE OF INOCULUM IN DIFFERENT GLASSHOUSES

V.4.a. *General*

In the years 1977-1979 the soil of 62 commercial glasshouses was investigated for the presence of *R. solani*. This was done in order to select trial plots on which to test candidate fungicides. The samples were taken either from soil that had been prepared for a lettuce crop, or from soil of the previous crop.

The procedure was as follows: Glasshouses were selected on the basis of regularly occurring a-specific bottom rot symptoms in lettuce. This choice was made on the assumption that the change was greater that *R. solani* would be found in these glasshouses rather than in those that had no problems.

Sampled units measured 9, 18 or 36 m^2. The total of investigated units per glasshouse varied from 1 to 18. Ten sub-samples per 9 m^2 were taken with an auger (see Chapter V.3.a.) and mixed making one composite sample. Depending on the number of the above mentioned units, composite samples contained 10, 20 or 40 sub-samples. The inoculum density in each composite sample was determined using the standard paper disk bait method. For each glasshouse the average inoculum density (corrected numbers of colonizations per 100 paper disks) and also the range of results of the separate composite samples are given.

The results of these samplings were also examined in order to answer the question to what extent, using the composite soil sampling procedure, a clustered spatial pattern of *R. solani* propagules (see Chapter V.2.b.) can be demonstrated. For this purpose the distribution of inoculum density data of separate samples from glasshouses of similar infestation degree was statistically analysed according to the method mentioned in V.2.a.

In order to relate observed inoculum densities to possible relevant farming characteristics, a questionnaire was filled

in by growers regarding soil type, crop management and fungicides in these glasshouses (Table V.3.). Eventual differences in average inoculum density between the various categories were statistically proved.

V.4.b. *Inoculum density*

Detailed figures on the results obtained for each investigated nursery are compiled in Table V.3.

In Table V.4. the average inoculum densities results per nursery are classified and their frequencies are given.

These data show that the average degree of infestation in the glasshouses was between 0 - 12.8 colonizations per 100 disks. In more than 50 per cent of the glasshouses the average inoculum density was less than 1.4 colonizations per 100 disks. Almost 90 per cent of all the glasshouses were in the category of less than 5.4 colonizations per 100 disks.

Calculated for numbers of propagules, using the equation as established in Chapter IV.4.c., these figures equal 0.4 and 1.4 *R. solani* propagules per 100 g of soil respectively. In the most infested glasshouse (No. 27, Table V.3.) the average inoculum density was 12.8 colonizations per 100 paper disks (3.3 propagules per 100 g of soil).

The data on the range of observed densities per separate sample (Table V.3.) show a great variation in infestation level of the soil within individual glasshouses. An example is nursery No. 39 where nine samples were taken (each from 18 square meters). The average inoculum density was low (1.3 colonizations per 100 paper disks, corresponding with 0.3 propagules per 100 g of soil). Of these nine samples one had an inoculum density of 16.3 colonizations per 100 paper disks (4.2 propagules per 100 g soil), while in the remaining eight samples no *R. solani* were observed. The range on the most heavily infested farm (No. 27) was 0 - 47.0 colonizations per 100 paper disks (12.2 propagules per 100 g of soil).

The occurrence of heavily infested composite samples next to samples that were practically free indicates a non-random distribution of *R. solani* propagules in these glasshouses. To

Table V.3.: Sampling data from 62 commercial glasshouses and the relative *Rhizoctonia solani* inoculum density in the soil (mean corrected number of colonizations per 100 paper disks and range)

No.	Year	Soil type	Sampling (month)	Sampling in		Previous crop	Soil treatment[1] before sampling		Quintozene treatment within last 2 years	Number of samples	Total sampled area (m^2)	Inoculum density[2]	
				Previous crop	Plant-bed		>1 Year	<1 Year				Mean	Range
1	77/78	clay	9	x						4	108	9.0	5.1-13.4
2	"	sand	9	x		tomato			+	4	144	0	-
3	"	sand	9	x		cucumber			+	4	144	0	-
4	"	organic	9	x		cucumber		Steam	-	4	144	0	-
5	"	sand	9	x		tomato	MBR '75		-	3	108	0	-
6	"	clay	9	x		cucumber	Steam '75		+	4	144	7.5	0-16.3
7	"	clay	9	x		cucumber		Steam	-	4	144	3.4	0- 7.8
8	"	organic	9	x		cucumber	MBR '75		-	4	144	1.0	0- 2.5
9	"	organic	9	x		lettuce			+	4	144	1.9	0- 2.5
10	"	sand	9	x		lettuce			-	2	72	2.5	2.5- 2.5
11	"	sand	9	x		tomato		MBR	-	2	72	5.0	0-13.4
12	"	sand	10		x	cucumber			+	3	108	1.7	0- 2.5
13	"	clay	11	x		tomato		MBR	-	18	162	0	-
14	"	sand	12	x		cucumber		MeNa	+	3	108	0	-
15	"	clay	1	x		endive			-	4	144	0	-
16	"	clay	1	x		endive	MBR '76		-	4	144	0	-
17	"	clay	1	x		bean		MBR	+	8	288	0	-
18	"	clay	2	x		lettuce		MBR	+	12	108	0	-
19	"	clay	2	x		lettuce		MBR	+	15	135	0	-
20	"	clay	2	x		lettuce			+	8	288	0.7	0- 2.5
21	"	clay	2	x		spinach		MeNa	-	3	108	1.1	0- 2.5
22	"	clay	2	x		lettuce	MeNa '76		+	18	162	5.4	0-35.7
23	"	organic	3	x		endive				4	144	0	-
24	"	clay	3	x						3	108	9.7	2.5-13.4
25	78/79	organic	9		x	cucumber		Steam	-	3	108	0	-
26	"	sand	10	x		bean		MeNa	+	9	162	0	-
27	"	clay	10	x		lettuce	Steam '76		-	9	162	12.8	0-47.0
28	"	clay	10	x		red pepper	Steam '76		-	9	162	0.9	0- 2.5
29	"	organic	10	x		cucumber		Steam	-	9	162	0	-
30	"	sand	10	x		tomato			-	9	162	0.9	0- 5.1
31	"	sand	10			tomato			+	9	162	1.2	0- 7.8

1) MBR = methyl bromide, MeNa = metam natrium, Steam = steam 2) Corrected number of colonizations per 100 paper disks

Table V.3. (continued): Sampling data from 62 commercial glasshouses and the relative *Rhizoctonia solani* inoculum density in the soil (mean corrected number of colonizations per 100 paper disks and range).

No.	Year	Soil type	Sampling (month)	Sampling in		Previous crop	Soil treatment[1] before sampling		Quintozene treatment within last 2 years	Number of samples	Total sampled area (m^2)	Inoculum density[2]	
				Previous crop	Plant-bed		>1 Year	<1 Year				Mean	Range
32	78/79	sand	10	x		tomato			+	9	162	7.0	2.5-13.4
33	"	sand	10	x		tomato		MBR	-	1	18	5.1	5.1- 5.1
34	"	clay	10	x		cucumber		MeNa	+	9	162	0	-
35	"	clay	10	x		tomato	MeNa '73		-	4	72	4.0	0- 7.8
36	"	clay	10	x		red pepper	? '74		-	9	162	4.5	0-19.2
37	"	sand	11	x		bean	Steam '76		+	9	162	0	-
38	"	sand	11	x		endive			-	9	162	4.2	0-16.3
39	"	sand	12		x	tomato	MBR '72		+	9	162	1.3	0-16.3
40	"	sand	12		x	spinach	MBR '74		-	9	162	1.0	0- 7.8
41	"	sand	12	x		tomato	Steam '71		-	9	162	3.9	0-13.4
42	"	sand	2	x		melon	Steam '73		+	9	162	1.1	0- 2.5
43	"	sand	2		x	spinach	MBR '72		+	9	162	0	-
44	"	clay	3	x		lettuce	MBR '77			10	90	3.6	2.5- 7.8
45	79/80	clay	9		x	cucumber	Steam '77		-	7	126	4.4	0-19.2
46	"	clay	9	x		tomato	MeNa '77		-	6	108	0	-
47	"	sand	9	x		bean			-	4	72	9.3	0-22.3
48	"	sand	9	x		cucumber	MBR '76		-	4	72	0	-
49	"	sand	11	x		chrysanthemum		MBR	+	9	162	2.2	0-28.8
50	"	sand	12	x		tomato			-	9	162	6.6	0-25.5
51	"	sand	12		x	chrysanthemum		MBR	+	8	144	3.3	0-10.5
52	"	organic	12	x		lettuce		Steam	-	6	108	0.8	0- 2.5
53	"	sand	1		x			MBR	+	4	144	0	-
54	"	organic	2		x	lettuce		Steam	-	4	72	1.3	0- 5.1
55	"	clay	2		x	lettuce		MBR	+	3	135	0	-
56	"	clay	2	x		tomato	MBR '78		-	5	90	0	-
57	"	clay	2	x		tomato			-	8	144	1.9	0- 5.1
58	"	sand	2	x		potato				4	144	5.2	0-10.5
59	"	clay	3	x		endive		?	-	5	90	1.9	0- 5.1
60	"	clay	3	x		spinach		MeNa	-	5	90	0	-
61	"	clay	3	x		spinach			-	5	90	2.4	0- 5.1
62	"	clay	3	x		endive			-	6	108	3.3	0-13.4

1) MBR = methyl bromide, MeNa = metam natrium, Steam = steam 2) Corrected number of colonizations per 100 paper disks

Table V.4.: Frequency table of inoculum density classes (mean corrected number of colonizations per 100 paper disks) from 62 glasshouses.

Corrected mean number of colonizations per 100 paper disks	Frequency[1]	
	number	%
0	24	38.7
< 1.4	11	17.7
1.5 - 2.4	6	9.7
2.5 - 3.4	4	6.5
3.5 - 4.4	5	8.1
4.5 - 5.4	5	8.1
5.5 - 6.4	0	0
6.5 - 7.4	2	3.2
7.5 - 8.4	1	1.6
8.5 - 9.4	2	3.2
9.5 - 10.4	1	1.6
10.5 - 11.4	0	0
11.5 - 12.4	0	0
12.5 - 13.4	1	1.6

Average: 2.3 colonizations per 100 paper disks (62 glasshouses)[2]

1) Frequency of glasshouses with specified number of colonizations.

2) Corresponding with 0.6 propagules per 100 g of soil.

confirm a clustered pattern and the fit to the negative binomial distribution of propagules, frequencies of the inoculum densities of 101 separate composite samples from 16 nurseries (average inoculum densities 1.5 - 4.5 colonizations per 100 disks - see Table V.3.) were statistically analysed. The results are shown in Table V.5.

It appears that the variance-to-mean ratio of the number of colonizations per 40 disks is significantly greater than 1. This indicates a departure from a pure random distribution of the inoculum density. This was confirmed by the goodness of fit of the observed frequency data for colonizations by the negative binomial distribution with a relatively small "k" value.

The results obtained indicate a clustering of *R. solani* propagules in the soil of the 16 glasshouses. It proves that the assumed clustered pattern of *R. solani* propagules in Chapter V.2.b. is confirmed by the results of the examination of composite soil samples from relatively large areas (9 - 36 m^2).

Table V.5.: Analysis for pattern of inoculum density values. Frequencies of the corrected number of colonizations per 40 paper disks of 101 soil samples from 16 nurseries, and the goodness of fit of expected frequencies for negative binomial distribution.

Corrected number of colonizations per soil sample[1)]	Frequency[2)]	Expected frequency for negative binomial
0	33	34.4
1	33	28.7
2	15	17.9
3	12	9.9
4	3	5.2
5	2	2.6
6	0	1.3
7	1	0.6
8	2	0.4

Mean corrected number of colonizations: 1.43
Variance: 2.71
Variance-to-mean ratio: 1.90
"k" value: 2.0
Goodness of fit for negative binomial distribution: chi-square value 2.06
Critical value 9.21, $P \leq 0.01$, 3 degrees of freedom

1) 40 Disks per soil sample.

2) Number of soil samples with specified number of colonizations.

V.4.c. *Inoculum density in relation to soil, cultural and sampling characteristics*

The questionnaire on data concerning soil type and crop management was received from all nurseries, although not all of them had been filled in completely. On the basis of the available information (Table V.3.), nurseries were grouped into relevant categories, for which the average inoculum density was calculated and occurring differences statistically analysed. Results are given in Table V.6.

There appears to exist a correlation between inoculum density and time of sampling. Glasshouses that had been sampled in the cold winter months had a level of infestation significantly half as high as nurseries that were sampled during the warmer spring and autumn. As expected, this phenomenon indicates the importance of temperature in population dynamics.

Also not quite unexpectedly, the level of infestation of the soil was related to the time of the previous soil treat-

Table 6: Inoculum density is relation to soil, cultural and sampling characteristics.

Factor	Number of glasshouses	Inoculum density[1]	Relation (S/N.S.)[2]
1. Sampling time			
autumn, 9-10-11 } spring, 3 }	39	2.81	
winter, 12-1-2	23	1.46	S($P \leq 0.05$)
2. Soil type			
sand	26	2.37	
clay	28	2.73	N.S.
3. Sampling crop			
plantbed for lettuce	10	1.30	
previous crop	52	2.50	S($P \leq 0.10$)
4. Previous crop			
lettuce, endive	17	2.26	
others	42	2.05	N.S.
5. Soil treatment			
>1 year ago	20	2.57	
<1 year ago	22	1.10	S($P \leq 0.10$)
6. Quintozene application			
yes	23	1.45	
no	34	2.42	N.S.

1) Mean corrected number of colonizations per 100 paper disks
2) S = significant at the indicated level
N.S. = not significant

ment. Soils treated with methyl bromide and metam-sodium or had been steam sterilized less that a year before sampling, had an average lower inoculum density than soils that had been treated more than a year ago. The number of nurseries was too low to analyse the influence of a single treatment. However, the data indicate that soil treatment has a controling effect on *R. solani*. The effect of methyl bromide will be further discussed in Chapter VII. Although the number of nurseries was limited, the impression was obtained that the effect of soil treatments was greater on clay than on sandy soil.

The observed significant difference of almost a factor two of the inoculum density in samples taken from the previous crop or from the plantbed of lettuce, can possibly be explained by the fact that the inoculum had been diluted by soil tillage, before the lettuce is planted.

