Neth. J. Pl. Path. 89 (1983) 223-228

Accumulation of hyperparasites of Rhizoctonia solani by addition of live mycelium of R. solani to soil

P.H.J.F. VAN DEN BOOGERT and G. JAGER

Institute for Soil Fertility, P.O. Box 30003, 9750 RA Haren (Gr.), the Netherlands

Accepted 30 Juny 1983

Abstract

Addition of live mycelium of *Rhizoctonia solani* to three slightly acid sands collected in winter from a field after a previous potato crop resulted in accumulation of hyperparasites and less infestation of sprouts of infected seed potatoes.

A similar effect was observed in alkaline marine sandy loam soils, but only after a second addition of live mycelium.

The predominant hyperparasite in these experiments was *Verticillium biguttatum.*

Additional keywords: Induced antagonism, *Verticillium biguttatum, Gliocladium roseum.*

Introduction

Jager et al. (1979) observed that the addition of live mycelium of *Rhizoctonia solani* to soil markedly stimulated growth of hyperparasitic fungi. *Gliocladium roseum* Bain. and *Verticillium biguttatum* W. Gams (Gams and Van Zaayen, 1982), predominated in activated soil. Because of their morphological as well as ecological similarity it was not clear so far which of the hyperparasites was the most effective one.

The present study was undertaken to determine whether a stimulated antagonism could protect potato sprouts against *R. solani* in different soils, and to determine the proportions of each hyperparasite in the activated soils.

Materials and methods

In pot experiments three pleistocene sands and three holocene marine sandy loam soils were used. The samples were taken from fields in January 1981 after a previous potato crop. Some properties of the soils are given in Table 1. The soils were passed through a 3-mm sieve and stored in plastic bags at 1 °C until use. Samples (6.0 kg) were either left untreated or mixed thoroughly with live mycelium of *R. solani.* For this purpose mycelium of a pathogenic isolate of *R. solani* was grown in malt peptone liquid culture (Jager et al., 1979). After washing in tap water the mycelial balls were fragmented in a Waring blender (five bursts, each for 10 s) and added to the soil as a thick slurry of coarsely fragmented hyphae (20 g fresh weight per kg of soil). After a 4 week incubation periode 1.5 kg portions of the soils were transferred to 1-l glass beakers. Pregerminated seed potatoes (cv Astarte) were disinfected with formaldehyde and half of

Table 1. some properties of the soils used.

Origin and type of soil	pH (in KCL)	Particles < 16 μm (%)	Organic matter (%)
Pleistocene sands:			
1 Haren	4.3	6	3.5
2 Zeijerveld	4.4	8	7.2
3 Borgercompagnie	4.6	4	7,9
Holocene marine sandy loam soils:			
4 Baflo	7.5	35	1.9
5 Sexbierum	7.4	24	3.2
6 Kloosterburen	7.8	10	1.9

Tabel 1. Enige eigenschappen van de gebruikte gronden.

them were re-infected each with three sclerotia of a pathogenic isolate of *R. solani.* Three potatoes were planted at a depth of 12 cm in each beaker with soil.

After emergence (after 3 weeks at 15 °C in darkness) the sprouts were rated for infestation (disease rating according to Jager and Velvis, 1980) and examined for the presence of hyperparasites and *R. solani*. The presence of hyperparasites in soil 1 and on sprouts from all soils was assessed by plating soil pellets and sprout segments (10 mm long) on plates with mycelium of *R. solani*. One week before, the plates had been prepared by inoculating malt peptone agar with a 3-mm agar disk from a young colony. Ten segments or pellets were placed in each of four plates. Soil pellets (5-mm diameter and 2 mm thick) were collected with a single-pellet soil-sampler, which is based upon the principle of Henis' soil-sampler (Henis et al., 1978).

