CODEN: IBBRAH (7-83) 1-34 (1983)

INSTITUUT VOOR BODEMVRUCHTBAARHEID

RAPPORT 7-83

BIOLOGICAL CONTROL OF *RHIZOCTONIA SOLANI* IN POTATO BY ANTAGONISTS. FIELD TESTING OF THE EFFECT OF INOCULATION OF SEED TUBERS WITH *VERTICILLIUM BIGUTTATUM* AND OTHER ANTAGONISTS IN 1981 AND 1982

by

G. JAGER and H. VELVIS

1983

Instituut voor Bodemvruchtbaarheid, Oosterweg 92, Postbus 30003,
9750 RA Haren (Gr.)

Inst. Bodemvruchtbaarheid, Rapp. 7-83 (1983) 34 pp.

CONTENTS

1. INTRODUCTION

In field experiments we compared the infestation and the formation of sclerotia on the tubers harvested from plants originating from seed tubers produced on a slightly acid pleistocene sand with those from seed tubers produced on a neutral holocene, marine loam soil (Jager and Velvis, 1983[b]). Plants from seed tubers produced on sand were found to be less infested by *R. solani* (at the same or a higher sclerotium index of the seed tubers) than plants from seed tubers produced on a marine loam soil. The sclerotium index of the yield obtained from a slightly suppressive sandy soil and from two marine loam soils was distinctly lower when the seed tubers had been produced on the sandy soil. We assigned a higher load of antagonists to these seed tubers. Since the antagonists - from a slightly acid sand - thrived in a neutral marine loam and gave some protection, we concluded that the antagonists are more strongly related to the plant than to the soil.

In earlier studies we found that *Verticillium biguttatum* (Gams and Van Zaayen, 1982) is a parasite of sclerotia of *R. solani*. This fungus is very common in acid sandy soils but, generally, less common in neutral marine soils (Jager and Velvis, 1980, 1983[a]). Velvis and Jager (1983) observed that some isolates of *V. biguttatum* killed sclerotia within seven weeks under conditions of high relative humidity (as in a normal moist soil) at temperatures of 15 °C or more. Amongst the isolates a variation in antagonistic strength was found. One isolate, M73, stood out positively.

In laboratory experiments that lasted five weeks, *Verticillium biguttatum* (isolate M73), inoculated on seed tubers, showed the ability to grow on the surface of emerging sprouts up to the soil surface. It protected the sprouts against infestation by *R. solani* from sclerotia on the seed tuber (Velvis and Jager, 1983). It also reduced the effects of soil-borne *Rhizoctonia* on emerging potato sprouts in similar experiments (Jager and Velvis, 1983[c]).

These properties appear to make *V. biguttatum* very suitable for biological control of *R. solani* in potato fields.

Van den Boogert and Jager (1984) showed that antagonistic fungi like *Gliocladium roseum, Hormiactis fimicola* and *Trichoderma* spp. gave little or no protection against infestation of stems and stolons. They did not reduce the amount of sclerotia on new tubers grown in a sandy soil. *V. biguttatum*, however, significantly lowered infestation and reduced production of sclerotia on the harvested tubers.

Results of efforts to biologically control *R. solani* in potato fields on different soils, by inoculating the seed with *V. biguttatum* and other antagonists, including *Azotobacter chroococcum* (Meshram and Jager, 1983), are described in this report.

2. MATERIALS AND METHODS

The experiments described were carried out in 1981 and 1982. Unless stated otherwise, the cultivar Bintje was used.

2.1. 1981

The seed tubers originated from a slightly acid sandy soil ("sand seed") and from a neutral clay loam ("clay seed"). The seed from both origins was divided into three lots, viz. clean tubers, lightly infected tubers and one lot was disinfected with formaldehyde (Butler and Jones, 1955). Half of the clean and infected seed tubers was inoculated with *V. biguttatum* (M73) plus *Gliocladium roseum* in a liquid inoculum, the other tubers were treated with the liquid without antagonists. The seed tubers pre-germinated in daylight in a greenhouse.

The liquid inoculum contained 1% carboxymethylcellulose (cmc) plus 300 g clay (subsoil) per litre of water. Five agar plates (ϕ 15 cm) overgrown with *V. biguttatum* and one with *Gliocladium roseum* were added to five litres of the liquid. The agar layers were dispersed in the liquid with a Silverson mixer-emulsifier.

After dipping the seed tubers in the inoculum, they were dried at room temperature in a forced air stream. The tubers were planted two days afterwards.

Tubers were grown for early (seed) and late (ware) harvesting. Foliage of the plants for early harvesting was desiccated chemically during the first week of August; that of the ware potatoes during the first week of September. Tubers were harvested three weeks after desiccation.

An experimental field comprised 54 plots in a random split plot design: nine treatments and six replicates. Of six replicates, three were harvested as seed and three as ware potatoes. Each plot consisted of six or four rows with five plants. From the plots with six rows, samples were taken in the course of the growing season to determine the disease index (Jager and Velvis, 1983[b]) and to assess the presence of

R. solani and its hyperparasites on pieces of stems and stolons. The methods were described by Van den Boogert and Jager (1984).

2.2. 1982

In the experiments of 1982 the potatoes were harvested as seed tubers, since the production of seed of high quality is most endangered by *R. solani*.

Seed tubers grown on a sandy soil were used. The treatments were: disinfected (see par. *2.1.*) (1); not-disinfected (2); not-disinfected and dipped in inoculum containing *V. biguttatum* M73 (3), or *V. biguttatum* M180 (4), or *Azotobacter chroococcum* (5^a, for soils with a pH of 7 or higher) or *Gliocladium nigrovirens* (5^b, for slightly acid sand soils) or a mixture of 3 + 4 + 5^a or 5^b (6), each at one third of the original concentration. The inoculum also contained 1% cmc plus 0.3% gelatin and 60 g clay per litre. The treated seed tubers were not dried after dipping, but were placed in a bucket and kept moist until planting the following day. This was done because experiments had shown that drying after dipping resulted in death of a large part of propagules. The experimental fields comprised 72 plots in a random split plot design, six treatments replicated twelve times. Each plot consisted of four or six rows with five plants, as described in par. *2.1.* Foliage killing and harvesting were carried out in the same way as in 1981.

Planting date. The minimum for growth of *V. biguttatum* is between 10 and 15 °C. Potato tubers, however, are planted from the end of March or early April on, when soil temperatures are usually below 15 °C. To examine whether this affected the result of inoculation*), an additional experiment (cv. Irene) was carried out on the sandy soil of Haren. Tubers were planted on 8 and 29 April and on 13 and 21 May. The tubers were harvested as seed and as ware potatoes. Foliage was chemically desiccated, as in 1981 at two different dates and the crop was harvested three weeks after desiccation. This field comprised 128 plots, sixteen treatments replicated eight times.

The disease index was calculated as given by Jager and Velvis (1983^b), but the value was divided by 5, thus giving a theoretical maximum of 100.

---

*) with *V. biguttatum* M73

Sclerotium index. Tubers were washed, dried, classified and weighed. The weight of each class was multiplied by a factor : clean (c) × 0; very lightly speckled with sclerotia (vl), × 1; lightly (l), × 2; moderately (m), × 3 and heavily (h), × 4.
The sclerotium index was calculated according to:

$$s.i. = \frac{(vl \times 1 + l \times 2 + m \times 3 + h \times 4) \times 100}{4 \times \text{total weight}}$$

The theoretical maximum value thus is 100.

The determination of the percentage of sclerotia infected with antagonistic fungi and streptomycetes was carried out as described by Jager and Velvis (1983[b]).

Some properties of the soils of the experimental fields of 1981 and 1982 are presented in tabel I.

