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Hyperparasites of Rhizoctonia solani in Dutch potato fields

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Abstract

Gliocladium roseum was found to be the most common and probably the most effective mycoparasite in potato fields in the northern parts of the Netherlands. It is able to parasitize and kill living hyphae at temperatures of 12°C and higher. Sclerotia of *R. solani* are often infected and killed by this fungus under suitable conditions, i.e. at temperatures of 16°C and more. Killing of sclerotia by other antagonistic organisms was also observed. It is also shown by not parasitic fungi and is caused by toxins produced by the antagonist.

The development of the *G. roseum* population was studied during the growth of a potato crop in two soils. In both soils its initial level was very low. In both a slightly acid sandy soil and a neutral sandy loam, suppression of *R. solani* can occur; *G. roseum* accumulated in the former mainly under continuous potato crops, *Colletotrichum coccodes* was the main antagonist in the latter.

Additional keywords: Antagonism, induced antagonism, sclerotia, suppressive soil, biological control, hyphal interaction, toxin production, *Gliocladium roseum*, *G. virens*, *G. nigrovirens*, *Volutella ciliata*, *Trichoderma* spp., *Penicillium* spp., *Nectria pityrodes*, *Pythium oligandrum*, *Colletotrichum coccodes*, *Hormiactis fimicola*, *Trichocladium asperum*, *Arthrobotrys oligospora*, *Acremonium verruculosum*, *Streptomyces* spp., *Verticillium* spec.

Introduction

Rhizoctonia solani Kühn occurs in Dutch potato fields, especially in light and sandy soils, and may cause severe yield reductions. Studies are going on at our institute, to assess and explain the phenomenon of suppressiveness of certain soils to this pathogen.

It is known that *R. solani* hyphae are parasitized by necrotrophic mycoparasites such as various *Trichoderma* species (Weindling, 1932; Boosalis, 1956; Hussain and McKeen, 1962; Ferrera-Cerrato, 1976), *Penicillium vermiculatum* (now bearing the name *Talaromyces flavus)* (Boosalis, 1956; Hussain and McKeen, 1962), *Gliocladium roseum* (Pugh and Van Emden, 1969), *Pythium oligandrum* (Drechsler, 1946; Deacon, 1976; Veselý, 1978a), *Pythium acanthicum* (Hoch and Fuller, 1977), *Papulospora stoveri* (Warren, 1948) and some unidentified fungi (De la Cruz and Hubbel, 1975). Naiki and Ui (1972) described a number of micro-organisms associated with sclerotia of *R. solani*, among which well-known antagonists such as *Trichoderma* and *Penicillium* species.

As it will be shown again here, one of the most important antagonists is *G. roseum*,

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about which Pugh and Dickinson (1965) presented an important review, which contains much interesting information. This species, however, is not a specific parasite of *R. solani*. Barnett and Lilly (1962) and Barnett and Binder (1973) mentioned many host fungi of *G. roseum* while, on the other hand *R. solani* was mentioned as a parasite of some other fungi.

From the work of Brian et al. (1951) it is known that *G. roseum* produces four substances with antibiotic properties, two of which, rubrogliocladin and aurantiogliocladin, are important (Bilai, 1963).

During this study mycoparasites were isolated from *Rhizoctonia* hyphae and sclerotia. Their possible significance for the reduction of inoculum density of *R. solani* is described.

Material and methods

The presence of mycoparasites of *R. solani* in a soil was tested by spreading 200 mg fresh soil in the centre of an agar plate overgrown with mycelium of a strain of *R. solani* pathogenic towards potato (plants) that had been isolated from the same soil. Barnett and Binder (1973) reported that the parasite and its host may vary considerably in their respective parasitic ability or sensitivity towards parasitism. Therefore the presence of mycoparasites in a soil was determined with a host from that soil.

The presence of mycoparasites on washed pieces (5 mm) of stems and stolons was tested in a similar way. The soil and the plant pieces were added to the plates one week after they had been inoculated with *R. solani*.

The presence of parasites on sclerotia of *R. solani* taken from potato tubers from the field was investigated by incubation of the sclerotia on a layer of moist pure sand or moist perlite in petri dishes (15 cm diam.). Perlite was used by preference as it has a better water holding capacity than sand and forms a firm layer when water-soaked. In the first trials, however, the sclerotia were placed on a nylon cloth overlying moist soil. Soil is unsuitable for this purpose since it often harbours organisms, e.g. mites, that seriously disturb the results by feeding on hyphae and conidia of *Gliocladium*.

The incubation temperature was generally 22 °C. Temperatures of 4, 10, 16 and 22 °C were chosen to study the influence of temperature on the development of other micro-organisms on sclerotia, while their growth on hyphae was studied at 12 and 22°C.

To stimulate the growth of mycoparasites of *R. solani* in soil, pieces of living mycelium were added. *R. solani* mycelium was grown in submerged culture and washed free of nutrient liquid by slightly squashing the balls and washing them in tap water. They were then divided into pieces and mixed with the soil.

