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Biological control of Rhizoctonia solani on potatoes by antagonists. 4. Inoculation of seed tubers with Verticillium biguttatum and other antagonists in field experiments

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Abstract

Inoculation of seed potatoes with the mycoparasite *Verticillium biguttatum,* isolate M73 (combined with *Gliocladium roseum* in 1981, either alone or mixed with isolate M180 plus antibiotics-producing isolates of *Azotobacter chroococcum* in 1982) repeatedly proved successful in reducing *Rhizoctonia solani* on stolons and stems. In field experiments, this ultimately led to a reduced formation of sclerotia on new tubers, particularly in neutral sandy loam and clay loam soils. In 1981 inoculation with antagonists led, when compared with no inoculation, to average reductions of 22 and 42% for the harvest from clean, and 15 and 26% for the harvest from infected seed tubers grown on slightly acid sandy soils and on neutral loam soils, respectively. The harvest from clean, inoculated seed tubers had the lowest sclerotium index. In 1982 inoculation of seed tubers planted in slightly acid sandy soils gave reductions of the sclerotium index of up to 22%. In the neutral marine loam soils considerable reductions were often achieved, viz., in slightly infected loams 51-68% and in rather heavily infected ones 4-43%. Chemical disinfection of seed tubers proved effective only in loam soils that were slightly infested with *R. solani.* In both years inoculation of seed tubers with antagonists led to significantly lower sclerotium indices of the harvest ($p = 0.1\%$ in 1981; $p = 5\%$ in 1982).

V. biguttatum was present more frequently and in greater densities on stems and stolons of plants from inoculated than from non-inoculated seed tubers. The latter were colonized by wild *V. biguttatum* strains from the soil, apparently less effective antagonists.

Early in the season, the soil temperature was too low for growth of *V. biguttatum.* Nevertheless, inoculation of tubers that were planted early resulted in a considerable control of *R. solani.*

Additional keywords: disease index, sclerotium index, suppression, mycoparasites, *Azotobacter chroococcum, Cylindrocarpon destructans, Gliocladium roseum, G. virens, G. nigrovirens, Hormiactis fimicola, Penicillium* spp., *Pyxidiophora* sp., *Streptomyces* spp., *Trichoderma* spp., *Volutella ciliata.*

Introduction

In field experiments we compared the infestation of plants by *Rhizoctonia solani* and the formation of sclerotia on new tubers of potato plants originating from seed tubers produced on a neutral, holocene, marine loam soil and from seed tubers produced on a slightly acid, pleistocene sand (Jager and Velvis, 1983b).

Plants from seed tubers produced on sand were less infested with *R. solani* than plants from seed tubers produced on a marine loam soil even when the sand seed tubers had more sclerotia. The sclerotium index of the tubers harvested from a slightly suppressive sandy soil and from two marine loam soils was definitely lower when the seed tubers had been produced on the sandy soil. We assumed that these seed tubers had a heavier load of antagonists. As the antagonists from this slightly acid sandy soil also thrived in a neutral marine loam and gave some protection, we concluded that the antagonists were associated more with the plant than with the soil.

In earlier studies we observed that *Verticillium biguttatum* was a parasite of sclerotia of *R. solani.* The fungus is very common in acid sandy soils, but in general less common in neutral marine soils (Jager and Velvis, 1980, 1983a). Velvis and Jager (1983) observed that certain isolates of *V. biguttatum* could kill sclerotia within seven weeks under high relative humidity and at a temperature of 15 °C or higher. The isolates varied widely in antagonistic activity. One isolate, M73, was outstanding; when it had been inoculated on germinating seed tubers it was found to be present on the surface of the sprouts up to the soil surface and gave protection to the sprouts against infection by *R. solani* from sclerotia on the seed tuber (Velvis and Jager, 1983) and from soil-borne *R. solani* (Jager and Velvis, 1984). These properties would make *V. biguttatum* very suitable for biological control of *R. solani* in potato fields.