TABLE I. Some properties of the soils of the experimental fields in 1981 and 1982.
TABEL I. Enkele eigenschappen van de gronden van de proefvelden in 1981 en 1982.

	pH (KCl)	Coarse sand 210-2000 (%)	Silt 2-50 (%)	Particles <16 (%)	Clay <2μm (%)	Org. matter (%)	K_2O (mg/kg)	MgO (mg/kg)	Pwater (mg/l)	$CaCO_3$ (%)
	1981									
Haren	4.7	10	35	7	-	4.6	160	104	15	-
Borger	4.9	17	15	4	-	4.7	120	92	31	-
Zeyerveld	4.3	18	34	7	-	6.3	100	38	38	-
Zuurdijk	7.4	0	71	34	-	1.7	210	126	18	-
Kloosterburen	6.0	0	43	14	-	1.6	400	109	71	-
Kimswerd	7.1	0	75	36	-	4.1	310	347	41	-
Sexbierum	6.3	1	63	24	-	4.2	180	339	71	-
Tollebeek	7.6	-	-	17	-	2.1	270	-	26	-
	1982									
Gasselte	5.4	12	18	5	-	5.9	210	93	73	-
Haren	7.2	14	33	10	-	4.0	150	95	49	-
Exl.mond	4.8	12	17	5	-	14.7	270	265	94	-
Zuurdijk	7.5	0	66	28	18	1.5	270	-	22	9
Kloosterburen	6.4	0	45	13	9	1.7	240	-	74	0
Usquert	7.2	0	56	17	-	2.4	180	66	53	3
Zandeweer	7.4	0	73	26	18	2.2	360	-	74	0
Schalsum	7.3	0	73	31	20	2.2	200	-	38	3
Sexbierum	7.3	1	38	22	14	4.3	340	-	85	2

3. RESULTS

3.1. 1981

3.1.1. Disease index

The infestation of stems (first sampling only) and stolons is given as the disease index. The sampling period lasted from early June until the first week of September (ware potatoes only). Each disease index was calculated as the average of 15 plants. Of some of the experimental fields the course of the disease index of plants from differently treated seed tubers, during the growing season, is presented in figure 1.

Figure 1 shows that plants from infected seed tubers were more severely infested than plants from disinfected or clean seed tubers.

Plants from disinfected seed tubers were more severely infested than plants from clean seed tubers at Haren and Zuurdijk, irrespective of their origin. The differences at the other fields were only small.

Plants from infected "sand seed" suffered less from infestation than those from infected "clay seed" at Haren, Zuurdijk, Kloosterburen, Kimswerd.

Clean seed tubers produced plants with rather low disease indices. The effect of inoculation with antagonists was variable.

Inoculation of infected seed tubers with antagonists distinctly reduced infestation of stems and stolons at Zuurdijk. At Kloosterburen it did so in plants from "sand seeds" but not in plants from "clay seeds". At Kimswerd plants from inoculated infected "clay seed" were less severely infested than the non-inoculated ones. Plants from infected "sand seed" showed a rather variable, but less severe infestation than plants from "clay seeds".

In the fields on sandy soil the variation was too large to permit conclusions.

3.1.2. R. solani on stems and stolons

The effect of inoculation of seed tubers with antagonists on the presence of *R. solani* on stems and stolons cannot be measured only by the disease

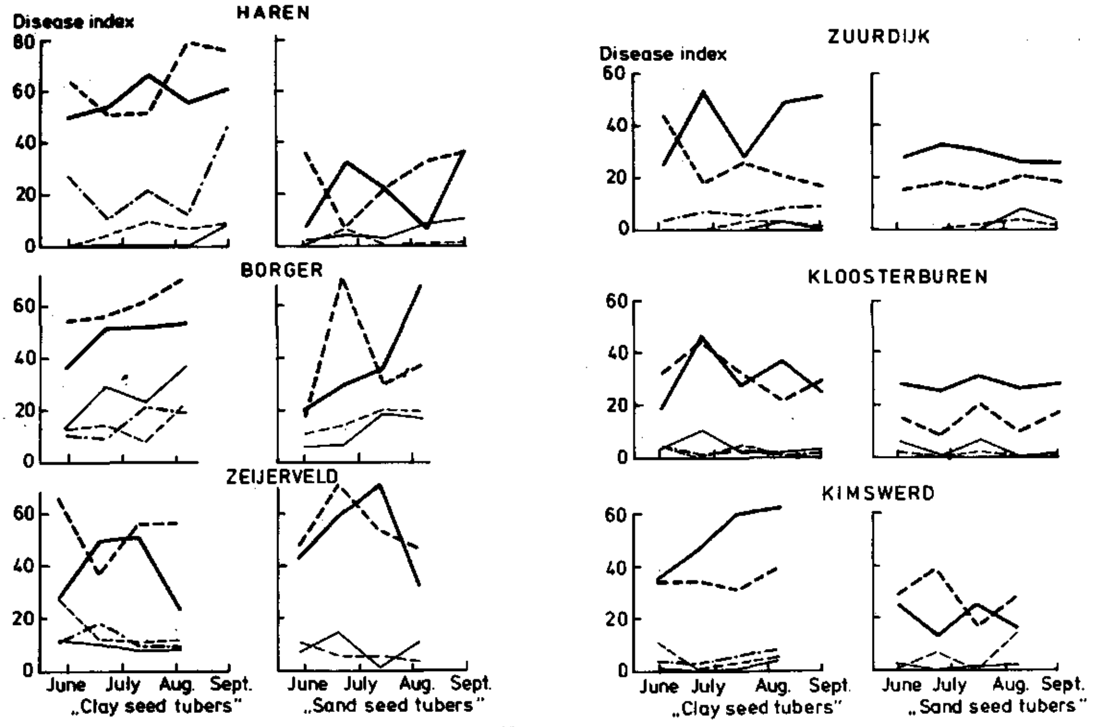

Figure 1. Disease indices of plants from seed tubers with sclerotia of *R. solani*, clean and disinfected seed tubers. Clean and infected seed tubers were inoculated with antagonists (*V. biguttatum* plus *G. roseum*) before planting or were left uninoculated. Seed tubers were produced on a slightly acid sand soil or on neutral sandy loam or clay loam soils ("clay seed tubers").

Plants from:

- disinfected seed tubers
- clean, not inoculated seed tubers
- clean, inoculated seed tubers
- infected, not inoculated seed tubers
- infected, inoculated seed tubers

Figuur 1. Aantastingsindices van planten uit met R. solani besmet, schoon en ontsmet pootgoed. Schoon en besmet pootgoed is wel en niet geënt met antagonisten van R. solani (V. biguttatum plus G. roseum). Het pootgoed werd verbouwd op licht zure zandgrond ("sand seed tubers") en op klei- en zavelgrond ("clay seed tubers").

Planten van:

- *ontsmet pootgoed*
- *schoon pootgoed, niet geënt*
- *schoon pootgoed, geënt*
- *besmet pootgoed, niet geënt*
- *besmet pootgoed, geënt*

index, which is an indication of only the pathogenic strains of *R. solani*. The percentage of stolon pieces colonized by *R. solani*, however, can also give information about a possible suppressive effect of antagonists on the surface of plant organs, such as stolons.

The percentage of stolon pieces (length 1 cm) of plants from the differently treated seed tubers colonized by *R. solani* in the course of the growing season is given in figure 2 for three fields.

Inoculation of the seed tubers with the antagonistic fungi reduced the percentage of stolon pieces colonized by *R. solani*. This is very clear for plants from infected seed tubers at Zuurdijk and Borger. Haren, in this respect, showed a rather irregular course.

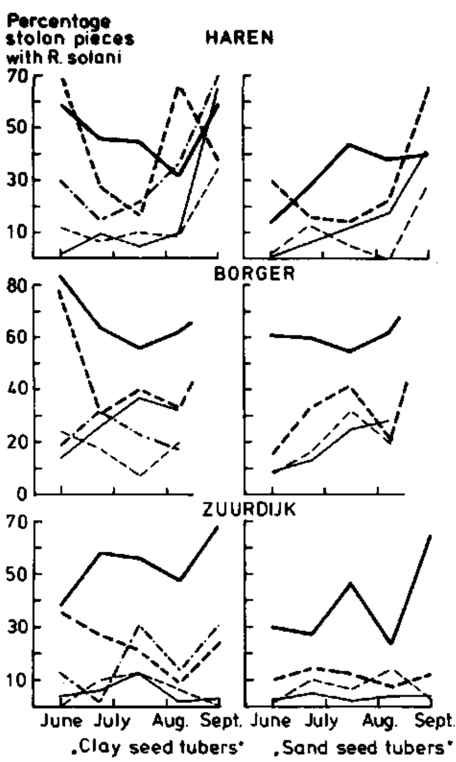

Figure 2. The percentage of stolon pieces with *R. solani* of plants from different seed tubers. See legends to fig. 1.

