

# Potato brown rot incidence and severity under different management and amendment regimes in different soil types

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**Abstract** *Ralstonia solanacearum* race 3 biovar 2, the causative agent of potato brown rot (bacterial wilt), is an economically important disease in tropical, subtropical and temperate regions of the world. In view of previous reports on suppression of the disease by organic amendments, and the expansion of organic agriculture, it was timely to compare the effects of organic and conventional management and various amendments on brown rot development in different soils (type: sand or clay; origin: Egypt or the Netherlands). Brown rot infection was only slightly

reduced in organically compared to conventionally managed sandy soils from Egypt, but organic management significantly increased disease incidence and pathogen survival in Dutch sandy and clay soils, which correlated with high DOC contents in the organic Dutch soils. There was no correlation between disease incidence or severity and bacterial diversity in the potato rhizosphere in differently managed soils (as determined by 16S DGGE). NPK fertilization reduced bacterial wilt in conventional Egyptian soils but not in Dutch soils. Cow manure amendment significantly reduced disease incidence in organic Dutch sandy soils, but did not affect the bacterial population. However, cow manure did reduce densities of *R. solanacearum* in Egyptian sandy soils, most probably by microbial competition as a clear shift in populations was detected with DGGE in these and Dutch sandy soils after manure amendment. Amendment with compost did not have a suppressive effect in any soil type. The absence of a disease suppressive effect of mineral and organic fertilization in Dutch clay soils may be related to the already high availability of inorganic and organic nutrients in these soils. This study shows that the mechanism of disease suppression of soil-borne plant pathogens may vary strongly according to the soil type, especially if quite different types of soil are used.

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## Introduction

*Ralstonia solanacearum* causes a vascular disease in many different host plants including many economically important crops and in more than 40 countries. Five races are distinguished: race 1, biovar 1, 3 and 5 having a broad host range, race 2, affecting bananas, and race 3 biovar 2 (phyllovar 1, sequevar 1) infecting primarily potato and tomato. The disease is generally referred to as ‘bacterial wilt’, but in potato it is also called ‘brown rot’ (Elphinstone 2005).

In some areas, naturally suppressive soils have been reported (see for a review e.g. Hayward 1991). The influence of soil type on disease severity has been repeatedly established (Moffett et al. 1983; van Elsas et al. 2000), but the actual effects are controversial and depend on geographical location, strains (race, biovar) of the pathogen, and crops involved. In some cases, bacterial wilt was more severe on well-drained sandy loams (French 1994; Hayward 1991), and the decline rate of the pathogen was higher in clay loam than in sandy loam (Moffett et al. 1983). In other cases, the disease was most severe on heavy clay-loam soil (Kelman 1953). Thus, suppressiveness of soil towards bacterial wilt may be related to other factors than soil texture, such as pH, organic matter content and microbial communities (van Elsas et al. 2005). Disease suppression was lost after soil treatment with methyl bromide or heat, indicating that a biological principle was the main factor.

Control of bacterial wilt in areas conducive to the disease is difficult: there are no curative chemicals, resistance breeding has only been reasonably successful against race 1 of the pathogen and diverse cultural measures (including the use of certified seed, wide rotations with non-host crops and careful water management) have had only limited success (Kelman 1953; Hartman and Elphinstone 1994; French 1994).

Inorganic soil amendments have sometimes shown promising results both in pot experiments and in the field, but in other cases there was little effect. An increase or decrease in pH reduced wilt in tobacco, tomato and eggplant when sulphur (acidification) or lime (neutralization) was added, especially in relatively acid, fine sandy soils (Kelman 1953; Michel and Mew 1998). However, for potato on sandy loam soils with high organic matter content this was not effective. A high nitrogen dose reduced bacterial wilt

in sandy soils; nitrates were more effective than ammoniacal compounds (Kelman 1953; Michel and Mew 1998). Soil amendment with NPK at 100 kg ha<sup>-1</sup> significantly decreased bacterial wilt incidence on potato and increased potato yield (Lemaga et al. 2005). Soil fertilization and amendment of soil with high inputs of urea and CaO or MgO also significantly decreased populations of *R. solanacearum* populations (Elphinstone and Aley 1993; Michel et al. 1997).

