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Biological control of Rhizoctonia solani on potatoes by antagonists. 5. The effectiveness of three isolates of Verticillium biguttatum as inoculum for seed tubers and of a soil treatment with a low dosage of pencycuron

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Abstract

In field experiments in 1983, inoculation of seed tubers with three isolates of *Verticillium biguttatum*, separately or mixed, was successful in reducing the amount of sclerotia of *Rhizoctonia solani* formed on new tubers. In holocene marine soils the sclerotium index (s.i.) was on average 50% of that of the harvest from untreated tubers.

In two slightly acid pleistocene sands an additional soil treatment with pencycuron at 20% of the recommended dosage improved the results of seed tuber inoculation in one soil (further reduction of the s.i. from 60 to 32%), but not in the other (reduction to 55%). The three isolates of *V. biguttatum* used in these experiments did not differ in their efficacy to suppress *R. solani.*

Lowering the number of spores in the inoculum fluid to 20% of the initial density (6 $\times$ 10^5 per ml instead of 3 $\times$ 10^6 did not influence the results.

Introduction

In previous studies we have shown that inoculation of sprouted seed tubers with a suspension of spores and hyphal fragments of *Verticillium biguttatum* (isolate M73) reduced disease symptoms and the formation of sclerotia on newly formed tubers (Velvis and Jager, 1983; Jager and Velvis, 1984, 1985; Van den Boogert and Jager, 1984). However, in soils with a high inoculum density of *R. solani*, where the harvest of a crop grown from disinfected seed tubers had many sclerotia, the effect of biological control in decreasing disease symptoms and the formation of sclerotia was often unsatisfactory (Jager and Velvis, 1985). We therefore examined whether a combined treatment, including inoculation of the seed tubers with *V. biguttatum* and a soil treatment with sublethal doses of a *R. solani*-inhibiting chemical would be more effective. The combination should be compatible, thus the chemical should not suppress the development of *V. biguttatum*. Pencycuron was found to meet this requirement (Jager, unpublished). It was applied at 20% of the recommended dosage.

In a laboratory screening test, two isolates of *V. biguttatum* were selected for their capacity to parasitize *R. solani* and to protect potato sprouts. Their efficacy in field trials was included in the programme. It was also studied whether it would be possible to economize on the amount of inoculant. The results are reported in this paper.

Materials and methods

The general procedures were the same as described earlier (Jager and Velvis, 1985). The inoculum was prepared by blending and homogenizing three agar layers (ϕ 15 cm) with sporulating mycelium of *V. biguttatum* in 4 l tapwater with carboxymethylcellulose (0.5%). The acidity was adjusted to pH 6.4 by adding 0.1% KH_2PO_4. The composition of the agar media was described by Van den Boogert and Jager (1984).

The number of spores in the different lots of inoculum fluid was about 3×10^6 ml^{-1}; in one treatment the amount was reduced to about 6×10^5 ml^{-1}.

Eight experimental fields were laid out comprising seven treatments in 12 replicates. The treatments of the seed tubers were:

0 - disinfected (formaldehyde);

1 - not disinfected;

2 to 6 as 1, but inoculated with isolates of *V. biguttatum* as follows: 2 - M73 (reduced amount); 3 - M73; 4 - Gas4; 5 - U55 and 6 - a mixture of M73, Gas4 and U55, each at one third of the amount when applied singly.

The cultivar Bintje was used on fields 1 to 7; cv. Spartaan was planted at Tollebeek, and cv. Prominent at Kloosterburen FH (experimental farm Feddemaheerd). The seed tubers were of commercial quality (class A) and not completely free from small sclerotia.

Experimental fields were a part of a larger field with ware potatoes. Plots that were used only to assess the amount of sclerotia on new tubers had four rows of five plants. Plants in the two middle rows were harvested for seed, three weeks after haulm destruction in the second half of July. Plots that were sampled twice in the growing season had two additional rows of plants. Plants were sampled to assess the disease index and the percentage of stolon pieces with living *R.solani* and *V.biguttatum* (Van den Boogert and Jager, 1984). The same pieces were used for assessment of *R. solani* and *V. biguttatum*. The tubers left in the field were harvested later as ware potatoes by the field's owner, who fertilized the field and took care of soil tillage. The sclerotium index (s.i.) of harvested seed tubers was determined as described by Jager and Velvis (1985).

The relevant properties of the soils on which the experiments were located are presented in Table 1. Potatoes had been grown on these soils 3 or 4 years ago. Pencycuron was given as a soil treatment on two opposite, nonadjacent quarters of a field; it was sprayed onto the field and raked in, one week before planting.

