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Potato plant response to seed tuber bacterization in the field in various rotations

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Abstract

The effects of a seed tuber treatment with antagonistic isolates of fluorescent *Pseudomonas* spp. were investigated on potato plants from 1981 to 1984. The experimental plots were located in fields in short and long rotations of potato. The short rotations are characterized by serious yield reductions which are caused by unknown microbial factors. The reductions varied from 30% in 1982 to only 3% in 1983 in the 3-year rotations. A statistically significant increase in yield (four to five months after planting) of ware potatoes varying from 9 to 20% was obtained in these plots through tuber bacterization, but only in 1981. In 1982 and 1983 initially significant improvements in shoot or tuber weight of seed potatoes were no longer detectable at ware potato harvest at the end of the growing period. Seed tuber bacterization had no effect on tuber yield in long rotations. Initial colonization of basal root parts by 53×10^4 colony forming units (cfu) of antibiotic-resistant mutants per gram of root (fresh) dropped significantly to 20×10^4 cfu per gram after three months. The bacterization effect on tuber yield depended on the development of harmful microbial activity and of introduced antagonists during the growing period. Seed tuber bacterization is more promising for seed potatoes than for ware potatoes in short rotations, the latter being harvested two months later.

Additional keywords: Pseudomonas spp., fluorescent pseudomonads, yield increase and decrease, short (narrow) rotation effect, microbial antagonism, root colonization.

Introduction

Considerable increases in yield can be obtained by treating seed tubers with selected fluorescent pseudomonads. In 1978, Burr et al. reported potato yield increases of up to 33% in field plots. Kloepper et al. (1980b) obtained yield increases of up to 17%. Both studies demonstrated that results are inconsistent with respect to location, soil type, potato cultivar, year and *Pseudomonas* sp. isolate. Plant growth and yield improvement by fluorescent pseudomonads have been ascribed to the antagonistic activity of the introduced pseudomonads, which modifies the composition and/or activity of the resident rhizosphere microflora in favour of the plant (Kloepper and Schroth, 1981; Suslow and Schroth, 1982). The most important mechanism of antagonism is supposed to be competition for iron, by the release of siderophores, between pseudomonads and harmful rhizosphere microorganisms. Siderophores are

metabolites with a high affinity for Fe(III) (Kloepper et al., 1980a; Schippers et al., 1985b). The influence on plant development and potato yield of a harmful microbial factor was demonstrated in long-term rotational experiments in the Flevopolder in the Netherlands. High frequency potato cropping induces yield reductions of approximately 15 to 30%, depending on cropping frequency (Hoekstra, 1981). They could not be fully ascribed to common potato pathogens nor to changes in chemical or physical soil fertility (Scholte et al., 1985). Similar yield reductions have also been reported and demonstrated for other important crops (Schippers et al., 1985a).

The accumulation, with potato cropping frequency, of the yield reducing microbial factor(s) permits the examination of the antagonistic potential of fluorescent pseudomonads at different levels of the target population. Suslow et al. (1979) mentioned that growth increases following bacterization were generally greater in field soils which had been previously grown to the same crop. This was not, however, studied in further detail. Our research resulted in the isolation of in vitro strongly antagonistic fluorescent pseudomonads (Geels and Schippers, 1983a). Considerable yield increases were obtained with several isolates in subsequent pot trials using soil from field plots with continuous potato cropping (Geels and Schippers, 1983b). In soil from the same fields but with no history of potatoes, no yield increase was obtained.

In this report, we present the results of field experiments from 1981 to 1984 on seed tuber bacterization in relation to cropping frequency-induced yield reductions. Antibiotic-resistant mutants of the antagonists were used to follow root colonization in relation to potato plant response in the field.

Materials and methods

Field plots. In 1981, plots were established in rotation fields in a clay-loam soil at the experimental farm 'De Schreef' and in a loam soil at the Research Station for Arable Farming and Field Production of Vegetables (PAGV) near Lelystad. The influence of potato cropping on potato yield has been studied at these locations for 23 and 13 years, respectively. These experimental stations are located in the Flevopolder, an area reclaimed from the IJsselmeer in 1958. As a result, the soil contains numerous shell fragments, is rich in lime and has a pH (KCl) of 7-8. Physical, chemical and agronomical characteristics of both sites are well documented (Hoekstra, 1981; Lamers, 1981). Yields in all rotations with potato have been determined every year at De Schreef and at PAGV. Other field plots were established on diluvial sandy soil at Wageningen-Hoog in 1981 and at Cantonspark, Baarn (sand) in 1982-1983. In Table 1, the most important characteristics of the soil at the experimental sites are listed. Six-year rotations (1:6) served as controls to assess the rate of yield reduction due to higher frequency potato cropping. At De Schreef, three different 3-year rotations (1:3) were used to examine the ability of seed tuber bacterization to limit yield reductions. These 3-year rotations include potatoes every third year, but differ as to the composition and sequence of the other two crops. Yield depressions in 3-year rotations account for up to 15% of yields in 6-year potato rotations (Hoekstra, 1981; Schippers et al., 1985a). Some experimental plots were established at PAGV in 6-year potato rotations and also in PAGV fields cropped continuously with potatoes (1:1). Yields in 1:1 fields are depressed 20-30% compared to those in 1:3 rotations (Lamers, 1981). In Wageningen-Hoog, plots were established in a 2-year rotation (1:2) and in a 6-year rotation (1:6).

