Neth. J. Pl. Path. 90 (1984) 117-126

Biological control of Rhizoctonia solani on potatoes by antagonists. 3. Inoculation of seed potatoes with different fungi

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Accepted 8 May 1984

Abstract

Inoculation of seed potatoes with *Verticillium biguttatum* and three other hyperparasitic fungi, alone or in combination, resulted in statistically significant reduction of infestation of potato plants by *Rhizoctonia solani. Gliocladium roseum, Trichoderma hamatum* and *Hormiactis fimicola* did not show prolonged protection against *R. solani* under farming conditions. *H. fimicola,* however, inhibited mycelial growth of *R. solani* in vitro, particularly in the lower temperature range where *V. biguttatum* did not show any growth. Combining these two antagonistic fungi may be advantageous as they cover the entire temperature range in which *R. solani* is active. At the end of the vegetation period, *V. biguttatum* was superseded by *G. roseum.*

Production of sclerotia on newly formed tubers from seed potatoes inoculated with *V. biguttatum* (alone or in combination with the other three antagonists) was significantly reduced.

Additional keywords: hyperparasitism, *Verticillium biguttatum, Gliocladium roseum, Hormiactis fimicola, Trichoderma hamatum,* suppressive soil, sclerotia.

Introduction

Biological control of *Rhizoctonia solani* has been the aim of several studies. Various antagonistic fungi have been used as control agents (Harman et al., 1980; Lewis and Papavizas, 1980; Odvody et al., 1980; Chet and Baker, 1981; Elad et al., 1981). Most investigations, however, were carried out under more or less controlled conditions in greenhouses or were confined to short-term effects on seedlings (damping-off).

Biological control of *R. solani* on potatoes is difficult because the subterranean parts of the sprouts and stolons are susceptible during the entire growing season; moreover, not only the seed potato, but also the soil constitutes a source of infection.

A useful antagonist should meet the following requirements: (1) good growth in a wide range of temperatures, (2) capacity to kill sclerotia on seed potatoes, (3) retention of high numbers of propagules, (4) high efficiency on susceptible organs under all environmental conditions which allow growth of *R. solani* and the host plant.

In this respect, *Verticillium biguttatum* appears to be a promising agent for biological control. This hyperparasite kills sclerotia and is able to grow on young sprouts of potato plants (Velvis and Jager, 1983; Jager and Velvis, 1984). It ac-

cumulates in soil enriched with live mycelium of *R. solani* (Van den Boogert and Jager, 1983).

Among the fungi which colonize sclerotia on potatoes, several other hyperparasites have been frequently found, viz. *Gliocladium roseum, Hormiactis fimicola,* and, less frequently, *Trichoderma hamatum* (Jager et al., 1979). *G. roseum* is a non-specific destructive mycoparasite (Barnett and Binder, 1973; Domsch et al., 1980) and according to Jager et al. (1979), it may be an important controlling agent of *R. solani* in Dutch potato fields. *H. fimicola* has rarely been mentioned in the literature, but it has been isolated repeatedly from *Morchella esculenta* and other discomycetes (W. Gams, personal communication).

The purpose of this study was to investigate the effects of the above-mentioned hyperparasites on *R. solani* in agricultural practice. The study is part of an extensive investigation programme on the possibilities of biological control of *R. solani* in potatoes on different soils.

Materials and methods

Media. Malt-peptone agar (MPA): 15 g malt extract, 0.25 g peptone (Oxoid, L72) and 12 g agar in 1000 ml deionized water (pH 6.5).
R. solani plates (RP): MPA plates overgrown with *R. solani* (isolate 06AHa)
Water agar (WA): 12 g agar in 1000 ml deionized water.

Fungal isolates. R. solani 06AHa from potato tubers grown at Haren; *R. solani* 09ABa from potato tubers grown at Baflo; *V. biguttatum* M 73 from a sclerotium of *R. solani* on potato tuber grown at Appelscha; *G. roseum* M 36 from soil at Haren; *T. hamatum* M 37 from soil at Haren; *H. fimicola* M 58 from a sclerotium of *R. solani* on potato tuber grown at Haren.

The isolates of *R. solani* were pathogenic to potato plants. The fungal isolates were maintained on MPA. Conidial masses were harvested from cultures on MPA.

