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Biological control of Rhizoctonia solani on potatoes by antagonists. 1. Preliminary experiments with Verticillium biguttatum, a sclerotium-inhabiting fungus

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Abstract

A common mycoparasite, *Verticillium biguttatum,* was found to kill sclerotia of *Rhizoctonia solani* placed on an inert material (perlite) as well as in soil at 15 °C and 20 °C, but not at 10 °C. Compared with the effectivity of *V. biguttatum,* that of *Gliocladium roseum, Gliocladium nigrovirens, Hormiactis fimicola* and *Trichoderma hamatum* on sclerotia was only low. In laboratory experiments, treatment of sclerotia-bearing seed potatoes with *V. biguttatum* reduced disease symptoms in the first stage of growth of the potato plant. *V. biguttatum* was found to occur on the subterranean part of the potato plant. On untreated plants the surface of the sprouts was colonised by *V. biguttatum* originating from the soil, presumably partly in response to the presence of *R. solani* mycelium. In a preliminary field experiment, *Verticillium* treatment did not reduce symptoms on the stem. However, there was a marked reduction in sclerotium formation on the newly formed potato tubers. This offers perspectives for a commercial use of *V. biguttatum* in the control of *R. solani.*

Additional keywords : Mycoparasites, *Gliocladium roseum, G. nigrovirens, Hormiactis fimicola, Trichoderma hamatum.*

Introduction

Biocontrol of *Rhizoctonia solani* by treatment of seeds or plant material with antagonistic bacteria or fungi has sometimes been successful. Price et al. (1971) treated seeds of wheat, oats and barley with *Bacillus subtilis* against *Rhizoctonia, Pythium* and *Fusarium,* and thus obtained higher yields. Merriman et al. (1974) were able to reduce symptoms of *R. solani* in wheat substantially by inoculating the seed with *Streptomyces griseus* or *Bacillus subtilis.* In cotton, Howell and Stipanovic (1979) obtained effective protection against *R. solani* by treating seeds with *Pseudomonas fluorescens.*

Biocontrol with mycoparasites also gave good results. In laboratory studies, Harman et al. (1980) successfully controlled *Rhizoctonia* infection of pea and radish by inoculating seeds with *Trichoderma hamatum.* They were able to improve this method by applying chitin and cell walls of *R. solani* to the seeds (Harman et al., 1981). Odvody et al. (1980) treated seeds of beet with an unidentified *Corticium* strain that was parasitic on *R. solani.* In this way they substantially reduced the effects of *Rhizoctonia* infection.

The literature cited above refers to control of infection by *Rhizoctonia* from the surrounding soil. In the case of potato, however, the disease may also be induced by sclerotia of pathogenic strains on the seed potato. In addition, potato plants are susceptible to *Rhizoctonia* infection during the whole growing season. About one month after planting, tuber formation starts on the stolons, which are very susceptible to infection by *R. solani*. Thus, the protection of the potato plant should last for the whole growing season.

Aluko (1968) succeeded in killing sclerotia of *R. solani* on potato tubers with *Gliocladium virens*. The treatment was effective only if performed before storage of the potatoes. Treatment just before planting had no effect, because *G. virens* disappeared rather soon after planting.

For an effective control of *R. solani* in potato, it might be important to employ common sclerotium-inhabiting mycoparasites. Literature dealing with the occurrence of mycoparasites on sclerotia in field soils is rather scarce. Naiki and Ui (1972) found *Trichoderma* and *Penicillium* spp. to be the most prevalent parasitic fungi on sclerotia of two isolates of *R. solani*. They also isolated *Fusarium, Mortierella, Cephalosporium, Mucor* and *Rhizopus* from the sclerotia. Jager et al. (1979) reported *Gliocladium roseum* as the most prevalent mycoparasite on sclerotia of *R. solani* in Dutch potato fields. Besides a number of other fungi they isolated a *Verticillium* species, indistinguishable from *G. roseum* when growing on sclerotia. Recently a large number of sclerotia was examined, obtained from potato tubers harvested from many potato fields in the northern part of The Netherlands (Jager and Velvis, 1980, 1983a, 1983b). The *Verticillium* species observed by Jager et al. (1979) was found to be the most common fungal inhabitant of *Rhizoctonia* sclerotia. It has recently been described as *Verticillium biguttatum* by Gams and Van Zaayen (1982).

