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Dynamics of *Rhizoctonia solani* (black scurf) in successive potato crops

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Abstract

The position of plants with *Rhizoctonia solani* sclerotia (black scurf) on progeny tubers was mapped for an experimental field at Haren where potatoes were grown continuously and in rotation with other crops for five successive years, and for another field at Borgercompagnie with a 1:2 frequency of potatoes during three potato crops. Initially, the distribution of plants with black scurf on both fields was rather dense and homogeneous. In the following years the distribution became heterogeneous and patchy. The local decline of *R. solani* AG 3 (the common potato pathogen) in Haren was apparently caused by an unknown factor selectively suppressing *R. solani* AG 3, while simultaneously *R. solani* AG 5 increased in mass. This AG 5 type proved to be an inferior competitor of AG 3 on the potato plant in a laboratory experiment. The specific *R. solani* antagonist *Verticillium biguttatum* did not play a role. A similar factor could have reduced the formation of black scurf in the experimental field at Borgercompagnie, where *V. biguttatum* was also too infrequent to account for the decline. *R. solani* AG 5 was not present here and could not indicate the presence of a selective factor against AG 3.

Introduction

Among the organisms in field soil antagonistic to *Rhizoctonia solani* on potato, the specific necrotrophic mycoparasite *Verticillium biguttatum* W. Gams [Gams and Van Zaayen, 1982] can play an important role in suppressing *R. solani* and reducing its damage to potatoes [Velvis and Jager, 1983; Van den Boogert and Jager, 1984; Jager and Velvis, 1985, 1986; Velvis *et al.*, 1989; Jager *et al.*, 1991]. In these studies damage by *R. solani* was measured as the amount of *R. solani* sclerotia (black scurf) on progeny tubers. Occasionally other antagonistic microorganisms present on sclerotia may have played a minor role [Jager and Velvis, 1983].

We repeatedly observed in different soil types (unpublished) that after a potato harvest strongly infected with black scurf, a harvest could follow (three or four years later) that was nearly free of black scurf.

A predator-prey relationship according to the Lotka-Volterra model between the host fungus *R. solani* and its specific mycoparasite *V. biguttatum* was observed in a pot experiment [Van den Boogert *et al.*, 1990; Van den Boogert and Velvis, 1992]. The question arose whether this system would be responsible for the regulation of *R. solani* in the field too.

Materials and methods

Experimental fields

Haren. The soil is a pleistocene sandy loam with 5% organic matter and a $pH_{(KCl)}$ of 5.3. In the three years before the experiment potatoes, wheat and barley were grown, respectively.

The experiment ran from 1986–1992. Fig. 1 shows the location of crops, i.e. potatoes, spring wheat, sugar beet and field bean (*Vicia faba*, var.) on the field in successive years.