The results were statistically evaluated according to the method of Kolmogorov-Smirnov (Lehmann, 1975). This method is suitable for comparison of two groups of data with an irregular frequency distribution.

Results

In Table 2 the disease index, the percentage of stolon pieces with living *R. solani*, and the *V. biguttatum* index of stolon pieces are presented for plants from fields 1, 3 and 4. Compared with untreated tubers, inoculation of the seed tubers with *V. biguttatum* resulted in a lower disease incidence. The effect was comparable to that of chemical disinfection of the seed tuber. Inoculation also reduced the percentage of stolon pieces with living *R. solani*. The effect was improved by an additional soil treatment with a low dose of pencycuron in the Haren soil as was shown both in the first and in the second sampling.

Table 1. Relevant properties of the soils[1] of the experimental fields.

Location	pH (KCl)	Org. matter (%)	Classification
1. Haren	5.3	5.0	sandy loam
2. Gasselte	5.0	5.4	loamy sand
3. Kloosterburen	5.4	1.6	loam
4. Zandeweer	6.7	2.0	silt loam
5. Uithuizen	7.2	3.0	silt loam
6. Schalsum	7.3	1.6	silt loam
7. Sexbierum	7.1	4.1	silt loam
8. Tollebeek	7.6	2.2	sandy loam
9. Kloosterburen, FH	7.6	1.3	silt loam

[1] Soils 1 and 2 are pleistocene soils; the others are holocene marine soils. The classification is according to the textural diagram of USDA (Alexander, 1961).

Table 2. Average disease indices, percentages of stolon pieces with living *R. solani*, and indices for the occurrence of *V. biguttatum* on stolon pieces of plants from disinfected (0), non-disinfected (1) and non-disinfected inoculated seed tubers on two sampling dates in 1983.

Treatment	First sampling[1]			Second sampling[1]		
	disease index	*R. solani* (%)	*V. biguttatum* index[3]	disease index	*R. solani* (%)	*V. biguttatum* index
1. Haren						
0	4	13	6	5	18	14
1	14	18	30	4	23	6
Inoculated[2]	7	12	33	5	6	21
0 +P	4	3	8	3	12	31
1 +P	8	5	11	5	7	30
Inoculated +P[4]	3	5	24	4	2	26
3. Kloosterburen						
0	9	11	46	14	3	28
1	9	13	48	20	6	47
Inoculated	8	6	74	12	1	39
4. Zandweer						
0	3	6	12	7	13	11
1	6	20	16	10	23	20
Inoculated	2	3	31	7	7	41

[1] Sampling dates: Haren 28 June and 18 July; Kloosterburen 12 June and 1 August; Zandeweer 25 July and 8 August.
[2] The data indicate the average values of all inoculated treatments.
[3] The maximum value of the indices is 100.
[4] +P: soil treatment with pencycuron (20% of the recommended dosage rate).

As observed earlier (Jager and Velvis, 1985), the incidence of *V. biguttatum* on stolon pieces was enhanced by inoculation of the seed tubers. Stolon pieces from disinfected tubers became colonized by the soil's population of *V. biguttatum*. Soil 3 obviously had a higher inoculum density of *V. biguttatum* than the other two soils. The effect of pencycuron on *V. biguttatum* is not clear in these experiments. In earlier studies pencycuron had no effect on the growth of *V. biguttatum* at the dose used here (Jager, not published).

The average sclerotium indices for each treatment of the seed tubers are shown in Table 3. There was no significant difference between the effect of the various isolates of *V. biguttatum* and that of a reduced amount of spores in the inoculation fluid on the formation of sclerotia on new tubers. Inoculation of sprouted seed tubers with *V. biguttatum* (average of all isolates) significantly reduced the sclerotium indices of the harvests from holocene soils ($P = 0.05$ or lower). Inoculation had no effect on sclerotium formation on the harvested tubers from field 8. This field was located in the corner of a large field which had been planted with inoculated seed tubers by way of experiment on a practical scale. Nevertheless, the harvested tubers had many sclerotia, which were found to be scarcely colonized by *V. biguttatum*. Only 2% showed a very poor growth of *V. biguttatum* after a relatively long incubation period. This is very exceptional for the soils of this farm. On inquiry it appeared that tulips had been grown in the preceding year and that the soil had been treated with quintozene (PCNB). This chemical and especially its conversion product pentachlorophenol is extremely toxic to *V. biguttatum* (Jager, unpublished). Most likely the quintozene had affected the results in field 8.