The presence of *R. solani* on stolon pieces without visible infection was investigated by placing ten pieces in a circle (about 1 cm from the margin) in a petri dish with a rather poor soil extract containing agar. Outgrowth of *R. solani* from the stolon pieces was visible after one day and marked.

For the detection of antagonistic micro-organisms on stolon pieces, the same plates were used. Three days after placing the stolon pieces, an inoculum disk (4 mm diam.) of agar with *R. solani* was placed in the centre of each plate. The outgrowth of an antibiotic organism was marked.

R. solani was grown on a malt-biotone medium (malt extract 15 g, special peptone or microbiotone 2.5 g and 1 l water for submerged cultures; 12 g agar was added to get a

solid medium). The soil extract agar was described by Jager and Bruins (1975). Oxoid preparations were used.

Results

Mycoparasites recovered from soil spread over R. solani mycelium

From soil spread on mycelium of *R. solani*, the most common organism that appeared after one or two weeks of incubation at 22°C was a fungal species with a white and abundantly sporulating mycelium. The fungus seemed to be a *Verticillium* species. In pure culture on malt agar, however, the verticillate conidiophores almost disappeared and most conidiophores were penicillate. The white colour, showing when grown on hyphae and sclerotia of *R. solani*, became pale pink after one or two weeks. This fungus was identified as *Gliocladium roseum* Bain.

The outgrowth of this and other mycoparasites from soil may, depending on the population density, be poor and only perceptible with the dissecting microscope or it may be more or less profuse and visible with the unaided eye. Since *G. roseum* did not appear on soil dilution plates, the estimation of the size of the population was based on the extent of the outgrowth.

G. roseum was the most common mycoparasite found in this way. Its hyphae coiled around hyphae of *R. solani* (Fig. 1), but only rarely penetrated the hyphae of its host (Fig. 2). It killed the hyphae of *R. solani* through hyphal coiling and clamping, as described by Barnett and Lilly (1962). Young hyaline hyphae of the host were killed faster than older ones with thicker walls encrusted with brown-black material.

The production of water-soluble substances inhibiting the growth of *R. solani* was shown by pairing the two fungi on a malt biotone agar plate. A temporary inhibition zone appeared. *R. solani* is inhibited and its growth stops, but *G. roseum* continues to grow after a short delay. It penetrates the mycelium of *R. solani*, parasitizing it and accumulating on the sclerotia, which may be killed.

G. roseum grows along infected hyphae through the soil and over the surface of potato tubers with germinated sclerotia. From observations of potato tubers we have the impression that infected hyphae of *R. solani* (largely) lost their ability to infect potato sprouts. The apical part of infected hyphae is cut off from its food base and presumably receives toxic substances instead of nutrients. Its growth stops and it dies.

Growth of the population of *G. roseum* in a soil was found to be stimulated by adding living mycelium of *R. solani* to the soil. Addition of dead mycelium did not have a stimulatory effect. In Table 1 the estimated quantity of *G. roseum* is shown for a number of soils together with the effect of adding living *R. solani* mycelium to these soils. This was positive in nearly all soils. In soil 5, however, no, or very little stimulation of *G. roseum* was observed.

The soil samples were taken in November and early December from fields on which potatoes had been grown as the preceding crop. On the field from which sample 5 was taken, however, no potatoes had been grown for three years.

The amount of outgrowth differed greatly. If the outgrowth was only visible with the dessecting microscope, it was assigned $\pm$; visibility with the unaided eye of a poor outgrowth on only one or two spots received $+$, a further outgrowth on more spots scored $++$, and an abundant outgrowth around the deposited amount of soil was given $+++$.

Fig. 1. *Gliocladium roseum* hyphae coiling around a hypha of *Rhizoctonia solani.*

Fig. 1. Hyfen van de mycoparasiet G. roseum slingeren zich rond eén hyfe van R. solani.

Fig. 2. A hypha of *Gliocladium roseum* inside a killed hypha of *Rhizoctonia solani.*