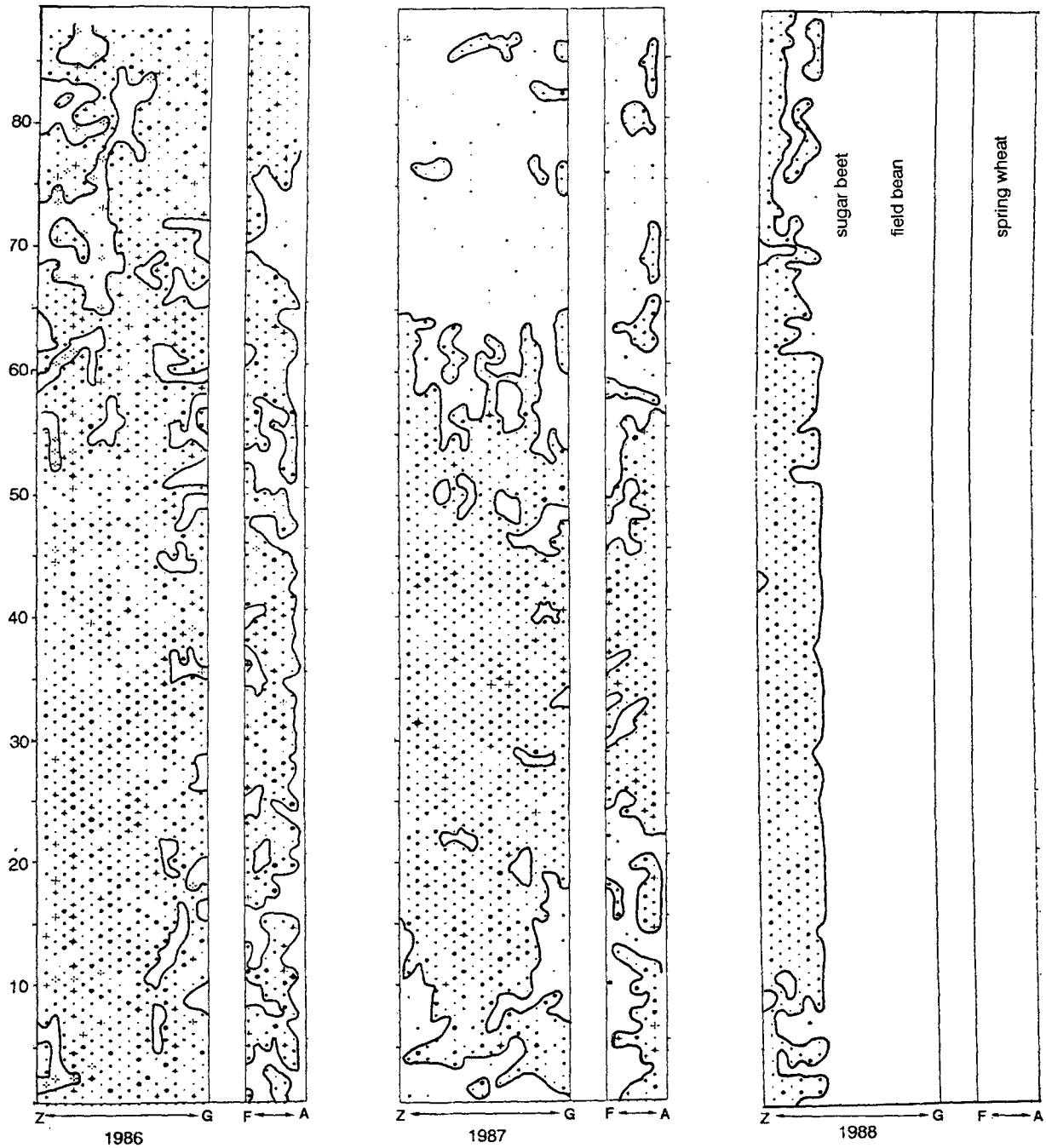

Fig. 1. Position of plants (dots; larger dots – larger amounts of black scurf) with black scurf on young tubers in the experimental field at Haren in successive years. Plants without black scurf have no dots except when the presence of *R solani* was observed (perfect state, lesions on stems or stolons, chats or malformed tubers) and denoted with -¦-. When black scurf was also present it was denoted with -¦- (in 1986 only). In 1990 the seed tubers in rows A–F and U–Z were inoculated with *Verticillium biguttatum*. Rows A–F and G–Z are separated by a track; plants in the rows are numbered 1–88.

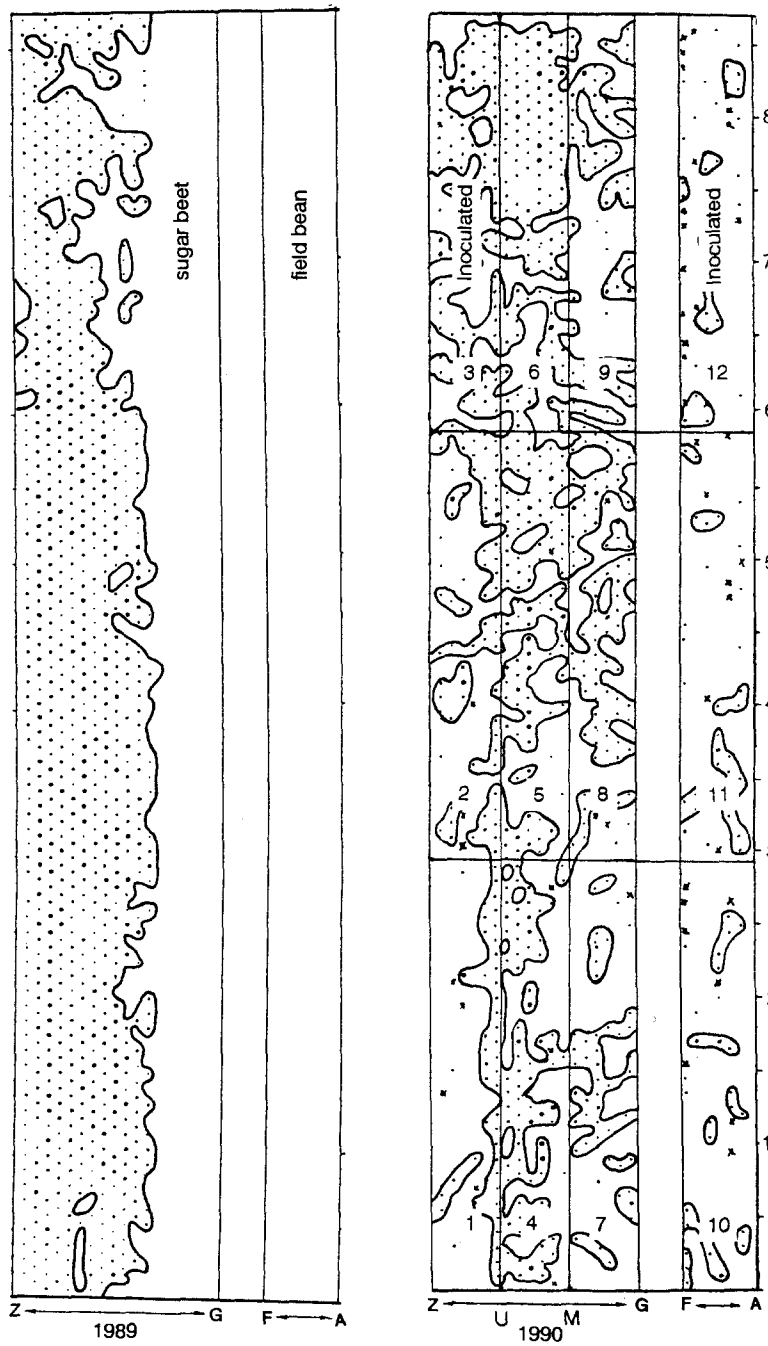

Fig. 1. Continued.

As we were interested in *R. solani* occurring in the soil, disinfected seed tubers were used. Seed tubers pregerminated in daylight were planted by hand in previously made rows, one meter apart to avoid mutual infection of the plants by *R. solani*. The distance between the rows was 75 cm. Plants alternated in adjacent rows. The rows were coded by letters (except I and Q) and the plants by numbers (1–88).

Soil disinfestation against potato cyst nematodes (p.c.n.) took place in autumn 1987 with metham

sodium (300 l ha^{-1}) and in spring 1989 with ethoprofos (10 kg a.i. ha^{-1}).