After incubation for 2 weeks at 21 °C, the intensity of outgrowth of hyperparasites was given a score from 1 to 4 (Jager et al., 1979), which was multiplied by the percentage of segments showing outgrowth. Occurrence of *R. solani* on sprouts was recorded after incubation of sprout segments (10 mm long) on water agar at 21 °C for 24 h. The outgrowth was also rated from 1 to 4 and the score was multiplied by the percentage of segments showing outgrowth (4 plates, 10 segments per plate).

Results and discussion

Non-activated soil 1 showed a weak development of hyperparasites. Only 5% of the pellets yielded *G. roseum. V. biguttatum* was only observed when the soil had been activated. In that case, 95% of the soil pellets showed growth and sporulation of *V. biguttatum,* but *Gliocladium* spp. were not present. After the soil had been stored for 3 months at 15 °C, it was re-examined for the presence of hyperparasites. The percentage of soil pellets with outgrowth of *V. biguttatum* had not decreased, but its density had diminished slightly and *Gliocladium* spp., in particular *G. roseum,* occurred more frequently. Fig. 1 shows an example of the outgrowth from pellets of non-activated and activated soils.

Fig. 1. Plates with mycelium of *R. solani* each of which were inoculated with ten pellets of sandy soil 1 (from location Haren) after 12 days of incubation. Left: non-activated soil; right: soil activated by adding live mycelium of *R. solani,* 4 weeks before the test.

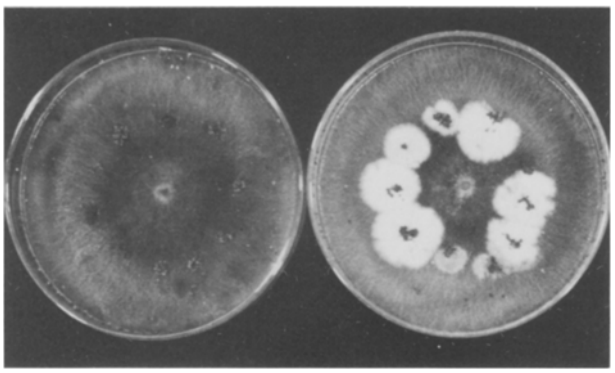

Fig. 1. Rhizoctonia-platen met grondpellets (Haren) na een incubatie van 12 dagen. Links: niet geactiveerde grond; rechts: grond geactiveerd door toevoeging van levens mycelium van R. solani, 4 weken voor de toets.

Table 2. Disease ratings of sprouts from non-infected or infected seed potatoes in untreated soil or in soil activated by adding live mycelium of *Rhizoctonia solani.*

Soil type	Infested sprouts (%)			
	untreated soil: seed potatoes		activated soil: seed potatoes	
	disinfected	re-infected	disinfected	re-infected
Pleistocene sands:				
1	25 (0.3)	100 (4.1)	100 (2.7)	86 (3.4)
2	28 (0.3)	100 (4.2)	0 (0.0)	28 (0.3)
3	11 (0.1)	100 (3.9)	17 (0.5)	70 (2.5)
Holocene marine sandy loam soils:				
4	17 (0.3)	100 (4.5)	100 (2.8)	100 (5.0)
5	0 (0.0)	100 (5.0)	100 (4.6)	100 (4.3)
6	0 (0.0)	100 (5.0)	100 (3.6)	100 (5.0)

[1] Between brackets: disease severity index, ranging from 0 (clean) to 5 (heavily infested).

Tabel 2. Ziektegetallen van aardappelspruiten van niet besmette en besmette poters geplant in onbehandelde grond en in grond geactiveerd door toevoeging van levend mycelium van Rhizoctonia solani.

Table 3. Disease ratings of sprouts from non-infected or infected seed potatoes in untreated soil or in soil activated by adding live mycelium of *Rhizoctonia solani.* (two applications in the case of sandy loam soils and one in the case of sands).