Assessment of occurrence of R. solani or hyperparasites on plant parts. The presence of hyperparasites of *R. solani* in soil and on plant parts was assessed by placing either cylindrical pellets of soil (5-mm diam., 2 mm high) or segments of sprouts or stolons (length 10 mm) on RP (10 segments on each plate of 90-mm diam.). The degree of outgrowth after 10 days at 21 °C was given a score ranging from 1 to 4 analogous to the method of Jager et al. (1979); the score was multiplied by the percentage of segments showing outgrowth (total range 0-400; N = 100). The density of *R. solani* was determined by placing segments of sprouts or stolons on WA (10 segments of 10 mm on each plate). After 20-24 h at 21 °C the percentage of segments showing outgrowth was recorded (N = 100).

Symptoms of *Rhizoctonia* infestation on potato plants (disease index) were visually evaluated according to Jager and Velvis (1982). A sclerotium index (SI) was calculated to describe the distribution of sclerotia on the tubers in various weight classes. Tubers were divided into five classes according to the degree of sclerotial contamination, ranging from clean (A kg), very light (B kg), light (C kg), moderate (D kg) to heavy (E kg). The SI was then calculated according to the formula

$$SI = \frac{(0 \times A + 0.25 \times B + 0.5 \times C + 0.75 \times D + 1 \times E) \times 100}{A + B + C + D + E}$$

Experiments on antagonism in vitro. The growth rate of the hyperparasites on MPA and RP was recorded. Radial outgrowth from 3-mm diameter agar disks taken from the edge of a young colony at 2-day intervals at different temperatures was measured.

Interactions between the hyperparasites and *R. solani* were tested in dual cultures at 21 °C. Pairs of 3-mm agar disks of different fungi were placed opposite each other 45 mm apart on MPA plates. The hyperparasites, except *T. hamatum,* were inoculated 5 days before *R. solani* was introduced. *T. hamatum* was inoculated simultaneously with *R. solani.* Inhibition zones and possible subsequent overgrowth were recorded. The effectiveness of the hyperparasites was tested by placing 3-mm agar disks of *R. solani* overgrown by a hyperparasite on WA plates for 20-24 h at 21 °C. Affected mycelium of *R. solani* was examined microscopically.

Experiments on biological control in the field. Field experiments were carried out on a pleistocene sand soil at the experimental farm of the Institute for Soil Fertility at Haren. The pH-KCl was 4.5. The preceding crop was wheat.

Pregerminated seed potatoes (cv. Irene), grown in clay soil (location Baflo) and infected with *R. solani* (SI varying from 25 to 75; average 61), were inoculated with hyperparasites. The hyperparasites were applied as a coating of a conidial suspension containing per liter 12 g carboxymethylcellulose (CMC) and 400 g clay (taken from a subsoil near Zuidwolde at a depth of 1 m). A mixture of four hyperparasites was prepared by combining equal parts of the various suspensions. The ultimate number of conidia per tuber determined by means of dilution plates was 16.7×10^6 for *V. biguttatum,* 8.0×10^6 for *G. roseum,* 37.0×10^6 for *T. hamatum* and 6.3×10^6 for *H. fimicola.* After being coated, the potatoes were allowed to dry for 3 days at room temperature; they were then planted out in plots (50 plants per plot). As controls (1) macroscopically clean seed potatoes, in addition disinfected in 0.3% formaldehyde for 10 min at 55 °C, and (2) seed potatoes naturally infected by *R. solani* (SI = 61) and coated with the CMC-clay slurry without hyperparasites, were used.

Each treatment was replicated eight times in a randomized block design. Infestation of five plants from each plot was assessed periodically, four replications on 10 June and 1 July, eight replications on the other dates. At the same time a subsample of sprouts or stolons was taken from the plants to examine for the presence of *R. solani* and hyperparasites. Normal cultivation practices were used including chemical haulm desiccation (dinoseb 1.6% a.i.; 800 $l.ha^{-1}$)at the end of the growing season. Four weeks after spraying, when sclerotium formation reached its maximum (Mulder et al., 1979), yield of tubers larger than 28-mm diam. was determined.