Borgercompagnie (experimental farm Borgerswold). The soil of this field is a pleistocene sandy loam, reclaimed from the sub-soil of cut-away sphagnum peat. The organic matter content ranges from 9 to 14%; about 14% loam is present in the upper 0–25 cm layer. The $pH_{(KCl)}$ is about 5.0.

The field is a production field for starch potatoes with three plants per meter row length. The field was investigated in 1987, 1989, and 1991. Potatoes were alternated with sugar beet in 1988 and wheat in 1990. In each year of potato cropping ten (uneven) rows in the area were sampled. Each third plant was taken, viz. plant 1 from row A, 2 from row B, 3 from row C etc. From each row a length of 50 m was investigated.

Soil disinfestation against p.c.n. was performed with metham sodium (300 l ha^{-1}) in the autumn preceding a potato crop.

In both fields, tubers were harvested three weeks after haulm destruction. The harvest of each plant was placed in a bag with the letter of the row and the number of the plant in the row, so giving its position in the field. Harvested tubers were washed and classified according the amount of black scurf.

Only the position of plants with black scurf is given in the maps. In 1986 plants in the Haren field visibly infected with *R. solani* (lesions, perfect state, chats) are also given in the maps (+).

Seed potatoes

Haren. The cultivar Bintje was used during the first three years of continuous cropping until p.c.n. became a problem. Bintje was then replaced by c.v. Producent, being resistant against the p.c.n. pathotype present. During the first three years seed potatoes were disinfected according to Butler and Jones [1955]; thereafter with validamycin (Solacol): ten seconds dipping in a 3% solution. Selected black-scurf-free tubers were used. In 1990 seed tubers planted in rows A–F and U–Z were inoculated with conidia of *V. biguttatum.*

Borgercompagnie. The cultivar Elles, resistant against the most frequently occurring p.c.n., was grown here. Seed was disinfected with validamycin (Solacol powder) before planting according to the manufacturer's instructions.

Sampling of soil for the assessment of *R. solani, V. biguttatum* and other antagonists took place on defined areas in the Haren field, where either black scurf had disappeared (in 1986 rows A–F, numbers 70–75) or where it was very common (rows N–W, numbers 25–30). From 1987 onward three other areas were sampled and compared: rows U–Z, where potatoes were continuously grown, plant numbers 65–70 (the R− area where black scurf had disappeared), plant numbers 40–45 (the R+ area where black scurf was frequently present) and plant numbers 15–20 (the R+/−, an intermediate area).

Samples were taken from newly formed rows before or just after planting and about one month after harvest in the old rows. Forty to fifty cores were taken with an auger (3 cm diameter, 30 cm long) from each area; the soil of each area was carefully mixed and homogenized by sieving. From this, subsamples were taken and analyzed. About the same amount of soil was sometimes collected from the 30–60 cm layer.

In October 1987 larger amounts of soil were collected from only the R− and R+ areas.

In 1990 the field was divided into 12 plots, i.e. plots 1, 2 and 3 in rows U–Z, 4, 5 and 6 in rows N–T, 7, 8 and 9 in rows G–M and plots 10, 11 and 12 in rows A–F. Each plot was sampled and analyzed in spring and autumn. In spring 1989 the field in Borgercompagnie was divided into 2 × 5 plots of 10 m length each. From each plot samples (about 5 kg) were randomly taken with an auger as described.

Assessment of R. solani. *R. solani* in soil was assessed by wet-sieving after Weinhold [1977], modified by Kooistra [1983], and is given as the number of propagules with *R. solani* per 250 g soil.

R. solani on roots, stolons and stems of potato plants was assessed by incubating 1-cm long parts of the organs for 24–48 h on water agar (WA, 2% agar) at 20 °C. Isolates from soil or plant parts were grown pure on malt extract (15) peptone (0.25) agar (12 g l^{-1}) (MPA), made with deionized water.

For the microscopical determination of the anastomosis group (AG) the isolates were paired with well-known AG types on malt extract agar (2.5 g malt extract l^{-1}).

Determination of Rhizoctonia-inhibiting factors. The presence of water-soluble substances in soil, sampled in 1987 from R− and R+ areas, inhibitory to hyphal growth of *R. solani* was assessed according to a modification by Velvis [1977] of the method of Sneh and Henis [1972], by measuring the colony diameter after three days growth at 20 °C on Whatman no. 1 filter

paper on wet soil. The mycelium was stained in lactophenol blue.

Soil factors inhibitory to *R. solani* on potato sprouts in the soil, sampled in 1987 from the R− and R+ areas, were determined as follows. Small seed tubers, sprouted in light, were placed on a 2-cm thick soil layer in a plastic tube (diam. 6 cm; height 20 cm) and covered with an 8 cm soil layer. After emergence of the sprouts, an inoculum disk of *R. solani* AG 3 (pathogen) was placed near each sprout and the tube was further filled with the soil under study. After 7, 14, 22 and 29 days five tubes were harvested. Sprout parts from the investigated soil were cut into 1-cm pieces and placed on water agar. The amount of living *R. solani* hyphae was assessed and expressed as a growth index (Gi).

$$Gi = \{p(1-5) \times 1 + p(6-10) \times 2 + p(11-25) \times 3 + p(>25) \times 4\} \times 100/4$$

where p(.−.) is the percentage of sprout pieces with outgrowth of .−. hyphae of *R. solani*.