Table 1. Characteristics of field experiments at different locations.

Location	Soil type	Soil pH	Commonly used potato cultivar	Relevant years
PAGV	loam	7.5	Saturna	1981-1984
De Schreef	clay-loam	7.9	Bintje	1981-1984
Wageningen-Hoog	sandy	5.2	Irene	1981
Cantonspark, Baarn	sandy	5.4	Bintje	1982
			Saturna	1983

Field plots of 1:1 and 1:6 potato cropping frequency were started in 1979 at Cantonspark, Baarn.

Plots were designed as randomized blocks. Each treatment consisted of a total of 16-20 plants in two, four or five rows and was replicated at least four times. Plant spacing was 33 cm within the row and 75 cm between the rows. Within each row, treatments were separated by two plants of a differently coloured potato variety, viz. cv. Cardinal or Irene. Between the rows, treatments were separated by at least two rows of 'gross' potatoes. Fertilizers plus weed, disease and pest control measures were applied to the plots according to common practice (Hoekstra, 1981; Lamers, 1981).

Isolates of fluorescent Pseudomonas spp. All isolates had been obtained from potato periderm or roots originating from cv. Bintje in De Schreef soil. They were selected for their antagonistic capacities as described by Geels and Schippers 1983a). Isolates were identified by Dr H.J. Miller (Plant Protection Service, Wageningen): WCS 358 and 361 as *Pseudomonas putida* and WCS 365 and 374 as *Pseudomonas fluorescens.* These isolates are strong in vitro antagonists and diminished the yield reductions of potatoes grown in short potato rotation field soil in pots in controlled environment chambers (Geels and Schippers, 1983a,b). WCS 512 and 522 are also fluorescent pseudomonads but remain unidentified to species level. They were isolated on King's medium B supplemented with 100 μg 8-hydroxyquinoline ml^{-1} to select for isolates producing siderophores with a strong iron (III)-binding capacity (Geels et al., 1985). Dr M.N. Schroth (University of California, Berkeley, USA) kindly provided us with the fluorescent *Pseudomonas* sp. isolate B 10, known for its production of the siderophore pseudobactin and its capacity to stimulate plant growth (Kloepper et al., 1980a).

Bacterization of seed potatoes. Pregerminated seed potatoes of excellent quality (NAK class S or SE) were coated with a 1% (w/v) carboxymethylcellulose solution (CMC) as controls or a cell suspension of fluorescent pseudomonads in CMC (Geels and Schippers, 1983a). Two and a half l of CMC suspension containing approximately 10^9 cells ml^{-1} was sufficient to treat four replicates of 20 tubers each (one treatment). Coated tubers were allowed to dry in situ before being covered with soil.

Assessment of plant response to bacterization. The effect of bacterization on plant development was studied at PAGV in 1981 in 1:1 and 1:6 rotation soil. Plant emergence was determined and expressed as a percentage of the number of potatoes planted per

replicate and was averaged per treatment. Crop height was estimated weekly per replicate half-way through the growing season as long as the stems were erect. The percentage crop cover per replicate was estimated. The shoot fresh weight of plants from duplicated plots, designed for root colonization, was measured in 1982 and 1983. Sixteen plants per treatment (four plants per replicate) were removed to assess shoot weight, total length and number of stolons, plus the weight and number of tubers. Sampling times (days after planting) were 33 and 75 days (1982), 63 and 91 days (1983) and 89 and 118 days (1984). In 1984 these dates coincided with the harvest of seed and ware potatoes, respectively. Final yields were assessed after grading according to tuber size (> 28 mm). By this procedure, loose soil was removed from the tubers.

Enumeration of bacteria on the root system. Root colonization by introduced pseudomonads was determined using mutants of isolates WCS 358 and 365, resistant to rifampicin (rif) and nalidixic acid (nal) Geels and Schippers, 1983 a). Two-cm distal, mid or proximal root segments from 4-10 plants per replicate were gently brushed with sterilized brush-pencils to remove adhering soil. Five segments per plant were obtained and transferred into culture tubes containing 5 ml of a sterilized 0.1% (w/v) proteose peptone (Difco) solution and 2 g of glass beads (3 mm diameter). After having shaken the tubes vigourously on a Vortex shaker for 2 min, serial dilutions in 0.1% proteose peptone were made from the supernatant. Appropriate dilutions were stored at 0 °C for up to 12 h and then plated onto selective media.