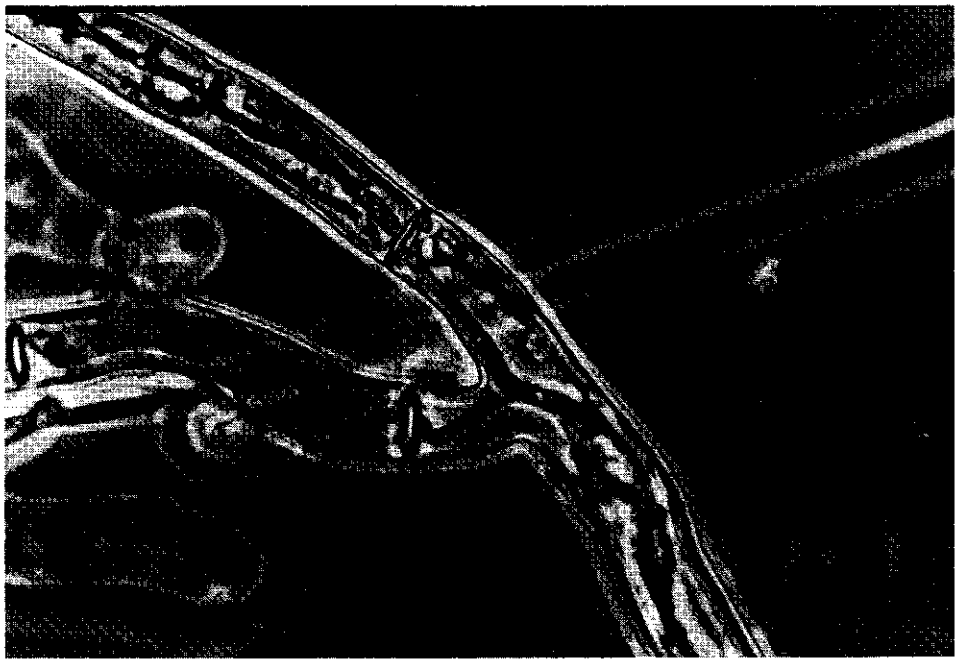

Fig. 2. Een hyfe van G. roseum in het inwendige van een gedode hyfe van R. solani.

Table 1. The occurence of *Gliocladium roseum* in soils of some Dutch potato fields, with and without addition of *Rhizoctonia solani*.

Soils	Percentage of plates assigned to the 4 classes of abundance (see text)								pH (KCl)
	no addition				living mycelium added				
	±	+	++	+++	±	+	++	+++	
Pleistocene soils:									
1. Sand, Borgercompagnie[1]	80	20						100	5.5
2. Sand, Haren	100				60	20	20		5.0
3. Sand, Rolde	10	50	40					100	5.0
Holocene coastal deposits:									
4. Sandy loam, Baflo	30	40	20	10	10		70	20	7.5
5. Sandy clay loam, Baflo	90	10			100				7.2
6. Sand, N.E. Polder	60	30	10					100	7.6
7. Sandy loam, N.E. Polder	90	10					20	80	7.5
8. Sandy clay loam, N.E. Polder	70	10	20		20	20		60	7.4
9. Sand, Wieringermeer		60	40		10	10	30	50	–
10. Sandy loam, Wieringermeer	80	20						100	–
11. Sandy loam, Wieringermeer	90	10						100	–

[1] Humus-rich, reclaimed from cut-away peat.

Tabel 1. De aanwezigheid van Gliocladium roseum in grond van enkele aardappelakkers in Nederland. Na toevoeging van levend R. solani mycelium aan grond treedt meestal een uitbreiding van de hyperparasiet G. roseum op.

G. roseum is present in soils of the northern part of the Netherlands and was also found in the young soil of the Flevopolder by Pugh and Van Emden (1969). It probably occurs in most arable soils of our country.

In the potato fields (Table 1) also small numbers of other mycoparasites of *R. solani* were found when soil or stolon pieces were spread on plates with mycelium of *R. solani*, viz. *Gliocladium virens*, *G. nigrovirens*, and rarely *Trichoderma koningii*, *T. hamatum* and *T. harzianum*.

Incidentally a *Chaetomium* species and the *Myrothecium* state of *Nectria pityrodes* was observed. The mycoparasite *Pythium oligandrum* was isolated from *Rhizoctonia* hyphae growing from pieces of rush stem (*Juncus effusus*) that were used as bait. *P. oligandrum* was the only mycoparasite which grew faster than *R. solani*. Data on mycoparasitism of *P. oligandrum* have been presented by Drechsler (1946), Deacon (1976) and Veselý (1978a, 1978b).

Among the hyperparasites, only one streptomycete was found on the hyphae of *R. solani*.

The effect of temperature on growth and sporulation of G. roseum on the hyphae of R. solani. Malt agar plates with *R. solani* mycelium were, one week after their inoculation, provided with 200 mg fresh soil and incubated at 22 and 12 °C, both temperatures commonly occuring in the field. For each soil and each temperature ten plates were used (soils 2 and 5 of Table 1). After an incubation period of three weeks at 22 °C and four weeks at 12 °C, the growth and sporulation at 12 °C were not less than those at 22 °C. Growth at 12 °C was slower than at 22 °C but the total amount of material obtained can be the same, if time is not a limiting factor. In soil 2 the outgrowth was abundant at both temperatures; in soil 5 only minimal. The rich outgrowth of *G. roseum* as a mycoparasite of *R. solani* at 12 °C suggests that good growth at 10 °C and even lower temperatures is also possible.

The epiphytic microflora of the sclerotia

The term epiphytic is used here instead of parasitic because only a part of the epiphytic flora has mycoparasitic properties. Others may show antibiotic acitivity, but do not parasitize the hyphae, or they are only saprophytes.