Competition between R. solani *AG 3 and AG 5.* Competition experiments between AG 3 and AG 5 of *R. solani* were performed in soil with an additional inoculation of both types. Amounts of 180 g soil from the Haren field were inoculated with agar disks (2 mm diam.) from cultures on MPA in the following densities for AG 3 and AG 5, respectively: 30 and 6 (ratio 5:1), 6 and 6 (ratio 1:1) and 6 and 30 (ratio 1:5). The soil was transferred to plastic tubes and covered with a 0.5 cm layer of uninoculated soil. A pregerminated seed tuber was placed on the soil surface and covered with another 10 cm uninoculated soil. The tubes were placed in a climate chamber with a day regime of 13 h light at 15 °C and 11 h dark at 10 °C. The proportion of AG 3 and AG 5 colonizing the sprouts was assessed four times in the course of seven weeks of growth. Both types clearly differed on MPA, as described below.

Assessment of degenerated R. solani *AG 3.* The possible presence of a degenerated type of *R. solani* AG 3 was studied by enriching soil from which *R. solani* AG 3 had disappeared with many healthy mini-sclerotia of *R. solani* AG 3 (2500–3000 sclerotia, size 0.5–1.0 mm, per kg soil). The activity in the samples was enhanced by adding rush baits (1.5 cm long; 50 baits per l soil), hoping to create circumstances to increase anastomosis and infection by a possibly occurring degenerated type. The rush baits were sieved out after two weeks, washed, placed on water agar and carefully inspected for growth of *R. solani*. Attention was focused on isolates showing irregular and slow growth. Fast-growing isolates were removed. Old baits were then fragmented and mixed again with the soil. New baits were added and the procedure was repeated six times. Incubation took place at 10 °C, below the minimum temperature for growth of *V. biguttatum* [Van den Boogert and Jager, 1984], to prevent its activity.

Assessment of V. biguttatum *and other mycoparasites.* The mycoparasites of *R. solani* were established on a specific medium, an MPA plate overgrown with *R. solani* (*Rhizoctonia* plate (RP); Jager *et al.*, 1979). In soil, *V. biguttatum* was assessed as colony-forming units (cfu) per g soil, using the Anderson sampler [Van den Boogert and Gams, 1988]. On subterranean plant parts and in sclerotia the presence of *V. biguttatum* was shown by incubation on RP. In 1990 seed tubers of the rows A–F and U–Z were inoculated with conidia of *V. biguttatum* to see whether the soil would contain factors detrimental to this mycoparasite of *R. solani*.

Statistical evaluation. Average values are given, together with the standard deviation of the mean. Student's-t-test was used to establish the statistical significance of the differences.

Results

Haren. The position of plants with black scurf on progeny tubers is shown in Fig. 1. In 1986 most plants showing signs of *R. solani* infestation, but with a harvest free from black scurf, were those with numbers higher than 60.

At the end of September soil was taken from rows in the R− and R+ areas. The numbers of *R. solani* propagules per 250 g soil for both areas were similar: 7.5 ± 3.3 for the R− and 8.3 ± 5.0 for the R+ area.

The 1987 map (Fig. 1) shows that plants with positions above 65 were almost free from black scurf. The density of *R. solani* in R− and R+ areas, however, proved not to be different. This could be due to qualitative differences in *Rhizoctonia* types. Eighty percent of the isolates of *R. solani* from soil of the R− area were found to differ from the normal type. In the R+ area nearly all isolates belonged to AG 3, the common pathogen of potato (Table 1). The isolates from R− could be placed in AG 5. The cultural appearances of this type on MPA was yellowish, showing a radial hyphal pattern. This type did not produce scle-

rotia, even after three weeks of growth on MPA (AG 3 formed sclerotia after about one week). Later it was observed that sclerotia of the AG 5 type (smooth, half spherical, 2–6 mm diam.) could be formed on tubers. Just like the AG 3 type, the AG 5 type could be parasitized by *V. biguttatum* [Jager and Velvis, 1989].

In the soil of the R− area in 1987 no factors could be demonstrated inhibitory or detrimental towards *R. solani* AG 3 that did not occur in R+ soil (Table 1). The frequent appearance of *R. solani* AG 5 was the most striking feature in the R− area where it seemed to have largely replaced *R. solani* AG 3 (the pathogen). Fig. 2 shows that *R. solani* AG 5 is inferior to *R. solani* AG 3 in competition on potato sprouts. Efforts to isolate a presumed degenerated type of *R. solani* AG 3 in the laboratory experiments described were unsuccessful. All *R. solani* AG 3 individuals were found to be normal fast-growing.

The pattern of plants with black scurf in 1988 showed a return of sclerotia-producing *R. solani* AG 3, which further expanded in 1989 and 1990 (Fig. 1). In 1990 the pattern was very erratic.

The density of propagules of *R. solani* in the soil of rows U–Z was assessed from 1986–1990. From 1988 onward the isolates were tested for their AG, and *R. solani* types were isolated from soil (0–30 cm), plant parts and sclerotia. In May 1988, 1989, 1990 and in September 1990 the 30–60 cm soil layer was also sampled. In the deeper layer *R. solani* was infrequently observed and in low densities. A comparison of the *R. solani* isolates (density and AG) from the 0–30 cm soil layer of the different plots is presented in Fig. 3. In 1986 *R. solani* propagules were estimated in the autumn in plots 2 and 3 only.

In spring 1987 the density decrease was found to be stronger in plot 3 than in plot 2. In 1987 the infestation of the potato harvest by black scurf had almost completely stopped in plot 3 (R−); the pathogen *R. solani* AG 3 in the soil was largely replaced by *R. solani* AG 5. In the following years the *R. solani* density increased and seemed to be kept at more or less the same level, just as the proportion of AG 3 in the population. In plot 2 (R+) the density of *R. solani* decreased to a rather low level. The proportion of AG 3 decreased as well, and AG 5 increased, until both were about equal. In plot 1 (R+/−) the density of *R. solani* was found to be unusually high in the autumn of 1988, after which it decreased to a low level. AG 5 seemed to increase until 1989. Due to the low density of *R. solani* the proportions of AG 3 and AG 5 observed were not reliable in 1990.