Figuur 2. Het percentage stolonstukjes met R. solani van planten uit verschillend pootgoed. Zie legenda bij fig. 1.

The percentage of stolon pieces from clean seed tubers colonized by *R. solani* was rather variable. The favourable effect of inoculation with antagonists was not evident.

Plants from disinfected seed tubers at Haren had a high percentage of stolon pieces with *R. solani*, especially towards the end of the season. At Borger they were similar to those of plants from clean seed tubers and at Zuurdijk the percentage of stolon pieces colonized by *R. solani* corresponded to that of plants from inoculated seed infected with *R. solani*.

It is striking that in many cases, at the end of the growing season, the percentage of stolon pieces with *R. solani* rose sharply. This may be due to the fact that senescent plants lose their resistance to *R. solani*. The disease index, however, only rose in a very few cases towards the end of the season, so that the increase in *R. solani* must mainly be due to saprophytic strains of *R. solani*. A rise of the disease index at the end of the growing season was observed earlier (Jager and Velvis, 1980, 1982), but apparently is not a generally occurring phenomenon.

3.1.3. Hyperparasites of R. solani on stolons

The average percentage of stolon pieces showing outgrowth of *V. biguttatum* and *G. roseum* and the average amount of *V. biguttatum* on stolons are presented in table II for the fields at Haren and Zuurdijk for each sampling during the course of the growing season. Stolons from disinfected seed tubers became infected with soil-borne *V. biguttatum* and *G. roseum*. The infection in the slightly acid sand of Haren was, on average, higher than in the neutral loam of Zuurdijk. The percentage of stolon pieces with *G. roseum* rose sharply at the end of the growing season in both soils. The amount of *V. biguttatum* on stolons of plants from disinfected seed tubers at Haren increased, especially during the second half of the growing season. At Zuurdijk the maximum amount was present at the end of June, followed by a sharp decrease and a second lower peak at the end of the season.

TABLE II. Percentage of stolon pieces colonized by *V. biguttatum* (V%) and *G. roseum* (G%), and the amount of *V. biguttatum* (V.am) on stolons during the course of the growing season (1981).

TABLE II. Het percentage stolon stukjes gekoloniseerd door V. biguttatum (V%) en G. roseum (G%) en de hoeveelheid V. biguttatum (V.am) op de stolonen in de loop van het groeiseizoen. Sand seed tubers = zandpoters; clay seed tubers = kleipoters. Clean = schoon, vrij van sclerotiën, clean + ant. = idem, geënt met antagonisten. Infected = geïnfecteerd, met sclerotiën; Inf. + ant. = idem, geënt met antagonisten.

Treatment	Sampling date														
	June 15			July 6			July 27			Aug. 17			Sept. 7		
	V%	G%	V.am	V%	G%	V.am	V%	G%	V.am	V%	G%	V.am	V%	G%	V.am
							Haren								
Sand seed tubers:															
Clean	78	9	144	60	6	148	39	15	58	85	39	142	65	74	149
Clean + ant.	74	80	132	100	91	249	75	23	123	76	45	123	100	57	288
Infected	79	13	128	86	3	156	94	43	180	75	86	125	100	34	310
Inf. + ant.	62	59	119	100	98	277	100	64	262	100	30	240	100	100	289
Disinfected	50	2	73	26	2	39	94	8	170	98	9	248	99	63	284
Clay seed tubers:															
Clean	69	28	132	87	46	208	57	15	82	66	55	106	86	68	207
Clean + ant.	98	91	220	92	43	226	96	28	208	61	65	93	98	100	292
Infected	85	0	230	100	45	274	99	53	239	99	91	218	100	54	306
Inf. + ant.	-	-	-	100	96	263	100	37	307	100	45	234	100	61	324

Treatment	Sampling date														
	June 9			June 30			July 20			Aug. 10			Aug. 31		
	V%	G%	V.am	V%	G%	V.am	V%	G%	V.am	V%	G%	V.am	V%	G%	V.am
							Zuurdijk								
Sand seed tubers:															
Clean	18	1	42	96	14	224	58	28	98	34	24	58	59	25	149
Clean + ant.	90	45	337	99	62	352	95	40	255	90	69	194	68	27	136
Infected	31	6	57	73	1	162	91	68	242	36	33	57	48	19	48
Inf. + ant.	100	76	258	100	88	339	98	44	279	98	79	227	98	36	240
Disinfected	40	4	79	97	15	258	28	2	44	13	5	17	47	78	109
Clay seed tubers:															
Clean	18	0	29	60	1	148	26	14	39	3	8	3	31	56	76
Clean + ant.	99	91	271	100	73	371	82	58	157	75	71	75	59	91	146
Infected	71	1	170	83	6	216	87	41	192	75	36	76	84	84	274
Inf. + ant.	99	87	241	100	55	327	96	57	250	98	71	98	98	69	275

A similar pattern occurred at Zuurdijk for stolons of plants from clean seed tubers. At Haren the amount of *V. biguttatum* on stolons of clean seed tubers more or less remained at the same level during the growing season. A minimum, however, was reached in June or July. Stolons of plants from infected seed tubers had higher amounts of hyperparasites than those from clean seed tubers.

Inoculation of seed tubers with antagonists led to high percentages of colonized stolon pieces and high amounts of *V. biguttatum* on stolons. Effectively high values were already present early and remained so to the end of the season. The strongest effect of inoculation, i.e. the largest difference between colonization of inoculated and non-inoculated tubers, was found on the neutral clay loam of Zuurdijk. At Haren the difference was smaller, possibly due to a stronger colonization of stolons by soil-borne *V. biguttatum*.

A higher percentage of stolons colonized by antagonists of *R. solani* or higher amounts of *V. biguttatum* on stolons does not necessarily mean a higher antagonistic activity. Stolons of disinfected seed tubers had, for instance, often higher amounts of (soil-borne) *V. biguttatum* than stolons of clean seed tubers, but the disease index was higher. The same is true for stolons of plants from infected clay seed with a higher disease index than plants from infected sand seed tubers, although the stolons of plants from clay seed tubers had higher amounts of *V. biguttatum* and a higher percentage of stolon pieces colonized by *G. roseum*. These discrepancies are caused by large interspecific differences with regard to antagonistic properties and competitive abilities, while also the time at which the antagonism really became active is important.

3.1.4. Sclerotium index of harvested seed and ware tubers

Table III shows the sclerotium indices of the yield of the seed tubers of the different treatments from the experimental fields. The results of seed tubers from clay and sand were combined, since they were not essentially different here. The standard deviation (s) is also given.

TABLE III. Average sclerotium indices (s.i.) of the yield obtained from seed tubers subjected to different treatments in 1981. Tubers were harvested as seed and ware potatoes. The last four columns show the lowest average s.i., as a percentage of the s.i. of the yield of disinfected and of clean seed tubers.

TABEL III. Gemiddelde sclerotiumindices van de oogst uit verschillend behandeld pootgoed op de verschillende proefvelden. De knollen zijn geoogst voor pootgoed en voor consumptie. De laagste sclerotiumindex van ieder veld is gegeven in percentages van de sclerotium-index van de oogst uit ontsmet en schoon pootgoed. S = pootgoed, W = consumptie. Tollebeek: schoon pootgoed plus: Trichoderma sp. (A), V. biguttatum (B), G. roseum (C) of de mix van deze drie (D). Exl.mond: (E:) schoon + Hormiactis fimicola, (F:) schoon + mix, (G:) besmet + H. fimicola, (H:) besmet + mix. De mix bevatte: Trichoderma sp., G. roseum, H. fimicola en V. biguttatum.