Sclerotia of *R. solani* on potato tubers are often infected with *G. roseum*. This can easily be demonstrated by keeping tubers with sclerotia in a moist atmosphere at room temperature for about two weeks. The black sclerotia turn white if they are infected with *G. roseum*.

The effect of temperature on the epiphytic microflora on sclerotia. Sclerotia were removed from potato tubers and incubated at different temperatures on moist perlite or on a nylon cloth overlying moist sand. Two hundred sclerotia were used per determination, 25 per petri dish. The sclerotia used were taken from tubers grown in the sandy soil of Borgercompagnie (soil 1 of Table 1).

At 4 °C, even after an incubation period of four months, no growth of *G. roseum* or other fungi was observed. Small, pinpoint, colonies of streptomycetes were found as white or grey tufts.

At 10°C, a slightly more abundant growth of similar streptomycetes took place and *G. roseum* did not appear even after four months. In a few cases *Trichoderma* spp. and also a *Verticillium* sp. (CBS 353.77 A-C and 360.77) (indistinguishable from *G. roseum* when growing on sclerotia), was present. The latter was not, or only slightly, parasitic and showed no antibiotic activity. It produced a characteristic bad smell when growing in pure culture on malt-biotone agar.

At 16°C G. roseum was the most common colonizer and destroyer of sclerotia in this soil. After five months of incubation at this temperature (the perlite was kept moist), 17% of the sclerotia still germinated, but the majority only produced one or two poorly growing hyphae. In the field such sclerotia are probably unimportant for the survival of *R. solani*.

At 22°C growth of *G. roseum* on sclerotia was even more abundant. Even large sclerotia were killed within 4–6 weeks. Eighty percent of the sclerotia from potato tubers sampled in 1976 were infected by *G. roseum*. Large sclerotia were more frequently infected than small ones. Killed sclerotia, however, are mostly soft and soon disintegrate. Consequently small dead sclerotia are removed with the soil and are not found on tubers, while the remaining part of large sclerotia, with viable cells, remains on the tuber.

Sclerotia on tubers in the field were found to be infected by *G. roseum* already in August. The proportion of sclerotia infected by antagonists seems to differ widely between various soils and even between different fields within the same soil type. This question will be studied further.

The composition of the epiphytic microflora. G. roseum was the main colonizer of sclerotia in soil 1 of Table 1. Sclerotia from other soils, as far as they were studied, often had a mixed population of epiphytes, of which *G. roseum* was still an important component.

In Table 2 antagonistic epiphytes of sclerotia from the slightly acid sand of Haren (soil 2 of Table 1) are listed. When more parasites were present on a sclerotium, they formed separate colonies and rarely penetrated each other. However, *Volutella ciliata* Alb. & Schw. Fr. was sometimes present in or near colonies of others. *V. ciliata* showed

Table 2. Composition of the epiphytic microflora of sclerotia from slightly acid sand (soil 2 of Table 1).

Antagonistic epiphytes	Sclerotia colonized (%)
Gliocladium roseum	22
Hormiactis fimicola	15
Gliocladium roseum, with *Hormiactis fimicola*, infrequently with *Volutella ciliata*	23
Volutella ciliata	6
Volutella ciliata, with *G. roseum* and *H. fimicola*	4
Streptomyces spp.	18

Tabel 2. Samenstelling van de microflora groeiend op sclerotiën van aardappelen uit een licht zure zandgrond.

Fig. 3. *Hormiactis fimicola* (left) paired with *Rhizoctonia solani* (right). An inhibition zone is still visible between both fungi after ten days of incubation. *H. fimicola* grows across, via 'bridge heads', after which it grows rapidly through the *R. solani* mycelium and accumulates on the sclerotia.