Other analysed factors did not show significant differences. Noteworthy was that the inoculum density in host crops such as lettuce and endive were not higher than in non-host crops.

There was no difference in the level of infestation between clay and sandy soils. The low number of observations did not allow paying attention to the effect of organic matter in the soil.

In soils treated with the persistent and for *R. solani* very toxic fungicide quintozene, the level of infestation was near to significantly lower ($P \leq 0.20$) than in non-treated soils.

V.5. DISCUSSION

According to Strandberg (1973) it seems proper to assume that spatial patterns of distribution of plant pathogens like other natural populations of organisms are seldom truly random or regular in nature. Results of our study show that also in the case of *R. solani* in lettuce glasshouses these horizontal patterns are not random, but clustered and can be adequately described by the negative binomial distribution. This is the first record of this pattern for *R. solani* in soil.

However, Campbell and Pennypacker (1980), in their studies on *R. solani* in snap bean, found a random distribution of diseased plants per 6 x 6 square meters. The distribution of lesions per plant was however clustered. Nevertheless, they concluded that on a small scale fungal propagules in soil may be dispersed non-randomly.

In this respect it is important that their study and Strandberg's results (1973) referred to the distribution of infested plants and lesions rather than the distribution of the pathogen itself. As the authors indicated, however, most epidemical studies are based on the assumption that lesions, disease damage and diseased plants give estimations of localized pathogen population densities.

Different distribution patterns may be expected with different cultural practices. Trujillo and Snyder (1963) reported an "uneven" distribution of *Fusarium oxysporum f.sp. cubense*. The au-

thors related this to the survival of the fungal propagules in host tissue combined with a lack of cultivation and ploughing in of banana stubble. On the contrary, practises for bean culture involve cultivation and subsoiling of bean plants after harvest. On this basis Trujillo and Snyder (1963) concluded that these practises would lead to an "even" distribution of *Fusarium solani f.sp. phaseoli* in such soils and cited the work of Nash and Snyder (1962) as evidence of even distribution of the pathogen in bean fields.

With respect to the lettuce glasshouses of our study, it may be assumed that the customary cultural practice (rotary tillage) usually causes little horizontal dispersion, leaving infested foci relatively undisturbed.

Soil treatment with methyl bromide could be a factor contributing to the observed clustering of propagules. In this connection some time after the disinfestion, local concentrations of *R. solani* propagules in the soil can be demonstrated, very probably related to the way of reintroduction of the fungus (Chapter VIII).

As far as the field sampling procedure is concerned, a non-random distribution of *R. solani* propagules is an important factor. The consequence is a possible reduction of the accuracy of inoculum density determinations. If sampling is executed randomly a mean value with a relatively high variance would be obtained. A larger sample size would be required, depending on the degree of clustering present, to obtain the same accuracy of the mean as at random distribution of the propagules in the soil. Also the shape of the sampling path (preferable W) and covering the entire field is important (Lin et al., 1979).

Data obtained on the vertical distribution of *R. solani* in soil indicate a strong decrease of the inoculum density with sampling depth. This is in good agreement with the literature data of Papavizas et al. (1975) and Naiki and Ui (1977).

For quantitative sampling purposes it is recommended that sampling depth be restricted to 5 cm in order to avoid undesirable dilution.

During investigations on changes in the inoculum density in two successive lettuce crops in three glasshouses, densities remained rather constantly low in glasshouses A and C. In glasshouse B a seasonal curve appeared as was also observed by Papavizas et al (1975) in bean fields and Herr (1976) in beet fields: a maximum in the warm period and a minimum in the cold months.

Fluctuations in the inoculum density of *R. solani* are affected mainly by the temperature and the availability of food material (Papavizas et al., 1975). During the first lettuce crop in glasshouse A, the soil temperature remained practically under 10°C, at which level little fungal growth can be expected (Chapter VII). During the second crop the temperature was much higher, but the water supply was minimal for *R. solani* growth because the grower kept the soil as dry as possible. Next to the above mentioned two factors influencing *R. solani* growth, the water supply is also a real factor for progress of the inoculum density (see also Chapter VII).

In glasshouse C no significant increase of the inoculum density was observed during the two crops. The rather low soil temperatures showed hardly any variation. Besides this, the glasshouse was on clay in which, sooner than in sandy soil, growth inhibition of *R. solani* due to CO_2 accumulation may occur (Papavizas, 1970). Herr (1976) also did not observe a seasonal density curve in clay.

In glasshouse B at the end of the preceding tomato crop (which was not attacked) a high inoculum density was found. Decaying leaf material probably served as a food supply. During glasshouse clearance in December, a decrease in the level of infestation was found. After drainage and tillage and during the next crop at low temperatures, the population level remained unchanged. At the end of the warm second lettuce crop the population density again increased. Besides a high temperature, decaying lettuce leaves which food supply may have been an important contributing factor.

Summarizing, it appeared from these investigations that the inoculum density of *R. solani* in the soil hardly changes during

winter and early spring crops when the soil temperature is about 10°C. In later crops when temperatures are higher the density may increase, provided their are no additional hampering factors such as drying out of the soil. In other crops, for example tomatoes (in practice non-host), high values are found possibly caused by decaying tomato leaves on the soil surface.

The average level of infestation in the soil of 62 glasshouses was low at sampling time (0.6 propagules per 100 g of soil). Because all nurseries concerned were familiar with bottom rot problems, the level of infestation in the total acreage (inclusive the glasshouses without structural problems) may have been even lower. There are few exceptions but within glasshouses infestation levels may vary considerably (see column "Range" in Table V.3.).

On average, observed density data are confirmed by the little information available in literature. Weinhold (1977) found that the population ranged from 0 - 15 propagules per 100 g soil in 60 cotton and potato growing fields. Almost 40 per cent of the sampled areas had an inoculum density of less than 0.5 propagules per 100 g of soil and 75 per cent less than 2.0 propagules per 100 g of soil. Clark et al. (1978) found fewer than 5 propagules per 100 g of soil in 50 per cent of 29 cotton fields.

There is also agreement between our findings and those by Jager (1979). In surveying 62 potato fields in the northern part of The Netherlands, using pieces of *Juncus effusus* as a bait, he found $\leqslant 1.4$ per cent of the baits colonized by *R. solani* in 50 per cent of the soil samples.

El Zarka (1965) using jew's mallow (*Chorchorus olitorius* L.) as a bait, found somewhat higher values in Dutch farm soils. From 24 fields with diseased potatoes, no colonizations were found in one field, in five fields 25-37 per cent of the baits were colonized, whereas in the other 18 fields it amounted less than 25 per cent.

In analysing the possible effects of nursery and sampling characteristics, higher infestation levels were found in samples taken in relatively warm periods than in those taken during the cold period. This indicates the expected positive correlation between *R. solani* activity and temperature (see also Chapter VII).

It was concluded with some reserve that lower inoculum densities occur after recent soil treatments especially on clay. Apparently the process of *R. solani* recolonization in sandy soils is quicker, therefore, the effect of soil treatment disappeared sooner.

A striking experience was the equally high inoculum densities in fields bearing host crops like lettuce and endive and fields bearing non-host crops. A possible explanation may be the sporadic occurrence of the *R. solani* disease in lettuce (see Chapter II). Besides, the application of fungicides which inhibit *R. solani* is common in all lettuce crops (Chapter I).

No difference in the level of infestation was found between clay and sandy soils. It is generally accepted that because of less aeration, clay could CO_2 concentrations that inhibit *R. solani* growth (Papavizas, 1970; Herr and Roberts, 1980). On this basis it may be assumed that clay soils show a lower average inoculum density. In our investigations the glasshouse clay soils involved were well aerated, therefore, this negative CO_2 factor for *R. solani* development was possibly not relevant.

The great differences in inoculum density between soils that had been treated and had not been treated with quintozene within two years previous to sampling were almost significant ($P \leqslant 0.20$). This observation indicates a longlasting influence on certain elements of the microflora (i.e. *R. solani*) by very persistent fungicides such as quintozene.

V.6. SUMMARY

Using the paper disk method the horizontal and vertical distribution of propagules of *R. solani* in soil of glasshouses was investigated. Based on results form horizontal soil samples

taken on planting space (about 25 x 25 cm) a departure from pure random distribution of the inoculum density appeared. The indicated clustering of propagules of *R. solani* could be well described by the negative binomial distribution. A clustered pattern of propagules of *R. solani* has also been proved to excist in composite soil samples of relatively large areas. This is the first record of such a pattern for *R. solani* in soil. The origin of this spatial distribution was related especially to the local reintroduction of the fungus after the soil had been disinfected with methyl bromide. The consequences of non-random distribution of propagules concerning size and path of sampling procedure have been discussed.

Data obtained on the vertical distribution of *R. solani* showed that the inoculum density markedly decreased with increasing depth of sampling. The maximum depth of occurrence of fungus in sandy soil was greater than in clay. To avoid an undesirable dilution, it is recommended that the sampling depth be restricted to 5 cm.

Inoculum density was stable during winter crops with soil temperatures up to about 10°C. Increasing inoculum density was observed in warm cropping periods and in the main crop tomato. In conformation of the literature concerned it was concluded that fluctuations of inoculum density are mainly affected by the availability of food, temperature and water supply.

On an average, the level of infestation in the soil of 62 glasshouses was low (about 0.6 propagule per 100 g of soil). There were few exceptions but within glasshouses local inoculum density may vary considerably. Higher infestation levels were found in samples taken during warm periods than in cold periods. Lower inoculum densities were found in glasshouses recently disinfected. The inoculum density of soils that had been treated within two years with the very persistent fungicide quintozene were nearly to significantly lower than in non-treated soils.

VI. INFECTION BY RHIZOCTONIA SOLANI IN RELATION TO INOCULUM DENSITY

VI.1. INTRODUCTION

In addition to information about the occurrence of *Rhizoctonia solani* in the soil and fluctuations of inoculum density during lettuce cultivation, data about the relationship between initial inoculum density and disease in the crop are important. Data available in the literature comes mainly from short-term damping off experiments under controlled conditions in artificially infested soil mostly with very high inoculum levels (Henis and Ben-Yephet, 1970; Benson and Baker, 1974 a, b; Geypens, 1974; Baker 1978). These data played an important role in comprehensive treatments of the general inoculum density-disease curve of soil-borne diseases (Baker, 1971; Vanderplank 1975; Baker, 1978; Ferris, 1982) and modelling proposals (Baker and Drury, 1981). From these laboratory results it was concluded that when the *R. solani* inoculum densities are low, the relationship to disease is close to linear.

The aim of the studies described here was to investigate this relationship under field conditions. The predictive value of the paper disk method to determine the initial inoculum density with respect to the occurrence of *R. solani* in lettuce was also evaluated. In addition, the type of epidemic progression of *R. solani* in lettuce is discussed. Relationships between inoculum density, disease incidence and disease severity were also studied in view of their possible use in assessing disease and loss.

VI.2. MATERIALS AND METHODS

The experimental data were obtained from two successive crops grown in the 1978-1979 season in greenhouses A, B and C, as mentioned in Chapter V.3., and also from two successive crops in greenhouse C grown in the 1979-1980 season. In the latter case experimental plots also remained in situ for three

Table VI.1.: Data on glasshouse, soil type, cropping period and temperature.

Glasshouse code	Soil	Growth period from	to	Days (number)	Mean temp. °C
A-1	Clayey sand	11- 2-79	18-4-79	66	10°
A-2		30- 4-79	6-6-79	37	19°
B-1	Sand	13- 1-79	9-4-79	86	6°
B-2		14- 4-79	28-5-79	44	14°
C-1	Clay	6-11-78	12-2-79	98	8°
C-2		24- 2-79	17-4-79	52	12°
C-3		1-12-79	27-2-80	88	9°
C-4		3- 3-80	23-4-80	51	11°

successive years.

Initial inoculum density data were determined as described in Chapter V.3.a. The final inoculum density was assessed by collecting soil from underneath 6-12 lettuce heads in each of the 6 plots (replicates) of 1 m^2 to a depth of 2 cm. The soil was transfered to petri-dishes, 9 cm diameter, to which 8 paper disks were added. Further analysis was carried out according to the standard method given in Chapter IV.2.a.

Disease incidence (percentage diseased heads) and disease severity were determined as described in Chapter II.4. Disease severity of all plants was calculated by dividing the total of severity grades of all plants by the total number of plants and the disease severity of diseased plants by dividing the severity grades of diseased plants by the number of diseased plants.

When 0-values occurred with the logarithmic transformation of data for regression analysis, the value 1 was added to each original value in the series.

Where necessary, the number of infections per 100 plants was obtained from the percentages diseased plants by correction for multiple infection according to Gregory (1948), see Chapter IV. 2.a.

Log-log transformation of the inoculum density and disease (infection) data were carried out after Baker (1971).

Temperatures in the crop were registered by a thermograph (Thies, Göttingen) and the mean temperature during the entire cropping period was calculated from the daily means.

General information on soil type, cropping period and mean temperatures and included in Table VI.1.

VI.3. RELATIONSHIP BETWEEN INITIAL INOCULUM DENSITY AND DISEASE

Initial inoculum densities and the percentages of diseased lettuce heads at harvest are given in the following table VI.2.

Results show that the initial inoculum density in the crops varied from 1.3 to 10.0 colonizations per 100 paper disks, with one exception in crop B-2. The percentage of diseased heads varied from 0 to 55.6.

Although a significant correlation appears to exist between both measurements ($\hat{y} = 1.29\ X$; $r = 0.80$; $P \leq 0.05$, where $\hat{y}$ = percentage diseased heads and X = initial inoculum level), the

Table VI.2.: Initial and final inoculum density, disease incidence and disease severity data from 8 lettuce crops in 3 glasshouses; the figures are means of 6 replicates.

Code[1]	Inoculum density[2]		Disease incidence[5]	Disease severity[6] average for	
	initial[3]	final[4]		all plants	diseased plants
A-1	2.1	7.9	0	0	0
A-2	2.1	5.8	3.3	0.1	2.0
B-1	10.0	41.6	13.3	0.4	3.0
B-2	48.7	128.2	55.6	1.6	3.0
C-1	1.3	1.8	0	0	0
C-2	4.7	9.5	25.0	0.8	3.3
C-3	2.5	2.6	4.2	0.1	1.7
C-4	7.9	13.3	41.7	1.8	4.2

1) Glasshouse and crop, see Table VI.1.
2) Corrected number of colonizations per 100 paper disks.
3) n = 40 paper disks each replicate.
4) n = 48-96 paper disks each replicate.
5) Percentage diseased heads (n = 6-12 heads each replicate).
6) Disease index (0 = not diseased, 5 = unmarketable).

extreme value in crop B-2 greatly influences both the slope of the regression line and the correlation coefficient.