Results of attempts to control *R. solani* in potatoes grown on various soils, by inoculation of the seed tubers with *V. biguttatum* and other antagonists, including *Azotobacter chroococcum* (Meshram and Jager, 1983; Meshram, 1984), are reported here.

Materials and methods

The cultivar Bintje was used in the field experiments in 1981 and 1982, unless otherwise stated. The seed tubers used in 1981 had been produced on slightly acid sand soils and on neutral marine sandy loam and clay loam soils. After washing, five lots of seed tubers from each origin were prepared:

1) selected clean seed tubers (i.e. without sclerotia of *R. solani)*;
2) selected clean seed tubers, inoculated with antogonists;
3) lightly infected seed tubers (natural infection);
4) lightly infected seed tubers, inoculated with antagonists;
5) disinfected seed tubers (tubers were disinfected with formaldehyde according to Butler and Jones (1955); the absence of live *R. solani* and antagonists was ascertained; when thick sclerotia proved not to be dead, they were removed manually).

In both years the seed tubers were pregerminated in daylight in a greenhouse. Thereafter the tubers of lots 2 and 4 were inoculated. Those of the other lots were treated with the carrier fluid of the inoculant only (1% carboxymethylcellulose (c.m.c.) in water, plus 30% clay (subsoil) in 1981). For inoculation five agar layers (15 cm diam.) with sporulating *V. biguttatum* (M73) plus one agar layer *Gliocladium roseum* were added to 5 1 carrier fluid in 1981.

In 1982, only seed tubers produced on a neutral loam soil were used, being a mixture of clean and lightly infected tubers. Inoculants were *V. biguttatum* M73, *V. biguttatum* M180, *A. chroococcum* (on neutral loam soil only), or *Gliocladium nigrovirens* (on sand soil and one sandy loam soil), or a mixture of three antagonists. One lot of the seed tubers was disinfected, to get an impression of the amount of soil-borne *R. solani.*

In 1981 the inoculated seed tubers were quickly dried in an air stream and planted two days afterwards. Drying, however, caused a considerable loss of live *V. biguttatum* propagules. In 1982, therefore, the inoculated tubers were kept moist in plastic containers and planted the next day. The carrier fluid was 1% c.m.c., plus 0.3% gelatin and 6% clay in water.

Aerial parts of the plants were killed chemically three weeks before harvest. The tubers were harvested both as seed and as ware potatoes in 1981 and as seed only in 1982.

From a number of fields plant samples were taken in the course of the growing season to determine the infestation of stems and stolons with *R. solani* and to assess the presence of *R. solani* and its hyperparasites on stem and stolon pieces. The methods were described by Van den Boogert and Jager (1984). The rate of infestation is presented as the disease index, calculated according to Jager and Velvis (1983b), but the values were now divided by 5, the theoretical maximum thus being 100.

The experimental fields were laid out in a randomized split plot design (nine treatments replicated six times in 1981, and six treatments replicated twelve times in 1982).

The minimum temperature for growth of *V. biguttatum* is 12 °C (Van den Boogert and Jager, 1984). Potato tubers, however, are planted from the end of March or early April onward, when soil temperatures are often lower. To examine whether early planting would affect the results of inoculation with *V. biguttatum* M73 an additional experiment was laid out on the sandy soil at Haren as a randomized split plot with four planting dates, inoculated and non-inoculated seed tubers, and a harvest of seed and ware potatoes. Each treatment was replicated eight times. A late-ripening cultivar, Irene, was used.

Harvested tubers were washed, dried, classified per plot and weighed per size class. The weight (kg) of each class was multiplied by a factor as follows: very lightly speckled with sclerotia (vl) x 1; lightly (l) x 2; moderately (m) x 3 and heavely (h) x 4. The sclerotium index (s.i.) was calculated according to:

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

The theoretical maximum value thus is 100.

Relevant soil properties are given in Table 1.