It is difficult to distinguish between *V. biguttatum* and *G. roseum* when sporulating on sclerotia. The primary conidiophores of *G. roseum* are *Verticillium*-like, whereas conidial heads of *V. biguttatum* often stick together forming one conidial mass. Microscopic examination gives a decisive identification, because the two fungi have different conidial forms.

The frequent appearance of the *Verticillium biguttatum* type on sclerotia of *R. solani* suggested that it could play an important role in the natural antagonism against *R. solani*. The relation between *R. solani* and *V. bigittatum* has been further examined in this study. The crucial question was the possible use of *V. biguttatum* as a biocontrol agent against *R. solani*.

Materials and methods

Isolates of mycoparasites. Three isolates of *V. biguttatum*, coded M 73, M 74 and M 75, were selected from sclerotia. For comparison, also a number of well-known other mycoparasites was taken from our collection: *Gliocladium roseum, Gliocladium nigrovirens, Hormiactis fimicola* and *Trichoderma hamatum*. The isolates of *G. roseum, G. nigrovirens* and *H. fimicola* had also been obtained from sclerotia of *R. solani*. The isolate of *T. hamatum* had been isolated from soil.

Media. The fungi used were grown on maltbiotone agar (MBA) containing 15 g maltextract, 2.5 g microbiotone and 12 g agar per liter demineralised water.

Sclerotia of *R. solani* treated with conidial suspensions of the mycoparasites were placed on moist perlite, to provide an inert basic layer.

Germination of the sclerotia was detected on 2% water agar, containing per ml 50 μg 7-chlortetracycline, 50 μg neomycin sulphate and 50 μg streptomycin sulphate, to prevent growth of bacteria and actinomycetes.

Determination of the effect of mycoparasites on sclerotia. Conidial suspensions of the mycoparasites were made in 1% aqueous solution of carboxymethylcellulose (CMC). Portions of 100 sclerotia, obtained from potato tubers, were surface-sterilised by placing them in 10% H_2O_2 for 2 min, followed by washing with sterile water. Subsequently, they were dipped in the conidial suspensions, placed on moist perlite, and incubated at 20 °C. On perlite the sclerotia showed initial germination. After 7 weeks the outgrown *Rhizoctonia* hyphae were removed with a scalpel, in order to obtain the proper effect of the antagonists on sclerotia. The sclerotia were placed on water agar with antibiotics for 1 day at 22 °C. Germination was examined with a Wild M 3 stereomicroscope.

Biocontrol assays. To check the value of *V. biguttatum* as a biocontrol agent of the germination of *Rhizoctonia* sclerotia on seed stock, infected seed potatoes (cv. Irene) were treated with a conidial suspension of *V. biguttatum* (M 73) in 1% CMC. After having been stored under moist conditions for 3 days at 15 °C, the seed potatoes were planted in soil.

The severity of sprout infestation was examined and represented in two figures, the first being the percentage of diseased stems, and the second the average severity of the disease of individual sprouts expressed on a scale of 1 to 5, where 1 = only one or two small spots or lesions and 5 = killed.

The colonisation of the sprout surface by *V. biguttatum* was determined by placing stem pieces on MBA plates overgrown with mycelium of *R. solani.* After 10 days, outgrowth of *V. biguttatum* was examined with a stereomicroscope.

In a preliminary field trial, also the percentage of potato tubers with sclerotia was determined at the end of the growing season.

Results

Effect of V. biguttatum and other mycoparasites on the viability of sclerotia. The effects of three isolates of *V. biguttatum* on the viability of sclerotia are shown in Table 1. A large number of *Verticillium*-treated sclerotia did not germinate on water agar, even after prolonged incubation. Most aggressive was isolate M 73, which killed 90% of the sclerotia.