Between initial inoculum density (X) and the number of infections per 100 plants (y, calculated from the percentage of diseased plants, using the correction factor for multiple infection) the relationship is also linear ($\hat{y} = 2.25\ X$; $r = 0.81$, $P \leq 0.05$) but again the extreme initial inoculum density value of B-2 predominates.

However, this value fits into the other observations when log-log transformation is applied. Linear regression analysis gives $\hat{y}_1 = 1.318\ X_1$; $r = 0.82$; $P \leq 0.05$, where $\hat{y}_1 = \log (y+1)$, (y = corrected number of infections per 100 plants) and $X_1 = \log (x + 1)$, (x = initial inoculum density, i.e. corrected number of colonizations per 100 paper disks).

The regression line is shown in Figure VI.1. The fact that the inoculum density-infection curve starts at the origin corresponds to the "experimental law of the origin" (Vanderplank, 1975). Likewise this is an extra indication for the suitability (see Chapter IV.5.) of the paper disk method in determining inoculum densities of *R. solani*.

It is worth mentioning that results hardly change when the extreme value of crop B-2 is not included ($\hat{y}_1 = 1.431\ X_1$; $r = 0.84$; $P \leq 0.05$). In both regression lines, slope values (1.318 and 1.431 respectively) do not differ from 1 significantly. According to Baker (1971), this value can be expected in the soil-borne disease category in which the pathogen can attack the plant from a distance. It is generally assumed that this is the case in *R. solani*. Therefore, the slope value obtained, agrees with Baker's view. Summarizing, the data obtained, indicate a linear regression between initial inoculum density of *R. solani* in soil and infection of lettuce under field conditions.

VI.4. RELATIONSHIP BETWEEN INITIAL AND FINAL INOCULUM DENSITY

Initial and final inoculum densities in the crops are given in Table VI.2. Final inoculum densities vary from 1.8 - 13.3

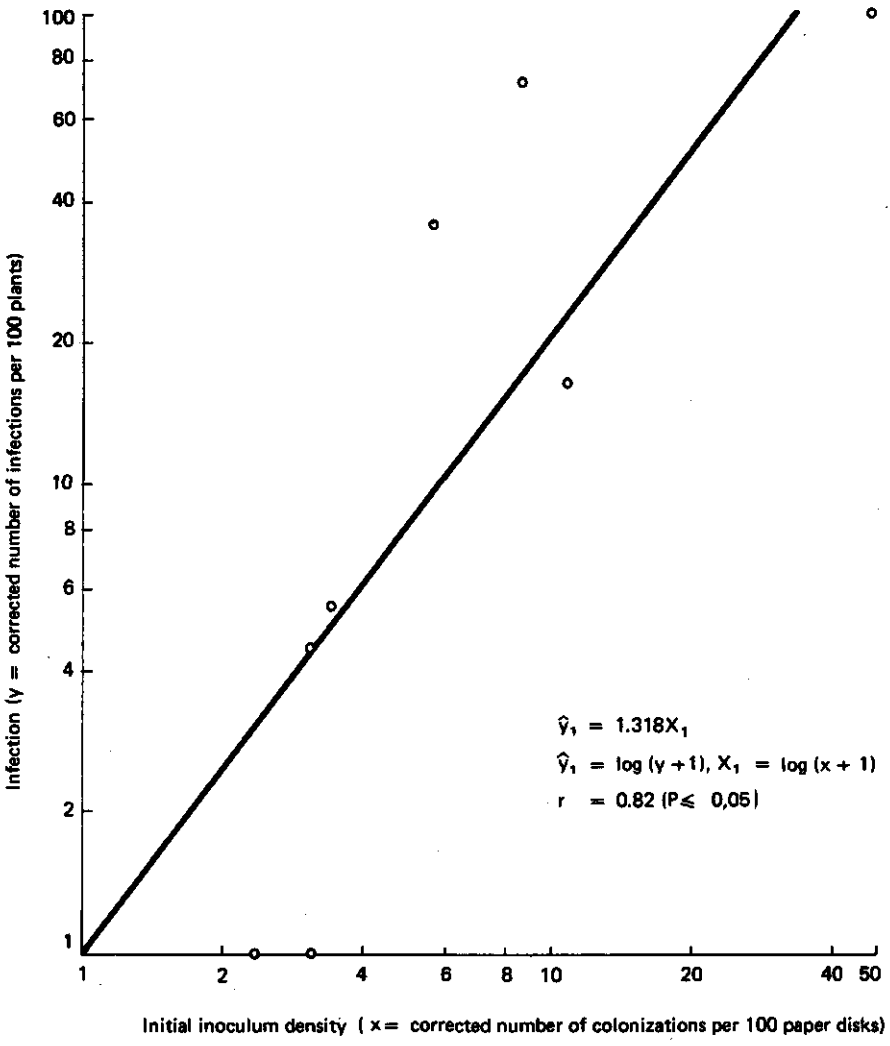

Figure VI.1.: Relationship between initial inoculum density and infection. Log-log transformation. Data from 8 crops. Data points represent the average of 6 replicates.

with two extreme values of 41.6 and 128.2 colonizations per 100 paper disks (crops B-1 and B-2). The relationship between both variables is shown in Figure VI.2. There proves to be a good linear regression between final (y) and initial (X) inoculum density ($\hat{y} = 2.622\ X$; $r = 0.99$; $P \leq 0.01$). The curve starts in the origin indicating the more the accuracy of the

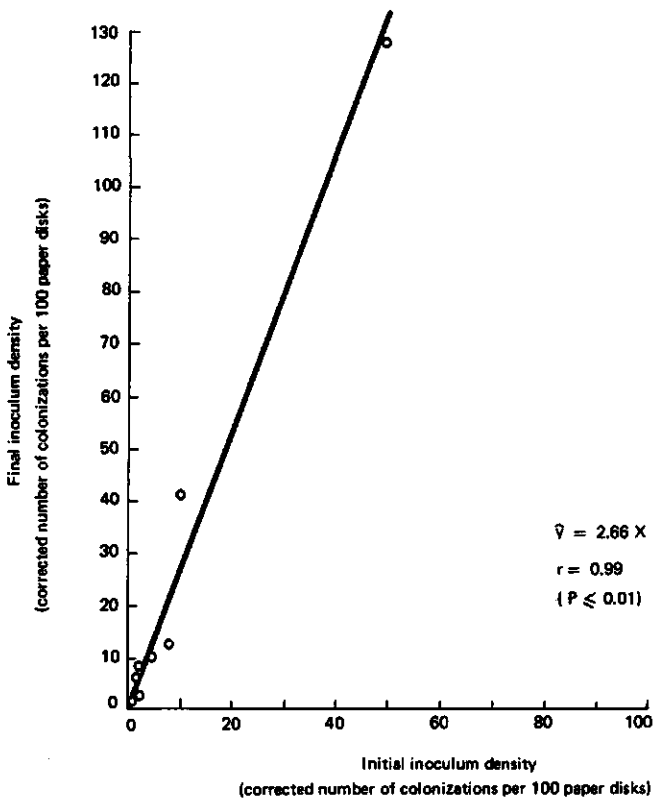

Figure VI.2.: Relationship between initial inoculum density and final inoculum density. Data from 8 crops. Density data points represent the average of 6 replicates.

employed paper disk method for isolating viable *R. solani* from soil (see Chapter IV.5.).

When the extreme values mentioned above are omitted, the correlation is maintained (n = 7: r = 0.88; n = 6: r = 0.87).

The value of the regression coefficient (2.66) indicates a certain increase of the inoculum density during cropping. This seems in contradiction to the results presented in paragraph V.3., where the conclusion from periodical sampling data was that there was a non-significant increase of the population during most crops. In this connection it should be noted that soil sampling at harvest was in the top two cm of the soil (see Chapter VI.2.) whilst pre-cropping sampling and

periodical sampling thereafter was at a depth of 5 cm (Chapter V.3.a.). It is likely that lower values would have been found if sampling at harvest time was done at a greater depth, because inoculum density decreases with soil depth (Chapter V.2. c.). Therefore, with respect to changes in inoculum density during cultivation, the regression coefficient value should not be emphasized too much.

VI.5. RELATIONSHIP BETWEEN DISEASE INCIDENCE AND DISEASE SEVERITY

Data on disease severity ratings of all plants, the ratings of diseased plants and incidence of disease (percentage of diseased plants) are given in Table VI.2.

Figure VI.3. shows a close linear regression between disease severity ratings of all plants (y) and disease incidence

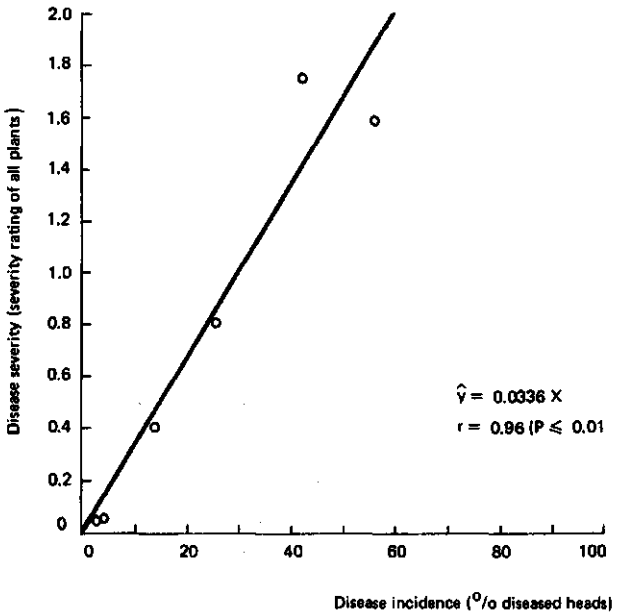

Figure VI.3.: The relationship between disease incidence and disease severity of all plants. Severity rates: 0 = not diseased, 5 = unmarketable. Data from 6 crops. Data points represent the average of 6 replicates.

(X) ($\hat{y}$ = 0.0336 X; r = 0.96; P $\leq$0.01). This indicates that the average severity of the disease increases proportionally with the percentage of diseased plants.

A general point in relation to the biological meaning of this relationship (severity rating of all plants-disease incidence) is that this severity parameter for arithmical reasons will be influenced by both disease incidence and disease severity of diseased plants, but not independently (Scott and Rosenkranz, 1981).

The relationship between disease severity ratings of diseased plants and disease incidence is given in Figure VI.4. Here the relationship is not linear. It appeared that the disease severity ratings of diseased plants (y) relates in a linear fashion to the logarithm of disease incidence x ($\hat{y}$ = 1.5 X_1 + 1.1; X_1 = log x; r = 0.85; P $\leq$ 0.05). This means that at

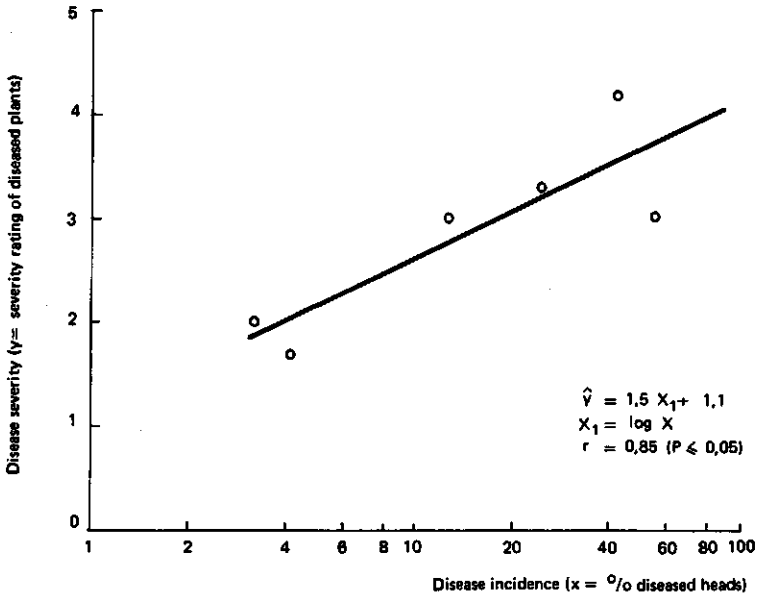

Figure VI.4.: Relationship between log disease incidence and disease severity of diseased plants. Severity rates: 0 = not diseased, 5 = unmarketable. Data from 6 crops. Data points represent the average of 6 replicates.

increasing numbers of diseased plants, the severity of the attack per diseased plant increases to a diminished degree.

This relationship can be used for estimating the threshold value of disease incidence causing economic loss. Crop losses in mature lettuce incited by bottom rot are mainly caused by the number of attacked bottom leaves per head. Lettuce heads with rating 1 (i.e. 1-2 diseased leaves) will mostly not be classified in a lower quality or weight class. Therefore, heads with this severity rating generally do not contribute to loss of yield, excluding the extra labour costs required for the cutting of the lettuce.

Assuming the validity of the regression line in Figure VI.4. (see Chapter VI.6.) the disease incidence at which the disease severity is less than 1, may be determined as 1-3 per cent diseased heads. Below this disease level no yield loss need be expected.

The question arises at which initial inoculum density value 1-3 per cent disease level will be reached. Using the regression formula in Chapter VI.3 (Figure VI.1.) it appears that 1-3 per cent disease (i.e. 1-3 infections per 100 plants) can be expected at an initial inoculum density of about 0.7-1.8 *R. solani* colonizations per 100 paper disks. Quantification of this relative figures with the formula of Chapter IV.4.c. gives 0.2-0.5 propagules per 100 g of soil. Economic loss can be expected if the infestation is above this level.

In Chapter V.4.b. it was found that the average infestation level of 62 glasshouses was 0.6 propagules per 100 g of soil, and therefore, above the mentioned threshold level for crop loss. This agrees with the fact that these holdings were familiar with bottom rot (*R. solani* inclusive) problems.