Tryptic soy agar (TSA), 1/10 strength, supplemented with 100 mg cycloheximide l^{-1} to suppress mould growth, was used for the enumeration of colony forming units (cfu) of the entire aerobic bacterial flora. Total cfu counts for fluorescent pseudomonads were assessed by plating onto King's medium B (KB) supplemented with 50 mg ampicillin, 12.5 mg chloramphenicol, 100 mg cycloheximide, 30 mg benomyl and 20 mg nystatin l^{-1}. Initially in 1981, KB medium including rifampicin (100 mg l^{-1}) and nalidixic acid (100 mg l^{-1}) in addition to the above-mentioned additional antibiotics, was used for the determination of rif/nal-resistant mutants of WCS 358 and 365. To enhance the recovery of the mutants, nalidixic acid was ommitted thereafter.

In 1983, cfu bacterial counts were assessed per gram root. Ten plants were sampled from each replicate. Per plant, 0,5 g (fresh weight) roots of approximately 1 mm diameter, 3-5 cm from the distal end, were brushed and transferred into 100-ml flasks containing 25 ml of 0.1% (w/v) proteose peptone, 0.1% (v/v) Tween 80 and 5 g of calcined silver sand. Following vigorous shaking on a Griffin shaker for 30 min at 4 °C, serial dilutions were plated onto media as described above. In 1981, root colonization was determined at harvest, 112 days after planting, in 1982 33 and 75 days after planting and in 1983 63 and 91 days after planting.

Disease rating. In 1981, the incidence of three soil-borne pathogens of potato was assessed at harvest time for ware potatoes. The relevant plant parts from all replicates of each treatment were sampled and examined or rated as described below.

Rhizoctonia solani. Stem base infection was examined per plant and classified: light (L) – no or few small lesions (≤ 3 mm diameter); moderate (M) – severe lesions which did not span the entire stem base; and heavy (H) – severe lesions which spanned the entire stem base. The following disease index was used:

$$\textit{R. solani} \text{ lesion index} = \frac{0L + 1M + 2H}{2\,(L + M + H)} \times 100$$

in which L, M and H are numbers of stems in class L, M and H.

Sclerotium density on tubers was assessed for 5 kg per replicate, randomly sampled from the 45-55 mm fraction of tubers (after grading and careful washing to remove adhering soil). Each 5 kg sample was rated using reference photographs ranging from completely healthy tubers (grade 10) to tubers which were completely covered with sclerotia (grade 0).

Streptomyces sp. (netted scab). According to Scholte and Labruyère (1985), netted scab is a related but distinct European form of russet scab. Its incidence was assessed as described for *R. solani* sclerotia.

Verticillium dahliae. Infection was assessed using an index based on stem length to the point of attachment of the youngest leaves with beginning symptoms of early dying disease such as one-sided wilting or yellowing. Measuring from the top of the plant downwards, five 10-cm regions were distinguished. Stems with only dead leaves were rated class 6. Stems with diseased leaves up to and including the top 10-cm region were rated class 5. Stems with no diseased leaves in any of the five regions were rated class 0 (healthy). In this way, a *V. dahliae* senescence index according to Lamers (1981) was obtained.

$$\text{Senescence index} = \frac{0w + 1x + 2y + \ldots . 6z}{6\,(\text{total number of stems})} \times 100$$

in which, w, x, y and z are numbers of stems in class 0, 1, 2 and 6.

Microsclerotia on the stems were also assessed: per replicate, all stems were collected at harvest and allowed to dry outside for at least one month. Stems infected with *V. dahliae* developed microsclerotia during this period. They were divided into four classes using photographs showing varying rates of stem occupation by microsclerotia: clean (C) without microsclerotia, light (L) moderate (M), and heavy (H) infection of stems. From this classification, a *V. dahliae* microsclerotia index was obtained.

$$\text{microsclerotia index} = \frac{0C + 1L + 2M + 3H}{3\,(C + L + M + H)} \times 100$$

in which C, L, M and H are the number of stems in the corresponding class of infection.

Results

The influence of cropping frequency on yields in different rotations. Deviating rates of final yield reductions for ware potatoes in various short (narrow) potato rotations (Table 2) should be taken into account when the effect of seed tuber treatment with isolates of fluorescent *Pseudomonas* spp. is assessed in field experiments. Compared to yields in long (6-year) rotations, yield reductions in 3-year rotations varied from 3% tot 17%, 7% to 23% and 7% tot 30% in the respective 3-year rotations at De Schreef over a 4-year period (Table 2). Yield depressions of approximately 30% in continuous cropping of potato (PAGV, 1:1 rotation) fluctuated less than those in other rotations.