Experiments on biological control under greenhouse conditions. The soil for pot experiments came from the same farm; the pH-KCl was 4.6, the previous crop was potatoes. In pot experiments performed in a greenhouse disinfected and pregerminated seed potatoes (cv. Irene) were each artificially infected near the buds with three sclerotia of *R. solani* (06AHa), which had been grown on MPA for 4 weeks. The hyperparasites were applied at the same time as described above. Disinfected and artificially

infected seed potatoes served as controls. Plants were grown in 15-1 buckets filled with a sand from Haren. On each sampling date six plants were used to assess the disease and record the occurrence of *R. solani* and hyperparasites on sprouts and stolons. At the end of the growing season, the tops were removed and 4 weeks later the tubers were harvested and rated for sclerotial density.

Statistical analysis. Effects of treatments were evaluated according to the Student's t-test and the sign test.

Results

Antagonism in vitro. In dual cultures, *G. roseum* and *H. fimicola* caused only temporary inhibition zones against *R. solani.* After a short arrest these hyperparasites overgrew the colony of *R. solani. V. biguttatum* and *T. hamatum* did not inhibit *R. solani* until hyphal contact occurred. After contact, *R. solani* stopped its radial growth and the hyphae of the hyperparasites grew alongside the host hyphae. *T. hamatum* and *G. roseum* typically coiled around the host hyphae, apparently without penetrating them. *V. biguttatum* produced only short side-branches from the main hyphae. These branches penetrated the walls of *R. solani* and grew over considerable distances within the hyphae. Appressoria, which are often formed by biotrophic mycoparasites (Barnett and Binder, 1973) were absent, as verified also by scanning electron microscopy. *H. fimicola* exhibited no parasitic behaviour, but empty and detached cells were often found. Although *V. biguttatum* coiled only infrequently around the host hyphae and *H. fimicola* did not coil at all, they were apparently effective, because only the hyperparasites grew from mycelial pieces taken from the parasitized zone. *T. hamatum* and *G. roseum* were less effective, since *R. solani* produced a greater or lesser amount of outgrowth from about 30% of the mycelial pieces on WA.

In dual cultures of the hyperparasites, all of them showed distinct inhibition zones, except the *G. roseum* – *H. fimicola* combination.

Growth rates measured at various temperatures are shown in Table 1. *G. roseum* and *T. hamatum* grew very irregularly and diffusely on *R. solani,* rendering consistent measurements impossible.

Biological control in the field and under greenhouse conditions. Results of the field trial are given in Table 2-6. Applications of *V. biguttatum, H. fimicola* and a mixture of the four hyperparasites decreased the incidence of disease caused by *R. solani* Table 2).

Plants grown from disinfected seed potatoes were only slightly infected at the end of the growing season. In general, the density of *R. solani* on sprouts and stolons decreased with time, although an obvious revival was noticetable shortly before harvest. In addition, the outgrowth from stolon segments was strongly reduced by *V. biguttatum, H. fimicola* and the mixture (Table 3).

Inoculation with *V. biguttatum* or with the mixture resulted in an exuberant outgrowth of the hyperparasites in early June. In the course of time the stolons were progressively colonized by hyperparasites from the soil, mainly *V. biguttatum* (Table 4).

Stolons from disinfected seed potatoes and seed potatoes inoculated with *H. fimicola* harboured relatively few hyperparasites. As shown in Table 5, among the

Table 1. Growth rate (diameter in mm per day) of four hyperparasitic fungi and the host fungus *Rhizoctonia solani* at different temperatures on MPA and on *R. solani* plates.

Fungus	Incubation temperature (°C)						
	0	5	10	15	20	25	30
On malt peptone agar (MPA)							
Verticillium biguttatum	0.0	0.0	0.0	1.0	2.5	4.0	1.5
Gliocladium roseum	0.0	1.5	2.0	3.5	4.5	5.0	5.0
Hormiactis fimicola	1.0	1.5	2.5	4.0	4.5	5.0	2.0
Trichoderma hamatum	0.0	0.0	2.0	6.5	14.0	18.5	17.1
Rhizoctonia solani (06 AHa)	1.0	1.4	6.8	10.8	15.2	15.5	11.0
Rhizoctonia solani (09 ABa)	1.0	1.0	6.8	11.3	15.8	15.5	6.3
Rhizoctonia solani cultures on plates (RP)							
Verticillium biguttatum	0.0	0.0	0.0	0.7	1.6	2.1	1.2
Gliocladium roseum	−[1]	+[2]	+	+	+	+	+
Hormiactis fimicola	0.1	0.7	1.8	2.3	3.2	3.3	0.5
Trichoderma hamatum	−	−	+	+	+	+	+

[1] − no growth.
[2] + irregular and diffuse growth.