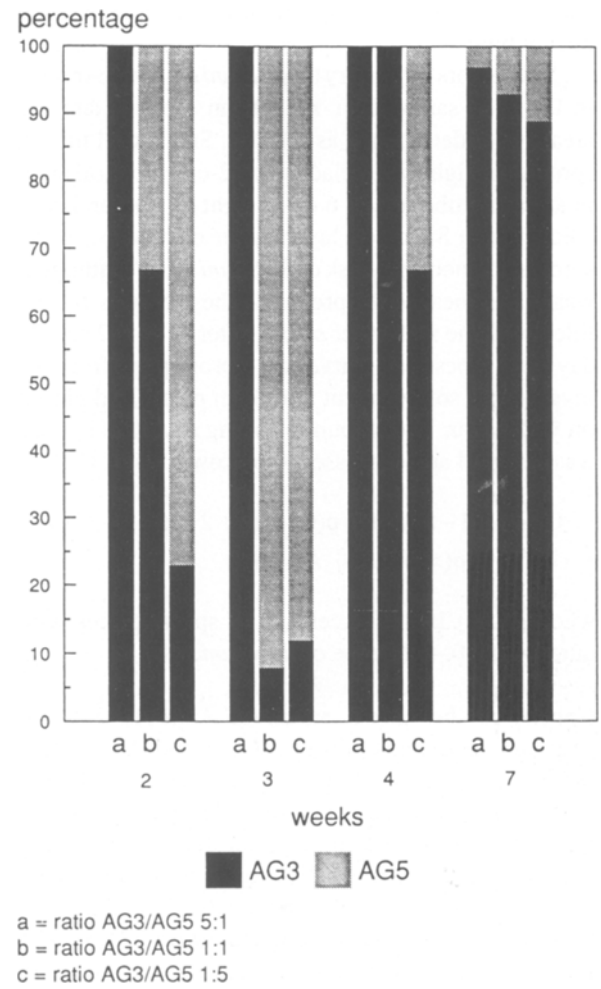

Fig. 2. Percentage of isolates of *R. solani* AG 3 and AG 5 on potato sprouts after inoculation with *R. solani* AG 3 and AG 5 in different proportions in the course of seven weeks' growth.

The AG 3 : AG 5 ratio on plant parts was about the same as in the soil. In sclerotia the proportion of AG 3 was usually higher than in soil. Two AG 2-1 sclerotia were found on a tuber from plot 1 and 3.

In spring 1990 the entire field was planted with potatoes. Samples were taken from 12 plots (Fig. 1). These were assessed individually for propagules of *R solani*. The results of the May and September sampling of plots 1, 2 and 3, on which potatoes had been grown for four years in succession, are given in Fig. 3. In May, the pathogen AG 3 was only found in four out of 12 plots. Deeper in the profile (30–60 cm) very low amounts of *R. solani* (AG 5 and AG 2-1) were found in plots 3, 6 and 12 only.

Table 1. Comparison of the activity of *R. solani* in R– (areas with little or no black scurf) and R+ (areas frequently infested with black scurf) soils and the presence of inhibitory diffusible factor in these soils (Haren, 1987)

Property	R–	R+	Comments
R. solani propagules			
per 250 g soil	2.3 ± 1.5	4.5 ± 4.5	not different
Isolates	7 out of 7	1 out of 10	no AG 3
Diameter of colony			
on soil (mm)	35.2 ± 11.5	38.8 ± 10.6	not different
Growth index of *R. solani* AG 3	62 ± 15	71 ± 17	not different
from sprouts on which it was	78 ± 13	82 ± 14	
inoculated in soil, after	67 ± 8	59 ± 15	
7, 14, 22 and 29 days	55 ± 15	48 ± 20	

The density of *R. solani* propagules in the soil after the potato harvest, in September, was not higher than in May, before the host crop was planted. The proportion of *R. solani* AG 3 seemed to have increased in three plots only (1, 3 and 5), but this AG was now not detected in plot 4. In the other plots of the field (7–12) 19 isolates of *R. solani* were obtained in September, two of which belonged to AG 3, seven to AG 5 and ten to AG 2-1. The latter was most numerous in plots 8 and 11 with other crops than potatoes in the previous two years.

In the Haren field three types of *R. solani* were detected. It is striking that the presence of the host plant did not always lead to an increase in numbers of the pathogen, *R. solani* AG 3, in the soil.

Verticillium biguttatum, as a specific mycoparasite of *R. solani*, was supposed to play a role in the dynamics of *R. solani* in the field [Van den Boogert *et al.*, 1990; Van den Boogert and Velvis, 1992]. The Haren field experiment was laid out to study this relationship. After the strong reduction of damage by *R. solani* in the R– area we expected a striking increase in cfu of *V. biguttatum*. In 1987, just after harvest, roots of plants from the R– and R+ areas were analyzed for the presence of *V. biguttatum*. Only ten isolates of *V. biguttatum* were gained from 600 root segments in the R– area (plot 3), while in the R+ area 41 isolates were obtained from the same amount.

In spring 1988 there was no accumulation of *V. biguttatum* in the R– area. On the contrary, the lowest values were found in this area (Table 2), where the percentage of stolon pieces with *V. biguttatum* was also lowest. In May 1989 *V. biguttatum* was hardly detected in the soil of the three plots. In 1990 the percentage of stolon pieces with *V. biguttatum* again showed very low values in plots where the sprouted seed tubers were not inoculated with conidia of *V. biguttatum* (plots 4, 5 and 6). After inoculation the percentages of stolon pieces with *V. biguttatum* were higher, but still low in comparison with values from earlier experiments [Jager and Velvis, 1985, 1986]. The numbers of cfu of *V. biguttatum* in soil of the rows in September were rather variable, but generally low. Very little *V. biguttatum* was observed in the 30–60 cm layer.