In the two fields on pleistocene sand pencycuron at a low dose rate (20%) had little or no effect when non-inoculated seed tubers were used. Inoculation with *V. biguttatum* (average of all isolates) proved not effective at Haren but significantly so at Gasselte ($P = 0.005$). An additional soil treatment with pencycuron markedly reduced the s.i. further at Haren ($P = 0.005$), but not at Gasselte.

Fig. 1 shows the distribution of s.i. classes of the harvest from non-inoculated (A) and inoculated seed tubers (B) from soil 3. The frequency distribution of s.i. classes in A is very irregular. Inoculation of seed tubers with *V. biguttatum* controlled the infection from soil and seed tuber, reduced the s.i. and led to a more regular frequency distribution. A similar picture is found when frequency distributions of s.i. classes are made for one of the other marine soils that were lightly or moderately infested with *R. solani*. In Fig. 2 the distribution of s.i. classes is compared for tubers harvested from uninoculated (A), inoculated (B) and inoculated seed tubers on plots where also a soil treatment with pencycuron (C) was given. The plots were on soil 1 (Table 1). Inoculation tended to reduce the s.i. However, the difference between treatments A and B was not significant. Combination of inoculation with soil treatment with pencycuron resulted in a significant difference between treatments A and C. This demonstrates that a combination of inoculation and application of a fungicide to the soil can be successful. The minimum dose rate that will sufficiently support biological control in various soils needs further study.

The frequency distribution of s.i. classes of different treatments in a field is often found to be irregular and not in accordance with the Poisson model. Therefore the method of Kolmogorow-Smirnov was used for statistical testing of differences.

Table 3. Average sclerotium indices of harvested seed tubers as affected by various treatments of the seed tubers.

Location	Treatment with pencycuron[1]	From disinfected seed tubers	From non-disinfected seed tubers (isolate of *V. biguttatum* used for inoculation)						Statistical significance of inoculation[2]
			none	M73 (20%)	M73	U55	Gas4	Mix	
1. Haren	—	21 ± 9	18 ± 8	13 ± 5	11 ± 2	14 ± 8	16 ± 7	13 ± 5	ns
	+	19 ± 14	18 ± 8	8 ± 5	6 ± 5	9 ± 6	8 ± 4	7 ± 2	+ +
2. Gasselte	—	37 ± 7	35 ± 5	20 ± 11	19 ± 5	—	22 ± 6	25 ± 5	+ +
	+	30 ± 5	33 ± 10	17 ± 6	17 ± 5	—	26 ± 5	23 ± 5	+ +
3. Kloosterburen	—	14 ± 13	18 ± 11	6 ± 7	9 ± 10	8 ± 9	6 ± 7	5 ± 4	+
4. Zandeweer	—	5 ± 6	17 ± 14	2 ± 3	0 ± 1	4 ± 7	4 ± 7	3 ± 4	+ +
5. Uithuizen	—	9 ± 9	15 ± 11	9 ± 9	4 ± 4	5 ± 6	—	8 ± 7	+
6. Schalsum	—	8 ± 9	10 ± 10	3 ± 7	6 ± 9	—	3 ± 5	1 ± 3	+
7. Sexbierum	—	22 ± 14	22 ± 7	10 ± 8	13 ± 7	11 ± 7	—	8 ± 8	+ +
8. Tollebeek	—	—	15 ± 11	17 ± 8	18 ± 12	18 ± 9	19 ± 9	23 ± 10	ns
9. Kloosterburen FH	—	—	45 ± 10	—	28 ± 9	—	—	—	+ +

1 +: soil treatment with pencycuron at a rate of 20% of the recommended dose; — no treatment with pencycuron.

2 The average effect of all isolates compared with the non-inoculated and non-disinfected control. ns: difference not significant; + and + + significantly different at P = 0.05 and P = 0.01 or lower.

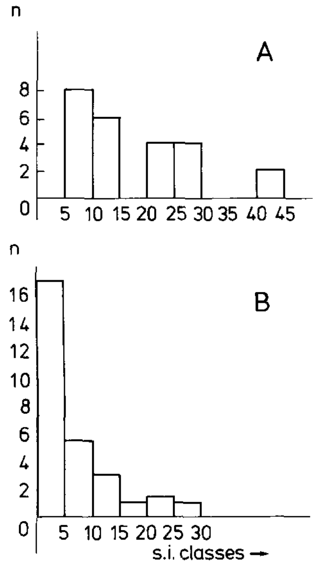

Fig. 1. Frequency distribution of the sclerotium index of the harvest obtained from non-inoculated (A) and inoculated (B) seed tubers grown in soil 3 (marine sandy loam, lightly infested with *R. solani* (see also Table 3). n = number of plots.