In comparison with other mycoparasites, *V. biguttatum* M 73 was by far the most lethal to sclerotia (Table 2). *G. roseum* and *H. fimicola* also affected the viability of the sclerotia, but the percentage of killed sclerotia was rather small. The effect of *G. nigrovirens* and *T. hamatum* was negligible.

Effect of temperature. Sclerotia that had been treated with a conidial suspension of *V. biguttatum* M 73 and untreated ones were incubated at 10, 15 and 20 °C. The ef-

Table 1. Viability of sclerotia of *R. solani* treated with conidial suspensions of three isolates of *Verticillium biguttatum.*

V. biguttatum isolate	Sclerotia (%) showing the indicated numbers of hyphae				
	0	1-5	6-10	11-25	>25
None (control)	12	3	6	30	49
M 73	90	8	1	1	0
M 74	62	34	4	0	0
M 75	54	26	12	5	3

Tabel 1. Levenskracht van sclerotiën van R. solani behandeld met conidiënsuspensies van drie isolaten van Verticillium biguttatum.

Table 2. Viability of sclerotia of *R. solani* treated with conidial suspensions of different mycoparasites.

Mycoparasite	Sclerotia (%) showing the indicated numbers of hyphae				
	0	1-5	6-10	11-25	>25
None (control)	4	3	5	14	74
Verticillium biguttatum M 73	95	5	0	0	0
Gliocladium roseum M 71	17	7	8	29	39
Gliocladium nigrovirens M 60	10	4	4	15	67
Hormiactis fimicola M 58A	16	7	11	34	32
Trichoderma hamatum M 37	11	8	6	18	57

Tabel 2. Levenskracht van sclerotiën van R. solani, behandeld met conidiënsuspensies van verschillende mycoparasieten.

Table 3. Viability of sclerotia of *R. solani* after treatment with *V. biguttatum* M 73 *(V.b.)* and incubation on perlite for 7 weeks at different temperatures.

Temperature	Inoculum	Sclerotia (%) showing the indicated numbers of hyphae				
		0	1-5	6-10	11-25	>25
10 °C	None	11	6	8	29	46
	V.b.	14	6	13	24	43
15 °C	None	15	5	13	18	49
	V.b.	96	4	0	0	0
20 °C	None	19	20	6	12	43
	V.b.	95	4	0	1	0

Tabel 3. Levenskracht van sclerotiën van R. solani na behandeling met V. biguttatum M 73 (V.b) en een incubatie op perliet gedurende 7 weken bij verschillende temperaturen.

Table 4. Viability of sclerotia of *R. solani* after treatment with *V. biguttatum* M 73 *(V.b.)* and incubation in different soils for 7 weeks at 15 °C.

Soil or substrate	Inoculum	Sclerotia (%) showing the indicated numbers of hyphae				
		0	1-5	6-10	11-25	>25
Peaty sand	None	90	1	0	3	6
	V.b.	100	0	0	0	0
Sandy soil Haren	None	60	4	1	10	25
	V.b.	98	1	1	0	0
Sandy soil Zeyerveld	None	89	6	2	1	2
	V.b.	100	0	0	0	0
Loamy sand	None	78	5	3	6	8
	V.b.	100	0	0	0	0
Clay loam	None	25	5	4	13	53
	V.b.	99	1	0	0	0
Perlite	None	15	8	5	17	55
	V.b.	100	0	0	0	0

Tabel 4. Levenskracht van met V. biguttatum M 73 (V.b.) behandelde sclerotiën van R. solani na een verblijf van 7 weken in verschillende gronden bij 15 °C.

fect of these temperatures on the effectiveness of *V. biguttatum* is shown in Table 3. Germination of untreated sclerotia after the incubation period followed a rather regular pattern at all three temperatures. At higher temperatures, especially 20 °C, there seemed to be a slight decrease in viability, presumably due to a more abundant primary germination on perlite. The lethal effect of *V. biguttatum* M 73 on *Rhizoctonia* sclerotia was equally strong at 15 °C and 20 °C, but was absent at 10 °C. Apparently, the fungus became active as an antagonist between 10 and 15 °C.