Table 2. Gross yields of ware potatoes as affected by cropping frequency.

Location	Cropping frequency[1]	Preceding crop	Relative yield[2]			
			1981	1982	1983	1984
De Schreef	1:6 (2a)	grass seed	100 (66,0)	100 (67.2)	100 (44.1)	100 (53,3)
(cv. Bintje)	1:3 (3c)	grass seed	83	87	97	91
	1:3 (5a)	spring barley	85	77	86	93
	1:3 (3b)	alfalfa	nd[3]	70	91	93
PAGV	1:6	grass seed	100	100	100	100
(cv Saturna)	1:1	potato	69	71	70	nd

[1] Plots formed part of rotations which were cropped with potatoes in different frequencies: every sixth year (1:6), every third year (1:3), every second year (1:2) or continuously (1:1). The number of these rotations in the rotation trial De Schreef is given in parentheses (Hoekstra, 1981).

[2] Expressed as a percentage of the yield 4-5 months after planting in the long (6-year) rotation. The underlined percentages refer to the rotations in which bacterization experiments were conducted. Figures in parentheses represent yields in tons per hectare.

[3] nd: not determined.

Effect of bacterization on shoot and tuber development. At a 1:1 cropping frequency, only isolate WCS 358 improved the rate of emergence one month after planting from 38% in the control to 50%. At a 1:6 cropping frequency, only WCS 374 improved the rate of emergence significantly. The two other isolates improved emergence, but not to significant levels (Table 3). A few days later, all plants had emerged. In June, half-way through the growing period, crop height in the 1:1 rotation was similar to that in the 1:6 rotation, indicating that growth in the 1:1 crop had not yet been retarded. At the end of the growing period, however, the percentage crop cover shows that plant senescence in the short rotation was enhanced considerably (Table 3). At De Schreef, plant development in the short 3-year rotations was retarded even half-way through the growing period in some years (Table 4). In 1982, the influence of bacterization on potato plant response was analyzed during the growing season in different soils and rotations (see also root colonization). Isolate WCS 358R, marked for resistance to rifampicin and nalidixic acid, was used in these experiments. Shoot weight was not significantly increased in plots at PAGV 33 days after planting (1:1 rotation, Table 5). By that time, no tubers had developed. Seventy-five days after planting, shoot weight, stolon length, number of tubers and tuber weight had increased over controls in 1:1 and 1:3 rotations at PAGV and De Schreef. Tuber quantity did not seem to be affected. In 1983, 63 and 91 days after planting (seed tuber harvest), shoot weight and tuber yield were significantly enhanced by WCS 358R. Tuber yield increases were no longer detected, however, in the final yields of ware potatoes (Table 6). In 1984, WCS 358R-treated plants did not differ from control plants when tuber yield was examined 89 days after planting at harvest time for seed potatoes (Table 5).

Table 3. Effect of seed tuber bacterization with fluorescent *Pseudomonas* spp. on plant development of potato cultivar Saturna in short (1:1) and long (1:6) rotations in 1981 at PAGV.

Seed tuber treatment	Emergence (%) 18 May		Crop height (cm) 15 June		Crop cover (%) 1 September	
	1:1	1:6	1:1	1:6	1:1	1:6
Control	38	43	40	39	12	87
WCS 358	50	57	42	41	13	78
WCS 365	39	63	43*	41	15	82
WCS 374	34	68*	40	42	18	80

* Significantly different from control using analysis of variance ($p = 0.05$).

Table 4. Crop height (cm) as affected by cropping frequency half way through the growing season at De Schreef.

Cropping frequency[1]	Preceding crop	1981 23 June	1982 30 June	1983 18 July	1984 5 July
1:6	grass seed	60	79	48	56
1:3	grass seed	55	71	50	55
1:3	spring barley	56	72	49	55
1:3	alfalfa	54	69	50	51

[1] See foot note 1 of Table 2.