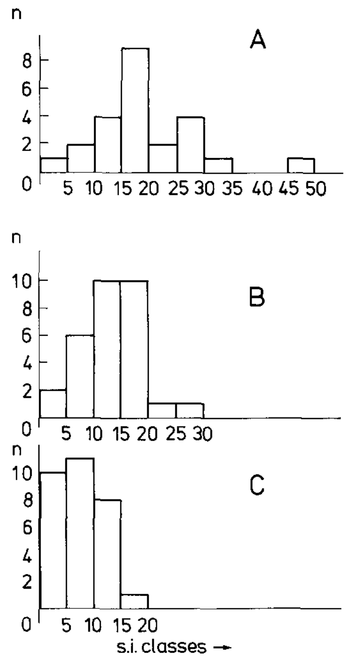

Fig. 2. Frequency distribution as in Fig. 1 for soil 1 (sand, moderately infested with *R. solani*). Treatments A and B as in Fig. 1; treatment C: seed tubers inoculated, plus a soil treatment with pencycuron (20%) (see also Table 3). n = number of plots.

Discussion

In the experiments on holocene marine soils, the biological control of *R. solani* by inoculation of sprouted seed tubers with *V. biguttatum* reduced the s.i. on average by about 50% compared with the s.i. of the harvest from non-disinfected, non-inoculated seed tubers. On slightly acid pleistocene sand soils the reduction was about 35%.

In soils with a high infection pressure of *R. solani* the efficacy of inoculation was not satisfactory, although significant reductions of the s.i. were obtained. Here integration with chemical control would be advantageous.

The heterogeneous spatial distribution of *R. solani* over a field probably is the main reason for large variations around the average s.i. values. The heterogeneous distribution and the patchy occurrence seem to be normal for *Rhizoctonia* spp. and other soil-inhabiting pathogens and also for soil pests as root-knot nematodes (Nicot et al., 1984). More knowledge of the factors involved in the heterogeneous spatial and temporal distribution of *R. solani* in the field is needed to improve the biological control of this pathogen. It will be the subject of further study.

From the results of this study it can be concluded that:
1. Biological control of *R. solani* in potato by inoculation of the sprouted seed tuber with *V. biguttatum* can be successful if the infection pressure of *R. solani* is not too high. Inoculation proved to be at least as effective as and in most cases even more effective than chemical disinfestation in reducing the amount of sclerotia on the harvest.
2. Integration of biological control and chemical control at low dosage rates appears to be promising when the infection pressure of *R. solani* is high.
3. The results obtained with three isolates of *V. biguttatum*, separately or mixed, in controlling *R. solani* were not signifcantly different.
4. Inocula containing 6×10^5 or 3×10^6 spores per ml were equally effective.

Samenvatting

Biologische bestrijding van Rhizoctonia solani in aardappelen door antagonisten. 5. Het effect van isolaten van Verticillium biguttatum als entmateriaal voor poters en het effect van combinatie met een grondbehandeling met een lage dosering pencycuron

Het beënten van voorgekiemde pootaardappelen met drie isolaten van *Verticillium biguttatum*, elk apart of als mengsel, bleek in veldproeven in 1983 gunstige resultaten te geven: de hoeveelheid lakschurft gevormd op nieuwe knollen was duidelijk minder dan in de onbehandelde controle. In zavelgronden werd de sclerotiumindex (maat voor de hoeveelheid lakschurft) gemiddeld teruggebracht tot de helft van de hoeveelheid die voorkwam op de oogst uit onbehandelde, niet ontsmette, poters.

In twee licht zure zandgronden werd een aanvullende grondbehandeling gegeven (volvelds) met pencycuron in een concentratie die 20% was van de aanbevolen dosering. In één grond werden de resultaten van de enting hierdoor verbeterd en trad een verdere reductie van de sclerotiumindex op van 60% (met alleen *V. biguttatum*) tot 30%. In de andere grond bleef de reductie beperkt tot het niveau van 55%. De dosering was hier waarschijnlijk te laag.

Het effect van enting met *V. biguttatum* op het verlagen van de hoeveelheid sclerotiën op de jonge knollen bleek minstens even goed als een chemische ontsmetting van het pootgoed en in de meeste gevallen zelfs beter.

De drie isolaten van *V. biguttatum* die in deze proeven gebruikt werden waren statistisch niet verschillend in hun vermogen *R. solani* terug te dringen. Evenmin was een verlaging van de sporendichtheid in de entvloeistof tot 20% (6×10^5 sporen ml^{-1}) van de oorspronkelijk gebruikte dichtheid van invloed op de resultaten.

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