Results

The results obtained in the two years will be given per year. If the year is not mentioned the results or conclusions are valid for both years.

Disease index (Table 2). As expected, plants from infected seed tubers were more severely infested than plants from disinfected or clean seed tubers. Plants from disinfected seed tubers were often more severely infested than plants from clean seed tubers. In general, plants from infected sand seed suffered less from infestation than those from infected clay seed. Clean seed tubers produced plants with low disease indices. The effect of inoculation with antagonists was variable. Inoculation of lightly

Table 1. Relevant properties of the soil of some of the experimental fields in 1981 and 1982.

	pH (KCl)	Coarse sand 210-2000 μm (%)	Silt 2-50 μm (%)	Particles <16 μm (%)	Clay <2 μm (%)	Org. matter (%)	K_2O (mg/kg)	MgO (mg/kg)	P_{water} (mg/l)	$CaCO_3$ (%)
1981										
Haren[1]	4.7	10	35	7	–	4.6	160	104	15	–
Borger[1]	4.9	17	15	4	–	4.7	120	92	31	–
Zuurdijk	7.4	0	71	34	–	1.7	210	126	18	–
1982										
Gasselte[1]	5.4	12	18	5	–	5.9	210	93	73	–
Usquert	7.2	0	56	17	–	2.4	180	66	53	3
Sexbierum	7.3	1	38	22	14	4.3	340	–	85	2

[1] Pleistocene sandy soils.

Table 2. Average disease indices of stems and stolons of plants from seed potatoes with various treatments[1].

	Disinfected seed tubers	Not disinfected seed tubers			
		clean	clean + antagonist	infected	infected + antagonist
1981					
Haren	24	6 (2)	2 (6)	21 (58)	27 (64)
Borger	15	12 (26)	15 (14)	38 (49)	39 (83)
Zuurdijk	7	2 (1)	1 (1)	28 (41)	17 (25)

	Disinf. seed tubers	Not disinf. seed tubers	Not disinf. seed tubers + antagonists			
			M73[2]	M180[2]	A3[3]	mix.
1982						
Gasselte	11	15	16	22	18	11
Usquert	1	23	6	5	7	9
Sexbierum	16	15	9	11	12	7

[1] Figures in brackets refer to plants from clay seed.
[2] M73 and M180 are isolates of *Verticillium biguttatum.*
[3] A3: *Gliocladium nigrovirens* at Gasselte and Usquert; *Azotobacter chroococcum* elsewhere.

infected seed tubers with antagonists *(V. biguttatum* plus *G. roseum)* reduced the infestation of stems and stolons on marine loam soils. On sandy soils the effect of inoculation was variable and often absent.

In 1982 observations were made only twice, in June. Inoculation of seed tubers with *V. biguttatum* led to a reduced disease index of plants in marine soils; in sandy pleistocene soils the results were variable.

R. solani on stems and stolons (Table 3). The effect of inoculation of seed tubers with antagonists on the presence of *R. solani* on stems and stolons cannot be measured by the disease index alone, because this concerns the pathogenic *R. solani* strains only; the percentage of stolon pieces colonized by *R. solani* is a better parameter, which can also provide information about a possible suppressive effect of antagonists on the surface of stolons.

Inoculation of seed tubers with antagonistic (i.e. mycoparasitic and antibiotic) fungi generally reduced the percentage of stolon pieces colonized by *R. solani.*

Stolons of plants from clay seeds were more densily colonized by *R. solani* than those of plants from sand seed, and stolons from disinfected seed more so than stolons from clean seed.

The highest colonization ratings were generally recorded on stolons of plants from infected seed tubers and of plants grown in soils heavily infested with *R. solani.* The effect of added antagonists was small or absent in these soils. The variation between fields – even with the same soil type – was large.

Table 3. Average percentage stolon pieces showing live *R. solani* obtained from plants grown from differently treated seed potatoes[1].

