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Suppression of Rhizoctonia solani in potato fields. 1. Occurrence

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Abstract

A search was made for *Rhizoctonia solani*-suppressive soils by establishing many small experimental plots, half of which were planted with *Rhizoctonia*-infected seed potatoes and the other half with disinfected seed stock. The sclerotium index of the harvested tubers was compared with that of the seed potatoes. In suppressive soils, the sclerotium index of the harvest is much lower than that of the seed potatoes.

None of the plots on holocene marine soils (loamy sand, sandy loam, clay loam and clay) proved to be suppressive in 1978 and 1979. Only on pleistocene, slightly acid sandy soil suppressiveness was observed. In 1978, four out of twelve plots showed suppressiveness when the plots were planted with seed potatoes produced on a sandy soil. In 1979, only two out of thirty-one plots were slightly suppressive when planted with seed potatoes produced on a young clay loam from a new polder.

A higher percentage of sclerotia on tubers from sandy soils proved to be infected with antagonistic fungi (73%) than of those on tubers from marine clay or loam soils (25%). Factors that influence suppressiveness are suggested.

Additional keywords: antagonists, sclerotia, holocene and pleistocene soils, saprophytic *R. solani* strains, *Gliocladium roseum, Hormiactis fimicola, Volutella ciliata, Streptomyces* sp., *Verticillium* sp., *Gliocladium* sp., *Penicillium* sp., *Trichoderma* sp.

Introduction

In the literature reference is made to soils in which certain soil-borne pathogens are suppressed. Menzies (1959) reported on a transferable biological soil factor suppressing potato scab. Gerlagh (1968) concluded that the decline of *Gaeumannomyces graminis* in soils of new polders is due to a biological factor. Shipton et al. (1973) confirmed this and also found a transferable biological factor that suppressed take-all in Eastern Washington. Alabouvette et al. (1979) found that *Fusarium oxysporum* is suppressed by a biological factor, presumably saprophytic strains of the same fungus and strains of *F. solani*. The existence of a potato field in the Dutch province of Groningen in which *Rhizoctonia solani* was suppressed was reported by Van Emden (1967) and later confirmed by Jager et al. (1979).

The finding of a *Rhizoctonia*- suppressive field prompted us to search for other fields having this property, and for the factors that may be responsible for it. Detailed results of this study are given in a report by Jager and Velvis (1980); the main points are presented here.

Materials and methods

For the experiments, 62 small plots were selected in 1978 and 63 in 1979, scattered over the northern part of the Netherlands. Half of each of the plots was planted with disinfected seed potatoes, the other half with seed potatoes infected with sclerotia of *Rhizoctonia solani*. All seed potatoes had been sprouted and exposed to light in a greenhouse prior to planting. Four times during the growing season, five plants in the outer rows were lifted to examine the damage done by *R. solani* to stems (first and second sampling) and to stolons (second, third and fourth sampling). The middle rows were used to harvest the tubers at the end of the growing season, three weeks after chemical desiccation of the aerial plant parts. Within this period of three weeks maximum development of sclerotia on the tubers may be expected (Mulder et al., 1979). The sclerotium index of the tubers was determined and compared with that of the seed tubers.

Sclerotium index. The tubers were classified according to the rate of infection by *R. solani*. The tubers of each class were weighed (kg) and the weights obtained were used for the calculation of the sclerotium index (s.i.) according to the following formula:

$$\text{s.i} = \frac{\text{hy}\times 1 + \text{l}\times 3.5 + \text{m}\times 5 + \text{h}\times 6}{\text{c} + \text{hy} + \text{l} + \text{m} + \text{h}}$$

in which c, hy, l, m and h are the weights (kg) of the tubers of the classes: clean, only hyphae (mainly near the buds), lightly, moderately and heavily set with sclerotia, respectively. In this formula the multiplication factors for each class were chosen arbitrarily. For the class 'clean' the multiplication factor 0 was chosen.

Soils. The plots were laid out on holocene, marine clay and loam soils and on pleistocene sandy soils. The pH-KC1 of the pleistocene soils ranged from 4.0 to 6.4, and their organic matter content, determined by loss on ignition, ranged from 3.3 to 9.9%. Their content of mineral particles $< 16\ \mu$m averaged 4.5%, with a maximum of 9.9%. A few peaty soils were included. They contained up to 36% organic matter. Two holocene soils had a pH-KC1 below 6, eleven had a value between 6 and 7 and the others had values above 7. Their organic matter content ranged from 2.1 to 6.6%, their content of mineral particles $< 16\ \mu$m ranged from 9 to 71%.