VI.6. DISCUSSION

The data presented in Figure VI.1. indicate at the investigated range a linear relationship between the initial inoculum density and infection of lettuce under field conditions. This conclusion - based on a relatively small number of observa-

tions - is supported by the results obtained from experiments mentioned in the literature with *R. solani* as damping-off disease under controlled circumstances (see Chapter VI.1.). Also Leach and Davey (1938), in studies of a comparable disease, *Sclerotium rolfsii* in beet, found a linear regression between the population of viable sclerotia and the percentage of disease in the subsequent crop in 22 commercial fields.

The determination of the initial inoculum density was carried out by the paper disk bait method measuring the competitive saprophytic ability of *R. solani* propagules. The observed good correlation with disease is in agreement with the close relationship between inoculum density measured with other bait techniques and the resulting disease by *R. solani* observed by Sneh et al. (1966), Geypens (1974) and Papavizas et al. (1975), see also Chapter IV.1. and 5.

The rather high correlation between initial inoculum density and disease indicates that environmental factors have been relatively constant or have had little effect on the disease incidence. The available data insufficiently prove this statement but a number of supporting remarks can be made.

As far as a-biotic factors such as conductivity of soils for *R. solani* is concerned, it can be noticed that the data are based on eight separate crops; four of these observations were made at one and the same holding, and the other four at two holdings. In each occasion two successive crops were involved.

Further the negative relationship between the average temperature and the duration of the crop is of importance (Table VI.1.). Crops that are grown when the average temperature is low take longer to incubate and grow than crops grown when the temperatures are higher.

It may be concluded that the initial inoculum density data from lettuce greenhouses obtained with the paper disk method have a disease forecasting value.

It appeared that inoculum density does not increase during lettuce cultivation (Chapters VI.4. and V.3.). Vanderplank (1963) defines diseases that result from a monocyclic process

in which an increase of disease occur without a corresponding increase of inoculum which is effective during that season, as "simple interest diseases" (SID).

Although disease progression during cultivation has not been investigated in the present study, the observation of a "static inoculum reservoir" makes the appropriateness of the SID model for *R. solani* in lettuce plausible.

In spite of the fact that few data were available, generally it was assumed that diseases induced by soil-borne pathogens progress according to the SID-model (Campbell et al., 1980[b]). However, from studies of these authors (1980[a, b]) and those of Campbell and Povell (1980) it appears that the assumption of SID-progression in soil-borne diseases is more frequently denied than confirmed. Mainly as a result of the appearance of secondary infection or increase of the inoculum during the growing season. Also with *R. solani* under very favourable conditions for disease development, a secondary attack on neighbouring plants may occur as a result of the well-known potentially fast spread of the fugus due to energy acquired from the primary host.

In view of disease control the assumed SID-character of *R. solani* in lettuce is of consequence for the strategy of control. As Vanderplank (1963) has pointed out, control of simple interest diseases can be effected by using initial inoculum reducing practices. In Chapter VIII this will be discussed in more detail

As shown in Chapter VI.5. good correlations exist between disease incidence and disease severity of all plants and disease severity of diseased plants respectively.

In most diseases little is known about the incidence-severity relationship (Seem and Gilpatrick, 1980). In their own investigations on apple mildew the authors found a correlation between incidence and the square root of the severity data. This relationship remained relatively constant for data from different locations (orchards) or cultivars, but varied according to different seasons.

According to these authors, disease assessment in large

field experiments or surveys can be made useful if a clear relationship between incidence and severity of disease can be established, thus requiring only incidence data to establish severity levels. Butt and Barlow (1979) made use of this relationship by basing the decision guidelines for supervised control in British orchards on mildew incidence threshold levels. Also in The Netherlands (Rijsdijk, in press), in a supervised control system of cereal diseases and pests, incidence-severity relationships are applied.

Relevant data on soil-borne diseases are very scarce (Campbell et al., 1980[a]; Marois and Mitchell, 1981). In the present study the relationship between disease incidence and disease severity of diseased plants was suitable to estimate the threshold values for economic crop losses of disease incidence and initial inoculum density respectively. The calculated figures, however, are more indicative than quantitative in view of the relatively small number of observations.

Considering the positive results obtained in the mentioned studies with apple and cereal diseases, time saving possibilities in the application of disease incidence data for *R. solani* in lettuce for crop loss assessments, and possibly, for disease management, must not be excluded. To this end, the number of experimental holdings has to be extended and also the effect of fungicidal treatment in the crop on the incidence-severity curve has to be studied. As for apple mildew, Seem et al. (1981) found a less sloping curve, depending on the type of fungicide. A similar picture for *R. solani* in lettuce is expected.

VI.7. SUMMARY

On a log-log scale a linear regression existed between initial inoculum density of *R. solani* in the soil and infection of lettuce. The slope of the curve did not differ from unity, indicating that *R. solani* attacks plants from a distance. It was concluded that initial inoculum density data obtained with the paper disk method has a disease-forecasting value.

The initial and final inoculum density data showed a very good correlation. From the rather low regression coefficient and the not varying inoculum density during the growth period a "static inoculum reservoir" was suggested, hence *R. solani* in lettuce would belong to the simple interest diseases. The consequence to strategy of control is mentioned.

The relationship between disease incidence and disease severity of all plants proved to be linear. In excluding not diseased plants the severity of the diseased plants was correlated to the logarithm of disease incidence. Based om this relationship the threshold values for yield losses of disease incidence and initial inoculum density respectively were estimated.

VII. INFECTION BY RHIZOCTONIA SOLANI IN RELATION TO TEMPERATURE AND RELATIVE HUMIDITY

VII.1. INTRODUCTION

In the earliest investigations on the epidemiology of *Rhizoctonia solani* in outdoor lettuce, Townsend (1934) established the significance of temperature and relative humidity. He observed that *R. solani* rot was a problem in New York mainly during periods of humid warm summer weather. From his experiments it appeared that *R. solani* rot was most severe when the mean daily temperature was above 20°C. Minimum daily temperatures as low as 10°C stopped disease development.

In a provisional survey on the occurrence of bottom rot pathogens of glasshouse lettuce in The Netherlands, it was found that *R. solani* mainly appears in lettuce heads in spring crops and scarcely in lettuce cropped in the colder winter months (Kooistra et al., 1974).

To confirm these findings, an extensive survey on the occurrence of bottom rot was carried out in co-operation with the Research Institute for Plant Protection and the Extension Service (Tichelaar, 1976 and 1977). Lettuce with bottom rot symptoms were examined on 82 holdings. The percentage glasshouses with *R. solani* diseased heads increased when harvesting took place in warmer periods: February 6.5 per cent, March 21 per cent and April 30 per cent in samples from 31, 24 and 27 glasshouses respectively.

As for the relative humidity, Townsend (1934) observed in outdoor lettuce that although humid weather favoured the disease this was not as essential as were high temperatures for the development of this disease. In his study on the effect of relative humidity on *R. solani* growth on agar, Townsend (1934) stated that some growth occurred at 81 per cent relative humidity but that rapid growth occurred only when the values were much higher.

In the other available study, Schneider (1953) classified

R. solani as a hygrophile. The linear growth rate decreased rapidly with decrease from 100 per cent relative humidity. The threshold value for growth was 95 per cent relative humidity.

Kooistra et al., (1974) observed that a high relative humidity is not only important for initiating the infection but also for further development. In an uncontrolled greenhouse artificially inoculated plants were rapidly infested by pathogenic strains when covered with plastic sheet. After removing the plastic, decay of leaves stopped and attacked leaves dried up.

Because of the above mentioned observations of the author, experiments under controlled conditions were set up to examine the influence of the temperature on *R. solani*. These experiments tested:

a. the minimum temperature for mycelium growth on agar and for infection of the lettuce leaf;
b. the effect of temperature on the length of the incubation period and the rate of infection of the leaf;
c. mycelium growth-temperature curves of a number of typical low temperature isolates.

Preliminary results of limited investigations on the effect of relative humidity on mycelium growth and infection will be discussed in Chapter VII.6. in relation to the literature data cited above.

VII.2. MATERIALS AND METHODS

Isolates were obtained from glasshouse soils using the paper disk method and from infected heads from different glasshouses.

In the mycelium growth experiments on cherry agar, agar punches (5 mm diameter) with young mycelium were plated out in 3 replicates. Outgrowth in two directions was measured periodically. The average daily growth at each temperature was calculated from the linear part of the growth curve.

Infection experiments were carried out either on slices of

lettuce leaf (4 cm diameter, surface 12.5 cm^2) or on young lettuce plants. Agar punches with young mycelium (5 mm diameter) were placed in the centre of the lower leaf surface. For each temperature six slices of leaf were used or three plants with one mycelium punch on two leaves.

Experiments on the rate of infection were carried out in six replicates on leaf slices. The percentage of attacked leaf area was estimated daily, from which the number of days required for 50 and 100 per cent infected leaf area at the different temperatures were estimated graphically.

For a limited number of isolates the linear spread of infection was established daily by measuring the diameter of lesions on 2 or 3 leaves of three young lettuce plants.

VII.3. MINIMUM TEMPERATURE

The minimum temperatures for growth on cherry agar and for infection of lettuce leaves were determined for 29 pathogenic isolates from 17 glasshouses (Table VII.1.).

The isolates from soil were tested at 9°C, some on lettuce leaf only. It appeared (Table VII.1.) that four out of eleven isolates did not infect leaves at this temperature but two of these isolates showed mycelium growth on cherry agar (one was not tested on agar).

The 18 isolates from attacked heads were collected in 14 glasshouses. The test temperatures were 9°C, 6°C and 3°C. The results (Table VII.1.) show that respectively 11 of eighteen, 3 of fourteen and 1 of three isolates infected the leaves. The minimum temperature for mycelium growth on cherry agar was always lower.

The results show that great differences in the minimum temperature requirements for infection of lettuce leaves exist between isolates, ranging from < 3°C to > 9°C. The minimum temperature for mycelium growth on cherry agar is at least three degrees lower than the minimum temperature for leaf infection.

The rate of increase of the infected area with three 409-isolates was observed for 12 days at 6°C in order to examine

Table VII.1.: Minimum temperature of 29 *Rhizoctonia solani* isolates (from soil and lettuce) for mycelium growth on cherry agar (CA) and for infection of lettuce leaf. - = no mycelium growth or no infection, + = mycelium growth for infection.

Isolate	3°C		6°C		9°C	
	CA	leaf	CA	leaf	CA	leaf
From soil:						
200-00-79					-	-
01						-
02						+
04						+
201-01-79					+	+
03						+
04					+	-
05					+	+
06					+	+
07					+	+
202-00-79					+	-
From lettuce:						
400-00-78						+
401-00-76			-	-	+	-
402-00-76			-	-	+	-
403-00-76						+
404-00-76			-	-	+	-
405-00-76			+	-	+	+
406-00-78			+	-	+	+
407-00-77			+	-	+	-
408-00-79			+	-	+	-
409-00-79			+	-	+	+
01			+	-	+	+
03			+	-	+	+
410-00-79			+	-	+	+
411-00-79						-
412-01-79					+	-
416-02-80	-	-	+	+	+	+
10	+	-	+	+	+	+
15	+	+	+	+	+	+

wether the minimum temperature (9°C) required to initiate an infection is higher than that needed to increase an established infection. Table VII.2. shows that the size of the infected

Table VII.2.: Mycelium growth on agar and increase of infected leaf area at 6°C, a temperature below the minimum for initiation of leaf infection (9°C) but above the minimum for mycelium growth on cherry agar (< 6°C).

Isolate	Mycelium growth on cherry agar at 6°C	Increase of infected leaf area at 6°C
409-00-79	+ [1]	- [2]
409-01-79	+	-
409-03-79	+	-

1) + : growth
2) - : no increase

area did not increase at 6°C, but, as was expected, mycelium growth on agar continued. From this it can be concluded that the minimum temperature for increase of an established infection is not lower than for initiating an infection.

In the infection experiments below the minimum temperature (Table VII.1. and 2.), mycelium growth on the leaves from the inoculum site were observed, but no typical complex infection structures for penetration of the cuticula and epidermal cell walls of lettuce leaf were formed such as at higher temperatures (lobate appresoria according to Townsend, 1934; Dodman and Flentje, 1970). Obviously, temperature requirement for the formation of infection structures is higher than for mycelium growth.

VII.4. INCUBATION TIME AND RATE OF LEAF INFECTION

Part of the isolates for which the minimum temperature for infection was shown in Table VII.1. were taken for the determination of the incubation time at different temperatures and the estimation of the rate of leaf infection (Table VII.3.). Seven isolates were obtained from soil from two glasshouses; the other ten were isolated from attacked lettuce from eight glasshouses.

Results (Table VII.3.) show that the incubation time at just above the minimum temperature (for most isolates 9°C,

for two 416-isolates 3°C) was 11-15 days. At higher temperatures the incubation time decreases rapidly (3-6 days), whereas at 20°C disease symptoms were evident within 3 days.

Temperature also had a distinct effect on the infection rate. At 9°C - just above the minimum temperature for most isolates - the infected leaf area increased from 50 per cent to 100 per cent in 3-4 days, at 15°C in 2-3 days and at 12°C in 1-2 days (Table VII.3.).

From these estimated infected rates the linear increase of the lesions in mm per day was calculated. This value was 2.2 mm per day at 9°C, 4.1 mm per day at 15°C and 8.2 mm per day at 20°C. Of the three 416-isolates which were already infective at 6°C, the linear increase of the lesions was measured: 1.4 mm per day at 6°C.

The above established rapid decrease of the incubation period and the rapid increase of the rate of infection of the lettuce leaf area at higher temperatures (15-20°C) confirms the observation by growers that the disease may rapidly increase during warm periods.

VII.5. MYCELIUM GROWTH CHARACTERISTICS OF STRAINS

Mycelium growth on agar in relation to temperature was measured for eight isolates from lettuce collected from a glasshouse in which the winter crop had been uncommonly heavily attacked (416-numbers). Two isolates (409-numbers) from lettuce collected from a glasshouse where attack during winter months was rare, were included for comparison.

Results (Figure VII.1) show that seven out of eight 416-isolates have a slowly increasing growth curve (maximum growth 5.5 mm per day) and a very wide range of the optimum temperature (12-25°C), with a minimum below 5°C and a maximum up to 34°C.

Both 409-isolates and one 416-isolate (416-02-80) show a steep growth curve (maximum growth up to 23 mm per day) with a rather sharp optimum at about 25°C and a minimum over 5°C and a maximum over 34°C.