	Disinfected seed tubers	Not disinfected seed tubers			
		clean	clean + antagonist	infected	infected + antagonist
1981					
Haren	35	16 (19)	10 (15)	33 (48)	30 (43)
Borger	23	19 (27)	19 (17)	60 (67)	28 (46)
Zuurdijk	18	4 (6)	7 (6)	38 (53)	11 (23)

	Disinf. seed tubers	Not disinf. seed tubers	Not disinf. seed tubers + *V. biguttatum*			
			M73[2]	M180[2]	A3[3]	mix.
1982						
Gasselte	14	22	23	22	25	19
Usquert	8	12	3	4	9	8
Sexbierum	17	14	7	14	11	6

[1] See footnotes in Table 2.

Mycoparasites of R. solani on stems and stolons. Observations were made on stolons of plants grown on soils mentioned in Table 1. Results are given in Table 4 for 1981 and Table 5 for 1982.

Stolons from disinfected seed tubers became colonized by soil-borne *V. biguttatum* and *G. roseum*. These wild strains proved less effective in suppressing *R. solani.* The colonization of stolons was on average higher in the slightly acid sands than in the neutral marine loams.

Stolons of plants from seed tubers with sclerotia usually had a higher density of mycoparasites than those from clean or disinfected seed tubers. Probably a part of the sclerotia had already been infected by mycoparasites.

Inoculation of seed tubers with antagonists led to high percentages of colonized stolon pieces and a more dense colonization of *V. biguttatum* on stolons. Effectively high values were already present in the beginning of the season and remained to the end. More detailed information has been given in a report (Jager and Velvis, 1983c).

In 1982, the most frequently observed hyperparasites on *Rhizoctonia* plates were *V. biguttatum* and *Gliocladium* spp. *(G. roseum, G. nigrovirens* and *G. solani).* Regularly, but less frequently and in smaller amounts, *Pyxidiophora* spp. occurred in one sand and one loam soil. It was quite rare in another loam soil in 1982. *Trichoderma* spp. usually occurred most frequently in acid sand but much less so in neutral clay loams. *Volutella ciliata, Penicillium* spp. and *Cylindrocarpon destructans* were present in sandy soil and least in the neutral marine loam.

Sclerotium index of the harvest. Table 6 presents the average sclerotium indices of seed tubers harvested from the different experimental fields in 1981. The results from the

Table 4. Density of *V. biguttatum* on stolon pieces in the course of the growing season and the average percentage of stolon pieces with *V. biguttatum* and *Gliocladium* spp. and the average density of *V. biguttatum* on stolon pieces in 1981.

Treatment of seed tubers	Sampling dates at Haren								Sampling dates at Zuurdijk							
	6/15	7/6	7/27	8/17	9/7	average[1]			6/9	6/30	7/20	8/10	8/31	average[1]		
						V%	G%	V.am						V%	G%	V.am
Sand seed tubers																
Clean	144	148	58	142	149	65	29	128	42	224	98	58	149	53	16	114
Clean + ant.	132	249	123	123	288	85	59	183	337	352	255	194	136	88	49	255
Infected	128	156	180	125	310	87	36	188	57	162	242	57	48	56	25	133
Inf. + ant.	119	277	262	240	289	92	70	237	258	339	279	227	240	99	65	269
Disinfected	73	39	170	248	284	73	17	163	79	258	44	17	109	45	21	101
Clay seed tubers																
Clean	132	208	82	106	207	73	42	147	29	148	39	3	76	28	16	59
Clean + ant.	220	226	208	93	292	89	82	208	271	371	157	75	146	83	77	204
Infected	230	274	239	218	306	97	49	253	170	216	192	76	274	80	34	186
Inf. + ant.	–	263	307	234	324	100	60	282	241	327	250	98	275	98	68	238

[1] V% = percentage of stolon pieces with *V. biguttatum; G% = idem with G. roseum;* V.am = density of *V. biguttatum* (maximum 400).