Potato varieties. In 1978 Bintje seed potatoes, obtained from a holocene sandy loam in Groningen, were used on holocene soils, and Ehud seed, produced on a pleistocene sand, was used on pleistocene soils. The sclerotium index of Bintje seed was 5.86, of Ehud seed 4.22.

In 1979 Bintje seed was used on all plots. It was obtained from the new Flevopolder. Its sclerotium index was 3.90.

Infection of sclerotia. Sclerotia were detached from harvested tubers and placed on wetted perlite in a large petri dish (25 in each dish). One hundred sclerotia were taken from each plot. Upon incubation at 20 °C, the sclerotia germinated usually within

48 hours and fungi and streptomycetes appeared within one to three weeks. The percentage of sclerotia infected was determined.

Results and discussion

The use of disinfected and *Rhizoctonia*-infected seed potatoes is a good way to discover conducive or suppressive soils. In a conducive soil the harvest from disinfected seed and that from infected seed will both carry many sclerotia, whereas in a suppressive soil the harvest from disinfected seed will be clean or nearly so, and the harvest from infected seed will be much cleaner than the seed used. The difference between the sclerotium indices of the seed tubers and that of the harvested tubers must be large if a soil is to be considered suppressive. The classification of tubers with sclerotia is visual and somewhat subjective as the classes are not sharply defined.

Fig. 1. Index for the amount of sclerotia on tubers from plants grown from disinfected seed potatoes (−R) plotted against the sclerotium index of tubers grown from infected seed (+R) potatoes in 1978. The numbers in the figures refer to the numbers of the plots.

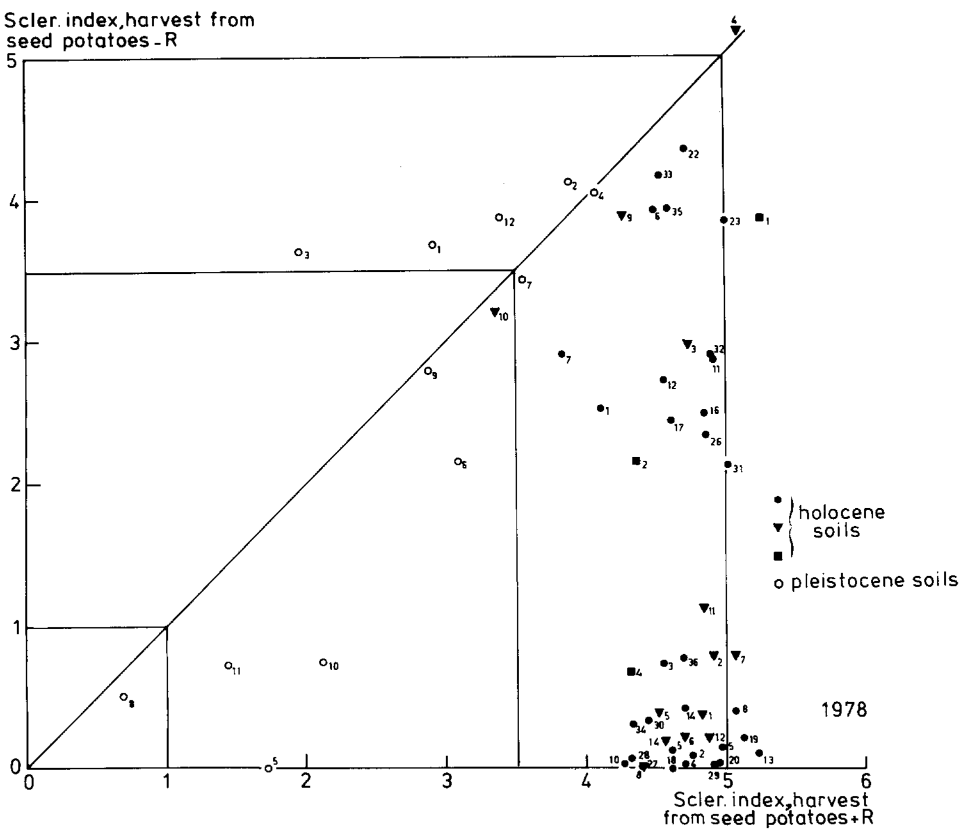

Fig. 1. De index voor de sclerotiënbezetting van geoogste knollen, verkregen uit ontsmet pootgoed (−R), uitgezet tegen die van besmet pootgoed (+R); 1978. De getallen bij de tekens in de figuur zijn de nummers van de proefplekken.