Table VII.3.: Temperature and length of incubation time (days) and relative infection rate (days required for 50 and 100 per cent infection of leaf area (12.5 cm^2) of 17 *Rhizoctonia solani* isolates.

Isolate	3°C	6°C	9°C			15°C			20°C		
	inc. time (days)	inc. time (days)	inc. time (days)	infected area 50% (days)	infected area 100% (days)	inc. time (days)	infected area 50% (days)	infected area 100% (days)	inc. time (days)	infected area 50% (days)	infected area 100% (days)
From soil:											
200-00-79						4	6	> 8	< 3	4	6
-01-			14	> 21	> 21				3	5	6
-02-			11	16	19				< 3	4	5
-03-			11	16	20				3	5	6
-04-			11	19	> 21				< 3	4	5
201-01-79						6	> 7	> 7	5	> 7	> 7
-03-			14	19	> 21				3	5	6
From lettuce:											
404-00-76		-[1]	15			< 3	6	8	< 3	4	6
405-00-76		-[1]	15								
406-00-78		-[1]	13			< 3	5	7	< 3	3	5
407-00-77		-[1]	15								
408-00-79		-[1]	15								
410-00-79		-[1]	13								
412-01-79		-[1]	13			< 3	6	8	< 3	3	5
416-02-80	-[2]	6	< 4								
-10-	11	6	< 4								
-15-	11	6	< 4								

1) observations ended after 20 days, 2) observations ended after 18 days.

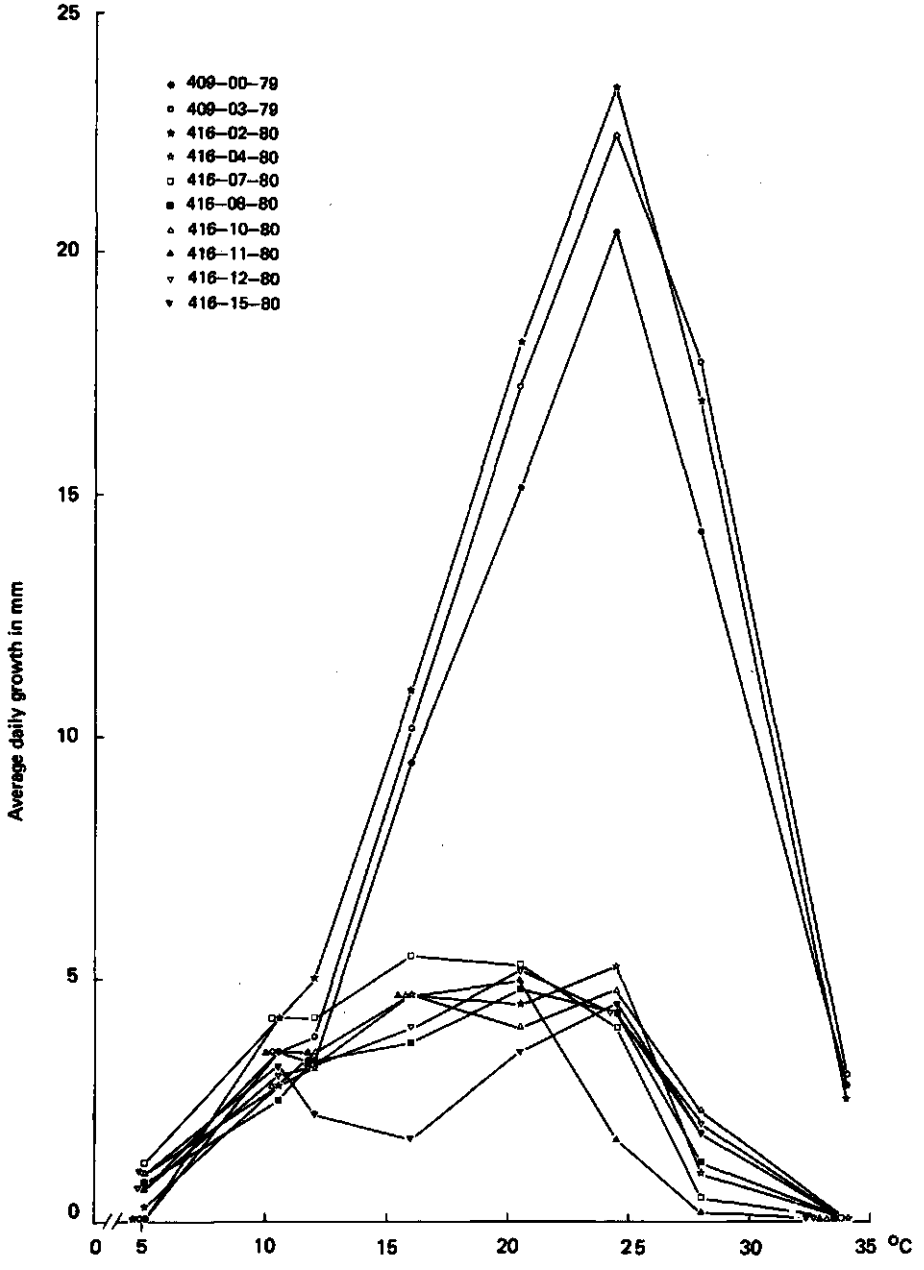

Figure VII.1.: The influence of temperature on the average daily growth on cherry agar of 10 *Rhizoctonia solani* isolates from lettuce of 2 glasshouses, 409- and 416-numbers respectively.

Two types of isolates can clearly be distinguished. One type, from the glasshouse with attack in cold crops, is characterized by a low linear growth rate, a low minimum temperature and a wide optimum temperature range. The other type has a steeper growth curve with a faster growth rate, a sharp optimum and higher minimum and maximum temperatures.

The established in vitro low temperature requirements of the tested 416-isolates are in agreement with the infection data of these isolates reported in the previous paragraph and with grower's experiences of recurring *R. solani* problems in winter lettuce.

In a general way *R. solani* attack in winter lettuce is scarce (Chapter VII.1.) and very low temperature requirements were not observed except for the 416-isolates. The history of the 416-glasshouse is also unusual compared to most of the lettuce holdings. It is located on moorland that was reclaimed 50 years ago. In the first 35 years only agricultural crops were grown, these were replaced by glasshouse crops and during the last few years lettuce has been the main crop; the soil was only sporadically superficially disinfected. Therefore it can be assumed that a relatively "natural" *R. solani* population is present, possibly with a great variation of strains. If this is the case "cold" strains could dominate in winter crops, causing *R. solani* bottom rot.

VII.6. DISCUSSION

In interpreting the obtained (minimum) temperature values for mycelium growth and infection of lettuce, it must be remembered that the experiments were conducted under artificial laboratory conditions. Pennypacker and Stevenson (1980) questioned the validity of applying such results to actual field situations. Moreover, requirements for temperature under field conditions are relative and vary in relation to the status of other factors such as food supply or humidity (compensation concept of Rotem, 1978).

From the data obtained it could be concluded that a great

part of the isolates tested did not infect lettuce at 9°C and that the few isolates with a lower minimum temperature requirement need a very long incubation time, and show a low rate of leaf infection at 9°C (Tables VII.1. and 3. respectively). Further, a glasshouse temperature regime of 6-7°C at night and 12°C during day time is usual for winter crops (Chapter I). This means that during the winter months the temperature often does not meet the minimum requirement for infection. Therefore it is very likely that the prevailing low glasshouse temperature is an important factor with respect to the observed minimum attack on winter crops (Chapter VII.1.).

In order to explore the possibility of controling the disease by regulating the temperature, knowledge about the occurrence of "low temperature" strains (Shephard and Wood, 1953; Parmeter and Whitney, 1970; Sherwood, 1970 and Doornik, 1981[a]) is important. In the present study isolates with a distinct low temperature character for mycelium growth and infection from only one glasshouse (416-numbers) were obtained (Chapter VII.4. and 5.). This was related to the "natural" *R. solani* population of this relatively young and seldom disinfestated holding (Chapter VII.5.).

In the important glasshouse district of Zuid-Holland most of the lettuce glasshouses have a long history of intensive (annual) disinfestation of the soil mainly with methyl bromide, which efficiently controls *R. solani* (Chapter VIII). It can be assumed that this practice reduces many strains including those that prefer low temperatures. Consequently, in the joint surveys in 1975 and 1976 (see Chapter VII.1.) practically no *R. solani* attack was found in lettuce glasshouses of the above mentioned district.

It was observed that the minimum temperature for mycelium growth on agar and leaves about 3°C lower than for infection of leaves (Chapter VII.3.). In evaluating the significance of the minumum temperature for *R. solani* in lettuce both threshold values should be considered. Should the glasshouse temper-

ature be too low for infection, saprophytic spread of the fungus over the soil or the leaf may occur, provided that the food base is sufficient. The increase of mycelium at low temperature can be a positive factor in the development of an epidemic. For refinement of the prediction of *R. solani* epidemics in lettuce it is, therefore, important to know what minimum temperature requirements are for both the saprophytic and the parasitic activity of isolates.

The literature and early experiments by the author (Chapter VII.1.) show that as far as the influence of the relative humidity on the attack of lettuce is concerned the fungus is hygrophylic. However, no free water is essential for infection as in some other *R. solani*-host plant systems (Baker and Martinson, 1970). It should be noted that Townsend (1934) and Schneider (1953) used one single isolate only. In preliminary unpublished experiments by the author at a relative humidity of 92 per cent and 15°C with 4 isolates mycelium growth on agar and infection of young lettuce plants hardly differed when compared with a relative humidity of 100 per cent.

Although thus no exact data on the threshold value of relative humidity for infection of representative strains are available, the following field observations also point out the hygrophile character of *R. solani*. In well-ventilated glasshouses, *R. solani* very rarely occurs in young, open crops. Moreover, attack is seldom found in outer leaves, but mainly in lower leaves and inside the heads, thus situations where the relative humidity is high. This agrees with the observations made by Pieczarka and Lorbeer (1975) in outdoor lettuce.

VII.7. SUMMARY

The minimum temperature for mycelium growth on cherry agar and for infection of lettuce leaf was determined for 29 *R. solani* isolates obtained form soil or heads of 17 glasshouses. The minimum temperature requirement for infection of most isolates was ≥ 9°C, one isolate infected at 3°C. The minimum tem-

perature for mycelium growth was about three degrees lower than for leaf infection. Temperature requirement for the formation of the lobate appressoria infection structures appeared to be higher than for mycelium growth.

Incubation time slightly above the minimum temperature amounted 11-15 days, at 20°C disease symptoms occurred within 3 days.

The linear increase of lesions of the isolates investigated was at 9°C 2.2 mm per day, at 20°C 8.2 mm per day.

Based on mycelium growth characteristics two types of isolates could be distinguished. One type, seldom occurring, has a low linear growth rate, a low minimum temperature and a wide optimum temperature range. The other type has a steeper growth curve with a faster growth rate, a sharp optimum and higher minimum and maximum temperatures.

It appeared that *R. solani* in lettuce must be considered hygrophilic, but free water is not essential for infection of the lettuce leaf.

VIII. CONTROL OF BOTTOM ROT

VIII.1. INTRODUCTION

Before 1978, the generally applied preventive control of the bottom rot fungi *Botrytis cinerea, Rhizoctonia solani* and *Sclerotinia spp.* in most instances consisted of one single pre-planting soil surface application of quintozene in combination with one single application of thiram up till seven days after planting. For further details, the need for quintozene replacing fungicides and the availability of dicarboximides see Chapter I.2.b.

This chapter first reports on in vitro experiments on the sensitivity of bottom rot pathogens to dicarboximides, followed by the results from characteristic field trials for the approval of dicarboximides in comparison to products of reference, quintozene and thiram. Also the efficacy of tolylfluanid against *B. cinerea* is examined. Finally the effect of soil disinfestation with methyl bromide on the population level of *R. solani* was investigated.

VIII.2. MATERIALS EN METHODS

The chemicals used were: AApirol Stuif (10 per cent thiram) AAgrunol B.V.; Asepta PCNB 20 per cent (20 per cent quintozene) Asepta B.V.; Brassicol Super (20 per cent quintozene), Hoechst Holland B.V.; Eupareen M (50 per cent tolylfluanid), Hoechst Holland B.V.; Ronilan (50 per cent vinclozolin), BASF Nederland B.V.; Rovral (50 per cent iprodione), Agriben Nederland B.V.; Sumisclex (50 per cent procymidone), AAgrunol B.V.

Bottom rot fungi for mycelium growth inhibition tests were isolated from attacked lettuce heads. Suspensions of the chemicals were mixed with melting cherry agar (50°C) in such a way that a range of six concentrations was obtained. From good growing colonies agar discs, 5 cm in diameter, were punched

out and plated out in duplo on the medium containing the fungicide. After one day, the radial growth was marked and then measured in two directions over three days. The daily growth was calculated and expressed as a percentage of growth in the untreated. These figures were plotted against the concentrations on logarithmic probability paper. From the estimated regression line the ED_{50} value in ppm active ingredient was determined.

Field trials were carried out in commercial glasshouses under usual cultural practices. The experiments were arranged in randomized blocks of 4-6 plots each. Plots measured 1.5 x 3 m, holding 20-25 plants per m^2. One glasshouse (Table III.2) was artificially inoculated with 1 kg per 100 m^2 *R. solani* infected grains of wheat. In analysing yields, 40-90 plants per plot were used; for the analysis of pathogens 15-90 plants per plot were examined. Assessment was carried out macroscopically or microscopically, as described in Chapter II.2. and II.4.

In most trials the percentage of lettuce heads of export quality class was determined. The maximum price is paid for these lettuce.

Experimental results were tested by analysis of variance..

Fungicides containing quintozene were applied over the soil just before planting; thiram was dusted and the other compounds were sprayed over the young crop.

Horizontal and vertical soil sampling before and after the application of methyl bromide was carried out as described in Chapter IV.2.a. and Chapter V.2.a. respectively. To avoid recolonization of the grower's soil no untreated plots were included in these disinfestation experiments.