Seed tubers: / Location	Disinfected		Clean		Clean + antag.		Infected		Infected + antag.		Lowest s.i. as % of s.i. of disinf.		clean	
	seed	ware	seed	ware	seed	ware	seed	ware	seed	ware	S	W	S	W
Haren	43.7± 5.4	35.5±13.2	31.0± 6.2	21.2±11.3	23.7±11.3	19.3±8.1	45.5± 6.5	35.3± 9.7	38.5± 8.4	24.8± 9.6	54	55	76	91
Borger	31.0±14.4	34.3± 2.5	32.2±11.4	27.5±12.5	23.9±13.9	21.8±8.7	41.5± 2.0	27.8± 8.6	33.8± 6.2	23.7± 8.5	77	64	74	79
Zeijerveld	37.3± 5.0	36.7± 8.6	35.5±11.4	26.7± 7.7	29.0± 9.8	26.0±8.9	41.7± 5.5	36.5± 5.3	36.8± 7.8	34.8± 4.4	78	71	82	97
Zuurdijk	15.7± 4.9	18.3±10.7	6.2± 5.0	4.3± 5.8	2.3± 2.3	2.0±2.8	39.7± 9.7	49.0± 3.7	22.2±13.5	18.2±11.3	15	11	37	47
Kimswerd	4.0± 4.5	15.7±13.7	7.3± 7.0	6.7± 6.7	7.5± 9.5	4.3±3.8	32.0±11.6	22.5±16.3	10.3± 2.6	14.2± 8.8	>100	27	>100	64
Kloosterburen	19.7± 5.5	15.0±14.0	14.5± 3.3	14.2± 7.2	13.8± 5.0	8.0±4.9	43.5± 4.2	40.7± 6.5	26.8± 8.3	17.5± 8.7	70	53	95	56
Sexbierum	18.7±11.5	27.3± 6.5	13.5± 4.4	18.7± 7.6	7.3± 2.3	14.3±5.2	19.7± 7.1	8.3± 6.7	19.3± 6.6	20.3± 0.6	39	52	54	76
Tollebeek	23.0± 5.6		24.0± 8.5 (A)		7.0± 7.8 (B)		23.0± 4.1 (C)		19.0± 8.5 (D)		30	-	-	-
Exl. mond		20.3± 7.3		18.2± 9.8 (E)		11.2±7.0 (F)		23.7± 4.3 (G)		16.1± 7.3	55	-	-	-

3.1.4.1. Sand soils. Disinfected seed tubers produced a yield with sclerotium indices similar to the yield of infected tubers. Inoculation of infected seed tubers with antagonists tended to produce tubers with a lower sclerotium index.

Clean seed tubers produced cleaner tubers, especially when they had been inoculated. At Haren and Borger the effect of inoculation of seed tubers with antagonists was significantly better than no inoculation ($p = 1\%$). Results at Zeijerveld were not significantly different. At Haren, inoculation with antagonists was significantly better than disinfection ($p = 0.1\%$).

3.1.4.2. Sandy loam and clay loam soils. The sclerotium index of the yield of ware potatoes from disinfected seed tubers was rather low and not significantly different from that of inoculated infected seed tubers.

Clean seed tubers produced a yield with a very low or low sclerotium index, which was significantly different from the sclerotium index of the ware tubers obtained from disinfected seed tubers at Zuurdijk and at Kimswerd.

Infected seed tubers produced yields with the highest sclerotium indices; inoculation with antagonists often led to significant reduction of the sclerotium index (Kloosterburen and Kimswerd seed tubers; Zuurdijk ware tubers).

The average effect of inoculation of seed tubers with antagonists on the sclerotium index of the harvested tubers was significantly different ($p = 0.1\%$) from the sclerotium index of the yield of infected seed tubers that had not been inoculated.

The overall effect of inoculation of seed tubers with antagonists, based on the sclerotium indices of all experiments, was favourable for clean ($p = 5\%$) and infected seed potatoes ($p = 1\%$).

The field at Sexbierum was planted with cv. Irene. The results were not essentially different from those of the other fields. It looks as if a mistake was made and infected seed tubers have been used in stead of infected seed tubers plus antagonists (ware tubers).

In Tollebeek the field was smaller; only seed tubers (cv. Civa) were grown. The seed tubers were treated as follows: disinfected; clean + *Trichoderma* sp. M165 (A); clean + *V. biguttatum* M73 (B); clean + *G. roseum* M163 (C) and clean + a mixture of the antagonists.

The foliage was not desiccated chemically but was removed mechanically. Three weeks later the tubers were harvested.

The strongest reduction of the sclerotium index was caused by *V. biguttatum*; the mixture was less effective and the *Trichoderma* and *G. roseum* isolates were not effective.

On the field at Exloërmond tubers for the potato starch industry were grown (cv. Prominent). Tubers were harvested after the foliage had died naturally. The seed tubers were treated as follows: disinfected; clean + *Hormiactis fimicola* (E); clean + a mixture of *Trichoderma* sp., *G. roseum*, *H. fimicola* and *V. biguttatum* (F); infected + *H. fimicola* (G) and infected + the mixture (H).

V. biguttatum was again responsible for the greatest reduction of the sclerotium index.

3.2. 1982

The disease index of stems and stolons was determined, only twice, in four fields in June. The indices did not exceed those of 1981 for plants from "sand seed". The highest values (average 16.5) were found on sand soils, the lowest (average 8.0) on clay loam soils. The lowest values were always found when the seed tubers had been treated with antagonists, either with *V. biguttatum* M73 or with a mixture also containing this antagonist.

3.2.1. R. solani on stems and stolons

The percentage of stolon pieces with *R. solani* is given in table IV. The highest values were observed on stolons grown in the sand soil of Gasselte, on which also the highest disease indices occurred. The values observed on a sandy loam (Sexbierum) were slightly lower, but the effect of the antagonists was more pronounced. The lowest values were observed on a clay loam (Usquert). The effect of the applied antagonists on the infection of the stolons is often clear compared with that on stolons from not disinfected tubers without antagonists; this, however, was not the case in the sandy soil. Infection by *R. solani* in this soil is too high.

TABLE IV. Percentage of stolon pieces with *R. solani* at different dates and treatments of the seed tubers in three field experiments in 1982.

TABEL IV. Het percentage stolon stukjes met uitgroei van R. solani op verschillende tijdstippen in het groeiseizoen bij verschillende poterbehandeling in drie veldproeven in 1982 (Ave = gemiddeld).

	Location and date of sampling														
	Gasselte					Sexbierum					Usquert				
	June		July		Ave.	June		July		Ave.	June		July		Ave.
Seed tubers:	14	23	5	14		21	30	12	21		16	28	7	19	
1 Disinfected	4	2	33	17	14	10	17	7	32	17	0	0	17	15	8
2 Not disinfected	16	23	23	25	22	12	4	2	36	14	12	4	12	19	12
3 Not d.+antag. 1	3	15	44	31	23	5	2	6	14	7	0	3	0	7	3
4 Not d.+antag. 2	12	2	32	40	22	5	28	9	15	14	7	0	1	9	4
5 Not d.+antag. 3	19	0	42	38	25	13	3	10	18	11	19	4	5	9	9
6 Not d.+antag. 4	0	15	35	27	19	4	9	3	7	6	5	9	8	8	8

3.2.2. *Hyperparasites on stems and stolons*

Stolons from plants originating from disinfected seed tubers were quickly colonized by *V. biguttatum* and *Gliocladium* species from the soil. The infection of stolons from seed tubers that were not disinfected, nor inoculated was, on average, slightly higher. The effect of inoculation with *V. biguttatum* was very clear in treatments 3, 4 and 6.

Table V presents the average percentage of stolon pieces showing outgrowth of *V. biguttatum* and *Gliocladium* species (*G. roseum*, *G. nigrovirens* and *G. solani*), as well as a measure of the abundance of these fungi on stolon pieces ("Mass").

Table V only shows the hyperparasites most frequently occurring. Regularly, but less frequently and in smaller amounts (on the *Rhizoctonia* plates), *Pyxidiophora* sp. occurred in June and July at Gasselte and Usquert. This fungus was quite rare at Sexbierum. *Trichoderma* spp. were usually present, most frequently in the acid sandy soil of the field at Gasselte, to a lesser extent in Sexbierum and almost none at Usquert. The presence of *Volutella ciliata*, *Penicillium* spp. and *Cylindrocarpon destructans* was observed in the sandy soil of Gasselte, to a lesser extent in Sexbierum and least in Usquert.

TABLE V. Average percentages of stolon pieces colonized by *V. biguttatum* (V) and *Gliocladium* spp. (G) during the growing season of 1982, and the average "mass" of these fungi (maximum = 500).