Moreover, it is impossible to distinguish between living and dead sclerotia in this way. Consequently the sclerotium index of seed potatoes may be overestimated. We observed that up to 40% of the sclerotia on seed tubers could be dead (Jager and Velvis, 1983).

In 1978, none of the 50 plots on holocene soils was suppressive to *Rhizoctonia* (Fig. 1). The harvest was far from clean and moderately set with sclerotia. Four plots out of twelve on sandy pleistocene soils showed suppresion. One of them (8) was strongly suppressive. This was unexpected, as sandy soils are usually infected with *R. solani*. It must be mentioned, however, that the sclerotia on Bintje seed potatoes were, on average, more numerous than on Ehud seed potatoes. On four plots on sand (indicated above the diagonal in Fig. 1) the harvested tubers of plants grown from disinfected seed had more sclerotia than those grown from infected seed.

In 1979, the Bintje seed potatoes were more uniformly and less severely infected

Fig. 2. Index for the amount of sclerotia on tubers from plants grown from disinfected seed potatoes (– R) plotted against the sclerotium index of tubers grown from infected seed potatoes (+ R) in 1979.

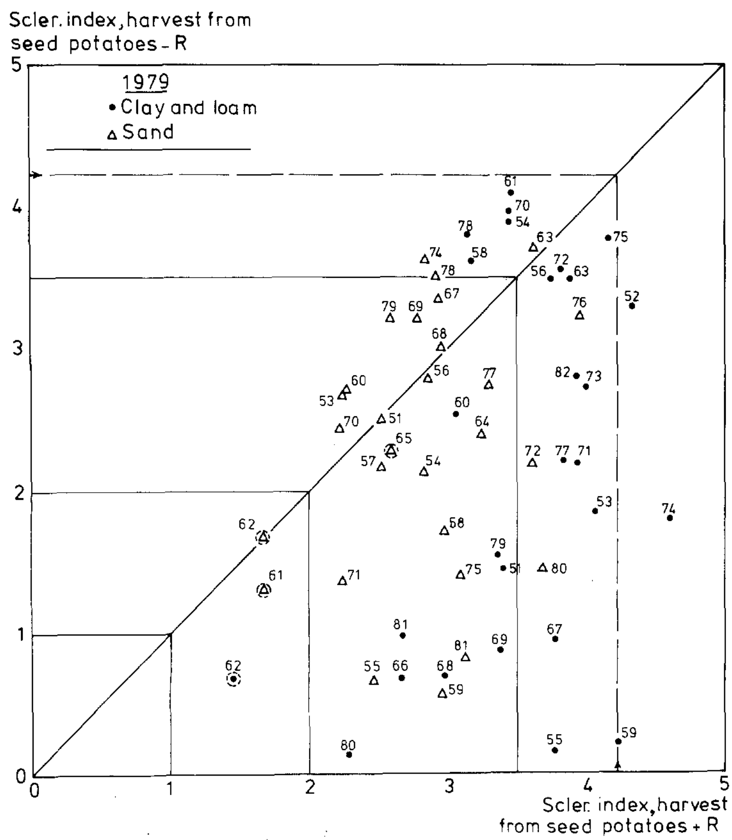

Fig. 2. De index voor de sclerotiënbezetting van de geoogste knollen uit ontsmet pootgoed (– R) uitgezet tegen die uit besmet pootgoed (+ R) in 1979.

than in 1978. None of the 63 plots showed an impressive ability to suppress *R. solani.* Only in a few plots a weak suppression was observed (Fig. 2).

On 25% of all plots, mainly on pleistocene sands, most sclerotia were formed on tubers of plants grown from disinfected seed potatoes. Plants from infected tubers were much more severely damaged by *R. solani,* but had less sclerotia (Jager and Velvis, 1980). In the former saprophytic strains of *R. solani* from the soils were involved. They contributed to the formation of sclerotia already early in the season but did not (or hardly) damage stems or stolons; on the contrary, we think they provided some protection from infection by pathogenic strains as they occupied space on stems and stolons, young tubers and roots, and behaved as competitive antagonists to pathogenic strains.