Effect of bacterization on yields in short and long potato rotations from 1981 to 1984. A considerable increase in tuber yield of ware potatoes was obtained in 1981 in three of the four fields frequently cropped with potatoes. Yield increases of up to 20% were obtained in a 2-year rotation in a sandy soil in Wageningen-Hoog, but only significantly with isolate WCS 374. At De Schreef, yield was significantly increased in two 3-year rotations but only by isolate WCS 358 (Table 7). Yield increases were not observed at PAGV in plots continuously cropped to potatoes (1:1 rotation), nor in long rotations (1:6) of potato. Different potato cultivars were used at PAGV and De Schreef in 1982, to find out if the origin of the *Pseudomonas* sp. isolate and or cultivar susceptibility were involved in bacterization effects. Apart from a lower yield level of cv. Saturna compared to cv. Bintje, both cvs Saturna and Bintje reacted proportionally similar with yield on preceding crop and on bacterization in the different 3-year rotations and in the 1:1 rotation (Table 8). In 1983, 'Bintje' and 'Saturna' showed similar yield reductions when the 1:1 rotation was compared with the 1:6 rotation (PAGV) or a 1:3 rotation with a 1:6 rotation (De Schreef, unpublished data). No yield increase was observed in treatments with any of the isolates originating from cv. Bintje in De Schreef soil, for both of these cultivars in 1:1 and 1:3 rotation soils. Several other isolates (not shown) isolated from cv. Saturna in PAGV soil, also did not improve tuber yield. In 1983 and 1984, the effect of the WCS isolates was compared with isolate B 10 used by Kloepper et al. (1980a). Two new *Pseudomonas* sp. isolates (WCS 512, 522), isolated

Table 5. Influence of seed tuber treatment with *Pseudomonas putida* isolate WCS 358R on shoot and tuber development[1] during the growing season in short potato rotations.

Year	Location	Days after planting	Cropping frequency[2]	Preceding crop	Average shoot fresh weight (g)		Average fresh weight of tubers (g)	
					control	WCS 358R	control	WCS 358R
1982	PAGV	33	1:1	potato	34	36	0	0
		75	1:1		1592	1663	270	290
	De Schreef	33	1:3	grass	36	42	0	0
		75	1:3	seed	878	1030*	614	703
		33	1:3	spring	33	38	0	0
		75	1:3	barley	919	951	600	647
1983	De Schreef	63	1:3	grass	364	403	410	472*
		91	1:3	seed	357	432*	923	1012*
1984	De Schreef	89	1:3	alfalfa	nd[3]	nd	543	543
		118	1:3		nd	nd	1045	995

[1] All figures are average values per plant per treatment (16 plants per treatment).
[2] See foot note 1 of Table 2.
[3] nd: not determined.
* Statistically significant at p = 0.05 over control.

Tabel 6. Gross yields of ware potatoes in short potato rotations, four to five months after seed tuber treatments with fluorescent *Pseudomonas* spp.

Year	Cultivar	Location	Cropping frequency[1]	Yield of control (kg are^{-1})	Rel.yield (% of control)				
					WCS 358	WCS 374	WCS 512	WCS 522	B10
1983	Bintje	De Schreef	1:3[2]	506	97	99	nd[4]	nd	104
	Saturna	PAGV	1:1	285	97	95	98	96	100
	Saturna	Cantonspark	1:1	360	106	nd	105	110	112
1984	Bintje	De Schreef	1:3[3]	466	95	nd	94	nd	nd

[1] See foot note 1 of Table 2.
[2] Preceding crop grass seed.
[3] Preceding crop alfalfa.
[4] nd: not determined.

on 8-hydroxyquinoline-supplemented media (Geels et al., 1985), were also used in these experiments (Table 6). The effect on ware potato tuber yield of seed tuber treatments with *Pseudomonas* sp. isolate B 10 or isolates WCS 512, 522, 358 and 374 was not significant (Table 6).

Table 7. Gross yields of ware potatoes in short and long potato rotations, four to five months after seed tuber treatments with fluorescent *Pseudomonas* spp. in 1981.

Cultivar	Location	Cropping frequency[1]	Yield of control (non-treated)		Relative yield (% of control)		
			kg are^{-1}	% of 1:6	WCS 358	WCS 365	WCS 374
Irene	Wageningen-Hoog	1:6	388	100	101	107	101
		1:2	198	51	109	100	120*
Bintje	De Schreef	1:6	595	100	104	91	98
		1:3[2]	495	83	111*	104	103
		1:3[3]	505	85	109*	102	100
Saturna	PAGV	1:6	552	100	104	98	104
		1:1	416	75	98	99	97

[1] See foot note 1 of Table 2.
[2] Preceding crop grass seed.
[3] Preceding crop spring barley.
* Statistically significant over control using analysis of variances ($p = 0.05$).

Table 8. Gross yields of ware potatoes in short potato rotations, four to five months after seed tuber treatments with *Pseudomonas* spp. in 1982.

Cultivar	Location	Cropping frequency[1]	Yield of control (non-treated)	Relative yield (% of control)		
			kg are^{-1}	WCS 358	WCS 361	WCS 374
Bintje	De Schreef	1:3[2]	589	97	101	103
		1:3[3]	534	100	98	101
Saturna		1:3[2]	430	95	nd[4]	nd
		1:3[3]	394	98	nd	nd
Bintje	PAGV	1:1	570	95	nd	nd
Saturna		1:1	398	94	97	103
Bintje	Cantonspark	1:1	420	nd	108	113

[1] See foot note 1 of Table 2.
[2] Preceding crop grass seed.
[3] Preceding crop spring barley.
[4] nd: not determined.