Table 2. Disease indices of potato plants grown in the field from seed potatoes naturally infected with *Rhizoctonia solani* and inoculated with hyperparasites (planting date 11 May 1981).

Hyperparasite applied	Date of assessment					Statistical significance[1]
	10 June	1 July	20 July	12 Aug.	9 Sept.	
none (disinfected clean potatoes)	0	5	0	6	5	*
none (*Rhizoctonia solani* control)	150	165	240	210	190	
Gliocladium roseum	138	230	187	190	214	ns
Verticillium biguttatum	110	112	163	95	80	*
Trichoderma hamatum	93	223	228	228	205	ns
Hormiactis fimicola	88	148	151	143	88	*
mixture of the four hyperparasites	68	103	97	34	59	*

[1] * and ns = Significantly different and not different at P = 0.05 from '*Rhizoctonia solani* control' in the course of time as determined by the sign test.

hyperparasites *V. biguttatum* predominated, whether it has been applied artificially or not. The proportion of *Gliocladium roseum* was found to increase at the end of the vegetation period, particularly on plants where it had not been applied artificially. In addition to the inoculated fungi, *Gliocladium* spp., *Volutella ciliata, Cylindrocarpon destructans, Pyxidophora* sp. and *Colletotrichum coccodes* were frequently observed; their presence may also affect the development of *R. solani.*

In plant-free soil, *V. biguttatum* could not be detected on RP, while *Gliocladium*

Table 3. Occurrence of *Rhizoctonia solani* on sprouts (10 June) and on stolons (other dates) of potato plants grown in the field from seed potatoes naturally infected with *Rhizoctonia solani* and inoculated with hyperparasites.

Hyperparasite applied	Date of assessment					Statistical significance[2]
	10 June	1 July	20 July	12 Aug.	9 Sept.	
none (disinfected clean potatoes)	2[1]	8	2	0	10	*
none *(Rhizoctonia solani* control)	70	44	23	14	29	
Gliocladium roseum	77	53	19	11	14	ns
Verticillium biguttatum	32	12	17	4	12	*
Trichoderma hamatum	67	35	12	12	31	ns
Hormiactis fimicola	36	8	11	12	14	*
mixture of the four hyperparasites	33	14	3	0	9	*

[1] Percentage of sprout or stolon segments showing outgrowth of *R. solani.*
[2] * and ns = Significantly different and not different at P = 0.05 from '*Rhizoctonia solani* control' in the course of time as determined by the sign test.

Table 4. Density of hyperparasites on sprouts (10 June) and stolons (other dates) of potato plants grown in the field from seed potatoes naturally infected with *Rhizoctonia solani* and inoculated with hyperparasites.

Hyperparasite applied	Date of assessment				
	10 June	1 July	20 July	12 Aug.	9 Sept.
None (disinfected clean potatoes)	7[1]	281	127	48	112
None *(Rhizoctonia solani* control)	185	352	298	262	275
Gliocladium roseum	145	227	166	276	289
Verticillium biguttatum	355	352	351	301	313
Trichoderma hamatum	190	262	149	240	293
Hormiactis fimicola	91	181	103	97	98
mixture of the four hyperparasites	298	353	283	260	294

[1] Degree of outgrowth (1-4) from segments of various species of hyperparasites (mainly *V. biguttatum)* × percentage of segments showing outgrowth (total range 0-400).

spp., including *G. roseum* (varying from 32 to 90%), and *T. hamatum* (varying from 80 to 100%) formed only scanty growth of hyphae and poor sporulation from the soil pellets.

V. biguttatum and the mixture of hyperparasites reduced the development of sclerotia on newly formed seed and ware potatoes to the same extent as chemical disinfection (Table 6).

The pot experiments did not support the findings of the field experiment: sclerotia obtained from an agar culture of an originally pathogenic isolate of *R. solani* did not

Table 5. Occurence of four hyperparasites on sprouts and stolons of potato plants grown in the field from seed potatoes naturally infected with *Rhizoctonia solani* and inoculated with these hyperparasites.