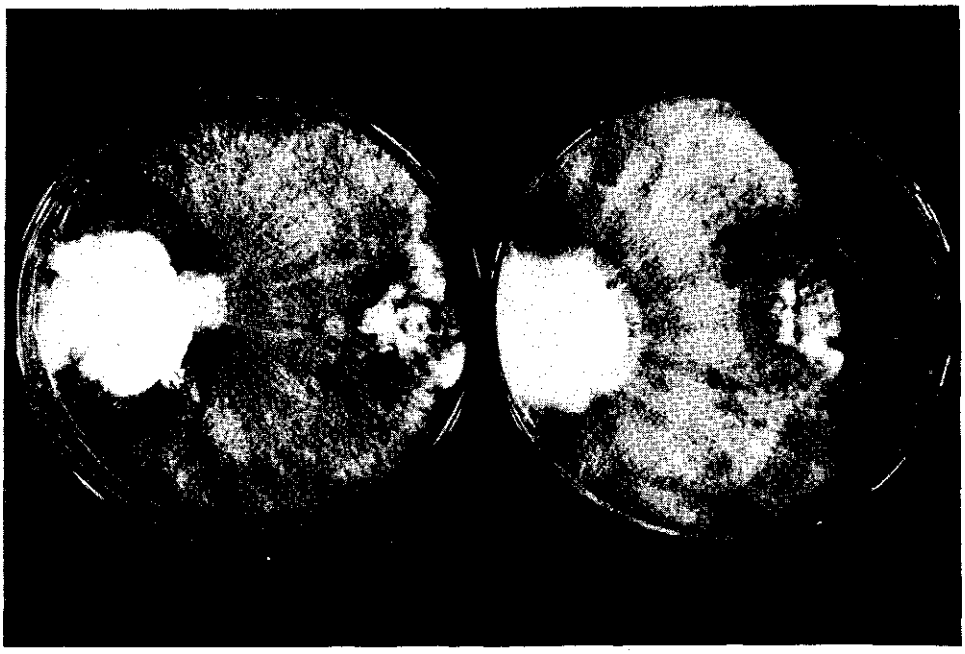

Fig. 3. Hormiactis fimicola tegenover R. solani op één plaat. Een remmingszone tussen beide schimmels is nog zichtbaar. Via enkele 'bruggehoofden' doorgroeit H. fumicola deze zone, groeit snel tussen de hyfen van R. solani door en concentreert zich op sclerotiën, die gedood kunnen worden. De groei van R. solani ligt volledig stil.

a weak antagonism, and is probably not very effective in soil. *Hormiactis fimicola* Sacc. & March. inhibited the growth of *R. solani* when placed with it on agar. After a few days of arrested growth, *H. fimicola* penetrated the inhibition zone, grew into the *R. solani* mycelium without attacking the hyphae and accumulated on the sclerotia (Fig. 3), killing them in about four weeks. Apparently *H. fimicola* can also kill sclerotia in the field, since some naturally infected sclerotia showed no germination, but produced an almost pure culture of *H. fimicola*.

Besides those listed in Table 2, the following species were isolated from sclerotia grown in other soils: *Gliocladium virens*, *G. nigrovirens*, *Trichoderma* spp. and the in vitro rather weakly antagonistic *Trichocladium asperum*, *Arthrobotrys oligospora*, *Nectria pityrodes* (*Myrothecium* state) and *Acremonium verruculosum*.

Bacteria were present mainly on sclerotia from soils with a pH of 7 or above; their antagonistic significance is not yet clear.

Colonization of sclerotia on tubers left in the field

Some observations were made on the fate of sclerotia on tubers left in the field after

harvest in the sandy soil of Borgercompagnie (soil 1 of Table 1). Ninety percent of the sclerotia collected from tubers lying on, or close to, the soil surface still germinated, at the end of October 1977; 42% proved to be infected by *G. roseum*, *G. nigrovirens* and by *Penicillium* and *Trichoderma* species.

The percentage of living sclerotia on (the remainder of) the tubers on the soil surface decreased during winter. In April very few sclerotia were collected, of which 38% were alive. From a few tubers buried deeply in the soil, however, all sclerotia germinated with many hyphae at that time.

The tubers at or near the soil surface were damaged by frost and decayed after some time and the viability of the sclerotia on these tubers declined quite rapidly. Tubers buried deeply in the soil were still in a perfect condition. These conditions were repeated experimentally, whereby tubers, naturally infected with sclerotia, were put in rather coarse nets of thin nylon, which were kept on the soil surface or buried in the soil at 15 and 30 cm depth, respectively. The experiment started at the end of November and had to be stopped in the first week of April. At that time the tubers had not decayed sufficiently. From the sclerotia of tubers at the soil surface, 38% were dead or germinated with a few poorly growing hyphae; from the tubers buried at 15 and 30 cm depth, 12% and 9% of the sclerotia were dead or in a very poor condition.

Coley-Smith and Cooke (1971) reported that the survival period of sclerotia of *R. solani* in soil varies from some months to many years. This presumably depends on soil type, soil depth and all factors affecting the composition and activity of the antagonistic microflora. The decay of the tuber seems to be a factor that strongly reduces the viability of sclerotia.

The development of the populations of G. roseum and R. solani during the growth of a potato crop, in a sandy soil and a sandy clay-loam

Observations were made on a slightly acid sandy soil and a neutral sandy clay-loam (soils 1 and 5 of Table 1). On the sandy soil, the observations were made on two plots of an experimental field. On one plot potatoes were grown for the fourth year in succession; on the other a three-year rotation (cereals, sugar beet and potatoes) was followed. The sandy clay-loam was managed in a four-year crop rotation (cereals, sugar beet, cereals and potatoes).

The seed potatoes used on the sandy soil were free from living sclerotia of *R. solani*. In the sandy clay-loam, two types of seed potatoes were planted: one from which all sclerotia were removed and the other on which five living sclerotia (about 5 mm diameter) were left on each tuber.

During the growing season the following parameters were determined a number of times:

1. The percentage of stolon pieces with a visible outgrowth of *R. solani* on soil extract agar.
2. The percentage of plates in which outgrowth of *G. roseum* was observed from rhizo- and stolonosphere soil and from soil free from roots and stolons placed on *Rhizoctonia* mycelium.
3. (as an addition to 2, and on the same plates) The abundance of the *G. roseum* outgrowth from the soil as a rough measure of its abundance in soil, especially for high population densities.