TABEL V. De percentages van stolonstukjes (stol.piec.) waarvan uitgroei werd verkregen van V. biguttatum (V) en van Gliocladium soorten (G) en een maat voor de massa aan uitgroei, gemiddeld over het groeiseizoen in afhankelijkheid van de poterbehandeling en de eigenschappen van de grond van de proefvelden in 1982.

Seed tubers	Location											
	Gasselte				Sexbierum				Usquert			
	% stol.piec.		mass		% stol.piec.		mass		% stol.piec.		mass	
	V	G	V	G	V	G	V	G	V	G	V	G
1 Disinfected	46	73	129	124	36	63	120	121	57	42	140	72
2 Not disinfected	75	73	233	131	48	69	167	135	60	17	171	23
3 Not d. + antag. 1	91	72	294	137	89	82	297	164	88	28	263	54
4 Not d. + antag. 2	96	80	363	162	59	68	205	140	92	47	332	93
5 Not d. + antag. 3	87	77	292	144	-	-	-	-	-	-	-	-
6 Not d. + antag. 4	89	71	308	134	85	76	292	147	80	48	252	93

Inoculation of seed tubers leads to, on average, higher amounts of the inoculated fungus on stems and stolons during the growing period. The percentage of stolon pieces with *V. biguttatum* proved to be rather variable during this period. In the sandy soil of Gasselte high percentages remained present up to mid-July, also on plants from disinfected seed tubers. This means that a continuous invasion of *V. biguttatum* from the soil takes place; these compete with the M73 isolate we inoculated and possibly have the ability to supplant it. The effect of inoculation in acid sandy soil is often negligible. Interspecific competition may be one cause, the high inoculum density of *R. solani* another. It is, however, not yet possible to distinguish between a good antagonist like M73 and the, on average, less valuable "wild" forms in the soil; therefore an exact interpretation of the observed phenomena cannot yet be given.

The amounts of *V. biguttatum* on the stolons seemed to be lower than in the corresponding periods of 1981.

3.2.3. Sclerotium index

The sclerotium index is an important index of quality of seed potatoes, determining the economic value. The effect of the various treatments of the seed tubers on the development of sclerotia on the new tubers in the soils of the experimental fields is presented in table VI. It is striking that the lowest average values were found in the harvested tubers of seed tubers inoculated with antagonists (treatments 3 or/and 6) and that were not disinfected. If there was an effect of disinfection of the seed tubers, it was rather small. In soils with a rather high inoculum density of *R. solani* (Haren, Gasselte, Exloërmond, Sexbierum and Schalsum) no positive effect of disinfection occurred. When the inoculum density of *R. solani* was low, the average sclerotium index of the tubers harvested from disinfected seed tubers was lower than that of the harvest of clean tubers that were not disinfected. The values of the sclerotium indices per treatment varied strongly. As a result the difference between the sclerotium indices of the yield of seed tubers that were disinfected and not disinfected proved to be insignificant.

TABLE VI. Average sclerotium indices of the harvested tubers from seed tubers from the different treatments of the experimental fields in 1982. (A = *Azotobacter chroococcum* in soils 4, 5, 7, 8, 9 and 10, *Gliocladium nigrovirens* in the other soils). The last two columns show the lowest average s.i. as a percentage of the s.i. of the yield of seed tubers that were disinfected (disinf.) or not disinfected, i.e. untreated.

TABEL VI. De gemiddelde sclerotium indices van de oogst uit verschillend behandeld pootgoed op de verschillende proefveldjes. De laagste gemiddelde waarde van ieder veldje is uitgedrukt in procenten van de waarde van onbehandeld (untr.) en ontsmet (disinf.) pootgoed (M73 en M180 zijn isolaten van V. biguttatum, A is voor klei en zavels Azotobacter chroococcum (een bacterie); voor zandgrond en Usquert Gliocladium nigrovirens, mix is een mengsel van verschillende antagonisten. (Zie Materials and methods.))

Location	Seed tubers						Lowest s.i. as a % of the s.i. of	
	disinfected	not disinfected	n.d. + M73	n.d. + M180	n.d. + A	n.d. + mix	disinfected	untreated
Haren (1)	17.1±12.7	17.3±13.7	13.1± 8.6	15.7±12.5	18.0±10.7	16.9±12.3	77	76
Gasselte (2)	27.2±15.0	25.6±15.4	20.3±15.8	25.5±14.0	32.0±10.5	16.7±11.9	61	65
Exloërmond (3)	47.3± 9.8	44.3± 9.6	40.2± 9.7	44.4±11.5	46.3± 6.5	42.9±10.0	85	91
Zuurdijk (4)	6.8± 9.4	13.8±10.9	2.6± 3.1	7.0± 6.7	6.8± 3.4	2.7± 3.5	38	19
Kloosterburen (5)	9.3±11.9	14.2±11.7	3.1± 2.3	7.7± 9.0	11.5±14.4	6.9± 7.4	33	22
Usquert (6)	5.1± 7.1	10.0±11.0	4.7± 5.1	2.0± 4.5	8.5± 8.4	5.9± 7.4	39	20
Zandeweer (7)	4.8± 7.8	6.5±11.0	1.6± 3.8	3.0± 3.3	4.3± 5.2	1.0± 1.9	21	15
Schalsum (8)	21.4±18.1	23.7±15.6	14.0± 8.1	22.7±12.2	26.2±16.3	10.1±10.1	47	43
Sexbierum (9)	30.2±13.1	23.6±12.6	19.9±13.7	22.9±11.6	23.4±12.4	16.7±11.8	55	71
Tollebeek (10)	8.9± 5.2	12.6± 6.6	6.7± 6.1	8.4± 8.1	15.3± 8.2	4.3± 3.0	48	34

3.2.4. Inhabitants of sclerotia on harvested tubers

Sclerotia were collected from tubers of the treatments that were not inoculated and incubated as described by Jager and Velvis (1983). Table VII gives the percentage of sclerotia of the different fields infected by various kinds of fungi and by streptomycetes.

TABLE VII. Percentage of sclerotia colonized by different fungi (mainly antagonists) and streptomycetes.

TABEL VII. Het percentage sclerotiën uit de niet geënte objecten (1 en 2) van de verschillende proefvelden dat gekoloniseerd is door verschillende schimmels (vnl. antagonisten) en door streptomyceten.

V.b. *Verticillium biguttatum*; G.r. *Gliocladium roseum*; G.spp. andere *Gliocladium* soorten *(G. nigrovirens, G. solani)*; H.f. *Hormiactis fimicola*; V.c. *Volutella ciliata*; Pyx.sp. *Pyxidiophora* sp.; Pen.sp. *Penicillium* spp.; Cyl.de. *Cylindrocarpon destructans*; Trich. *Trichoderma* spp.; Strep. *Streptomyces* spp.

Location	V.b.	G.r.	G.spp.	H.f.	V.c.	Pyx.sp.	Pen.spp.	Cyl.de.	Trich.	Strep.
Haren	61	1	3	0	8	7	3	1	0	68
Gasselte	94	0	0	0	0	7	2	0	0	18
Exloërmond	72	1	11	9	6	0	8	0	0	30
Zuurdijk	2	0	1	0	1	0	1	0	0	89
Kloosterburen	76	1	2	0	4	0	6	0	1	73
Usquert	53	2	2	0	2	0	3	0	0	78
Zandeweer	60	0	0	0	0	15	0	0	1	75
Schalsum	83	0	4	0	12	7	1	1	0	77
Sexbierum	77	0	1	0	6	3	2	0	0	87

V. biguttatum colonized a high percentage of the sclerotia. At Zuurdijk a very low percentage of sclerotia was colonized by fungi. Of the fungi, *Verticillium biguttatum* is the most important colonizer - and destructor - of sclerotia of *R. solani*. The other fungi colonize a percentage of the sclerotia that varies, but is much lower. The species present as colonizers of sclerotia depend on the composition of the mycoflora of the field involved. *Trichoderma* and *Cylindrocarpon* species only occurred seldom. *Hormiactis fimicola* was only found in one field on an acid soil, where it is often present (Jager and Velvis, 1980). In 1982 *Pyxidiophora* species proved to be more common than in earlier years. *Gliocladium roseum* did not occur as general as it did on stolons or stems. *Volutella ciliata* occurred in most soils and colonized a variable but low percentage of the sclerotia. This was also the case with *Penicillium* species.