Hyperparasite applied	*V. biguttatum*		*G. roseum*		*T. hamatum*		*H. fimicola*	
	10 June	9 Sept.	10 June	9 Sept.	10 June	9 Sept.	10 June	9 Sept.
None (disinfected clean potatoes)	6[1]	77	0	71	0	0	0	0
None *(Rhizoctonia solani* control)	96	100	0	77	0	0	0	1
Gliocladium roseum	94	99	80	63	18	0	0	1
Verticillium biguttatum	100	100	0	86	10	40	3	2
Trichoderma hamatum	97	100	0	72	100	100	0	2
Hormiactis fimicola	77	74	5	31	0	0	24	1
mixture of the four hyperparasites	100	95	68	55	20	70	17	2

[1] Percentage of segments showing outgrowth of one of four applied hyperparasites.

Table 6. Yield (kg) from 15 plants and sclerotium index of harvested seed and ware potatoes grown in the field from seed potatoes naturally infected with *Rhizoctonia solani* and inoculated with hyperparasites.

Hyperparasite applied	Seed potatoes[1]		Ware potatoes[1]	
	yield	sclerotium index	yield	sclerotium index
None (disinfected clean potatoes)	6.46 ± 3.11	9.3 ± 9.8**[2]	19.78 ± 1.50	10.5 ± 4.3
None *(Rhizoctonia solani* control)	7.67 ± 2.26	28.8 ± 9.0	20.03 ± 2.51	32.0 ± 11.0
Gliocladium roseum	6.66 ± 1.94	31.8 ± 15.0	19.19 ± 1.61	30.8 ± 16.3
Verticillium biguttatum	7.63 ± 2.65	9.3 ± 6.3**	19.89 ± 3.09	14.0 ± 8.9**
Trichoderma hamatum	7.33 ± 1.96	21.3 ± 12.0	20.37 ± 2.10	27.3 ± 12.8
Hormiactis fimicola	7.48 ± 3.05	29.0 ± 7.8	17.71 ± 2.67	30.0 ± 12.5
mixture of the four hyperparasites	7.66 ± 2.92	9.3 ± 6.7**	19.96 ± 2.26	15.5 ± 10.7*

[1] Harvest dates of seed and ware potatoes 9 Sept. and 3 Oct., respectively.
[2] ** and * = Significantly different at P = 0.01 and P = 0.05 from '*Rhizoctonia solani* control' as determined by the Student's t-test.

produce any visible symptoms nor was any living mycelium noticed upon subterranean plant parts. The same occurred when the trial was repeated.

Inoculation with *V. biguttatum* or the mixture again resulted in a distinct initial increase in hyperparasites on sprouts. In the course of the growing season, the density of hyperparasites, mainly *V. biguttatum,* on the stolons decreased markedly. *G. roseum* however, generally increased. *H. fimicola* was only found on sprouts of potatoes treated with this species or the mixture (5 and 15%, respectively). *T. hamatum* occurred irregularly on sprouts and stolons from all treatments.

In plant-free soil, *Gliocladium* spp., including *G. roseum,* and *T. hamatum* were often observed; *V. biguttatum* again did not appear from soil pellets on RP.

The harvested potatoes showed very few sclerotia of *R. solani* (SI varied from 4.3 to 9.2). It was concluded that the sample of this soil harboured little *R. solani.*

Discussion

Our findings indicate that *V. biguttatum* has a potential for controlling *R. solani* in agricultural practice when applied to seed potatoes. The success of *V. biguttatum* (alone or in mixed application) in controlling *R. solani* is believed to be based upon: (1) reduction of sclerotial germination on seed potatoes and (2) maintenance of an adequate level of the hyperparasite population on stolons which prevents effective colonization by *R. solani.*

A high density of hyperparasites, mainly *V. biguttatum,* did not always control *R. solani.* Isolate M 73 of *V. biguttatum* proved to be very effective; it seems that this isolate has some specific properties that enable it to compete with local strains of the same species and to parasitize *R. solani* agressively.

In plant-free soil, the population density of *V. biguttatum* was so low that the fungus could not be detected. Soil must have been the main inoculum source of *V. biguttatum* as seed potatoes originating from marine clay soil (pH > 7.0) were virtually free of this fungus (Jager and Velvis, 1982). A high initial density of *V. biguttatum* in soil is considered to be an important prerequisite for a rapid colonization and multiplication on the subterranean plant parts.

Our results indicate that *R. solani,* as a host, can create conditions favourable to the development of *V. biguttatum* (Van den Boogert and Jager, 1983).

