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Biological control of Rhizoctonia solani on potatoes by antagonists. 2. Sprout protection against soil-borne R. solani through seed inoculation with Verticilium biguttatum

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Abstract

Verticillium biguttatum isolate M73, when inoculated on the seed tuber, was found to protect young potato sprouts against *Rhizoctonia* infection from the soil and also, as was demonstrated before, against infection from sclerotia on seed tubers. The experiments were performed under laboratory conditions and lasted a few weeks.

V. biguttatum colonizes the surface of the sprouts and may be regarded as a natural inhabitant of the surface of sprouts and presumably also of stems and stolons in a later stage. This fungus may be of value for biological control of *R. solani.*

Additional keywords: sclerotia.

Introduction

Verticillium biguttatum occurs regularly in sclerotia of *Rhizoctonia solani* on potato tubers. The percentage of sclerotia infected with this fungus is usually higher in slightly acid soils than in neutral soils (Jager and Velvis, 1980, 1983). *V. biguttatum* is able to kill the sclerotia, although the efficiency of different isolates is rather variable (Velvis and Jager, 1983). When inoculated on seed potatoes, *V. biguttatum* largely prevents infestation of emerging sprouts with *R. solani* from sclerotia on the seed tuber. *V. biguttatum* grows on the surface of sprouts in the soil and probably is a natural inhabitant of the subterranean surface of the potato plant (Velvis and Jager, 1983).

The present study was performed to determine if *V. biguttatum* could also protect emerging sprouts from infestation with soil-borne *R. solani.*

Materials and methods

An acid sand from Zeijerveld was used in the first experiment and an acid sand from Haren in the second one (Table 1). The soil was sieved (3 mm), homogenized and divided into two portions. One portion got additional *R. solani.*

The soil additionally supplied with *R. solani* received 20 sclerotium fragments (1-2 mm) from agar plates of a pathogenic isolate per 600 ml soil. In the first experiment, each portion was used to fill 40 plastic tubes (diameter 7 cm; height 20 cm). After 8

Table 1. Some properties of the soils used.

	pH (KCl)	Clay particles < 2 μm (%)	Silt particles 2-50 μm (%)	Coarse sand 210-2000 μm (%)	Organic matter (%)	K_2O (mg/100 g)	MgO (mg/kg)	P_{water} (mg P_2O_5/l)
Zeijerveld	4.4	0	42.4	17.5	6.8	19	95	42
Haren	4.1	0	37.1	11.4	4.5	11	42	15

days two small germinating tubers (cv. Irene) were planted in each tube, about 13 cm deep. These treatments, with ten tubes each, were:

A. Sclerotia-free seed tubers;

B. Seed tubers with sclerotia;

A+V. Sclerotia-free seed tubers, inoculated with *V. biguttatum;*

B+V. Seed tubers with sclerotia, inoculated with *V. biguttatum.*

V. biguttatum isolate M73, a good antagonist (Velvis and Jager, 1983), was used for inoculation. A suspension of conidia in an aqueous solution of 1% carboxymethylcellulose (c.m.c) was applied to the seed tubers to be inoculated; the others received 1% c.m.c. only. The sprouts were removed from the tubers 19-20 days after planting. The degree of infestation was assessed and a disease index was calculated for each treatment by multiplying the percentage of infested sprouts by the average severity of infestation. The severity was given a score according to Sneh et al. (1966), from 0 for healthy sprouts to 5 for dead or completely girdled ones. The percentages of 1-cm long sprout pieces colonized by *R. solani* or *V. biguttatum,* going from the base upwards, were determined as described by Velvis and Jager (1983).

The soil of the second experiment was pasteurized (30 minutes at 60 °C) to kill *R. solani.* After pasteurization the soil was inoculated with a filtered suspension of the same fresh soil and left for 8 days. Thereafter the soil was mixed and divided into two portions. To one portion *R. solani* (100 ml of a sclerotia-containing culture in perlite per 10 l of soil) was added. After 6 days each portion was split into ten sub-portions which were each put into a glass beaker of 1 litre. Three small *Rhizoctonia*-free seed tubers (cv. Irene) were planted about 15 cm deep. In summary: the treatments, consisting of five replicates, were:

I. Soil without *R. solani* added; seed tubers not inoculated;

II. Soil without *R. solani* added; seed tubers inoculated with *V. biguttatum;*

III. Soil plus additional *R. solani:* seed tubers not inoculated;

IV. Soil plus additional *R. solani:* seed tubers inoculated with *V. biguttatum.*

Twenty-five days after planting the sprouts were harvested and their infestation was determined. The disease index was calculated, as described above, from the pooled sprouts of each treatment.

Both experiments were performed at 15 °C. The moisture content of both soils was near field capacity.

Results and discussion

The results of the first experiment are given in Fig. 1 (cf. Velvis and Jager, 1983). The