Streptomycetes colonized a rather high percentage of sclerotia, especially on sandy loam and clay loam soils with a neutral pH. They usually form small colonies that grow very slowly. Their effect on *R. solani* is not yet clear.

Notwithstanding the unfavourable soil conditions in 1982 (long spell of drought), *V. biguttatum* was able to colonize sclerotia and survived on subterranean parts of the potato plant, although its antagonistic activity seemed to be reduced substantially.

It is probable that *V. biguttatum* preferentially survives in soil in sclerotia, once formed on stolons, stems, roots and possibly tubers.

3.3. Yield

In 1981 the best yield of seed potatoes was produced from clean seed tubers, inoculated or not inoculated by antagonists. Infected seed tubers inoculated with antagonists then produced the lowest yield.
In 1982 the best average yield was obtained from non-disinfected seed tubers inoculated with *V. biguttatum* M73. Usually seed tubers inoculated with antagonists produced a better yield than the non-inoculated or disinfected. Calculated differences between the yield of disinfected and the best of inoculated non-disinfected seed tubers ranged from 0.1 to 4 tonnes per ha in 1982.

The total yields from the different fields varied in 1982 from about 21 to 44 tonnes per ha.

3.4. Inoculation at different dates

Table VIII presents the average sclerotium indices of seed and ware tubers, obtained after planting at different dates, harvested in early August and early October, respectively.

The weather in April and early May was cold and dry and the first leaves of the tubers planted on the first, second and third date emerged approximately on the same date in mid May. Notwithstanding these unfavourable weather conditions following the early planting date the inoculated propagules of *V. biguttatum* survived in sufficient numbers to give a protective effect.

The overall effect of inoculation with *V. biguttatum* M73 on the presence of *R. solani* differed significantly (p = 0.1%) from non inoculation, irrespective of dates of planting and harvesting (seed- or ware tubers).

TABLE VIII. Sclerotium indices of the harvest of inoculated (M73) and non-inoculated seed tubers, planted at different dates and harvested for seed- and ware potatoes.

TABEL VIII. Sclerotiumindices van de oogst van wel en niet geënt pootgoed, gepoot op verschillende data, geoogst voor pootgoed en consumptie (seed potatoes en ware potatoes).

Planting date	Yield			
	seed potatoes		ware potatoes	
	inoculated	not inoculated	inoculated	not inoculated
April 8	4.2 ± 2.3	13.8 ± 9.2	17.3 ± 11.1	22.8 ± 11.6
April 29	9.7 ± 5.0	9.7 ± 7.1	14.2 ± 14.5	24.6 ± 16.8
May 13	8.4 ± 5.7	11.3 ± 8.1	10.2 ± 10.5	19.6 ± 13.1
May 21	4.9 ± 5.6	8.2 ± 8.5	12.8 ± 15.5	20.9 ± 11.5

During the dry summer of 1982 the sclerotium indices of ware tubers were higher than those of seed tubers. In slightly acid sandy soil the reverse is often true (table III), due to a longer time of antagonistic activity. In this dry summer the antagonistic activity presumably was more inhibited than the growth of *R. solani*.

4. DISCUSSION

Inoculation of seed tubers with *Verticillium biguttatum* (M73), a hyperparasite of *R. solani* and inhabitant of the subterranean parts of the potato plant, proved to be effective in reducing infestation of plant parts by *R. solani* and the formation of sclerotia on new tubers.

In the field experiments of 1981 the variation of the sclerotium indices per treatment was rather low and the effect of the use of antagonists was statistically significant ($p = 0.1\%$). On one sandy soil, with a high inoculum density of *R. solani*, inoculation had no effect.

The results of the field experiments of 1982 showed a much greater variety. This was possibly due to the unfavourable effect of dry and warm weather on the activity of *V. biguttatum*. The activity of the antagonist may vary by different moisture contents of the different plots of the same field. The distribution of *R. solani* in a field is usually rather heterogeneous. Both factors combined can give a large variation in the values of the sclerotium index in a field at the end of the growing season.

Nevertheless, the average effect of inoculation of seed tubers with *V. biguttatum* M73 and with a mixture of *V. biguttatum* (M73 + M180) plus antagonistic *Azotobacter chroococcum* on average reduced the formation of sclerotia on new tubers on marine sandy loam and clay loam soils in 1982 ($p = 5\%$). In sandy soils the average effect of inoculation of seed tubers with *V. biguttatum* M73, alone or in combination with *V. biguttatum* M180, caused a significant reduction ($p = 5\%$) of the sclerotium index of the harvested tubers.

The effect of inoculation of seed tubers with *V. biguttatum* M73, however, is small or absent in acid sandy soils with a high inoculum density of *R. solani*. The inability of *V. biguttatum* M73 to reduce *R. solani* may be caused by two factors: *1*. the inoculum density of *R. solani* is too high, due to very favourable soil conditions and *2*. competition with *V.biguttatum* from the soil. The "wild" *V. biguttatum* from the soil probably consists of less effective antagonists which compete with M73 and displace it largely from the subterranean plant organs. (Fig. 1 and 2 show that, on sandy soil, inoculation of (infected) seed tubers at first reduces the disease index and the percentage of stolon pieces colonized by *R. solani*. This reduction disappeared at a later stage.) The antagonistic ability of *V. biguttatum* isolates proved to differ strongly (Jager and Velvis, unpublished). Apart from agressive hyperparasites, such as M73, we isolated up to now only two *V. biguttatum* individuals from sclerotia which, on agar, were completely inhibited by *R. solani*. (M73 and the latter presumably represent antagonistic extremes of the population in soils.) As M73 and the "wild" forms cannot be distinguished microscopically, the replacement of an inoculated effective antagonist by inferior wild types cannot be checked.

4.1. Effect on sclerotia and hyphae

The number of fungal species (or individuals within a species) that can grow on and kill metabolically inactive resting stages, such as sclerotia, is higher than the number that can kill living hyphae or control the disease caused by the pathogen involved. Aluko (1968) observed that *Gliocladium virens* could kill sclerotia of *R. solani* under suitable conditions of humidity (above 80%) and temperature (25 °C) within 3 - 4 weeks, but it proved, however, unsuitable for biological control when inoculated on seed tubers.

Fungal parasites of sclerotia of *R. solani* are known from studies of Naiki and Ui (1972) and of Jager *et al.* (1979) and Jager and Velvis (1980, 1982). The presence of hyperparasites on sclerotia of *Sclerotia* spp, *Botrytis cinerea* and *Claviceps purpurea* was studied by Karhuvaara (1960) and by Makkonen and Pohjakallio (1960). Rai and Saxena (1975) and Walker and Maude (1975) studied hyperparasites on sclerotia of *Sclerotinia sclerotiorum* and *Botrytis allii*, respectively. Most hyperparasites belonged to the genera *Penicillium, Trichoderma, Gliocladium, Aspergillus* and much less frequently *Fusarium, Cephalosporium* and *Acrostalagmus*. *Gliocladium roseum* was often mentioned.

A few hyperparasitic fungi, which were able to kill *R. solani* on agar plates, were used by Van den Boogert and Jager (1983) for biological control of *R. solani* on potato by inoculation on seed tubers. *G. roseum*, and *T. hamatum* proved to have no value as a control agent. *Verticillium biguttatum* (Gams and Van Zaayen, 1982) proved to be the only effective antagonist.

4.2. Methods of inoculation

The antagonists can be inoculated into the soil, but also on seed, tubers, bulbs, stecklings, etc. The first method needs large quantities of inoculation material and much labour to mix it homogeneously into the soil. Inoculation of seeds etc. is the most economic way.

Both methods are used to control damping off of germinating seeds and seedlings with success (Dunleavy, 1955; Merriman *et al.*, 1974[a], 1974[b]; Dhingra and Khare, 1973; Goel and Mehrotra, 1974; Wu Wen Shi, 1976; Kommedahl and Windels, 1978; Henis *et al.*, 1978; Howell and Stipanovic, 1979; Hadar *et al.*, 1979; Chet *et al.*, 1979; Odvody *et al.*, 1980; Harman *et al.*, 1980; Elad *et al.*, 1982). The control of damping off only lasts a relatively short period of about two weeks. Inoculated antagonists usually are present during two or three weeks and a rich choice of antagonists is often available (fungi, streptomycetes and bacteria).