Soil type	Infested sprouts (%)			
	untreated soil: seed potatoes		activated soil: seed potatoes	
	disinfected	re-infected	disinfected	re-infected
Pleistocene sands:				
1	8 (0.1)	100 (3.8)	0 (0.0)	46 (0.5)
2	55 (0.5)	100 (3.4)	20 (0.2)	40 (0.5)
3	0 (0.0)	100 (2.5)	0 (0.0)	0 (0.0)
Holocene marine sandy loam soils:				
4	12 (0.1)	100 (4.7)	15 (0.1)	66 (1.5)
5	40 (0.9)	100 (4.0)	40 (0.6)	55 (0.8)
6	15 (0.1)	100 (3.8)	0 (0.0)	91 (2.1)

[1] Between brackets: disease severity index, ranging from 0 (clean) to 5 (heavily infested).

Tabel 3. Ziektegetallen van aardappelspruiten van niet besmette en besmette poters geplant in onbehandelde grond en in geactiveerde grond (in kleigronden twee toevoegingen en in zandgronden één toevoeging van levend mycelium van R. solani).

Fig. 2. Potato sprouts grown in soil 6 (Kloosterburen). From left to rightz; Soil untreated and disinfected seed potatoes ($K^- R^-$); soil untreated and infected seed potatoes ($K^- R^+$); soil with live mycelium of *Rhizoctonia solani* and infected seed potatoes ($K^+ R^+$ 1 ×); soil with two additions of live mycelium of *R. solani* and with infected seed potatoes ($K^+ R^+$ 2 ×).

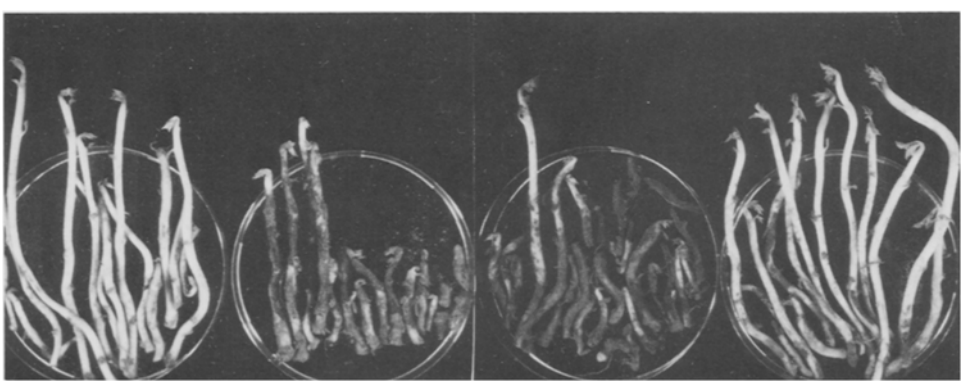

Fig. 2. Aardappelspruiten gekweekt in grond 6 (Kloosterburen). Van links naar rechts: onbehandelde grond en ontsmette poters ($K^- R^-$); onbehandelde grond en besmette poters ($K^- R^+$); grond plus levend mycelium van Rhizoctonia solani en besmette poters ($K^+ R^+$ 1 ×); grond met twee toevoegingen van levend mycelium van R. solani en met besmette poters ($K^+ R^+$ 2 ×).

In the first series of experiments (planting date 23 February) the two types of soil behaved differently (Table 2). In the sandy loam soil and, to a lesser extent, in sandy soil 1 the added *Rhizoctonia* fragments acted as an inoculum and aggravated the infestation of sprouts. In sandy soils 2 and 3 the infestation of sprouts from infected seed potatoes grown in activated soils was less serious than observed in non-activated soils.

To stimulate accumulation of the hyperparasites, the sandy loam soils were given another treatment with live mycelial fragments of *R. solani.* After a 3-week incubation period, again seed potatoes were planted in all beakers (planting date 11 May). The disease ratings of the sprouts are shown in Table 3. In this second series the seed potatoes, whether infected or not, produced healthy or only slightly infested sprouts in activated soils (see also Fig. 2).