VIII.3. SENSITIVITY OF BOTTOM ROT PATHOGENS TO DICARBOXIMIDES IN VITRO

From ED_{50} values given in Table VIII.1. it appears that dicarboximides are active against the *R. solani* isolates tested. Iprodione was most effective; vinclozolin least. The difference in activity between both these compounds is in agreement

Table VIII.1.: Effects on radial growth of *Rhizoctonia solani*, *Botrytis cinerea*, *Sclerotinia sclerotiorum* and *Sclerotinia minor* isolates on cherry agar by iprodione (ipr.), vinclozolin (vincl.) and procymidone (proc.) at two temperatures. ED_{50} in ppm a.i.

	ED_{50} (ppm a.i.)					
	15°C			25°C		
Fungus/isolate	ipr.	vincl.	proc.	ipr.	vincl.	proc.
R. solani						
401-02-76	0.6	1.5	0.8	0.5	0.9	1.0
402-01-76	0.7	1.5	0.9	1.1	1.4	1.4
403-00-76	0.6	1.5	0.8	0.8	2.3	1.1
404-00-76	0.4	0.6	0.6	1.0	3.0	1.4
(average	0.6	1.3	0.8	0.9	1.9	1.3)
B. cinerea	-	-	-	0.4	0.3	0.4
S. sclerotiorum	-	-	-	0.3	0.2	0.3
S. minor	-	-	-	0.2	0.1	0.2

with results by Fisher (1979) obtained in in vitro experiments with a single *R. solani* isolate. Some small differences in sensitivity between isolates were found, particularly at 25°C and especially with respect to vinclozolin.

On an average, in all compounds the ED_{50} value at 25°C was 1.5 x greater than at 15°C. Hans et al. (1981) found that also the systemic compound carbendazim and Kataria and Grover (1976) the systemic compounds benomyl, thiophanate-methyl and chloroneb, inhibited *R. solani* more at the lower than at the higher temperatures, when compared to the non-systemic fungicide quintozene which was more active at the higher temperature.

The sensitivity of *B. cinerea* was practically the same for all three compounds. The resulting ED_{50} values correspond with data by Leroux et al. (1977), Pappas and Fischer (1979), Grindle (1981) and Davis and Dennis (1981).

S. sclerotiorum and *S. minor* were more sensitive than *B. cinerea*. Vinclozolin seems to be slightly more active than both other compounds. Reilly and Lamoureax (1981) in their experiments on the effect of iprodione against *S. sclerotiorum* also found similar ED_{50} values for the inhibition of mycelium growth.

Out four fungi tested, *R. solani* was least sensitive for dicarboximides. This is in agreement with Fischer's results (1979).

VIII.4. FIELD EXPERIMENTS

VIII.4.a. *Rhizoctonia solani*

Table VIII.2. shows the results of a field experiment in which the effectivity of dicarboximides against *R. solani* was tested under extremely favourable conditions for the pahogen: high (artificial) inoculum density and high temperature (summer crop). One high dose of Ronilan and Sumisclex was applied and 3 doses of Rovral. The dosage of the reference products Asepta PCNB 20 % and AApirol Stuif was as recommended.

Only 5 per cent of the lettuce on the untreated plots were marketable due to heavy attack, especially by *R. solani*. The yield in plots treated with the products of reference was low: 39 per cent marketable heads. Ronilan scored even lower. Rovral and Sumisclex were much more effective, with a significant dosage effect in Rovral. The effect of the fungicides is also expressed in the disease index value. In the analysis of pathogens *R. solani* proved to be the most important fungus. Many heads were attacked by more than one pathogen.

Tables VIII.3. and 4., show the results of treatments in a glasshouse with natural *R. solani* infestation. In the untreated plots the percentage of heads of export quality class was fairly high: 81 per cent. This percentage was somewhat higher in the treated plots, but differences were not significant. There was a distinct effect of the compounds on the disease index value. Leaves in the Ronilan treated plots were significantly less attacked than in "untreated", but the control

Table VIII.2.: Effect of fungicides on percentage of marketable heads, disease index and occurrence of bottom rot pathogens. Means of 4 replicates, each 45 plants. Cv "Ostinata". Sandy soil, artificially inoculated with *Rhizoctonia solani*. Planting 17-6-1977, harvest 27-7-1977. Asepta PCNB 20 % and AApirol Stuif applied on 16-6-1977, the other fungicides on 1-7-1977. R = *Rhizoctonia solani*, B = *Botrytis cinerea*, P = *Phythium species*, C = combination of different pathogens.

Treatment	Dosage g/m^2	Percentage marketable heads	Disease index [1] (0 - 5)	Number of heads[2] with R	B	P	C
Untreated		5	4.9	13	3	3	4
Asepta PCNB 20 % + AApirol Stuif	15 10	39	4.0	8	6	6	5
Ronilan	0.5	21	4.5	14	3	1	3
Rovral	0.5	69	2.7	6	2	8	1
"	0.25	52	3.4	-	-	-	-
"	0.125	33	4.1	-	-	-	-
Sumisclex	0.5	73	2.9	-	-	-	-
LSD 0.05		14.4	0.6				
LSD 0.01		19.8	0.8				

1) 0 = no bottom rot, 5 = not marketable

2) n = 15 analysed heads from one plot

Table VIII.3.: Effects of fungicides on the percentage of marketable heads, the percentage heads of export quality class, and the disease index. Means of 4 replicates, each 40 plants. Cv "Ostinata". Sandy soil, naturally infestated with *Rhizoctonia solani*. Planting 4-4-1978, harvest 17-5-1978. Brassicol Super + AApirol Stuif applied on 4-4-1978, the other fungicides on 11-4-1978.

Treatment	Dosage g/m^2	Percentage marketable heads	Percentage heads of export quality class	Disease index (0 - 5) [1]
Untreated	-	98.8	81.0	2.6
Brassicol Super +	15	100	92.5	0.9
AApirol Stuif	10			
Ronilan	0.3	100	90.0	1.5
Rovral	0.3	100	88.1	0.8
Sumisclex	0.3	100	90.6	0.7
LSD 0.05		-	N.S.	0.6
LSD 0.01		-	N.S.	0.8

1) 0 = no bottom rot, 5 = not marketable

obtained with this compound was somewhat less than that with the other products. From the analysis of pathogens in the untreated plot (Table VIII.4.) it appeared that over 40 per cent of the heads were attacked by *R. solani* (alone or in combination with other pathogens).

From both experiments it can be concluded that Rovral and Sumisclex are at least as effective against *R. solani* as the products of reference at the dosage used. Ronilan is less effective when conditions for development of *R. solani* are very favourable.

VIII.4.b. *Botrytis cinerea*

In Chapter I.2. it was stated that *B. cinerea* usually occurs in maturing heads. Also starting from more or less weakened young plants there is a great chance for fall out due to *B. cinerea* attack. Fungicides should protect the crop sufficiently in all stages of development.

Two field trials are described in which the effect of Ronilan and Eupareen M against *B. cinerea* are compared. In the first experiment no attack was observed at the beginning of the cropping period; at harvest time it appeared that about 4 leaves of the heads from the untreated plots were diseased (disease index 2.2, Table VIII.5.), a rather moderate attack by *B. cinerea*. In the treated objects the percentage of heads

Table VIII.4.: Pathogen analysis of 15 heads from the untreated object of the fungicide trial of Table VIII.3.

Pathogens present	Percentage heads
Botrytis cinerea (B)	37.8
Pythium spp. (P)	8.9
Rhizoctonia solani (R)	26.7
B + R	15.6
B + P	2.2
R + P	2.2
No pathogen	6.7

Table VIII.5.: Effect of fungicides on percentage of marketable heads, on percentage of heads of export quality class and on disease index. Means of 6 replicates, each 40 plants. Cv "Miranda". Sandy soil. Planting 2-2-1980, harvest 9-4-1980. The fungicides applied on 8-2-1980.

Treatment	Dosage g/m^2	Percentage marketable heads	Percentage heads of export quality class	Disease index (0-5) [1]
Untreated	-	97.1	68	2.2
Eupareen M	0.3	96.0	89	0.5
Ronilan	0.2	100	78	1.0
LSD 0.05		-	N.S.	0.7
LSD 0.01		-	N.S.	0.9

1) 0 = no bottom rot, 5 = not marketable

of export quality class was higher than in "untreated", but the difference was not significant. The disease index in both treated objects was significantly lower than in "untreated". Based on these results it can be concluded that in this trial Eupareen M controls *B. cinerea* in maturing lettuce.

The picture changes if young weakened plants must also be protected by the fungicides, as it appears from the experiment of which the results are given in Table IX.6. and Figure VIII. 1. Due to unexpected circumstances the plants used in this trial had to be stored for three weeks before planting. They became elongated and the pot soil dried out, so that the plants weakened. It is generally known that under these conditions *B. cinerea* will easily attack, which causes the young plants to die off.

The latter appears from Figure VIII.1.; 14 days after planting about 5 per cent of plants in "untreated" had disappeared; a week later this percentage had increased to 40. Two months after planting only 25 per cent of all plants had remained. Although Eupareen M had given some protection, the effect was far behind that of Ronilan. At harvest time 55 per cent of all the plants in Eupareen M plots had disappeared whereas only 2

Table VIII.6.: Effects of fungicides on the percentage of marketable heads, on the percentage of heads without disease and on the disease index. Means of 6 replicates, each 90 plants. Cv. "Miranda". Sandy soil. Planting 14-12-1979, harvest 12-3-1980. The fungicides applied on 21-12-1979.

Treatment	Dosage g/m^2	Percentage marketable heads	Percentage heads without disease	Disease index (0-5)[1]
Untreated	-	24.7	16.4	3.9
Eupareen M	0.3	54.3	32.9	3.0
Ronilan	0.2	98.1	86.7	0.2
LSD 0.05		-		0.8
LSD 0.01		-		1.1

1) 0 = no bottom rot, 5 = not marketable

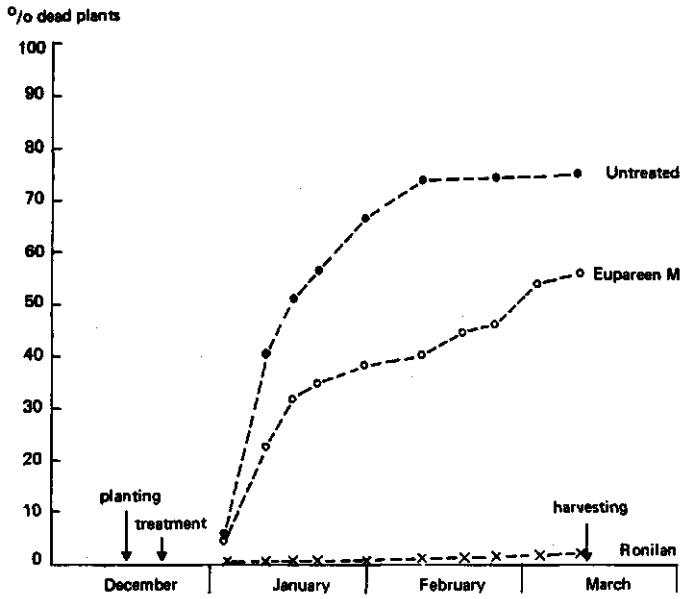

Figure VIII.1.: Percentage lettuce plants killed due to *Botrytis cinerea* infection during the growth season in relation to treatment with Ronilan (0.2 g/m^2) and Eupareen M (0.3/m^2). Data points are based on 6 replicates (plots), each 90 plants. For data on cultural practices see Table VIII.6.

per cent in plots treated with Ronilan. The difference in effectiveness also appears from the disease index values (Table VIII.6.). These data show that the *B. cinerea* infection was at the beginning of the cropping period. Plants present at harvest time in all plots were predominantly free from attack.

It can be concluded that Eupareen M has failed under these conditions and that Ronilan was very effective.

VIII.4.c. *The bottom rot complex*

The effect of fungicide treatments was investigated in a culture of lettuce which lasted as long as three months. The results are shown in Table VIII.7. Attack was rather heavy on the untreated plots (disease index 2.8). All treatments had a positive effect on the number of harvested heads. Products of reference Brassicol Super and AApirol Stuif had somewhat less effect than the dicarboximides, but differences were not significant. The percentage of heads of export quality class from

Table VIII.7.: Effects of fungicide treatments on the percentage of marketable heads, the percentage of heads of export quality class, and on the disease index. Means of 4 replicates, each 40 plants. Cv "Parmante". Planting 7-10-1977, harvest 10-1-1978. Brassicol Super and AApirol Stuif applied on 7-10-1977, the other fungicides on 14-10-1977.

Treatment	Dosage g/m^2	Percentage marketable heads	Percentage heads of export quality class	Disease index (0-5) [1]
Untreated	-	84.8	51.5	2.8
Brassicol Super +	15	92.0	83.0	1.6
AApirol Stuif	10			
Ronilan	0.3	98.1	85.4	1.1
Rovral	0.3	95.0	78.3	1.6
Sumisclex	0.3	99.0	87.3	0.9
LSD 0.05		7.8	16.9	0.6
LSD 0.01		10.8	23.7	0.9

1) 0 = no bottom rot, 5 = not marketable

Table VIII.8.: Pathogen analysis of heads from the untreated plots of the fungicide trial of Table IX.7. The figures are based on 4 replicated plots, each 15 heads.

Pathogen(s) present in the untreated plots	Percentage heads
Botrytis cinerea (B)	31.7
Pythium spp. (P)	0
Rhizoctonia solani (R)	16.7
Sclerotinia spp. (S)	5
B + P	6.7
B + R	26.7
B + S	10
P + S	1.7
B + P + R	1.7
No pathogen	0

all the treated plots was much higher than from the "untreated". This was also expressed by the disease index values. Plants treated with Sumisclex were significantly less attacked than when treated with Rovral and the products of reference. The results with Ronilan were intermediate. It can be concluded that the effectiveness of dicarboximides at least equalled that of the products of reference. From the analysis of pathogens from lettuce heads in the untreated plots (Table VIII. 8.) it appeared that attack was mostly caused by *B. cinerea* alone and to a lesser extent by *B. cinerea* and *Sclerotinia spp.* Results from this trial show a distinct effect of dicarboximides against three important bottom rot pathogens in a longlasting crop.

Table VIII.9. shows the results of a trial in which, according to macroscopical observation, *Sclerotinia spp.* were the most common pathogens, followed by *B. cinerea* and *R. solani*. Data from "untreated" show a rather heavy attack (disease index 3.0). All treatments had a positive effect on the number of marketable heads, on the percentage of heads of export quality class and on the disease index. Differences between treatments were not significant. This experiment also proves the

Table VIII.9.: Effects of fungicides on percentage of marketable heads, on the percentage of heads of export quality class, and on the disease index. Means of 6 replicates, each 40 plants. Cv "Miranda". Sandy soil. Planting 7-3-1980, harvest 17-4-1980. Brassicol Super and AApirol Stuif applied on 7-3-1980, the other fungicides on 14-3-1980.