Effect of soil. In previous experiments, optimal conditions for infection of sclerotia by the mycoparasite were maintained: any unfavourable effect from the soil microflora was eliminated. To determine whether *V. biguttatum* M 73 is able to maintain itself on sclerotia also in soil, sclerotia were treated with a conidial suspension of the parasite, and immediately after treatment they were placed on soil in a petri dish (15 cm diam.) and covered with nylon gauze and a second layer of soil. The same was done for untreated sclerotia. After an incubation time of 7 weeks at 15 °C, re-germination of the sclerotia was investigated. Five types of soil were used, viz., a peaty sand, two sandy soils, a loamy sand and a clay loam. For comparison with the first experiments, treated and untreated sclerotia were placed on perlite. In all soils the moisture content was maintained at 15% (w/w). It should be kept in mind that equal amounts of water in different soils may give unequal water potentials. The resulting water potentials were supposed to be characteristic for each soil, as well as the ecological consequence of this for *V. biguttatum.*

In the various soil types, *V. biguttatum* was sufficiently active at 15 °C to kill nearly all sclerotia (Table 4), but a large fraction of the untreated sclerotia also died in all soils but one, viz., clay loam. This soil presumably lacked a population of effective antagonists. Yet it is known that sclerotia of *Rhizoctonia solani* may survive in soils from a few months to several years, depending on soil type and other factors affecting the composition and activity of the antagonistic microflora (Coley-Smith and Cooke, 1971).

Biocontrol with V. biguttatum in laboratory experiments. Thirty infected seed potatoes treated with *V. biguttatum* M 73 and 30 potatoes treated with a CMC suspension only were planted in plastic cylinders, filled with a sandy soil from Haren. The tubers were covered with a 15 cm layer of this soil, and incubated at 15 °C. The moisture content was maintained at 18% (w/w). After 1, 2, 3 and 5 weeks, six plants were harvested from both treatments. The severity of the *Rhizoctonia* disease was determined, along with the length of sprouts on which *Rhizoctonia* hyphae could be detected. To establish the position of *V. biguttatum*, the sprouts were cut into pieces of 1 cm from the sprout base upward, which were placed on MBA plates overgrown with mycelium of *R. solani*.

In Table 5 the severity of infestation is shown for the young potato sprouts. *V. biguttatum* M 73 considerably reduced the percentage of infested stems as well as the severity of the disease during the whole period of investigation (5 weeks). An illustration of it is given in Fig. 1.

The development of *Rhizoctonia* hyphae on the subterranean stems of the untreated plants was also considerable during the whole period of investigation (Fig. 2). *Verticillium*-treated plants had fewer *Rhizoctonia* hyphae, which agreed with the reduced severity of the disease. Average stem length was much greater in the *Verticillium*-treated plants, which was due to the fact that in the untreated series a number of sprouts and growing tops had been killed by *R. solani.* The final decrease in average length in the treated series was the result of growth of new sprouts.

One week after planting, *Verticillium* was detectable on 50% of the young sprouts of the *Verticillium*-treated plants; on untreated plants, however, it was present only in a few cases. *V. biguttatum* grew along with the sprout during the period of investigation. The number of *Verticillium*-carrying sprouts of the untreated plants also increased during the test period, since the stems were colonised by the fungus present

Table 5. Effect of treating sclerotium-bearing seed potatoes with *V. biguttatum* M 73 on the severity of disease of potato sprouts[1].

Inoculum	Time after planting (weeks)			
	1	2	3	5
None (control)	20; 2.6	76; 4.7	83; 4.7	73; 4.7
V. biguttatum M 73	7; 2.0	20; 5.0	38; 3.7	38; 3.5

[1] The first figure represents the percentage of diseased stems, the second one the severity of the disease expressed on a scale or 1 to 5, where 1 = only one or two small spots or lesions and 5 = killed.