Root colonization. Mutants of isolates WCS 358 and 365, marked for resistance to rifampicin and nalidixic acid, were used to monitor root colonization during the growing season. Cfu counts for marked mutants (per cm root) tended to increase with time on the basal root parts during a period of up to 75 days after planting (Table 9). Beyond

that period in 1983, cfu (per gram root fresh weight) dropped from 53×10^4 to 20×10^4, at 63 and 91 days after planting, respectively.

One hundred and twelve days after planting, however, the introduced mutants could still be reisolated from the root system (Table 9). In most cases, the total number of pseudomonads on the roots after seed tuber treatment increased considerably (Table 9). This suggests that these pseudomonads were predominantly the introduced antibiotic-resistant mutants, However, this assumption is not reflected in the relatively small increase in cfu on medium containing the two antibiotics. Lowering the concentrations of both rifampicin and nalidixic from 200 to 100 mg l^{-1} improved the recovery (Geels and Schippers, 1983a) as did the total omission of nalidixic acid in the isolation medium. Due to the characteristic colony form of WCS 358 and in vitro experiments with other isolates, we could confirm that the omission of nalidixic acid was not associated with a loss of selectivity by the isolation medium. The introduced mutant WCS 358R accounted for 0.02 - 0.60% of the total number of fluorescent pseudomonads, which accounted for 0.3 - 58% (controls) or 0.8 — 81% (after bacterization) of the total number of bacteria (Table 9).

Incidence of soil-borne pathogens as related to bacterization effects on yields. In 1981, the incidence of three well-known soil-borne pathogens of potato was assessed. *R. solani* infection was very prominent in the sandy soils of the short rotations in Wageningen-Hoog and Cantonspark, Baarn. At De Schreef, we noticed little difference between the incidence of *R. solani* in the 1:6 and the 1:3 rotations with respect to sclerotia on tubers. This makes it very unlikely that *R. solani* was involved in the observed yield reductions of 17 and 15% in the respective 1:3 rotations (Tables 2 and 10). The incidences of *R. solani* in the 1:1 rotation at PAGV was, though not serious, clearly enhanced by increasing the cropping frequency. *R. solani* was effectively absent in the 1:6 rotation. The effect of bacterization on the incidence of *R. solani* was insignificant in all cases, according to an analysis of variance of the results (Table 10). *Streptomyces* sp. (netted scab) was very prominent at De Schreef in short rotations, but not in the 1:1 rotation at PAGV as 'Saturna' is a resistant variety (whereas 'Bintje' is susceptible). No effect of bacterization was found on the incidences of netted scab (Table 10). Incidence of *V. dahliae* was high in the 1:1 rotation at PAGV. It was less serious, but sporadic, in the short 1:3 rotations at De Schreef. Isolate WCS 374 diminished a *V. dahliae* stem infection significantly, but this was not reflected in a yield increase (Tables 7 and 10). Conversely, the statistically significant yield increases by isolates WCS 358 and 374 in Wageningen-Hoog and De Schreef were not accompanied by a reduced incidence of any of the pathogens mentioned (Tables 7 and 10).

Discussion

This field study confirms observations with pot experiments (Geels and Schippers, 1983b) that tuber yield increases caused by antagonistic fluorescent pseudomonads are only obtained in soils in which yields are seriously reduced by predominantly unknown harmful microbial activity as a result of frequent potato cropping. This study, however, als demonstrates that a serious yield reduction is no guarantee that seed tuber bacterization will stimulate plant growth and increase tuber yields. In the field, other factors, such as soil water availability and temperature, will affect the

Table 9. Root colonization[1] of potato cv. Bintje by fluorescent *Pseudomonas* spp. isolates WCS 358R and 365R, marked for resistance to rifampicin and nalidixic acid.

Year	Location and cropping frequency[2]	Days after planting	Treatment	Number of rif/nal resistant mutants per cm root length (A)		Total number ($\times 10^2$) of fluorescent pseudomonads per cm root length (B)		Total number ($\times 10^4$) of aerobic bacteria per cm root length (C)
				A	A, as % of B	B	B, as % of C	C
1981	Cantonspark (1:3)	112	control	0		0.5		nd[3]
		112	WCS 365R	60		1		nd
1982	PAGV (1:1)	33	control	0		426	0.4	1018
		33	WCS 358R	390	0.6	666	0.8	812
		75	control	0		2862	48	60
		75	WCS 358R	472	0.2	2412	60	40
1982	De Schreef (1:3)	33	control	0		284	0.3	1014
		33	WCS 358R	324	0.6	548	0.9	632
		75	control	0		1328	58	23
		75	WCS 358R	614	0.4	1620	81	20

[1] Basal root parts, 3 to 5 cm from their attachment to the stem base, were examined and the colonization rate expressed as colony forming unit (cfu) count per cm root length.
[2] See foot note 1 of Table 2.
[3] nd: not determined.