Hyperparasites were numerous on sprouts from activated soils and the density of *R. solani* on these sprouts was strongly reduced. A second culture of potato plants from re-infected seed potatoes, particularly in soil 2, already gave rise to accumulation of hyperparasites (Table 4). Nearly all outgrowing hyperparasites were *V. biguttatum* (a similar outgrowth as shown in Fig. 1). Occasionally *Gliocladium* spp. (mainly *G. roseum)* were observed within the white collars of sporulating mycelium of *V. biguttatum;* more rarely *Trichoderma* spp. and *Volutella ciliata* occurred.

The results of these pot experiments show that it is possible to induce suppression of *R. solani* by adding live mycelium fragments of *R. solani* to the soil. A similar method was used by Gerlagh (1968) and by Zogg and Joeggi (1974) to induce antagonism against *Gaeumannomyces graminis,* although a specific antagonist was not found. In our case, specifically *V. biguttatum* appears to accumulate, which therefore may play a role in natural control of *R. solani.* Field experiments in which seed potatoes were inoculated with, among others, *V. biguttatum* were carried out. The results will be presented in a subsequent paper.

Table 4. Densities of *Rhizoctonia solani (R.s.)* and *Verticillium biguttatum (V.b.)* on the potato sprouts in Table 3 (total range 0-400).

Soil type	Untreated soil: Seed potatoes				Activated soil: seed potatoes			
	disinfected		re-infected		disinfected		re-infected	
	R.s.	V.b.	R.s	V.b.	R.s.	V.b.	R.s.	V.b.
Pleistocene sands:								
1	3	100	323	139	1	395	1	397
2	3	2	237	342	1	384	1	392
3	0	67	231	120	1	381	1	394
Holocene marine sandy loam soils:								
4	0	144	393	94	0	395	13	380
5	49	25	323	20	96	383	14	383
6	2	55	326	112	1	387	56	365

Tabel 4. Dichtheden van Rhizoctonia solani (R.s.) en van Verticillium biguttatum (V.b.) op de aardappelspruiten van Tabel 3 (dichtheid kan variëren van 0 tot 400).

Samenvatting

Ophoping van hyperparasieten van Rhizoctonia solani door toevoeging van levend mycelium van R. solani aan de grond

Toevoeging van levend mycelium van *Rhizoctonia solani* aan licht zure zandgronden, in de winter verzameld van velden na een aardappelteelt, leidde tot een ophoping van hyperparasieten en een afneme van *R. solani* en resulteerde uiteindelijk in bescherming van aardappelspruiten tegen *R. solani.* Dit effect werd ook in mariene zavels waargenomen, echter pas na een tweede toevoeging van mycelium.

Verticillium biguttatum bleek de meest voorkomende hyperparasiet te zijn.

References

Gams, W. & Zaayen, A. van, 1982. Contribution to the taxonomy and pathogenicity of fungicolous *Verticillium* species. I. Taxonomy. Neth. J. Pl. Path. 88: 57-78.

Gerlagh, M., 1968. Introduction of *Ophiobolus graminis* into new polders and its decline. Neth. J. Pl. Path. 74 (suppl. no. 2), 97 pp.

Henis, Y., Ghaffar, A., Baker, R. & Gillespie, S.L., 1978. A new pellet soil-sampler and its use for the study of population dynamics of *Rhizoctonia solani* in soil. Phytopathology 68: 371-376.

Jager, G., Hoopen, A. ten & Velvis, H., 1979. Hyperparasites of *Rhizoctonia solani* in Dutch potato fields. Neth. J. Pl. Path. 85: 253-268.

Jager, G. & Velvis, H., 1980. Onderzoek naar het voorkomen van *Rhizoctonia*-werende aardappelpercelen in Noord-Nederland. Inst. Bodemvruchtbaarheid, Rapp. 1-80, 62 pp.

Zogg, H. & Joeggi, W., 1974. Studies on the biological soil disinfection, 7. Contribution to the take-all decline *(Gaeumannomyces graminis)* initiated by means of laboratory trials and some of its possible mechanisms. Phytopath. Z. 81: 160-169.