A low percentage of sclerotia from inoculated seed tubers was infected with *V. biguttatum* when compared with earlier results [Jager and Velvis, 1983a].

Borgercompagnie. The position of plants with black scurf are shown in Fig. 4. In 1987 the tubers of only six plants out of 500 were free from black scurf; in 1989 and in 1991 this number had increased to 61 and 115, respectively. In 1987 black scurf was uniformly distributed over the field, in 1989 it tended to be less uniform, while in 1991 the pattern of black scurf free plants was irregular, especially in one half of the field. (This is true for the uneven sampled rows; the even rows were not sampled and their inclusion might have led to (minor) changes in the maps. The maps are, however, drawn as if the even rows did not exist.) Sampling soil (May) and plant parts (June) in 1989 in the different plots gave the following results. The density of propagules of *R. solani* ranged from 0.5 to 5.0 per 250 g of soil in the 0–30 cm layer and from 0

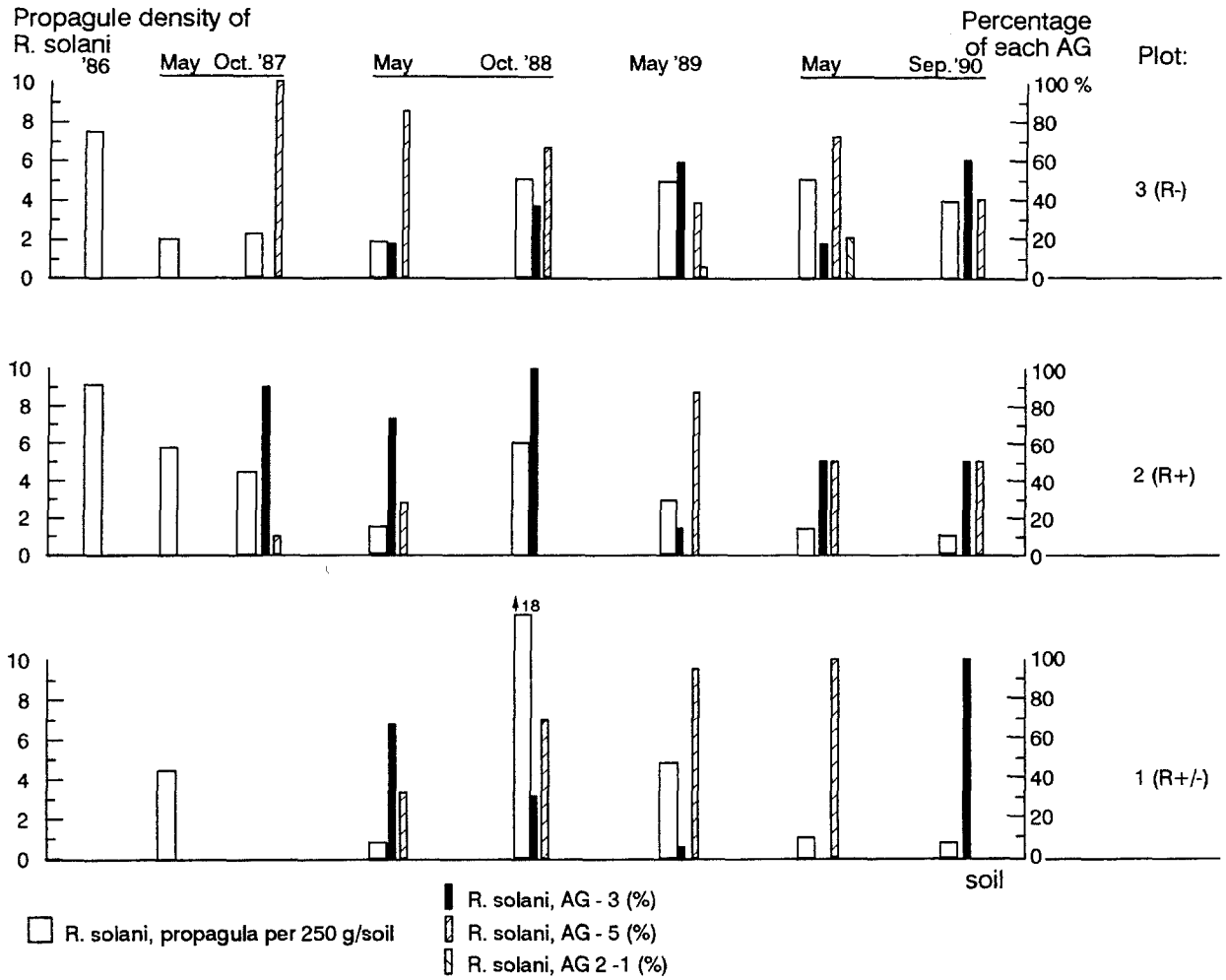

Fig. 3. Density of *R. solani* propagules and AG composition (%) of the isolates in soil from three separate parts (R−, R+ and R+/− areas) of the experimental field at Haren in successive years (0–30 cm).

to 1.0 in the 30–60 cm layer (1 propagule was found in only one plot).

Rather little *V. biguttatum* was detected in the 0–30 cm layer, ranging from 0 to 10 cfu per g soil (0 in only one plot). In the 30–60 cm layer 0 to 3 cfu per g soil were detected (0 in five plots).

The percentage of stolon pieces with living *R. solani* ranged from 10 to 33% (average 22%); for root pieces it was 7 to 30% (average 17%).