Table 10. Incidence of soil-borne potato pathogens after seed tuber treatment with different isolates of *Pseudomonas* spp. in 1981.

Pathogen	Location	Rotation	Treatment			
			Control	WCS 358	WCS 365	WCS 374
Rhizoctonia solani						
a) Stem base infection[1]						
	Wageningen-Hoog	1:2	84	83	85	80
	Cantonspark	1:1	54	53	48	nd[4]
b) Sclerotia on tubers[1]						
	Wageningen-Hoog	1:2	7.3	7.3	7.0	8.0
	PAGV	1:6	9.8	10.0	10.0	10.0
		1:1	7.0	7.3	6.3	7.1
	De Schreef	1:6	9.8	9.8	9.8	9.8
		1:3[2]	7.0	8.3	8.0	8.0
		1:3[3]	9.0	9.0	9.0	8.8
Streptomyces sp. (netted scab)						
Tuber infection[1]						
	PAGV	1:6	10.0	10.0	9.8	10.0
		1:1	9.8	9.8	9.5	9.5
	De Schreef	1:6	8.8	9.0	9.0	9.0
		1:3[2]	7.0	7.0	6.8	6.8
		1:3[3]	6.3	5.8	5.5	6.5
Verticillium dahliae						
a) Senescence index[1]						
	PAGV	1:1	22	26	28	25
b) Microsclerotia index[1]						
	PAGV	1:6	20	18	19	24
		1:1	77	83	81	68*
	De Schreef	1:6	24	30	31	28
		1:3[2]	71	83	63	66
		1:3[3]	39	48	30	35

[1] See Material and methods.
[2] Preceding crop grass seed.
[3] Preceding crop spring barley.
[4] nd: not determined.
*Statistically significant over control using analysis of variance ($p = 0.05$).

harmful microbial activity and the growth-promoting pseudomonads. The overall influence of bacterization on yield therefore fluctuates from year to year. At least two factors, i) the level of harmful microbial activity and ii) the in situ effect of the introduced plant growth-stimulating pseudomonads, determine the final outcome. The average yield reduction of 15% for 1978-1980 in 3-year potato rotations at De Schreef (Hoekstra, 1981) was also observed in 1981 and coincided with statistically significant ware potato yield increases of 9 and 11% obtained with isolate WCS 358 in two dif-

ferent 3-year rotations. The same isolate failed to increase ware potato yield in the following 3 years. The relative yield depression (narrow rotation effect) in these years, however, was serious (23%) in only one 3-year rotation and moderate to negligible (13, 7 and 3%) in three of the other 3-year rotations (see underlined figures in Table 2). A tuber yield increase after two and three months (seed potato harvest), however, was observed more frequently (Table 5). In 1985, a significant increase by *P. putida* isolate WCS 358 of 12% in seed tuber yield 86 days after planting was obtained at De Schreef. Non-treated controls in this 3-year rotation showed a yield reduction of 13%, compared to the 6-year rotation. Again, no yield increase was obtained in the 6-year rotation (Bakker et al., 1986). Observations at the PAGV plots need special attention. In spite of seriously decreased yield in plots continuously cropped with potatoes, introduced antagonists were unable to raise the final yield. The long-term rotational experiments (Hoekstra, 1981; Lamers, 1981), at De Schreef and PAGV, have demonstrated that rotational effects usually develop later in the growing season. In contrast with the situation at PAGV, where plant development in the 1:1 rotation is maintained fairly well until mid July, plant development at De Schreef is, in certain years, retarded early in the season.

Decreasing numbers or activity of introduced pseudomonads during the season, may fail to suppress the possible late development of the harmful microorganisms in the second half of the growing season (situation at PAGV). Early onset of harmful microbial activity at de Schreef, however, can be counteracted resulting in reduced harmful activity in the second half of the season. Another explanation may be that our isolates which originate from cv. Bintje in De Schreef soil, do not thrive on cv. Saturna, the common cultivar used in the 1:1 rotation at PAGV. Fluorescent pseudomonads isolated from cv. Bintje did not appear to be able to improve yield of cv. Saturna in the field. Host specificity by plant growth-promoting pseudomonads has also been suggested by Kloepper et al. (1980b). They showed that of four isolates capable of increasing yield in radish, only one improved potato growth significantly.