Table 3. The occurrence of *Rhizoctonia solani* and its antagonist on stolon pieces and in the soil during the growth of a potato crop on two different soils.

Soil	Date	Percentage stolon pieces with			Percentage plates with *G. roseum*[1] from	
		R. solani	*G. roseum*[1]	other antagonists	root-free soil	rhizosphere soil
Sandy clay loam (5)	23 May				70 ±	
	14 June		60 ± (75 ±)		45 ±	80 ± (30 ±)
	15 Aug.	5 (7)[2]	5 ± (8 ±)	65 (43)[2]	60 ±	30 ± (30 ±)
	5 Sept.	2 (0)	– –	73 (48)	10 ±	10 ± (0 ±)
	26 Sept.	12 (0)	75 ± (75 ±)	96 (75)	0	50 ± (40 ±)
Sandy soil (1)	23 May				75 ±	
Plot with potatoes	28 June	30	–	8	100 ±	100 ±
for four years in	15 Aug.	40	100 +	31	100 +	100 +
succession	12 Sept.	15	90 + +	45	100 + +	100 + +
	3 Oct.	8	–	45	10 +	100 + + +
Crop rotation plot, potatoes	22 Aug.	12	27 +	60	100 +	100 +
after sugar beet	3 Oct.	25	53 +	50	0	100 +

[1] For *G. roseum* the classes of abundance are given (average of the number of positive cases). See text.
[2] Figures in brackets refer to plants grown from seed potatoes without living sclerotia of *R. solani*.

Tabel 3. Het voorkomen van R. solani en zijn antagonisten op stolonstukjes en van G. roseum op stolonstukjes en in grond gedurende de groei van een aardappelgewas in een tweetal gronden.

4. The percentage of stolon pieces, from which *G. roseum* was obtained when placed on agar plates with *R. solani*, together with its abundance.
5. The percentage of stolon pieces with antagonistic fungi other than *G. roseum*, on the plates mentioned under 1.

A. The sandy soil. a) The plot with potatoes for the fourth year in succession: The stolons were infected with *R. solani* from the soil. At the end of June, 30% of the stolon pieces yielded colonies of *R. solani;* in mid-August this was 40%. This percentage decreased to about 15% in mid-September and to less than 10% in early October (Table 3). The percentage of stolon pieces with antagonistic fungi increased from less than 10% at the end of June to 44% in September and October.

G. roseum appeared on 75% of the plates with soil in May. The outgrowth could only be perceived with the dessecting microscope; 100% of the plates still showed a poor outgrowth at the end of June. In mid-August a visible outgrowth of *G. roseum* occurred on all plates. The abundance of *G. roseum* increased further in September in rhizo- and stolonosphere soil. In root- and stolon free soil the abundance decreased and the distribution in the soil became more irregular.

In mid-August 100% of the stolon pieces yielded *G. roseum*, the outgrowth being abundant. In September the percentage of stolon pieces with outgrowth was slightly lower. At the end of the vegetation period of the potatoes, few stolons were in a good condition and the percentage of stolon pieces with *G. roseum* could not be determined. As the rhizo- and stolonosphere soil proved to be very rich in *G. roseum*, it may be assumed that the stolons were also rich in *G. roseum* at that time.

b) The 1 : 3 crop rotation plot: The percentage of stolon pieces yielding *R. solani* was highest at the end of the growing season, and higher than in the plot with potatoes as a continuous crop. The abundance of *G. roseum* in rhizo-and stolonosphere soil and on stolon pieces was lower than in the plot with potatoes for the fourth year in succession.

In the four year potato plot, the decrease of *Rhizoctonia* on the stolons started when *G. roseum* became abundant on the stolons as well as in the rhizo- and stolonosphere soil. From mid-August on, this plot became *Rhizoctonia*-suppressive. This suppressiveness at the end of the growing season is also reflected in the higher percentage of clean and nearly clean potatoes at harvest when compared with the 1 : 3 rotation plot. Details of the distribution of sclerotia on tubers in both plots are given in Table 4.

Table 4. Contamination by sclerotia of *Rhizoctonia solani* of potato tubers harvested from two plots of the sandy soil of Table 3 (Percentage of the number of tubers).

Proportion of the tuber surface occupied by sclerotia (%)	Plot with 4 years potatoes	Plot with potato in 1 : 3 rotation
0 (clean)	21	14
0–0.5 (nearly clean)	52	44
0.5–1	23	32
1–5	4	11

Tabel 4. Bezetting van aardappelknollen, geoogst uit de zandgrond van Tabel 3, met sclerotiën van R. solani (in procenten van het aantal knollen).