Table 5. Density of *V. biguttatum* on stolon pieces in the course of the growing season and the average percentage of stolon pieces with *V. biguttatum* and *Gliocladium* spp. and the average density of *V. biguttatum* on stolon pieces in 1982.

Treatment of seed tubers	Sampling dates at Gasselte						
	6/15	6/24	7/5	7/14	average[1]		
					V%	G%	V.am
Disinfected	20	285	6	206	46	73	129
Not disinfected	120	228	208	375	75	73	233
Idem + *V.big.* M73	277	457	173	268	91	72	294
Idem + *V.big.* M180	411	376	318	345	96	80	363
Idem + *Gliocladium nigrovirens*	401	171	280	315	87	77	292
Idem + mix[2]	250	422	282	277	89	71	308

Treatment of seed tubers	Sampling dates at Usquert						
	6/16	6/28	7/7	7/19	average[1]		
					V%	G%	V.am
Disinfected	369	25	87	–	36	63	120
Not disinfected	404	142	113	9	48	69	167
Idem + *V.big.* M73	480	322	211	174	89	82	297
Idem + *V.big.* M180	437	91	276	17	59	68	205
Idem + *Gliocladium nigrovirens*	–	–	–	–	–	–	–
Idem + mix[2]	490	263	273	142	85	76	292

Treatment of seed tubers	Sampling dates at Sexbierum						
	6/27	7/2	7/12	7/21	average[1]		
					V%	G%	V.am
Disinfected	51	122	180	208	57	42	140
Not disinfected	150	62	283	188	60	17	171
Idem + *V.big.* M73	414	119	266	254	88	28	263
Idem + *V.big.* M180	469	212	249	396	92	47	332
Idem + *Gliocladium nigrovirens*	–	–	–	–	–	–	–
Idem + mix[2]	283	217	138	371	80	84	252

[1] V% = percentage of stolon pieces with *V. biguttatum;* G% = idem with *G. roseum;* V.am = density of *V. biguttatum* (maximum 500).
[2] The mixture used at Gasselte and Usquert consisted of the three fungi mentioned; at Sexbierum *Azotobacter chroococcum,* strain J4, was used instead of *G. nigrovirens.*

Table 6. Effect of disinfection or inoculation with antagonists of planted tubers on the average sclerotium indices of the seed potato harvest from experimental fields on slightly acid pleistocene sandy soils and on neutral holocene sandy loam and clay loam soils in 1981.

	Seed tubers with sclerotia of *R. solani*			Selected clean seed tubers	
	disinfected	untreated	inoculated	untreated	inoculated
Three sandy soils					
Sclerotium index	37.3 ± 9.3	42.9 ± 5.0	36.4 ± 7.5	32.9 ± 10.0	25.5 ± 11.8
Reduction (% of untreated)	13	–	15	–	22
Relative s.i. (disinfected = 100)	100	115	98	88	68
Four loam soils					
Sclerotium index	14.6 ± 7.2	33.7 ± 8.6	19.7 ± 8.7	10.4 ± 5.1	7.7 ± 5.6
Reduction (% of untreated)	57	–	41	–	26
Relative s.i. (disinfected = 100)	100	231	135	71	53

fields on slightly acid sands and on neutral marine loam soils are presented separately, as the soils behave differently towards *R. solani* (Jager and Velvis, 1983b). The reductions in the sclerotium index due to the application of antagonists and due to disinfection are shown. Relative sclerotium indices, using the sclerotium index of disinfected seed tubers as a reference, are also given. The results for clay and sand seed tubers were combined as they were not essentially different here.