Treatment	Dosage g/m^2	Percentage marketable heads	Percentage heads of export quality class	Disease index (0-5) [1]
Untreated	-	85.5	74.6	3.0
Brassicol Super +	15	98.3	94.5	1.5
AApirol Stuif	10			
Ronilan	0.4	98.3	94.5	1.4
Rovral	0.4	94.6	91.2	1.6
Sumisclex	0.4	94.2	91.6	1.6
LSD 0.05		10.3	5.3	0.6
LSD 0.01		N.S.	7.2	0.8

1) 0 = no bottom rot, 5 = not marketable

controlling effect of dicarboximides on the bottom rot complex.

VIII.4.d. *Dicarboximides and the incidence of Pythium spp.*

In Table VIII.10. the results are given of a field trial with regard to the analysis of pathogens from lettuce heads taken from untreated, quintozene (Brassicol Super) treated and dicarboximides treated plots respectively. *B. cinerea* proved to be the pre-dominant fungus, followed by *Pythium spp.*, whether or not in combination with *B. cinerea*. In this trial *R. solani* scarcely occurred.

Pythium spp. were the least found in the untreated plots (14 per cent). In the treated plots 20-34 per cent of the lettuce heads were attacked by *Pythium*. These figures are an indication that the application of specific fungicides such as quintozene and dicarboximides may give rise to an increased incidence of insensitive *Pythium spp.*. Lockwood (1970) demonstrated a similar phenomenon for quintozene. An increase in the occurrence of *Pythium spp.* in lettuce after treatment with dicarboximides was also observed by Tichelaar (1978).

Table VIII.10.: Effect of fungicides on the incidence of bottom rot pathogens at harvest. Means of 2 replicates, each 15 heads. Cv "Ravel". Sandy soil. Planting 6-10-1977, harvest 12-12-1977. Brassicol Super and AApirol Stuif applied on 6-10-1977, the other fungicides on 14-10-1977. No path. = no pathogen(s), B = *Botrytis cinerea*, P = *Pythium species*, R = *Rhizoctonia solani*.

Treatment	Dosage g/m^2	Percentages of heads infected with					
		no path.	B	P	R	B+P	P+(B+P)
Untreated	-	0	87	7	0	7	14
Bassicol Super +	15	7	70	10	0	13	23
AApirol Stuif	10						
Ronilan	0.3	20	50	10	3	17	27
Rovral	0.3	3	59	3	3	31	34
Sumisclex	0.3	17	63	13	0	7	20

VIII.4.e. *Effect of methyl bromide disinfestation on Rhizoctonia solani in soil*

The effect of soil disinfestation with methyl bromide on the population of *R. solani* in plots with lettuce and subsequently tomato are presented in Table VIII.11. Just before disinfestation, soil sampling results show a light to moderate *R. solani* infestation. Up till four months after the treatment no *R. solani* was found in any of the samples. This is an indication of the controlling effect of methyl bromide against *R. solani* (as indicated in Chapter VIII.2., no untreated plots were included).

Nine months after disinfestation the soil in one plot proved to be lightly infested. Two months later *R. solani* was found in three plots. These plots were all situated along the central path in the glasshouse. After the second disinfestation, one year after the first, again no *R. solani* was found in the soil samples, therefore this treatment had also been effective.

Before plots in a second glasshouse (Table VIII.12.) were treated, a heavy infestation was found in some plots and a lighter infestation in others. Immediately after disinfesta-

Table VIII.11.: Glasshouse A. Effect of soil disinfectation with methyl bromide (two treatments, 100 g/ 100 m^2) on the level of *Rhizoctonia solani* in a glasshouse with sandy soil. Samples taken horizontally to a depth of 5 cm, vertically at a depth of 2.5, 5, 7.5 and 12.5 cm. The dimensions of the six replicates were 1.5 x 3 m.

Sampling	Date	Crop	Number of colonized disks										
			horizontal samples						vertical samples				
			replicate No.						depth (cm)				
			1	2	3	4	5	6	2.5	5	7.5	10	12.5
Just before disinfestation [1]	09-10-1978	-	4[2]	4	5	3	1	4			-		
1 month after disinfestation	13-11-1978	lettuce	0	0	0	0	0	0			-		
4 months after disinfestation	28-02-1979	lettuce	0	0	0	0	0	0	0[3]	0	0	0	0
9 months after disinfestation	24-07-1979	tomato	0	0	0	0	0	1	4[4,5]	0	0	0	0
11 months after disinfestation	24-09-1979	tomato	0	0	4	0	1	5			-		
½ month after disinfestation [6]	08-11-1979	-	0	0	0	0	0	0			-		

1) date of disinfestation: 16-10-1978

2) 40 disks per sample (plot)

3) 10 disks per sample level, 4 samples per plot

4) 10 disks per sample level, 2 samples per plot

5) from one sample of plot No.6

6) date of disinfestation: 31-10-1979

tion with methyl bromide, no *R. solani* could be found in any soil sample. One and a half month after treatment a light infestation was found in one plot. In this plot the population density rapidly increased also at greater depths. At the end of the observation period, five months after disinfestation, no *R. solani* was detected in the remaining plots.

From the results in both experiments it can be concluded that *R. solani* in soil can be controlled well by methyl bromide but that local re-infestation may occur rather rapidly.

VIII.5. DISCUSSION

The sensitivity of in vitro tested bottom rot pathogens to dicarboximides indicated a promising fungicidal activity of the compounds under practical conditions. It was found that the inhibition of mycelium growth of the *R. solani* isolates tested was stronger at 15° than 25°C. The question arise, whether this phenomenon, observed in vitro, might have practical consequences for the control of this fungus in warm crops in which chances for attack by this fungus are greatest (Chapter VII). In this connection the high efficacy of Rovral against *R. solani* in the field trial under warm conditions (Chapter VIII.4.a.) must be mentioned. This may be explained as follows. Since the duration of the culture is shorter at higher temperatures, a greater amount of fungicide might be present during the most critical last period of the crop, at least if we assume that these temperatures do not cause a faster breakdown of the residue.

In field experiments, carried out on different types of crops with different bottom rot pathogens, the dicarboximides Ronilan and Rovral proved to be good substitutes for the compounds of reference quintozene and thiram. The somewhat lower activity of Ronilan against *R. solani* in these and in in vitro-experiments, will in most instances not lead to economically lower yields (Tables VIII.3. and 7.).

In the trial with weakened young plants, Ronilan proved to give excellent protection against *B. cinerea* (Table VIII.6.).

Table VIII.12.: Glasshouse B. Effect of soil disinfestation with methyl bromide (100g/m^2) on the level of *Rhizoctonia solani* in a glasshouse with moisty sandy soil. Samples taken horizontally to a depth of 5 cm, vertically at a depth of 2.5, 5, 7.5 . . . . . 27.5 and 30 cm. The dimensions of the 6 replicates were 1.5x3 m.

Sampling	Date	Crop	Number of colonized disks											
			horizontal samples						vertical samples					
			replicate No.						depth (cm)					
			1	2	3	4	5	6	2.5	5	7.5	10	12.5	...30
Just before disinfestation [1]	28-05-1979	lettuce	14[2]	0	10	3	15	8				-		
½ month after disinfestation	21-06-1979	tomato	0	0	0	0	0	0	0[3]	0	0	0	0	0
1½ months after disinfestation	24-07-1979	tomato	0	0	0	0	2	0	0	0	0	0	0	0
2½ months after disinfestation	24-09-1979	tomato	0	0	0	0	10	0	0	0	0	0	0	0
5 months after disinfestation	08-11-1979	tomato	0	0	0	0	17	0	2[4]	0	0	0	0	0
									6[5]	0	2	2	1	0

1) data of disinfestation: 07-06-1979

2) 40 disks per plot

3) 10 disks per sample level, 2 samples per plot

4) Replicate No. 5., sample 1

5) Replicate No. 5., sample 2

Although growers do their utmost to start from healthy young material, late planting of weakened seedlings cannot always be avoided.

In some countries dicarboximides resistant strains were isolated from crops, where these fungicides had been used frequently (Beever and Byrde, 1982). However, in most cases the disease was controlled. In recent experiments (not published) *B. cinerea* isolates from a lettuce glasshouse the author found a moderate level of iprodione resistance. The ED_{50} value for mycelium growth on agar was about 5 ppm, which is about ten times as high as that for sensitive isolates. In this case the disease was also controlled. There is still no evidence that disease control in lettuce by dicarboximides is reduced, but this should be watched carefully. The topic will be discussed further in a broader context in the next Chapter IX "General Discussion".

The increased incidence of *Pythium spp.* after application of specific fungicides, observed in other crops (Lockwood, 1970, quintozene; Williams and Ayanaba, 1975, benomyl and related products) also occurred after application of dicarboximides and quintozene in lettuce (Table VIII.10.). The results obtained do not permit the conclusion that this increase of *Pythium* incidence caused so many more damaged leaves in the heads, that economic loss occurred. This also depends on the pathogenicity of the *Pythium spp.* in the different glasshouses.

Apart from sporadic cases when nematode attack is anticipated, no soil disinfestation is specifically practised for the culture of lettuce. Soil fumigation with methyl bromide is commonly carried out for the benefit of tomato cropping, but because of cultural reasons applied just before the preceding crop, i.e. lettuce. See also Chapter I.1.b. The effects of methyl bromide on fungi are well-known. *R. solani* is one of the fungi which are moderately sensitive to this compound (Vanachter, 1979; Munnecke et al., 1978).

So far, the disinfecting effect on this fungus in glasshouse soil in The Netherlands had not been investigated. In the experiments, presented in Tables VIII.11. and 12., methyl bromide caused a strong reduction of the *R. solani* population in soil. Thereafter, local re-colonization may in some instances very soon occur, but the soil may also remain free from *R. solani* for a year. Therefore, the yearly nematicide application of methyl bromide in the glasshouse district of Zuid-Holland must be considered an important sanitary measure against *R. solani*. The rare occurrence of this fungus in lettuce from the mentioned district (Tichelaar, 1976, 1977) is probably connected with this practice.

From the results in Chapter V.4.e. it also appeared that the inoculum density of *R. solani* in glasshouse soils treated with methyl bromide within one year before lettuce planting was only half to that in glasshouses that had been treated more than a year ago or had not been treated regularly.

From the results of Chapter V.4.c. it also appeared that t can be concluded that re-colonization occurs in foci, i.e. local high inoculum densities may occur. It is possible therefore, that the observed clustered distribution of *R. solani* propagules in soil (Chapter V.2.b. and 4.b.) is connected to regular soil disinfestation.

VIII.6. SUMMARY

In vitro, bottom rot pathogens *B. cinerea*, *R. solani*, *S. minor* and *S. sclerotiorum* are sensitive to low dosages of the dicarboximides iprodione, procymidone and vinclozolin. The latter compound appeared less active against *R. solani*.

The activity of dicarboximides against *R. solani* in vitro was at 25°C less than at 15°C.

In field trials, carried out in different cropping periods during which different bottom rot pathogens were present in the crops, the effect of dicarboximides proved to be at least equal to that of the products of reference quintozene and thiram. Ronilan (vinclozolin) and Rovral (iprodione) have been

therefore officially registered as substitutes for quintozene and thiram for the control of bottom rot in lettuce. Sumisclex (procymidone) was somewhat phytotoxic when used for this purpose.

It was observed that after the application of both quintozene and dicarboximides *Pythium spp.* attacks increased.

Soil disinfestation with methyl bromide for control of nematodes proved to be a good sanitary measure in reducing the population level of *R. solani* in soil. The significance of this nematicide used in the culture of tomatoes, is discussed in connection with the lower incidence of *R. solani* in the glasshouse district of Zuid-Holland and the clustered distribution of propagules of this fungus in soil.

IX. GENERAL DISCUSSION

Economically, the most important harvesting periods for lettuce are winter and early spring. In Chapter I, Introduction, the difficulties are mentioned, of complying with the strict regulations with regard to pesticide residues, and on the other hand growing a lettuce head that is sufficiently heavy and compact to be graded export quality. This means that high priority is given to a minimum use of pesticides.

The above gives rise to two questions firstly, whether the *Pythium spp.* damage as described in Chapter II and VIII require chemical control. Secondly, which of the present fungicides controlling *Oomycetes* are acceptable. There was little information available with regard to the economic importance of these fungi in the bottom rot complex (see also Blok and Van der Plaats - Niterink, 1978).

Further investigation is also required into the reasons for the increase of *Pythium spp.* on plots treated with dicarboximides or quintozene when compared with untreated plots (Chapter VIII.4.d.). It is not known whether this increase of *Pythium spp.* is a general phenomenon or only occurs in special soils under certain conditions. Pythiaceous fungi are known to be tolerant to the fungicides mentioned. The increase of *Pythium spp.* could be due to differential fungicide sensitivity between these pathogens and their antagonists (Bollen, 1979).

It is possible that, because of the general use of quintozene in the recent past, as well as the use of benzimidazoles and dicarboximides in the tomato crop that precedes the lettuce, the pathogenic significance of *Pythium spp.* has increased, as has been demonstrated in other crops by Papavizas and Lewis (1979 b).

In Chapter VIII.4.e. the effectiveness of controlling *R. solani* with methyl bromide has been demonstrated. According to Van Assche et al. (1968, 1969) the population density of *Sclerotinia spp.* is also reduced by this chemical especially in the upper soil layer.

In a literature review, Vanachter (1979) reports favourable results in controlling *Pythium spp.* with methyl bromide in different crops. Sclerotia of *Botrytis cinerea* are also killed (Van Assche et al., 1968), therefore methyl bromide can be expected to have a controlling effect on the initial soilborne inoculum which contributes to the infection by these fungi. It can be assumed that the annual treatment with methyl bromide applied shortly before planting lettuce, but indended for the succeeding tomato crop (see Chapter I.1.b.) has a favourable side effect by preventing bottom rot in lettuce. When evaluating compounds to replace methyl bromide, this aspect should also be considered.