B. The sandy clay loam. Stolons of plants grown from seed potatoes without sclerotia were free from *Rhizoctonia* after August. Stolons of plants grown from seed potatoes with sclerotia had a surprisingly low percentage of pieces from which *Rhizoctonia* grew (3–12% from mid-August onward). (At least 90% of the sclerotia on the seed potatoes were alive at planting time in mid-May).

The *Rhizoctonia*-suppressing effect of this soil was also clear from the amount of sclerotia on the tubers at harvest. In the harvest from clean seed potatoes 98% (w/w) was clean; on 2% of the tubers 0–0.5% of the surface was covered with one or two small and thin sclerotia. Of the harvest from the 100%-infected seed potatoes, 58% was clean, on 35% of the tubers 0–0.5% of the surface was covered with a few small and thin sclerotia and on 7% of the tubers 0.5–1% of the surface was covered with larger, but also thin, sclerotia. The sclerotia on the seed potatoes were rather thick and convex and did not resemble the thin and flat sclerotia on the harvested tubers.

The percentage of stolon pieces with antagonistic fungi (other than *G. roseum*) increased from mid-August to the end of September. The percentage was higher on stolons from seed potatoes with sclerotia and amounted to 95% at the end of the growing season. Of the antagonistic fungi, 90–95% belonged to *Colletotrichum coccodes* (Wallr.) Hughes, a species, which is known as a pathogen of potatoes (Butler and Jones, 1961). From clean seed potatoes the percentage of stolon pieces showing antagonistic fungi was approximately 20% lower. This may be due to the mechanical removal of the sclerotia of *Rhizoctonia*, whereby also sclerotia of *C. coccodes* were removed.

G. roseum was present in this soil throughout the growing season but only at a very low level.

C. coccodes was common on the stolons in both soils. Damage to the stolons caused by this pathogen was neither observed in the sandy soil, nor in the sandy loam soil.

Discussion

Significance of hyperparasites in the field

Hyperparasites of *R. solani* are present in most Dutch potato fields. The most common and probably the most effective of these, found in our study, is *G. roseum*. This fungus can attack and kill hyphae as well as sclerotia.

Jouan and Lemaire (1974) found the fungus in potato fields in France; they suggested that the presence of *G. roseum* was stimulated by potatoes. This may be an indirect effect, i.e. it may result from the presence of *R. solani* on the potatoes in the field.

In laboratory experiments, growth of *G. roseum* on hyphae of *R. solani* was considerable at 12 °C, but at 22 °C a more rapid growth was observed. The total amount of sporulating mycelium was simular at both temperatures after four weeks of incubation. Presumably growth on hyphae even occurs at temperatures below 10 °C. For growth on Dox's and potatodextrose agars, Isaac (1954) found a minimum temperature of 10 °C, a maximum of 40 °C and the optimum at 25 °C. For mycelial growth, Morquer et al. (1963) found a minimum temperature of 6–7 °, a maximum of 35 and an optimum of 24 °C.

For the development of conidia Morquer et al. (1963) found slightly different cardinal temperatures, viz., a minimum of 8 °C, an optimum at 24 °C and a maximum

of 29 °C. Thus, it may be concluded, that the development of *G. roseum* populations in Dutch potato fields will be better in warm (and usually dry) summers, like 1975, 1976 and 1977, than in cold (often wet) summers.

The minimum temperature for the attack of *G. roseum* on sclerotia is between 10 and 15 °C. The decay of sclerotia of *R. solani* in winter can therefore, probably not be ascribed to *G. roseum*. An increasing activity of *G. roseum* can be expected from May onwards, when the temperature generally exceeds 12 °C.

Isaac (1954) mentions an optimum pH-range for the growth of *G. roseum* of 6.4–8.6, with slightly less growth at pH 9.6 and 5.3, with a minimum pH of 2.0. Morquer et al. (1963) found mycelial development at a pH ranging from 3.2 to 10.5, with the optimum between pH 6.1 and 6.6. The optimum pH for conidiogenesis was found to be between 5 and 6.

In the acid sand (soil 1 of Table 1), *G. roseum* could indeed build up a large population in the plot where potatoes were grown for the fourth year in succession. In the plot, with a 3-year crop rotation, the population of *G. roseum* did not build up as much and consequently a suppression of *R. solani* did not occur.

In the sandy clay loam (soil 5 of Table 1), suppression of *R. solani* was not caused by *G. roseum*. The fungus was present, but at a low level. In this soil, *Colletotrichum coccodes* is one of the organisms suppressing *R. solani;* other antagonists included *Bacillus*, *Pseudomonas* and *Streptomyces* species present in the soil surrounding the stolons.

More field observations are necessary to ascertain to what extent these results, obtained in one year only, are representative for different soil types.

The destruction of sclerotia probably depends more on the production of toxic substances rather than on direct parasitic activity. *Hormiactis fimicola*, for instance, kills sclerotia, but does not parasitize the hyphae. It exerts antibiotic activity against *R. solani*, like *G. roseum* and many other antagonists associated with the sclerotia of *R. solani* (Naiki and Ui, 1972). Sclerotia of other fungi than *R. solani* can be destroyed by 'parasites' which are known to produce toxic substances (Karhuvaara, 1960; Makkonen and Pohjakallio, 1960; Rai and Saxena, 1975; Walker and Maude, 1975).