The percentage stolon and root pieces with *V. biguttatum* ranged from 20 to 40% for stolon pieces and from 5 to 20% for root pieces; in general the density was low.

71 out of 72 *R. solani* isolates obtained from plant parts belonged to AG 3. The remaining isolate did not belong to AG 5.

In 1991 sclerotia were taken from tubers from plants growing alongside areas with plants free from black scurf. Four percent of the sclerotia (33 out of 873) were dead. Seven of the 33 dead sclerotia harbored *V. biguttatum* and were probably killed by it. *V. biguttatum* was present in only 3% of all sclerotia, a very low value.

Other microorganisms present on or in sclerotia included: *Gliocladium roseum, Volutella ciliata, Penicillium* sp. and *Hormiactis fimicola* (one only). *G. roseum* was present on nearly 2% of the sclerotia. None of these organisms, however, is known to cause a seri-

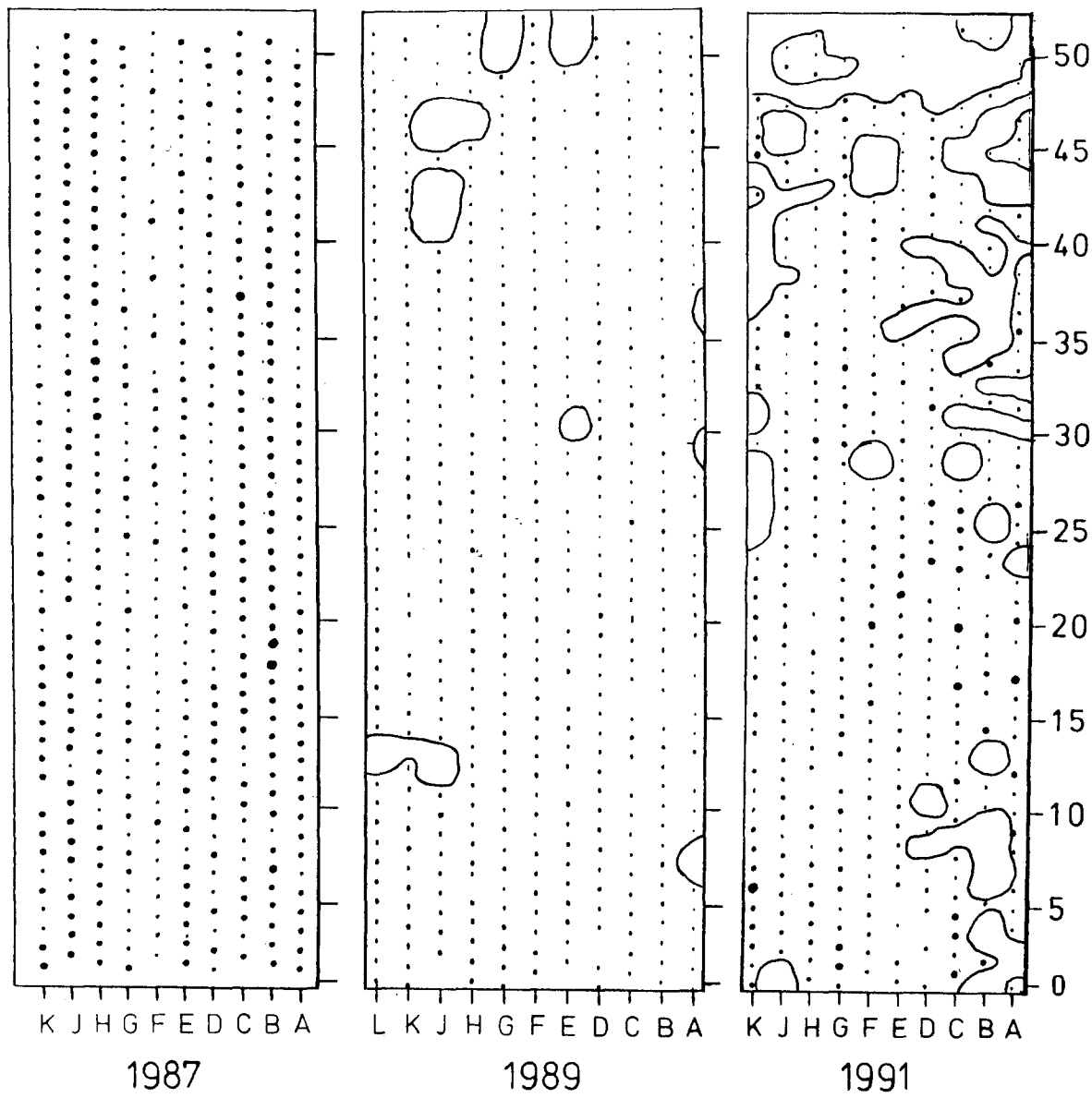

Fig. 4. Plants with black scurf in the experimental field at Borgercompagnie in 1987, 1989 and 1991.

ous threat to black scurf [Velvis and Jager, 1983; Jager, unpublished].

From 385 plants with black scurf at harvest, 36 also harboured *V. biguttatum* (9%). These plants were apparently randomly distributed throughout the field. No relationship with spots free from black-scurf was recognizable.

V. biguttatum thus was present in low densities in the soil of this field; it was present on plants, rather infrequent and in small quantities, and only a very low percentage of the black scurf was infected by it.