On sandy soils, the harvest from disinfected seed tubers had a fairly high sclerotium index due to *R. solani* infection from the soil. Clean seed tubers produced a harvest with a significantly lower sclerotium index (p = 1%) on one field only when antagonists had been applied to the seed. The harvest from infected seed tubers had the highest sclerotium index, which was reduced by the application of antagonists. The effect of inoculation with antagonists was only slight or absent in the soils that were rather heavily infested with *R. solani.* On sandy loam and clay loam soils, the sclerotium index of the harvest obtained from disinfected seed tubers was rather low. Clean and especially inoculated clean seed tubers produced harvests with low sclerotium indices. Infected seed tubers produced a harvest with the highest sclerotium index, which was markedly reduced by the application of antagonists (Table 6). The great value of clean seed tubers for the production of a healthy crop is clear from these experiments.

The overall effect of inoculation of seed tubers with antagonists calculated for the sclerotium indices of all experiments was positive for clean (p = 5%) and infected seed tubers (p = 1%). The influence of the various treatments of the seed tubers on the average sclerotium indices of the harvest in 1982 is presented in Table 7.

The lowest average sclerotium indices were found where the seed tubers were inoculated with *Verticillium biguttatum* M73, alone or in a mixture. The effect of M180

Table 7. Average sclerotium indices of harvested seed potatoes, reduction of the sclerotium index by either disinfection or by inoculation with antagonists in 1982.

	Disinfected seed tubers	Not disinfected seed tubers			
		untreated	inoculated with		
			V. bigutt. M73	*V. bigutt.* M180	mixture
Two sandy soils					
Sclerotium index	22.2 ± 13.9	21.5 ± 14.6	16.7 ± 12.7	20.6 ± 13.3	16.8 ± 12.1
Reduction (%)	−3	–	22	4	22
Relative s.i.	100	103	75	93	76
One sandy soil[1]					
Sclerotium index	47.3 ± 9.8	44.3 ± 9.6	40.2 ± 9.7	44.4 ± 11.5	42.9 ± 10.0
Reduction (%)	−7	–	9	0	3
Relative s.i.	100	94	85	94	91
Five loam soils					
Sclerotium index	7.0 ± 8.6	11.4 ± 10.4	3.7 ± 4.3	5.6 ± 6.7	4.2 ± 5.2
Reduction (%)	39	–	68	51	63
Relative s.i.	100	163	53	80	60
Two loam soils[1]					
Sclerotium index	25.8 ± 15.8	23.7 ± 14.2	17.0 ± 11.3	22.8 ± 11.9	13.4 ± 11.0
Reduction (%)	−9	–	28	4	43
Relative s.i.	100	92	66	88	52

[1] Heavily or rather heavily infested with *R. solani.*

was on average not as good as that of M73, although in most cases the differences were insignificant. The treatments had no effect in a heavily infested sand soil. In rather heavily infested loam soils inoculation of seed tubers with the individual antagonists, and especially the mixture of antagonists, reduced the average sclerotium index. Inoculation significantly reduced the sclerotium index in the experiments in 1982 ($p = 5\%$).

Disinfection proved to be effective in producing a harvest with less sclerotia if the infection from the soil with *R. solani* was very low, as was already observed in earlier experiments (Jager and Velvis, 1980, 1983a, b).

Inoculation and planting on various dates. Table 8 presents the average sclerotium indices of seed and ware tubers harvested after planting and inoculation on different dates. Seed tubers were harvested in early August and ware tubers in early October. Notwithstanding the unfavourable weather conditions prevailing when the seed tubers were planted early, the inoculated propagules of *V. biguttatum* survived in sufficient numbers to give a protective effect.

The overall effect of inoculation with *V. biguttatum* M73 was significant ($p = 0.1\%$), irrespective of planting and harvest dates (seed or ware tubers). Ware potatoes

Table 8. Sclerotium indices of the harvest from inoculated and non-inoculated seed tubers planted at different dates and harvested as seed and ware potatoes. *Verticillium biguttatum* M73 was the inoculant.