Tabel 5. De mate van aantasting van aardappelspruiten van wel en niet met V. biguttatum M 73 beënte sclerotiëndragende pootaardappelen,

Fig. 1. Effect of treatment of *R. solani*-infected seed potatoes with *V. biguttatum* at 3 weeks after planting. Left: untreated; right: treated with *V. biguttatum.*

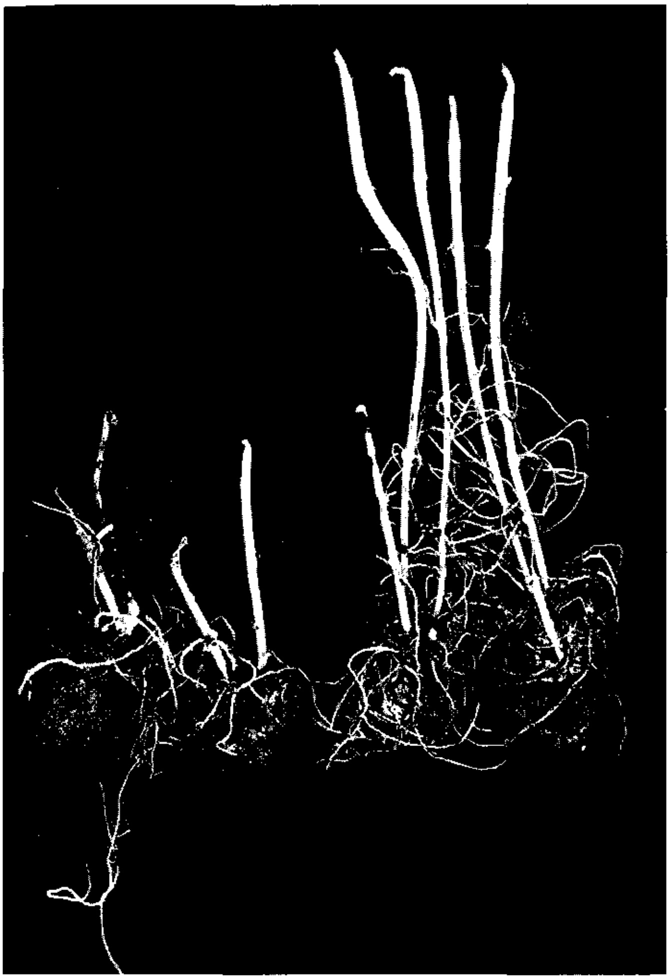

Fig. 1. Het effect van een behandeling van met R. solani geïnfecteerde pootaardappelen met V. biguttatum 3 weken na pootdatum. Links: onbehandeld; rechts: beënt met V. biguttatum.

in the soil. Although *V. biguttatum* is commonly found on the potato stem, its abundance in presence of *Rhizoctonia* may indicate an interaction between the fungus and *R. solani.* This is supported by the observation that *V. biguttatum* grew well from stem pieces on MBA plates in the presence of *Rhizoctonia* mycelium.

Biocontrol with V. biguttatum in the field. Finally, an experiment was conducted to determine if *R. solani* could be controlled in the field. Four plots were laid out on sandy soil in Haren. *Verticillium*-treated seed potatoes were planted in two plots, untreated tubers in the other two. Ten plants of each treatment were harvested 1 and

Fig. 2. Colonisation of the subterranean sprouts of potato by mycelium of *R. solani* and *V. biguttatum.* – = untreated; + = treated with *V. biguttatum.*