The position of the plots in Figures 1 and 2 is rather different. In Fig. 2 no strongly or moderately suppressive plots are shown (soils of plots 61 and 62 are exceptions). Another type of plots shown in Fig. 1 is not or hardly represented in Fig. 2, viz. plots where disinfected seed potatoes produced a clean or nearly clean harvest, but where infected seed potatoes produced a harvest with many sclerotia. In these soils the population density of *R. solani* was low, presumably due to a weak suppression, but the soils had no defence against a strong infection. These soils are present in the plots 55, 59, 69, 67, 59 and 81 (Fig. 2). The soils are conducive, but have a (very) low population density of *R. solani* and a high sensitivity to infection with *R. solani.* To distinguish these soils from conducive soils with a high population density of *R. solani* we define then as *Rhizoctonia* sensitive soils. In Figures 1 and 2 the suppressive soils are indicated near the origin. In strongly suppressive soils the sclerotium indices of the harvest of disinfected and infected seed potatoes are lower than 1; in moderately suppressive soils between 1 and 2 and in weakly suppressive soil 2 to 2.5. Conducive soils are those which always produce a more or less 'dirty' harvest.

From each plot one hundred sclerotia were taken from the tubers and incubated on moist perlite in large petri dishes. Most sclerotia (95-100%) were alive and germinated with many hyphae. A variable portion was infected with antagonistic fungi and streptomycetes. In 1979, on average, 75% of the sclerotia from the harvested 'sand potatoes' were found to be infected, mainly by fungi (73%) and by streptomycetes (17%). The most important fungus was a *Verticillium* species belonging to the *Verticillium – Gliocladium* complex; *Gliocladium roseum, Hormiactis fimicola* and *Volutella ciliata* were rather common. A smaller percentage of sclerotia from 'clay potatoes' was infected, viz., 64% and mainly by streptomycetes (50%). Fungi were present on 25% of the sclerotia (Table 1). More than one species of antagonists could often be observed on one sclerotium. The fungi mentioned were already known as antagonists of *R. solani* (Jager et al., 1979).

The streptomycetes formed small and very slowly growing colonies, which covered – in contrast to most common fungi – only a small part of a sclerotium. Sometimes they were present on dead hyphae only. Presumably they are not always important as antagonists. Most important are the fungi, especially those of the *Verticillium-Gliocladium* complex.

It seems contradictory that Fig. 2 did not show any plot with a considerable suppression, while sometimes high percentages of sclerotia were infected with antagonistic fungi. This apparent contradiction is caused by the very low population density of antagonistic fungi in spring and its slow development during summer

Table 1. Percentage of sclerotia on harvested tubers from different experimental plots, overgrown with different microorganisms (mainly antagonists) in 1979. D = pleistocene; G = holocene).

Plot	Sclerotia infected with indicated microorganisms[1]						
	Glio/Vert	Glio	Vol	Hor	Others	Strept	Total
D 51	60	1	0	8	5	1	68
52	62	2	1	14	13	8	80
53	58	0	1	23	0	5	82
54	79	3	0	12	2	4	89
55	79	1	2	2	1	3	80
56	66	1	5	16	9	10	87
57	56	0	3	4	2	32	80
58	78	1	0	4	38	41	86
D 70	44	3	0	11	13	2	70
71	65	0	0	0	5	3	69
72	63	1	2	4	1	25	82
74	84	0	4	0	6	62	99
75	33	0	0	0	21	31	62
D 80	39	4	0	2	5	46	84
81	64	4	1	1	5	7	77
G 54	84	0	1	0	2	29	97
G 51	54	0	0	0	1	4	60
52	20	3	1	0	4	5	31
53	29	0	0	0	7	13	44
55	11	0	0	0	4	33	44
56	27	4	0	5	5	72	87
58	12	1	0	0	14	14	40
59	9	2	0	0	0	7	18
G 77	29	0	4	1	2	92	97
78	11	0	1	0	1	86	90
79	96	0	0	1	3	90	99
G 80	1	0	0	0	0	92	91
81	47	1	0	0	0	76	94
82	86	3	4	0	3	88	99

[1] Glio/Vert: *Gliocladium roseum* and/or *Verticillium* species, which cannot easily be distinguished when growing on sclerotia (probably the majority was *Verticillium*). Glio: clearly recognizable *Gliocladium roseum*, Vol: *Volutella ciliata*, Hor: *Hormiactis fimicola*, 'Others': fungi not belonging to the species mentioned, among others *Penicillium-*, *Trichoderma-*, *Gliocladium-*, and unidentified species, Strept: *Streptomycetes.*

Tabel 1. Het percentage sclerotiën van geoogste knollen afkomstig van de verschillende proefplekken, dat begroeid is met andere micro-organismen (vnl. antagonisten) in 1979. D = pleistocene en G = holocene gronden.