To achieve a long lasting control, addition of large amounts of selected antagonists in food containing substrates (pellets) to the soil can be a successful method. Elad *et al.* (1980[b]) used wheat bran cultures of *Trichoderma harzianum* to control *R. solani* and *Verticillium dahliae* in potato fields. Integrated control (addition of an antagonist after solar heating or fumigation with methylbromide) proved very successful. Elad *et al.* (1980[b]) observed that *T. harzianum*, added to the soil, was effective against *R. solani* and *Sclerotium rolfsii*; its introduction reduced disease symptoms in beans, cotton and tomatoes and significantly increased yields of beans. In strawberry fields *T. harzianum* (in wheat bran culture added to the soil) also controlled *R. solani* and gave an increase in valuable early yield.

The addition of large amounts (400-600 kg/ha) of antagonists to the soil in a certain formulation can lead to killing of resting stages and hyphae, and spores of all species of pathogens they can attack. Since the antagonist is specialized in this "substrate", it can maintain itself for a long time without competing for food with the existing and offer protection to the crop.

This method, however, cannot be used on heavy soils.

4.3. Relation between plant and antagonist

Organisms reducing damping-off need to be present during the sensitive period of the young plant. They disappear afterwards because they have no special bond with the plant they protect; they can be regarded as *antagonists* that are *not associated with the plant involved.*

The relation between a *plant-associated* antagonist and its plant is specific. *Verticillium biguttatum*, for instance, is a natural inhabitant of the subterranean phytoplane of the potato plant. It colonizes the subterranean parts from the soil and from the tuber. Inoculation is quite simple and requires relatively small amounts of inoculum. Its presence on the organs that can be attacked by a pathogen leads to a reduced infestation, and gives protection that can last as long as the plants lives.

Liu Shan da and R. Baker (1980) carried out interesting experiments, whereby the suppression of the infestation of radish by *R. solani* was achieved by *Trichoderma* spp., of which the number increased in Fort Collins clay loam by growing radish in succession in the same (small amounts of) soil. The increase in *Trichoderma* could be due to an increase in *R. solani* as a result of a more frequent growth of radish, but could also be due, as we think, to the fact that *Trichoderma* is a radish-associated antagonist. Another interesting observation, of Liu Shan da and Baker, offers more clarity: the suppression of *R. solani* by *Trichoderma* spp. could be achieved by growing radish or cucumber in succession, but not by growing sugarbeet, alfalfa or wheat in monoculture. This may point to the fact that *Trichoderma* spp. are antagonists associated with radish and cucumber. Or, in other words: radish and cucumber possibly are plants *supporting the antagonist Trichoderma* in the soil used.

From earlier studies (Jager and Velvis, 1980) and later observations we conclude that oats possibly is a crop supporting antagonists, among which *Verticillium biguttatum*. *V. biguttatum* can thrive well on the roots of oats and can increase in mass. This relation and its significance for the sclerotium index of potatoes grown after oats (inoculated with *V. biguttatum* or not) will further be studied in field experiments. Meanwhile we continue to search for other crops and plants with properties supporting *V. biguttatum*. These could be used as crops preceding potatoes or as green manure crops grown before potatoes. Inoculation of these crops with agressive antagonistic isolates of *V. biguttatum* might lead to success.

4.4. Gliocladium roseum versus Verticillium biguttatum

In 1979 Jager *et al.* reported that *G. roseum* was the most important antagonist of *R. solani* in fields in the northern parts of the Netherlands. During the warm and dry summer of 1977 we isolated *G. roseum* from soil and parts of plant. As a result of the weather conditions *G. roseum* was more abundant than in normal cool and moist years. Colonies of *G. roseum* and *V. biguttatum* often were close together and isolates of conidia often were a mixture. The faster growing *G. roseum* overgrew the slow *V. biguttatum*, the presence of which was not further noticed. *G. roseum*, when growing on poor substrates, often has verticillate sporophores, which further enhanced confusion. Later we found that a colony can contain conidia of both fungi. The conidia can easily be kept apart and in this way the presence of the fungi can be established.

Contrary to our first statements (Jager *et al.*, 1979), not *G. roseum* but *V. biguttatum* is the most important antagonist of *R. solani* in potato fields in the Netherlands.

The role of *V. biguttatum* in biological and integrated control of *R. solani* in potato fields and in the destruction of sclerotia in heavily contaminated lots of seed tubers will be the subject of following reports.

5. SUMMARY

The inoculation of seed potatoes with the hyperparasitic fungus *Verticillium biguttatum*, isolate M73 (combined with *Gliocladium roseum* in 1981, alone or in a mixture with the isolate M180 plus the antibiotic *Azotobacter**) isolate J4 or J6 in 1982) often proved successful in repressing or even suppressing *R. solani* on stolons and stems. On average, this ultimately led to a significantly reduced formation of sclerotia on new tubers in field experiments, particularly in neutral sandy loam and clay soils. Compared with the sclerotium index of the tubers harvested from disinfected seed tubers, the inoculation with antagonists led to maximum reductions per field between 22 and 46% for tubers grown in slightly acid sands and from 0 to 89% for those grown in neutral sandy loam and clay loam soils in 1981. In 1982 the reductions varied from 9-35% and from 29-85%, respectively. Reasons for the often smaller reductions on acid sandy soils are discussed.

In 1981 the values for the sclerotium indices generally were higher than in 1982 for the tubers harvested from disinfected seed tubers. In 1982, however, the range of the sclerotium indices of replicates per field was much larger than in 1981. In both years the sclerotium index of the yield from inoculated seed tubers was, on average, nevertheless significantly lower than that of seed tubers that were not inoculated ($p = 0.1\%$ in 1981; $p = 5\%$ in 1982).

V. biguttatum was much more frequently present on stems and stolons of inoculated seed tubers than on those from tubers that were not inoculated. These became colonized by "wild" *V. biguttatum* from the soil; these antagonists often were inferior in controlling *R. solani*.

Inoculation of tubers that were planted early, at a time that the soil temperature did not yet permit growth of *V. biguttatum*, nevertheless, proved effective.

The possibility to enhance the population of *V. biguttatum* in field soil with the aid of "crops supporting *V. biguttatum*" is suggested.

---

*) *Azotobacter chroococcum*

6. SAMENVATTING

Biologische bestrijding van Rhizoctonia solani in aardappelen met Verticillium biguttatum en andere antagonisten in veldproeven in 1981 en 1982

Het enten van poters met de op *Rhizoctonia solani* parasiterende (hyperparasitaire) schimmel *Verticillium biguttatum* (isolaat M73) in combinatie met *Gliocladium roseum* in 1981 en met *V. biguttatum* (M73) alleen, of in mengsel met isolaat M180 plus antibiotische bacteriën, nl. *Azotobacter chroococcum* (isolaten J4 en J6) in 1982 bleek gunstig voor het terugdringen of het onderdrukken van *R. solani* op stengels en stolonen en het verminderen van de aantasting (figuren 1 en 2).

De meest effectieve enting leidde op zandgrond in 1981 tot een gemiddelde sclerotiumindex, die per veld varieerde van 54-78% van die waarde die werd verkregen als werd uitgegaan van ontsmette poters; op klei- en zavelgronden varieerde dit percentage van 100 (waar de sclerotiumindex van de oogst uit ontsmet pootgoed al zeer laag was) tot 11. In 1982 varieerden deze percentages van 65-91% op zandgrond en van 15-71% op klei- en zavelgronden (tabellen 3 en 6).

In 1981 was de vorming van sclerotiën (lakschurft) op de oogst uit ontsmet pootgoed groter dan in 1982. In 1982 was de spreiding tussen de herhalingen van eenzelfde behandeling per veld aanzienlijk groter. In beide jaren leidde enting van het pootgoed met antagonisten tot waarden voor de sclerotiumbezetting van de oogst die niettemin gemiddeld betrouwbaar lager waren dan die van niet geënt pootgoed ($p = 0,1\%$ in 1981; $p = 5\%$ in 1982).