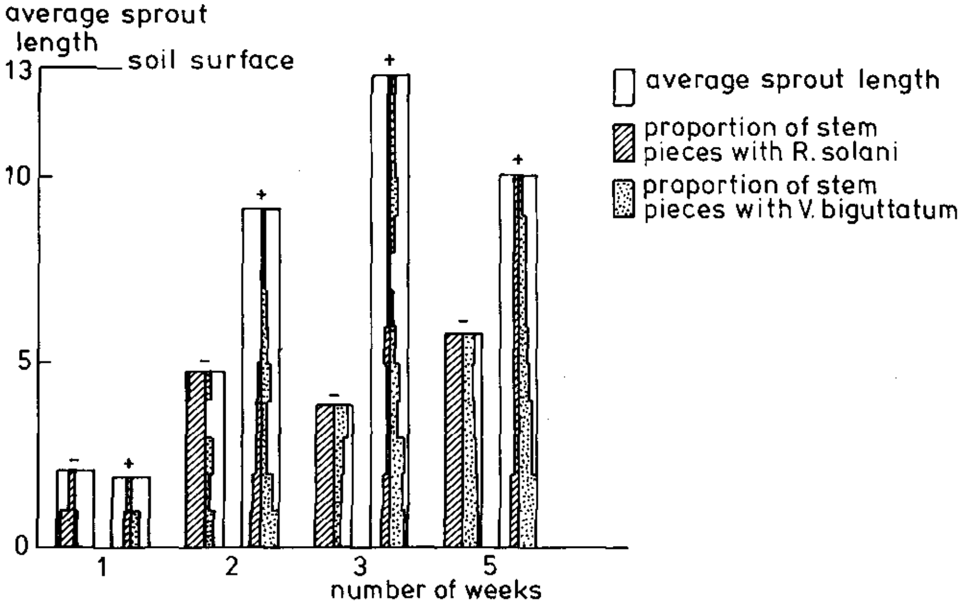

Fig. 2. De bezetting van de ondergrondse stengels van aardappel door mycelium van R. solani en V. biguttatum. – = onbeënt; + = beënt met V. biguttatum.

2 months after planting and examined for disease. The first sample was only slightly diseased, without any difference between plants from *Verticillium*-treated and untreated seed potatoes. The disease symptoms in the second sample were somewhat more severe, but again there was no marked influence of the *Verticillium* treatment.

Yet there was a distinct effect of the *Verticillium* treatment on the number of new tubers with sclerotia. In the untreated series, 53% of the 632 harvested tubers had sclerotia, in the *Verticillium*-treated series only 10% of 759 tubers. *V. biguttatum* growth could be clearly seen on newly formed sclerotia on potatoes in the field (Fig. 3).

Discussion

The results of our experiments demonstrate that *V. biguttatum* was effective in decreasing the viability of sclerotia of *R. solani,* and the parasite may be useful for biocontrol of *R. solani* in the field. Unlike the treatment with *G. virens* (Aluko, 1968), tubers can be treated with *V. biguttatum* before planting, because the latter organism continued to be active on the sclerotia even in soil.

Temperature is a limiting factor in cold springs, for the antagonistic activity decreased at temperatures below 15 °C, and was absent at 10 °C.

Trichoderma hamatum, being effective in biocontrol of *Rhizoctonia* on pea and

Fig. 3. Growth of *V. biguttatum* upon sclerotia on a newly formed potato tuber.