(Jager et al., 1979). The consequence is that the population has reached its maximum antagonistic activity when it is too late to suppress *R. solani* and to prevent damage, but not too late for mass infection of sclerotia. This state of high activity is not achieved in all soils. Furthermore it is probably easier to infect a metabolically inactive sclerotium than active and antibiotics-producing hyphae.

The sandy soil of plot 8, for instance, showed a clear suppression when planted with seed potatoes produced on sand (Fig. 1). These seed potatoes and their sclerotia were probably infected with antagonistic fungi (Table 1). Antagonists from both sources, soil and seed potato, contributed to an initial higher population density and

Fig. 3. Tubers with sclerotia of *Rhizoctonia solani* from the harvest of plot 75 on sand (Δ 75) after one week's storage under humid conditions at 20 °C. A large part of the sclerotia is overgrown with an antagonistic fungus, forming white spores. The antagonist can kill sclerotia and hyphae.

Fig. 3. Met sclerotiën van Rhizoctonia solani bezette knollen uit de oogst van plek 75 op zandgrond (Δ 75) nadat ze ongeveer een week onder vochtige condities bij 20 °C waren bewaard. Een groot deel van de sclerotiën is begroeid met een antagonistische schimmel die witte sporen vormt. Deze antagonist kan sclerotiën en hyfen (schimmeldraden) doden.

a faster development of antagonism which led to suppression. The same soil (plot 75, Fig. 2) did not show any suppression in 1979 when it was planted with seed potatoes from a young marine clay loam from Flevoland. These seed potatoes were probably not or only slightly infected with antagonistic fungi. The sclerotia on tubers from the harvest of this plot were strongly infected with antagonistic fungi showing an abundant white sporulation (Fig. 3). The sclerotium serves as a source of food and is killed by an abundantly sporulating antagonist. Tubers as shown in Fig. 3 would be good seed material to promote antagonism in a "suitable" soil, leading to suppression of *R. solani*.

We tentatively conclude that the suppression of *R. solani* in a potato field is conditioned by a combination of two factors:

1. The abundance of antagonists in the soil. These antagonists must be able to thrive and multiply on the surface of the subterranean parts of the potato plant, and
2. the 'load' of antagonists on the skin and in and on sclerotia of the seed potatoes fitting in with the microflora of the soil. Antagonists of both sources contribute to the suppression.

Apparently this combination of factors seldom occurs in the north of the Netherlands. The hypothesis is further tested in additional experiments.

Samenvatting

Onderdrukking van Rhizoctonia solani in aardappelpercelen. I. Vóórkomen

Met behulp van proefplekken, waarop zowel met *Rhizoctonia* besmet als schoon pootgoed is gezet, is geprobeerd *Rhizoctonia*- onderdrukkende gronden te vinden.

In 1978 bleek op geen van de 50 proefplekken op klei- en zavelgrond in Groningen, Friesland of de N.O.P., bepoot met Bintje pootgoed uit Noord-Groningen, het optreden van de ziekte onderdrukt te worden. Van de 12 proefplekken op het zand, bepoot met Ehud pootgoed van het zand, bleek één sterk, twee matig en één licht onderdrukkend te werken. In 1979 werden 63 plekken op klei-, zavel- en zandgrond bepoot met Bintje pootgoed uit Flevoland. Slechts op twee plekken op het zand en één op de klei werd een lichte onderdrukking geconstateerd. De plek naast die welke in 1978 met zandpoters een sterke onderdrukking vertoonde, bleek geen onderdrukking te vertonen wanneer het veld was bepoot met kleipoters. Wel bleken de sclerotiën sterk geïnfecteerd te zijn met een antagonistische schimmel.

In 1979 bleek 73% van de sclerotiën gevormd op aardappelen in zandgrond met antagonistische schimmels geïnfecteerd te zijn en slechts 25% van de sclerotiën op aardappelen in klei- en zavelgronden.

Geconcludeerd werd, dat de onderdrukking van *Rhizoctonia* in aardappelakkers veroorzaakt kan worden door de combinatie van twee factoren, nl.:

1. de (overvloedige) aanwezigheid van antagonisten in de grond. Deze moeten behalve in de grond ook groeien op het oppervlak van de ondergrondse delen van de aardappelplant, en
2. de antagonisten aanwezig op het oppervlak van de pootknol en op en in de sclerotiën. Verondersteld wordt, dat deze moeten 'passen' in de microflora van de grond. Samen met antagonisten uit de grond kunnen ze bijdragen aan de onderdrukking van *R. solani*.

Kennelijk is deze combinatie van factoren in onze proeven zelden bereikt. Verder onderzoek is gericht op het toetsen van deze veronderstelling.

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