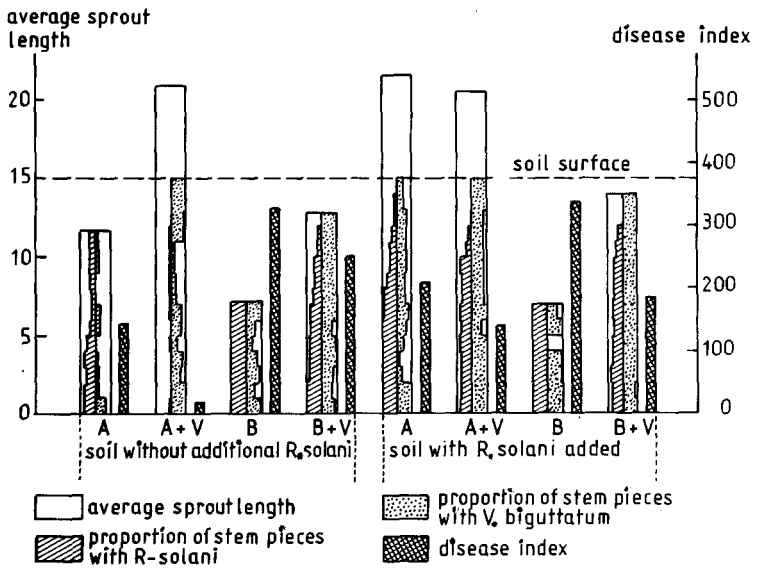

Fig. 1. Development of mycelium of *R. solani* and *V. biguttatum* on the subterranean sprouts of potato. The colonization of the sprouts by *Rhizoctonia* and *Verticillium* is indicated. The infestation of sprouts is separately expressed in the disease index.
A: 'clean' seed tubers; B: seed tubers with sclerotia of *R. solani;* +V: seed tubers inoculated with *V. biguttatum* (M73).

disease indices of sprouts from inoculated tubers (+V) were always lower than those from corresponding noninoculated seed tubers. The average length of sprouts emerged from inoculated seed tubers was in most cases greater than that of noninoculated seed tubers.

Soil without R. solani added. The sprouts of treatment A were apparently infected with *R. solani* in an early stage: *R. solani* was present on at least one third of the sprouts over their whole length and the sprouts were relatively very short. Moreover, their disease index was high too. Possibly some tubers were not free from *R. solani.*

Sprouts in treatment A+V hardly had any *Rhizoctonia,* and a very low disease index. Sprouts were long.

The use of infected seed tubers (B) led to severely infested and stunted sprouts and relatively high disease indices. Inoculation of infected seed tubers with *V. biguttatum* M73 led to a reduced disease index and longer sprouts.

Soil plus additional R. solani. Sprouts of clean seed tubers in soil plus additional *R. solani* were infected at a later stage than sprouts of infected seed tubers (B) and consequently were longer (A and A+V). Inoculation with *V. biguttatum* resulted in a reduction of the disease index.

Sprouts of infected seed tubers (B) planted in soil plus additional *R. solani* had about the same disease index as those in soil without *R. solani.* Soil infection did not further aggravate an already severe infestation of sprouts caused by sclerotia on the

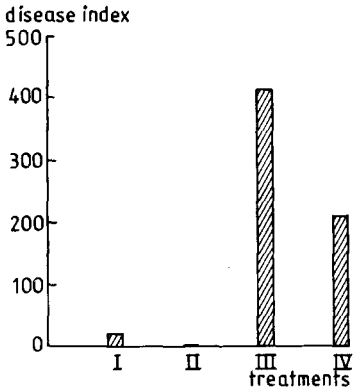

Fig. 2. The effect of inoculation of *Rhizoctonia*-free seed tubers with *V. biguttatum* (M73) on the infestation of sprouts by *R. solani* originating from soil. I: clean soil, seed tubers not inoculated; II: clean soil, seed tubers inoculated; III: soil infected, seed tubers not inoculated; IV: soil infected, seed tubers inoculated.

seed tubers in these experiments.

Inoculation of seed tubers with *V. biguttatum* proved beneficial as was evidenced by a lower disease index.

Sprouts of seed tubers not inoculated with *V. biguttatum* proved to be colonized by soil-borne *V. biguttatum.* The soil had been taken from a potato field and at the end of the growing season contained a rather high population of *V. biguttatum.* Suppression of *R. solani* hardly occurred, although colonization of sprouts by *V. biguttatum* from the soil was rather high. This was probably due to the fact that the antagonistic power of the *V. biguttatum* population in the soil, on the average, was less than that of isolate M73 which was especially selected for its strong antagonistic qualities, and/or to the fact that *V. biguttatum* from the soil appeared too late on the sprouts to prevent or inhibitit infestation.

Fig. 2 gives the disease indices for the sprouts in the second experiment. In pasteurized soil (I and II), sprouts of seed tubers without *V. biguttatum* (I) had a low disease index, 6% of the sprouts being severely infested. If *V. biguttatum* M73 was present (II) infestation did not occur.

In soil plus additional *R. solani,* sprouts from noninoculated seed tubers (III) had a very high disease index, 92% of the sprouts being severely infested. Inoculation of seed tubers with M73 (IV) lowered the disease index. The percentage of infested sprouts was reduced to 67, while also the severity of infestation was lowered to moderate.

The reduced disease indices of sprouts emerged from seed tubers inoculated with *V. biguttatum* M73 demonstrated that this necrotrophic hyperparasite of *R. solani* may be a valuable tool in biological control. It was able to reduce the infestation of sprouts resulting from infected seed tubers (Velvis and Jager, 1983) or from soil infection of *R. solani.*

The value of *V. biguttatum* M73 and other antagonists in biological control of *R. solani* will be further tested in field experiments.

Samenvatting

Biologische bestrijding van Rhizoctonia solani op aardappel met behulp van antagonisten. 2. Bescherming van de spruit tegen R. solani-aantasting vanuit de grond door beënting van het pootgoed met Verticillium biguttatum

Isolaat M73 van de schimmel *Verticillium biguttatum,* indien geënt op de pootknol, blijkt de aantasting van aardappelspruiten door *Rhizoctonia solani* te kunnen verminderen. Zowel de aantasting vanuit de grond en, zoals reeds eerder aangetoond, vanaf sclerotiën op de pootknol worden minder.

Verticillium biguttatum koloniseert het oppervlak van de spruiten en kan beschouwd worden als een natuurlijke bewoner van het oppervlak van spruiten en wellicht ook van stengels en stolonen in een later stadium. In kort durende proeven, als hier beschreven, lijkt *V. biguttatum* goed bruikbaar voor een biologische bestrijding van *R. solani.*

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