Scholte et al. (1985) showed that *V. dahliae* may cause yield reductions of up to 8% (pot experiments) in cv. Bintje, which is even more susceptible than cv. Saturna. However, we know from other short rotations at PAGV that considerable discrepancy between yields of 3-year rotations and 1:1 rotations does not necessarily coincide with significant differences in the stem microsclerotia index. The same reason accounts for differences between the two 3-year rotations sampled at De Schreef (Table 10). Therefore, it is not likely that *V. dahliae* is the main causal agent of the serious yield reductions in continuous potato cropping. Potato favours the production of resting structures (microsclerotia), whereas sugar beet, being a host as well, fails to do so. This phenomenon is of interest when rotations with or without sugar beet are involved (Van der Spek, 1985). Our results show, that *V. dahliae, Streptomyces* sp. and *R. solani* are sensitive to potato cropping frequency, depending on potato cultivar susceptibility. Yield increases due to bacterization, but which did not coincide with significant suppressions of one of the three pathogens, support the general opinion (Schippers et al., 1985) that the observed yield reductions in short rotations are mainly due to other unknown microbial factors. The significant suppression of *V. dahliae* at PAGV by isolate WCS 374 is confirmed by results of Wadi and Easton (1983), who reported a reduced stem infection after bacterization with isolates of fluorescent *Pseudomonas* spp., but no increased yield in field plots.

Colonization densities of the introduced antibiotic-resistant mutants of WCS isolates show that cfu per cm root did not exceed 10^3 on basal root parts, two months after planting (Table 9). Their numbers gradually declined during the season, probably because, with time, the basal root parts became less suitable for pseudomonads. Nevertheless, mutants were still detectable 112 days after planting. Assessment of cfu on young, growing root parts would have been more valuable but we did not obtain sufficient quantities of this material from the field. Quantitative variability of bacterial populations on individual plant roots (Loper et al., 1985) demands sufficient material to obtain reliable results. A relative increase in the total fluorescent pseudomonad cfu count was not reflected in a proportionally high cfu count for the mutants (max. 0.6% in this study). Factors affecting recovery of rif/nal mutants were discussed earlier (Geels and Schippers, 1983a).

In conclusion, the unique information on decreasing yields with increasing cropping frequency provided by the long-term rotational field experiments at De Schreef and at PAGV was indispensable to analyze the potential use and limitations of seed tuber treatments with fluorescent pseudomonads.

The degree of colonization of the roots by the introduced fluorescent pseudomonads seems to be a determining factor for the degree of stimulation of potato yields. Their root-colonizing abilities are expressed most clearly under field conditions and need further examination.

Our results indicate that bacterization with wildtype isolates of *Pseudomonas* spp. in short rotations is more promising for the cultivation of seed potatoes than for ware potatoes, as seed potatoes are harvested one to two months earlier than ware potatoes.

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Samenvatting

De invloed van pootgoedbehandeling met bacteriën op de teelt van aardappelen in nauwe rotaties

De invloed van pootgoedbehandeling met antagonistische isolaten van fluorescerende *Pseudomonas*-soorten op de aardappelteelt, werd onderzocht in de periode van 1981 tot en met 1984. De proefvelden maakten deel uit van zowel ruime als nauwe rotaties met aardappelen. Kenmerkend voor de nauwe rotatie is, dat de opbrengst aanzienlijk gereduceerd wordt als gevolg van de aanwezigheid van nog onbekende microbiële factoren. Deze opbrengstverlaging varieerde van 30% in 1982 tot slechts 3% in 1983 in de 3-jarige rotaties. Pootgoedbacterisatie had in deze proefvelden een significante toename van de eindopbrengst (vier tot vijf maanden na pootdatum) van consumptieaardappelen tot gevolg, die varieerde van 9 tot 20%, echter alleen in 1981. In 1982 en 1983 werd het effect van bacterisatie ook in de loop van de groeiperiode onderzocht. Aanvankelijk significante toenames van zowel spruit- als knolgewicht waren aan het

einde van het groeiseizoen niet meer aantoonbaar. Pootgoedbacterisatie bleek geen effect te hebben op aardappel in ruimte rotaties. Aanvankelijk werden de basale wortelgedeelten gekoloniseerd door antibioticum-resistente mutanten met 53×10^4 kolonievormende eenheden (kve) per gram wortel (vers); dit aantal liep (drie maanden na pootdatum) echter significant terug tot 20×10^4 kve per gram. Het effect van bacterisatie op de eindopbrengst werd bepaald door de ontwikkeling van de schadelijke microbiële activiteit en de ontwikkeling van de geïntroduceerde antagonisten tijdens het groeiseizoen. Pootgoedbacterisatie in nauwe rotaties biedt meer mogelijkheden voor de teelt van pootaardappelen dan die van consumptieaardappelen, die geruime tijd later geoogst worden.

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