Planting date	Harvested seed potatoes from		Harvested ware potatoes from	
	inoculated seed	non-inoculated seed	inoculated seed	non-inoculated seed
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

had higher sclerotium indices than the seed potatoes. This is presumably due to drought, during which *R. solani* was still active, whereas the antagonists lost their activity.

Discussion

The sclerotium indices in 1981 showed a narrower range than in 1982. The average effect of inoculation was significant in both years. The greater variation in 1982 could be due to the use of not uniformly infected seed and the unfavourable effect of warm and dry weather on the activity of *V. biguttatum*. Moreover, the distribution of *R. solani* in a field is usually heterogeneous and densities beyond certain, hitherto unknown, limits cannot be controlled. These factors combined can result in large variations in the sclerotium index of the harvest from different plots in a field.

In sandy soils the effectiveness of inoculation of seed tubers in reducing the sclerotium index of the harvest is often low or even absent. Usually, this effect is smaller in sandy soils than in marine clay or loam soils with a similar *R. solani* infection from the soil. The inability of *V. biguttatum* M73 to repress *R. solani* in some sandy soils may be caused by two factors: (1) a too high inoculum density of *R. solani,* and (2) competition of wild *V. biguttatum* strains from the soil with *V. biguttatum* M73. The wild *V. biguttatum* often are less effective antagonists, which possibly largely displace M73 from the subterranean parts of the potato plant. Van den Boogert and Jager (1984) observed that plants from non-inoculated seed tubers can harbour a dense population of *V. biguttatum* which was not effective in reducing the sclerotium index of new tubers. This interspecific competition possibly is a great drawback in biological control of *R. solani* in soils containing a relatively dense population of less effective *V. biguttatum* strains.

Attack of sclerotia and hyphae. The number of fungi being able to grow on and to kill metabolically inactive resting structures of a pathogen, such as sclerotia, is higher than the number that kill living and antibiotics-producing hyphae and control the disease caused by the pathogen involved. Fungal parasites of sclerotia of *R. solani* are known from studies of Naiki and Ui (1972), Jager et al. (1979) and Jager and Velvis (1980, 1982).

A few mycoparasites, which were able to kill *R. solani* on agar plates, were inoculated on seed tubers by Van den Boogert and Jager (1984) for biological control of *R. solani* on potato. *Gliocladium roseum, Hormiactis fimicola* and *Trichoderma hamatum* isolates were found to be without value as control agents. *Gliocladium virens* was earlier found to be unsuitable for biological control of *R. solani* in the field by Aluko (1968). Only *V. biguttatum* was effective in our experiments.

Methods of inoculation. The antagonists can be introduced into the soil or on seeds, tubers, bulbs and stecklings. Soil inoculation can be used successfully in light soils, but it requires much inoculum and much labour to mix it homogeneously with the soil. Inoculation of planting material is the most economical way. Both methods are used to control damping-off in germinating seeds and seedlings (Merriman et al., 1974a, b; Chet et al., 1979; Harman et al., 1980; Elad et al., 1982). The control of damping-off is effective for about two weeks only. Inoculated antagonists are present at least during that period. Many antagonists are available for this purpose, among which fungi, streptomycetes and bacteria.

For long-term control, addition to the soil of large amounts (440-600 kg/ha) of selected antagonists in substrates that contain the proper nutrients can be successful (Elad et al., 1980 a, b). Such additions can lead to the killing of resting structures, hyphae and spores of all species of pathogens subject to attack. As the antagonist is specialized on the living substrate, it can maintain itself for a long time without competing for food with the existing microflora and offer protection to the crop.

Plant-associated and non-plant-associated antagonists. Organisms that reduce damping-off need to be present during the susceptible period of the young plant. Usually they disappear afterwards, because they have no special association with the plant they protect.

The relation between a plant-associated antagonist and its associate is specific. *V. biguttatum,* for instance, seems to be a natural inhabitant of the subterranean phytoplane of the potato plant. It colonizes the subterranean parts from the soil and from the tuber. Inoculation of planting material with this type of antagonist is quite simple and requires relatively small amouts of inoculum and can be used in all kinds of soil. Its presence on the organs that can be attacked leads to a reduced infestation and can give long-term protection of the plant.