The need for compounds to replace quintozene, the direct occasion for the present study, was supplied by the introduction of dicarboximide fungicides which were active against pathogens causing bottom rot with the exception of *Pythium spp.* Because bottom rot usually starts in leaves that are in contact with infested soil, the total soil surface has to be treated shortly after planting. If the fungicide is applied later, expanding lettuce leaves may cover part of the soil surface, thus preventing complete protection. The efficacy of this method, which has been for many years common practice in The Netherlands, was recently demonstrated in England to control *R. solani* with iprodione (Clark and McPherson, 1981).

Frequent applications of dicarboximides may increase the risk of developing resistance to fungicides as has been particularly demonstrated for *B. cinerea* in strawberry and vineyards (Beever and Byrde, 1982). In Swiss vineyards, serious attack of *B. cinerea*, associated with vinclozonlin resistance, lead to the advice that this product should be used for the first sprays only and other fungicides for the further applications (Pezet, 1982). The resistance level however, is usually not so high that the control with dicarboximides fails (Beever and Byrde, 1982; Lorentz and Eichhorn, 1982; Leroux et al., 1982). Evidence was obtained that moderately resistant strains, which appeared to have normal fitness, still can

be controlled by the usual doses of the fungicide. Highly resistant strains appear to have a reduced fitness, and apparently do not readily build up a resistant pathogen population. Losses due to disease control failure are therefore not expected. This reduction is probably related to the abnormal sensitivity to high osmotic pressure of the highly resistant strains (Beever and Byrde, 1982).

In 1982 the author also obtained a low-resistant *B. cinerea* isolate from glasshouse lettuce (unpublished), but no failure of control was observed. In this connection it should be mentioned that dicarboximides in lettuce are applied only once. In the preceding tomato crop dicarboximides are used frequently. To date in The Netherlands, breakdown of control of *B. cinerea* has never been reported. Besides, resistant *B. cinerea* strains quickly disappear with infrequent treatments (Lorentz and Eichhorn, 1982).

Leroux et al. (1977) reported cross-resistance to dicarboximides, quintozene and dicloran of *B. cinerea* laboratory strains. In The Netherlands, the latter compound is still used in lettuce against *B. cinerea* and *Sclerotinia spp.* In the past, when quintozene and dicloran were generally applied, no resistance was reported.

The author has no information on resistance of *R. solani* or *Sclerotinia spp.* to dicarboximides. The change that resistance builds up till disease control breaks down, is considered to be small because of low selection pressure as a result of infrequent applications, the survival of sensitive forms in the soil, the slow spread of these fungi and the very restricted sporulation (Dekker, 1982). Should *B. cinerea* not be controlled by dicarboximides, these compounds will remain useful in controlling the other pathogens.

The risk of resistance can be reduced by the alternate use of fungicides with a different mechanism of action (Dekker, 1982), especially against *B. cinerea* in the preceding tomato crop.

Cultural measures can be very effective in controlling bot-

tom rot, e.g. keeping the relative humidity in the glasshouse as low as possible checks all pathogens. Bottom rot is also reduced by choosing less sensitive or, even better, "upright" cultivars. *R. solani* is sensitive to a temperature regime below 10°C, therefore winter crops are less risky. *B. cinerea* is a weak parasite, healthy plant material and regular plant development are important, especially immediately after planting in a correct glasshouse climate.

Further investigations should prove whether the application of fungicides can be optimized by cultural practices. A possibility is to reduce the dose in less sensitive "upright" cultivars. In winter crops and on disinfected soil where the chance the attack by *R. solani* and *Sclerotinia spp.* is small, it may be possible to restrict chemical control to the use of compounds that control *Botrytis* only.

The possibilities of the biological control of bottom rot were investigated by Newhook (1951). He tried to control the attack of the basal leaves by *B. cinerea* in outdoor lettuce with antagonists (mainly bacteria). Although some positive results were obtained, the investigations were not continued, probably because effective fungicides became available. Kollmorgen (1971) controlled *S. sclerotiorum* in lettuce by the use of small quantities of stable manure. It is believed that here the attack is limited by the stimulation of the antagonism in the soil. Efficient natural biological control of *Sclerotinia minor* is assumed by Imolehin and Grogan (1980) in many fields of an important lettuce growing area in the USA.

Although there has been a great deal of research into biological control of soil-borne pathogens in the last ten years in other crops, no biological agents have become commercially available. Recently, some progress has been reported in the biocontrol of the pathogens *Pythium spp.* and *R. solani* in seedlings (Harman et al., 1980). Papavizas and Lewis (1979, b) have also experimented with integrated control of *R. solani* in beans, using a combination of ploughing, antagonistic microorganisms and fungicides.

Developments in the near future are likely to effect the treatment of bottom rot. Firstly the use of methyl bromide as a soil disinfectant is likely to be restricted or even prohibited. Unless equally effective compounds should become available, the inoculum density of *R. solani* and *Sclerotinia spp.*, possibly also of *Pythium spp.*, will increase. It is questionable if the single soil treatment with dicarboximides will under these circumstances be sufficient. Increased doses, as well as more frequent applications will increase the risk of exceeding the residu tolerance. The use of nutrient film as a growing medium for lettuce, by which typical soil-borne pathogens are practically eliminated, does not fit into the Dutch cropping plan (tomato-lettuce).

Secondly the use of plastic screens to limit the energy costs will encourage pathogens growth. Because of this screens the relative humidity is higher and the light intensity is less, which results in an unbalanced plant growth and conditions that are favourable for the increase of *B. cinerea*.

A positive development is the increasing interest in the more tolerant "upright" cultivars. However, the assortiment is yet too limited and the market value too low to enable commercial cropping on a large scale.

Finally, investigations on the integrated control of soil-borne pathogens are expected to continue and eventually may result in future perspectives for the cultivation of lettuce. At short notice, breeding of more "upright" cultivars together with the application of lower doses of fungicides and appropriate cultural measures are the best solution for the optimization of bottom rot control.

SUMMARY

The basal parts of maturing glasshouse lettuce can be attacked by several soil fungi, which cause bottom rot. Until recently quintozene was generally applied against this disease complex. The study of the causal fungi - especially *Rhizoctonia solani* - and their control was undertaken in view of the need for quintozene replacing fungicides.

A survey revealed that *Botrytis cinerea* was the most frequently observed pathogen, especially in winter crops. The incidence of *Sclerotinia minor, Sclerotinia sclerotiorum, Pythium spp.* and *Rhizoctonia solani* was much lower. These fungi were more prevalent in autumn and late spring crops than in winter crops. Many of the attacked heads were infected by more than one pathogen. On an average disease severity caused by *R. solani* was the highest and by *Pythium spp.* the lowest. Trials showed that on untreated areas the average loss of lettuce heads of export quality class caused by bottom rot was 17 per cent, whereas on plots treated with appropriate fungicides this loss was 5 per cent.

For the detection of *R. solani* in the soil a relatively simple and fast paper disk bait method was improved and standardized. In addition a time saving quantitative technique was developed by plating out sieved soil clumps on a selective medium.

The paper disk method proved that the horizontal dispersion of *R. solani* propagules in the soil of lettuce glasshouses is not random, but clustered. This pattern could be characterized by the negative binomial distribution. Propagules were found throughout the whole ploughlayer but the inoculum density decreased remarkly as the depth of sampling increased.

The average inoculum density on 62 holdings was low: 0.6 propagule of *R. solani* per 100 g of soil. The individual glasshouse values were related to the period of sampling, soil disinfestation and the use of quintozene.

During winter crops the inoculum density remained low, in

spring time the values were higher, especially at the end of the cropping period. An attack of *R. solani* in most lettuce crops can be characterized as a "simple interest disease" sensu Vanderplank. This implies that the reduction of the initial inoculum in the soil is the most important factor in the control strategy.

On a log-log scale there appeared to exist a linear relationship between the initial inoculum density of *R. solani* determined with the paper disk method and the infections of lettuce heads. This indicates that this method of detection has a disease forecasting value. A tentative threshold value of initial inoculum density of 0.2-0.5 propagules per 100 g of soil was established, if the value is less than this no economic loss need be expected.

Most of the *R. solani* isolates from lettuce leaves that were examined showed that the minimum temperature required for infection was at least 9°C.

The incubation period at this temperature was from 11 - 15 days, whereas at 20°C less then 3 days. The linear extension of the lesions on infected leaves was at 20°C about 8 mm per 24 hour. These data confirm the observations that on lettuce, cropped during winter months with a low temperature regime, the attack is minimal, and that in late spring crops with periods of high temperature the attack can progress very quickly. Isolates were obtained with specific low temperature requirements from a glasshouse with a winter crop which had been severely attacked.

Rhizoctonia solani in lettuce appeared to be hygrophylic, but free water is not necessary for the initiating of the infection process.

All seventeen glasshouse crops tested, in a host range experiment, were attacked by *R. solani*.

In isolates obtained from diseased heads, three out of four known European anastomis groups of *R. solani* occurred. The other, anastomis group 3 ("potato group") was only found once among isolates from soil.

The dicarboximides, iprodione and vinclozolin, have proved

in field and in vitro experiments, to be good replacements for quintozene to control attacks of *B. cinerea*, *R. solani* and *Sclerotinia* spp. The infection by *Pythium* spp. appears to increase when quintozene and dicarboximides are used. Further research is necessary in order to establish whether the chemical control of *Pythium* spp. will be required economically.

Soil disinfestation with methyl bromide caused a large reduction of the inoculum density of *R. solani*.

Finally it has been discussed that the control of bottom rot should be optimized by integration of the culture of the more tolerant "upright" cultivars, the management of appropriate cultural measures and a reduced dose of fungicides.

SAMENVATTING

Aanslag of smet van de onderste bladeren van rijpende kassla kan veroorzaakt worden door verschillende bodemschimmels. Tot voor kort was quintozeen het belangrijkste fungicide voor de bestrijding van dit schimmelcomplex. De noodzaak tot het vervangen van quintozeen was de aanleiding tot het onderzoek van smet veroorzakende schimmels, in het bijzonder van *Rhizoctonia solani*.

Botrytis cinerea werd het meest geïsoleerd, vooral uit winterteelten. *Sclerotinia minor* en *S. sclerotiorum*, *Pythium* spp. en *R. solani* kwamen veel minder voor. Deze pathogenen werden meer in de herfst- en late voorjaarsteelten aangetroffen dan in winterteelten. De kroppen waren vaak door meer dan één pathogeen aangetast. Gemiddeld tastte *R. solani* de kroppen het zwaarst aan, *Pythium* spp. het minst.

Het verlies aan kroppen in de export-kwaliteitsklasse als gevolg van smet bedroeg op proefveldjes die niet met fungiciden waren behandeld gemiddeld 17 procent, op behandelde veldjes 5 procent.

Een betrekkelijk eenvoudige en snelle methode voor isolatie van *R. solani* uit grond (met papierschijfjes) werd verbeterd en gestandaardiseerd. Aansluitend werd een kwantitatieve bepalingsmethode ontwikkeld door gezeefde grond op een selectief medium uit te leggen.

Met de papierschijfjes-methode werd aangetoond dat propagula van *R. solani* in de bovenste grondlaag niet volgens het toeval zijn verdeeld, maar in "clusters". Dit patroon kan door de negatief binominale verdeling worden beschreven. Door de hele bouwvoor werden propagula gevonden, maar de inoculumdichtheid nam sterk af naarmate dieper bemonsterd werd.

De gemiddelde inoculum-dichtheid op 62 bedrijven was slechts 0,6 propagula per 100 g grond. De waarden op de afzonderlijke bedrijven varieerden met het bemonsteringstijdstip, de uitgevoerde grondontsmetting en de toepassing van quintozeen.

Tijdens de winterteelten bleef de inoculum-dichtheid laag, in het voorjaar werden hogere dichtheden gevonden, vooral tegen het einde van de teelt. In de meeste teelten kan de aantasting door *R. solani* als een "simple interest disease" sensu Vanderplank worden gekarakteriseerd. Dit houdt in dat verlaging van het aanvangsinoculum in de grond de belangrijkste factor voor de bestrijding is.

Het aantal infecties van slakroppen bleek lineair gecorreleerd met de inoculum-dichtheid van *R. solani* in het begin van de teelt, bepaald volgens de papierschijfjesmethode (beide parameters na log-transformatie). Deze detectiemethode heeft derhalve ziektevoorspellende waarde. Als voorlopige drempelwaarde werd 0,2-0,5 propagula per 100 g grond berekend; beneden deze waarde hoeft geen economisch verlies verwacht te worden.

Van de meeste *R. solani* isolaten, afkomstig van sla, was de minimum infectie-temperatuur boven 9°C. De incubatietijd bedroeg dan 11-15 dagen, bij 20°C minder dan 3 dagen. De lineaire uitbreiding van de lesies op geïnfecteerde bladeren was bij 20°C gemiddeld 8 mm per 24 uur. Deze gegevens bevestigen de waarneming dat de aantasting minimaal is in sla, geteeld in de wintermaanden bij een laag temperatuursregime, maar dat in perioden met hoge temperaturen de aantasting zeer snel kan verlopen.

Rhizoctonia solani van sla bleek weliswaar hygrofiel te zijn, maar vrij water is geen voorwaarde voor infectie.

In een besmettingsproef werden alle 17 onderzochte kasteeltplanten door *R. solani* aangetast.

Drie van de vier Europese anastomose-groepen werden in isolaten uit aangetaste kroppen aangetroffen. De andere, ("de aardappel-groep") werd slechts eenmaal uit de grond geïsoleerd.

In laboratorium- en veldproeven bleken de dicarboximiden iprodion en vinchlozolin het fungicide quintozeen voor de bestrijding van *B. cinerea*, *R. solani* en *Sclerotinia* spp. goed te kunnen vervangen. Bij toepassing van quintozeen en dicarboximiden bleek de infectie door *Pythium spp.* toe te nemen. Verder onderzoek zal moeten uitwijzen of chemische bestrijding van *Pythium*

spp. economisch noodzakelijk is.

De inoculum-dichtheid van *R. solani* werd aanmerkelijk verlaagd door grondontsmetting met methylbromide.

Tenslotte wordt geargumenteerd dat de bestrijding van smet geoptimaliseerd zou kunnen worden door integratie van de teelt van de minder gevoelige, rechtopstaande cultivars, adequate cultuurmaatregelen en de vermindering van de hoeveelheid fungicide.

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