The stolons of potato plants were generally free of *G. roseum* at the beginning of the growing season, except where inoculated deliberately. Higher temperature later in the year may have favoured the build-up of *G. roseum* in soil (Jager et al., 1979). Another factor to be considered in this respect is senescence of the host plant; in general, senescent subterranean plant parts are more strongly colonized by this fungus than young ones (Domsch et al., 1980). As *G. roseum* did not prove to be an effective antagonist against *R. solani,* this shift might be detrimental to other hyperparasites in the stolonosphere as was the case with *V. biguttatum* in dual culture. A slight increase of *R. solani* on stolons later in the year (Table 3) could be due to antagonism of *G. roseum* to *V. biguttatum.*

T. hamatum was not found to be an effective hyperparasite in these experiments. Appropriate amendments in the seed coating material might improve its efficiency (Harman et al, 1981).

Jager et al. (1979) and Velvis and Jager (1983) reported that *H. fimicola* reduced the

viability of sclerotia. This fungus proved to be an effective antagonist at lower temperatures at which *V. biguttatum* did not grow (Table 1). Therefore a mixed inoculation with these two fungi should be considered since a combination of their temperature ranges corresponds with that of *R. solani.*

In a mixed inoculum, antagonistic relationships among the hyperparasites hardly reduced the effect of *V. biguttatum* alone. However, *H. fimicola* in single application appeared to prevent the colonization by *V. biguttatum* from soil (Table 4). No explanation of this long-term effect is available, which finally resulted in a distinct loss of antagonistic potential (Table 6).

The application of hyperparasites had no adverse effects on plant growth and yield. *R. solani* itself did not reduce the yield but decreased the quality of potatoes by formation of sclerotia.

The sclerotium indices of the harvested tubers were lower than those of the original plant material, which points to a suppressive power of this Haren soil (Jager and Velvis, 1980). The failure of sclerotial germination of *R. solani* in the pot trials could be due to the suppressive property of the Haren soil, but this is in contrast with the results of previous pot experiments without clay-coating procedures in the Haren soil (Van den Boogert and Jager, 1983). Stotzky (1973) and Filip (1979) observed adverse effects of clay minerals on various fungi of the soil microflora. So, germination of in vitro produced sclerotia in Haren soil might have been inhibited by components of the clay used for coating.

It is concluded that *V. biguttatum* appears to be an effective antagonist of *R. solani* in the moderately suppressive Haren soil. Many questions concerning relationships between *V. biguttatum* M 73 towards *G. roseum* and towards other local strains of *V. biguttatum* have yet to be answered before *V. biguttatum* can be recommended to growers as a universal biocontrol agent against *R. solani.*

Acknowledgement

The authors want to thank Dr W. Gams for his valuable criticism of the manuscript.

Samenvatting

Biologische bestrijding van Rhizoctonia solani op aardappel met antagonisten. 3. Inoculatie van pootaardappelen met hyperparasitaire schimmels

Inoculatie van pootaardappelen met *Verticillium biguttatum*, apart of samen met drie andere hyperparasieten, had een gunstig effect op het onderdrukken van *Rhizoctonia solani* op de plant; *Trichoderma hamatum, Gliocladium roseum* en *Hormiactis fimicola,* ieder apart toegediend, boden de plant op lange termijn geen bescherming tegen *R. solani* onder praktijkomstandigheden. *H. fimicola* bleek bij lage temperatuur, waarbij *V. biguttatum* geen groei-activiteit meer vertoonde, op hyfen en sclerotiën van *R. solani* te kunnen groeien. Toepassing van deze schimmel en *V. biguttatum* in een gemengde inoculatie zou over een breder temperatuurtraject effectief kunnen zijn.

Tegen het einde van het groeiseizoen vond er op de ondergrondse plantedelen een verschuiving plaats, waarbij *G. roseum* meer op de voorgrond trad. Verondersteld wordt dat de afname van *V. biguttatum* op stolonen hiervan een gevolg was.

De produktie van sclerotia op nieuwe aardappelen afkomstig van met *V. biguttatum* behandeld pootgoed (alleen of met andere hyperparasitaire schimmels) bleek sterk verminderd te zijn. Vooral dit gegeven maakt de biologische bestrijding van *R. solani* interessant: *V. biguttatum* blijkt ook op lange termijn effectief te zijn.

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