Discussion

Tables 1 and 2 show that no antagonistic factors could be demonstrated in R− soil that were not present in R+ soil too. Most striking was the replacement of *R. solani* AG 3 in R− soil by *R. solani* AG 5. As AG 5

Table 2. Presence of *Verticillium biguttatum* in soil, on stolons and in sclerotia from the different plots of the field at Haren

	Plot number					
	1	2	3	4	5	6
1987						
root segments (%)	–	7	2			
1988						
Soil, cfu[1], May	14.0	14.4	0.8			
Stolon pieces (%)	–	4	0			
Sclerotia (%)	33	35	2			
1989						
Soil, cfu, May	0	1.2	0			
	Seed potatoes					
1990	*Inoculated*			*Not inoculated*		
Soil, cfu, May	18.4	15.2	7.2	7.2	2.4	1.6
Stolon pieces I[2] (%)	25	18	25	0	0	0
Stolon pieces II[3] (%)	17	28	36	0	2	0
Soil, 0–30 cm, cfu, Sept.	1.6	16.8	4.8	4.8	0.8	0
Soil, 30–60 cm, cfu	0	0	0.8	1.6	1.6	0
Sclerotia (%)	21	16	35	–	–	–

[1] Cfu per g soil; [2] Stolon pieces I, sampled June 22, [3] Stolon pieces II, sampled July 19. – = not assessed.

is a weak competitor of AG 3 on the potato plant, it could only replace AG 3 when the latter had already disappeared for other reasons.

The density of *V. biguttatum*, the specific mycoparasite of *R. solani*, was too low to account for the disappearance of AG 3. Also the percentage of infected sclerotia was very low in comparison with previous results [Jager and Velvis, 1980]. Besides, if *V. biguttatum* had controlled AG 3, it would have been impossible for AG 5 to increase in mass as *V. biguttatum* parasitizes all AGs of *R. solani* [Jager and Velvis, 1989; Van den Boogert *et al.*, 1989].

The presence of a specific antagonistic activity against *R solani* AG 3 in the field at Haren was concluded from these observations.

A similar mechanism may have played a role in the experimental field at Borgercompagnie as well. However, as no indicative organism as *R. solani* AG 5 or a representative of another AG that could have replaced the eliminated AG 3 has been found, the nature of the selective mechanism remains unknown. We do not know whether the selective elimination as observed in the field at Haren is a general phenomenon or only an exception. More research is needed to resolve this problem.

The decline of a soil-borne disease and the simultaneously generated situation of suppressiveness is usually ascribed to the activity of antagonistic microorganisms [Cook and Baker, 1983]. In the R– soil at Haren *R. solani* AG 3 declined but the soil was not suppressive against AG 3 (Table 1); this also suggests that microbial antagonists were not involved.

The real cause of the disappearance of *R. solani* AG 3 from the soil of the field in Haren is still a matter of speculation. A specific virus or virus-like particle, lethal to *R. solani* AG 3, could be a cause [Hollings, 1982; Nuss and Koltin, 1990].

Castanho and Butler [1978] think that an injuring or lethal principle could be transported from a diseased to a healthy *R. solani* AG 3 by anastomosis. As a consequence of the infection, *R. solani* AG 3 becomes disabled: it grows very slowly and irregularly; it should, however, be able to anastomose, it looses its pathogenicity and the ability to form sclerotia [Castanho and Butler, 1978; Hashiba *et al.*, 1984] and thus vanishes from the population.

The presence of virus-like particles in (pathogenic) fungi is a general phenomenon [Hollings, 1982; Nuss and Koltin, 1990]. These particles are very diverse in genetic respect and can be responsible for various degrees of hypovirulence but also for hypervirulence.

Attempts to isolate a slowly growing diseased or otherwise deviating *R. solani* AG 3 from soil and from roots and stolons after harvest were unsuccessful. The visibly diseased type is short-lived [Castanho and Butler, 1978] and slow growth does not always seem to be a characteristic of hypovirulence.

The antagonistic activity and the protective effect of binucleate *Rhizoctonia*-like fungi (BNR) against the *Rhizoctonia* pathogen of some crops is mentioned by Burpee and Goulty [1984], Sneh *et al.* [1986], and Cardoso and Echandi [1987]. It seems not very probable that BNR played a role in the decrease of *R. solani* AG 3 in the R– area of the Haren field as we isolated here only *R. solani* AG 3 and AG 5 in 1987 and 1988.

Precise knowledge of the processes in our experimental fields is lacking.

Although *V. biguttatum* is a specific, frequently occurring, density-dependent mycoparasite of *R. solani*, there are not many examples from the field pointing clearly to *V. biguttatum* as the cause of suppression [Jager and Velvis, 1980, 1983]. Addition of living hyphae or sclerotia of *R. solani* to the soil led

to marked increases in *V. biguttatum* inoculum and to suppressiveness [Van den Boogert and Jager, 1983]; this effect was more pronounced on slightly acid sandy soil than on neutral marine sandy loam. Enhancement of the initial density of conidia of *V. biguttatum* on the plant by inoculation led – on average – to fewer sclerotia on young tubers, especially in marine loams [Jager and Velvis, 1985, 1986; Jager *et al.*, 1991].

Neither in the field of Haren nor in that of Borgerswold could *V. biguttatum* have been the cause of the elimination of *R. solani* AG 3. In both fields factors detrimental to *V. biguttatum* seem to be active and responsible for its low densities [Jager and Velvis, 1994].

Nematodes and springtails can reduce the deleterious effect of *R. solani* on its host plants [Barker, 1964; Curl and Harper, 1979; Caubel *et al.*, 1981; Hofman, 1988; Bollen *et al.*, 1991]. It is, however, improbable that these soil animals would specifically eliminate *R. solani* AG 3.

As the unknown mechanism supposed in this study may drastically reduce *R. solani* AG 3, its precise nature deserves investigation; this might result new in possibilities for the control of *R. solani* AG 3 in potato [Hashiba, 1987].

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