V. biguttatum was veel vaker aanwezig op ondergrondse stengeldelen en stolonen van planten uit geënt pootgoed dan op die van niet geënte. De laatste werden gekoloniseerd door "wilde" *V. biguttatum* vanuit de grond; dit waren vaak minder effectieve antagonisten.

Enting van vroeg gepote knollen - bij een temperatuur die nog onvoldoende hoog is voor de groei van *V. biguttatum* - leverde toch gunstige resultaten (tabel 8) op.

De mogelijkheid om de populatie van *V. biguttatum* in de grond te versterken door de verbouw van, bij voorkeur geënte "*V. biguttatum* (of andere antagonisten) ondersteunende gewassen" wordt genoemd.

7. LITERATURE

Aluko, M.O., 1968. Microbial antagonists in the control of *Rhizoctonia solani* Kühn on potatoes. Thesis Univ. Nottingham.

Boogert, P.H.J.F. van den and Jager, G., 1984. Biological control of *Rhizoctonia solani* on potatoes by antagonists. 3. Inoculation of seed potatoes with different hyperparasitic fungi. Neth. J. Plant Path. (in press).

Butler, E.J. and Jones, S.G., 1961. Plant Pathology. Macmillan, London, 979 pp.

Chet, I., Hadar, Y., Elad, Y., Katan, J. and Henis, Y., 1979. Biological control of soil-borne plant pathogens by *Trichoderma harzianum*. In: B. Schepers and W. Gams (eds.), Soil borne plant pathogens, Academic Press, London, pp. 585-591.

Dhingra, O.D. and Khare, M.N., 1973. Biological control of *Rhizoctonia bataticola* on urid bean. Phytopath. Z. 76: 23-29.

Dunleavy, J., 1955. Control of damping off of sugar beet by *Bacillus subtilis*. Phytopathology 45: 252-258.

Elad, Y., Chet, I. and Katan, J., 1980[a]. *Trichoderma harzianum:* a biocontrol agent effective against *Sclerotium rolfsii* and *Rhizoctonia solani*. Phytopathology 70: 199-121.

Elad, Y., Katan, J. and Chet, I., 1982[b]. Physical, biological and chemical control integrated for soi-borne diseases in potatoes. Phytopathology 70: 418-422.

Elad, Y., Kalfon A. and Chet, I., 1982. Control of *Rhizoctonia solani* by seed coating with *Trichoderma* spp. spores. Plant Soil 66: 279-281.

Gams, K.W. and Van Zaayen, A., 1982. Contribution to the taxonomy and pathogenicity of fungiculous *Verticillium* species. I. Taxonomy. Neth. J. Plant Pathol. 88: 57-78.

Goel, S.K. and Mehrotra, R.S., 1974. Biological control of the root and collar rot of okra (*Abelmoschus esculentus*, L, Moench). Ann. Inst. Pasteur (Paris) 125[a]: 365-370.

Hadar, Y., Chet, I. and Henis, Y., 1979. Biological control of *Rhizoctonia solani* damping-off with wheat-bran culture of *Trichoderma harzianum*. Phytopathology 69: 64-68.

Harman, G.E., Chet, I. and Baker, R., 1980. *Trichoderma hamatum* effects on seed and seedling disease induced in radish and pea by *Pythium* spp. or *Rhizoctonia solani*. Phytopathology 70: 1167-1172.

Henis, Y., Ghaffar, A. and Baker, R., 1978. Integrated control of *Rhizoctonia solani* damping-off of radish: Effect of successive plantings, PCNB, and *Trichoderma harzianum* on pathogen and disease. Phytopathology 68: 900-907.

Howell, C.R. and Stipanovic, R.D., 1979. Control of *Rhizoctonia solani* on cotton seedlings with *Pseudomonas fluorescens* and with an antibiotic produced by the bacterium. Phytopathology 69: 480-482.

Jager, G., Hoopen, A. ten and Velvis, H., 1979. Hyperparasites of *Rhizoctonia solani* in Dutch potato fields. Neth. J. Pl. Path. 85: 243-268.

Jager, G. and Velvis, H., 1980. Onderzoek naar het voorkomen van Rhizoctonia-werende aardappelpercelen in Noord-Nederland. Inst. Bodemvruchtbaarheid, Rapp. 1-80, 60 pp. (With an English summary: Occurrence of *Rhizoctonia solani*-suppressing potato fields in the nothern part of the Netherlands).

Jager, G. and Velvis, H., 1982. Aantasting van aardappel door *Rhizoctonia solani* in afhankelijkheid van de herkomst en de mate van besmetting van het pootgoed. Inst. Bodemvruchtbaarheid, Rapp. 4-82, 23 pp. (With an English summary: Infestation of potato with *Rhizoctonia solani* as affected by origin and severity of infection of the seed stock).

Jager, G. and Velvis, H., 1983[a]. Suppression of *Rhizoctonia solani* in potato fields. 1. Occurrence. Neth. J. Plant Path. 89: 21-29.

Jager, G. and Velvis, H., 1983[b]. Suppression of *Rhizoctonia solani* in potato fields. 2. Effect of origin and degree of infection with *Rhizoctonia solani* of seed potatoes on subsequent infestation and on formation of sclerotia. Neth. J. Plant Path. 89: 141-152.

Jager, G. and Velvis, H., 1983[c]. Biological control of *Rhizoctonia solani* on potato by antagonists. 2. Sprout protection against soil-borne *R. solani* through seed inoculation with *Verticillium biguttatum*. Neth. J. Plant Path. (in press).

Karhuvaara, L., 1960. On the parasites of the sclerotia of some fungi. Acta Agric. Scand. 10: 127-134.

Kommedahl, T. and Windels, C.E., 1978. Evaluation of biological seed treatment for controlling root diseases of peas. Phytopathology 68: 1087-1095.

Liu Shan da and Baker, R., 1980. Mechanism of biological control in soil suppressive to *Rhizoctonia solani*. Phytopathology 70: 404-412.

Makkonen, R. and Pohjakallio, O., 1960. On the parasites attacking the sclerotia of some fungi pathogenic to higher plants and on the resistance of these sclerotia to their parasites. Acta Agric. Scand. 10: 105-126.

Merriman, P.R., Price, R.D. and Baker, K.F., 1974[a]. The effect of inoculation of seed with antagonists of *Rhizoctonia solani* on the growth of wheat. Aust. J. Agric. Res. 25: 213-218.

Merriman, P.R., Price, R.D., Kollmorgen, J.F., Pigott, T. and Ridge, E.H., 1974[b]. Effect of seed inoculation with *Bacillus subtilis* and *Streptomyces griseus* on the growth of cereals and carrots. Aust. J. Agric. Res. 25: 219-226.

Meshram, S.U. and Jager, G., 1983. Antagonism between *Azotobacter chroococcum* isolates and *Rhizoctonia solani*. Neth. J. Plant Path. (in press).

Naiki, T. and Ui, T., 1972. The microorganisms associated with the sclerotia of *Rhizoctonia solani* Kühn in soil and their effect on the viability of the pathogens. Mem. Fac. Agric. Hokkaido Univ. 8: 252-265.

Odvody, G.N., Boosalis, M.G. and Kerr, E.D., 1980. Biological control of *Rhizoctonia solani* with a soil-inhabiting basidiomycete. Phytopathology 70: 655-658.

Rai, J.N. and Saxena, V.C., 1975. Sclerotial mycoflora and its role in natural biological control of white-rot disease. Plant Soil 43: 509-513.

Velvis, H. and Jager, G., 1983. Biological control of *Rhizoctonia solani* on potatoes by antagonists. I. Preliminary experiments with *Verticillium biguttatum*, a sclerotium-inhabiting fungus. Neth. J. Pl. Path. 89: 113-123.

Walker, Jane A. and Maude R.B., 1975. Natural occurrence and growth of *Gliocladium roseum* on the mycelium and sclerotia of *Botrytis alii*. Trans. Br. mycol. Scc. 65: 335-337.

Wu Wen Shi., 1976. Biological control of seed- and soil-borne fungi with wheat and oats. Bot. Bull. Acad. Sin. (Taipeh) 17: 161-168.