A similar association seems to exist between *Trichoderma* spp. and radish and possibly cucumber (Shan-da Liu and Baker, 1980). The association radish – *Trichoderma* spp. also appears from the work of Kuter et al. (1983).

Earlier studies (Jager and Velvis, 1980) and later observations indicate that oats can create conditions in the soil that are unfavourable to *R. solani.* A potato crop following oats appears to suffer less damage and to be occupied by fewer sclerotia than a crop preceded by wheat or sugar beet. Only in a few cases *V. biguttatum* was clearly involved. This study will be continued and meanwhile we are searching for crops and plants with properties supporting *V. biguttatum* or other antagonists of *R. solani.*

G. roseum versus V. 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. In the warm and dry summer of 1977 we frequently isolated *G. roseum* from soil and plant parts, especially towards the end of the growing season. A mass of conidia from soil or stolons on *Rhizoctonia* plates gave rise to mixed colonies of both fungi when inoculated on maltagar plates. The more vigorous *G. roseum* overgrew the slow-growing *V. biguttatum* which then seemingly disappeared. *G. roseum,* when growing on poor substrates, often had verticillate sporophores, which further confused identification. Later we found that a colony often contained conidia of both fungi. The conidia can easily be distinguished and the presence of the fungi can thus be established. In contrast 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 destruction of sclerotia in heavily contaminated lots of seed tubers will be the subject of subsequent reports.

Samenvatting

Biologische bestrijding van Rhizoctonia solani in aardappelen door antagonisten. 4. Beënting van poters met Verticillium biguttatum en andere antagonisten in veldproeven

Het beënten van poters met de op *Rhizoctonia solani* parasiterende schimmel *Verticillium biguttatum* isolaat M73 in combinatie met *Gliocladium roseum* (1981) of met *V. biguttatum* M73 alleen of in combinatie met isolaat M180 plus antibiotische isolaten van de bacterie *Azotobacter chroococcum* (1982), bleek effectief in het terugdringen of het onderdrukken van *R. solani* op stengels en stolonen en het verminderen van de aantasting.

Beënting van het pootgoed leidde tot een vermindering van de sclerotium (lakschurft)-vorming op de nieuwe knollen, vooral in klei- en zavelgronden. In 1981 leidde beënting van poters tot reductie in de sclerotiumvorming van gemiddeld 22 en 42% voor de oogst uit schoon en 15 en 26% voor de oogst uit besmet pootgoed geteeld op respectievelijk zandgrond en klei- en zavelgrond.

In 1982 leidde beënten van de poters uitgeplant in licht zure zandgrond tot een gemiddelde reductie van de sclerotiumindex van de oogst van 22%. In zwaar besmette zandgrond trad evenwel geen reductie op; de infectiedruk was hier te groot. In de neutrale zavel- en kleigronden, vaak ook in de zwaarder besmette percelen werden aanzienlijke reducties bereikt, in de licht besmette gemiddeld 51-68% en in de zwaarder besmette 4-43%. Ontsmetten van pootgoed bleek alleen effectief in percelen die licht met *R. solani* waren besmet.

In beide jaren bleek beënten van pootgoed met antagonisten te resulteren in een significant lagere sclerotiumindex van de oogst (p = 0,1% in 1981; p = 5% in 1982).

V. biguttatum was veel vaker en meer aanwezig op de ondergrondse stengeldelen en stolonen van planten uit beënt pootgoed dan op die van niet beënte poters. De laatsten werden gekoloniseerd door wilde stammen van *V. biguttatum* uit de grond, die vaak minder effectieve antagonisten waren. Beënting van vroeg gepote knollen – als de temperatuur nog te laag is voor de groei van *V. biguttatum* – leverde toch gunstige resultaten op.

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