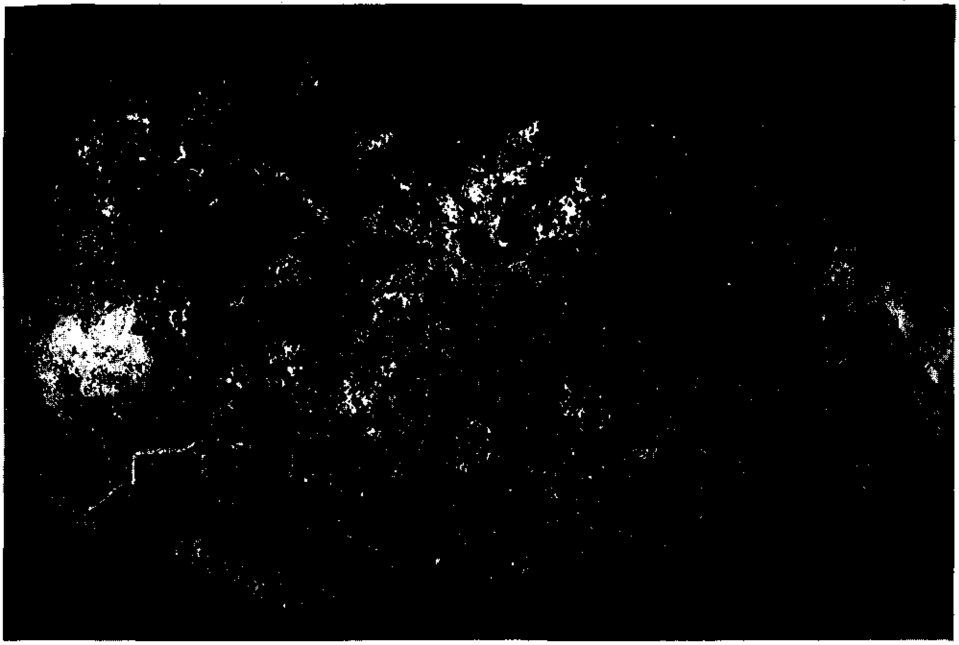

Fig. 3. Groei van V. biguttatum op sclerotiën op een nieuwgevormde aardappelknol.

radish (Harman et al., 1980), was not successful in killing sclerotia, neither were *Gliocladium roseum, G. nigrovirens* and *Hormiactis fimicola.*

It was found that *V. biguttatum* grew along with the young potato sprout in a slightly acid soil, and it also became abundant on potato sprouts from untreated seed potatoes that were heavily infected with *Rhizoctonia.* Presumably there was a mycoparasitic interaction between *R. solani* and *V. biguttatum* on the surface of the stem. This offers an interesting basis for a further study of the protective effect of *V. biguttatum* against *Rhizoctonia* infection from the soil.

Samenvatting

Biologische bestrijding van Rhizoctonia solani op aardappel met behulp van antagonisten. 1. Eerste proeven met Verticillium biguttatum, een sclerotiumkoloniserende schimmel

Sclerotiën van *Rhizoctonia solani,* die bij het rooien van aardappelen op de knollen zitten, blijken veelvuldig geïnfecteerd te zijn met de schimmel *Verticillium biguttatum.*

In dit onderzoek is aangetoond dat *V. biguttatum* in staat is de sclerotiën van *R. solani* te doden. Dit effect wordt bereikt zowel wanneer de beënte sclerotiën geïncubeerd worden op perliet als in grond. *V. biguttatum* kan zich ook in het bodemmilieu goed op de sclerotiën handhaven.

In vergelijking met de doding van sclerotiën door *V. biguttatum* is het effect van andere bekende mycoparasieten als *Gliocladium roseum* en *Hormiactis fimicola* vrij gering, en dat van *Gliocladium nigrovirens* en *Trichoderma hamatum* zelfs te verwaarlozen.

De temperatuur blijkt voor de activiteit van *V. biguttatum* een belangrijke factor. Pas tussen 10 en 15 °C treedt groei en doding van sclerotiën op.

Onder laboratoriumomstandigheden bij 15 ° C resulteerde een behandeling van sclerotiëndragende pootaardappelen in een vermindering van de *Rhizoctonia*-aantasting van de jonge spruiten.

De ontwikkeling van *Rhizoctonia*-mycelium op de ondergrondse stengels was bij de onbehandelde aardappelen aanzienlijk groter.

V. biguttatum groeide bij de behandelde serie met de spruiten mee. Ook bij de onbehandelde objecten trad een sterke kolonisatie van het stengeloppervlak door *V. biguttatum* vanuit de grond op. Hoewel *V. biguttatum* ook zonder *R. solani* voorkomt als bewoner van aardappelstengels, lijkt het frequente voorkomen van *V. biguttatum* in aanwezigheid van *R. solani* te duiden op een interactie op het stengeloppervlak.

In een kleine veldproef kon vermindering van de stengelaantasting door behandeling van het pootgoed met *V. biguttatum* niet worden aangetoond. De onderdrukking van de sclerotiumbezetting op de eindoogst bleek evenwel aanzienlijk.

In latere veldproeven, waarover nog gepubliceerd zal worden, werd ook een effect op de stengelaantasting geconstateerd.

De uitkomsten bieden perspectief voor de toepassing van *V. biguttatum* als biologische bestrijder van *R. solani* in de praktijk.

De vraag of *V. biguttatum* de aardappelplant ook bescherming biedt tegen *Rhizoctonia*-infectie vanuit de grond dient nader te worden onderzocht.

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