Many sclerotia from potatoes in the field proved to be infected by antagonists, which were shown to destroy them under laboratory conditions. Under favourable conditions, these also are likely to kill a large part of the sclerotia in the field. Even if, however, 95% of the sclerotia in an infested soil were heavily infected or killed, the remaining 5% in the soil would be sufficient to damage a following sensitive crop.

The antagonism against *R. solani* in the early stage of growth of a potato crop is mostly too weak to control the disease. The buildup of an antagonistic microflora takes much time and suppression of the pathogen does not occur before the second half, often only towards the end, of the growing season. The chance of suppression is better if the initial population of antagonists is sufficiently high and if growing conditions for these antagonists are favourable during the growth of the crop. Much work still needs to be done to obtain a better insight into the relation between *R. solani* and its antagonists in different soil types and under different weather conditions.

Possibilities of using mycoparasites in biological control

Aluko (1968) obtained good results using *G. virens* for biological disinfection of seed

potatoes. Unfortunately, this fungus disappeared rather soon after planting. In the soils used in our studies, *G. virens* was not very common. Since *G. roseum* is more common in our soils and presumably has better chances to survive, application of this fungus might be more promising. An effective control of *R. solani* can be expected if the population of *G. roseum* can start at a higher density early in the growing season under suitable conditions for growth and spreading over the surface of stolons, stems and young tubers.

Moody and Gindrat (1977) and Gindrat et al. (1977) reported a moderate control of *Phomopsis sclerotioides*, causing cucumber black rot by *G. roseum* inoculated in greenhouse soils.

For the appropriate use of *G. roseum* as a tool in biological control, more detailed knowledge is needed, especially on the ecological behaviour of the fungus with regard to the pathogen in particular soils and under different weather conditions and agricultural practices. *G. roseum* can, however, kill the eyes of seed potatoes and cause tuber rot when the conditions for germination and growth are very bad (too cold weather) and when seed potatoes with physiologically old and weak eyes are used (Langerfeld, 1977).

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Samenvatting

Hyperparasieten van Rhizoctonia solani in aardappelvelden in Nederland

In de meeste Nederlandse aardappelakkers komen schimmels voor die *Rhizoctonia solani* kunnen aantasten en doden. De meest algemene, en waarschijnlijk ook de meest belangrijke, die we tot nu toe vonden, is *Gliocladium roseum* (Tabel 1). Het is bekend, dat deze schimmel stoffen produceert die voor *R. solani* giftig zijn. Met behulp hiervan kan *G. roseum*, evenals andere antibiotisch actieve micro-organismen, ook de sclerotiën doden (Tabel 2). Voor doding door *G. roseum* is de temperatuur een factor van belang. Hyfen worden nog gedood bij een temperatuur van 12 °C, waarbij de sclerotiën niet meer aangetast kunnen worden. Gedurende het winterseizoen worden sclerotiën door deze schimmel naar alle waarschijnlijkheid niet gedood.

De ontwikkeling van de populatie van *G. roseum* en andere antagonisten van *R. solani* werd gevolgd in aardappelvelden op een licht zure zandgrond en op een neutrale zware zavel. Op de zandgrond werden twee proefplekken bemonsterd: één waarop voor het vierde achtereenvolgende jaar aardappelen werden geteeld en één met een vruchtwisselingsschema van graan, bieten en aardappelen.

In de zandgrond nam in het groeiseizoen de populatie van *G. roseum* toe. Op de proefplek waar voor het vierde jaar achtereen aardappelen stonden werd *R. solani* vanaf half augustus onderdrukt, evenwel niet volledig. Ook in het vruchtwisselingsstuk breidde *G. roseum* zich flink uit, doch een onderdrukking van *R. solani* werd niet bereikt.

In de zware zavel nam de populatie van *G. roseum* niet toe. Hier werd *R. solani* – uit

besmet pootgoed – onderdrukt door *Colletotrichum coccodes* (zelf een pathogeen van stolonen) en antagonistische bacteriën. De resultaten zijn vermeld in Tabel 3.

De besmetting van de geoogste knollen met sclerotiën, zoals die voorkwam op de zandgrond, is in Tabel 4 vermeld. Op de zavel leverde schoon pootgoed een bijna schone oogst (2% van de knollen was zeer licht bezet met sclerotiën). Besmet pootgoed leverde een oogst met 58% schone knollen, 35% met een zeer lichte en 7% met een iets zwaardere sclerotiënbezetting. Hoewel uit 100% besmet pootgoed een veel schonere oogst werd verkregen, was eerder toch een beschadiging van het gewas opgetreden. Pas tegen het eind van het groeiseizoen werd *R. solani* flink onderdrukt.

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