Apart from inorganic amendments, organic amendments can affect both survival of the pathogen in soil and infection of the host. Amendment of soil with compost or manure (Schönfeld et al. 2003) or pig slurry (Gorissen et al. 2004) reduced soil pathogen populations depending on soil type (Domínguez et al. 2001; Kelman 1953; Michel and Mew 1998). These effects have been attributed to abundance of antagonistic microbial populations (Schönfeld et al. 2003; van Elsas et al. 2005), a microbial community shift (Gorissen et al. 2004), or ammonia toxicity (Michel and Mew 1998).

Management of bacterial wilt is especially important for small farmers in tropical countries, where the disease is endemic, including the Nile Delta area in Egypt, where the ‘potato-specific’ race 3 biovar 2 is common since the 1940s (Farag et al. 1999). Frequent interception of this quarantine pathogen after import of Egyptian early consumption potatoes has prompted the EU to ban importation of potatoes from the Nile Delta area (Anon. 1998). Export of Egyptian potatoes to the EU, however, is allowed from specially designated pest-free areas (PFAs) known as uncontaminated areas. These are mostly recently developed desert areas with sandy soils, which are pivot-irrigated mainly with ancient water from a large uncontaminated aquifer. Of course, in the case of accidental introduction of *R. solanacearum* into the desert area, it would be important to know what the disease risk would be and what farmers could do to reduce this risk.

In view of the potential suppressive effects of soil amendments, it would be interesting to know if organic management could lead to a reduction in bacterial wilt. Root diseases and wilts caused by fungi are frequently well controlled in organically-managed soils (van Bruggen and Termorshuizen 2003), but this has not been studied for bacterial diseases.

We previously studied the effects of organic versus conventional management, different soil types, and amendment with NPK fertilizer or cow manure on survival of *R. solanacearum* race 3 biovar 2 (Messiha 2006). The decline in *R. solanacearum* was faster in sandy than in clay soils; management effects were much smaller than effects of soil type. Application of NPK fertilizer or cow manure enhanced the decline rate of *R. solanacearum*. Survival of the pathogen in soil was positively correlated with total soluble organic matter and negatively with bacterial diversity as estimated from results of denaturing gradient gel electrophoresis (DGGE) of eubacterial 16S rDNA directly extracted from soil (Messiha 2006).

The main aim of this study was to investigate if soil management could affect potato brown rot development. Both management types (organic and conventional) and individual management components (fertilization of conventional soil with NPK and amendment of organic soil with manure or compost) were compared in sandy and clay soils from Egypt and the Netherlands. Various factors that could possibly explain differences in potato brown rot development in the different soils were assessed, e.g. soil physical and chemical characteristics and bacterial diversity in the bulk soil and rhizosphere of healthy potato plants grown in the various soils inoculated with *R. solanacearum*.

## Materials and methods

### Soils and amendments

Four pairs of soil were used: two pairs originated from Egypt, the two others were from the Netherlands. For each country half of the soils were sandy and half were clay. Two management regimes were selected, conventional versus organic. (Table 1). For repetition of the experiments the soils were sampled three times, in July 2003, March 2004, and February 2005 (experiments 1, 2 and 3, respectively). Twenty-five kilogram of soil was collected at random sampling points in 100 m<sup>2</sup> plots down to 15–25 cm with an augur and mixed. Samples were transported in plastic bags in containers with ice. Egyptian soils were sent to the Netherlands in plastic bags in styrofoam boxes by overnight express airmail. Upon arrival in the laboratory each soil sample was thoroughly mixed and plant parts and earthworms were removed. The soils were stored at 4°C until the start of the experiment.

To test the effect of different soil amendments (experiments 2 and 3), soils were left non-amended or were supplemented with 170 kg N ha<sup>-1</sup> (0.1 g N kg<sup>-1</sup> soil), the maximum amount that could be applied in manure according to EU regulations. Conventional soils were amended with NPK fertilizer (12% N = 7% ammonium + 5% nitrate; 10% P; 18% K; 0.09 g·100 g<sup>-1</sup> soil) or left non-amended. Organic

**Table 1** Origin of soil samples

Sample	Code	Country	Soil type	Management	Previous and actual crops at time of sampling	Location
1	ESC <sup>a</sup>	Egypt	Sand	Conventional	Potatoes <sup>b,c,d,e</sup>	Nubaria, desert area
2	ESO	Egypt	Sand	Organic	Potatoes <sup>b,c,d,e</sup>	Nubaria, desert area
3	ECC	Egypt	Clay	Conventional	Beans <sup>b</sup> , potatoes <sup>c,d,e</sup>	Behera, Delta area,
4	ECO	Egypt	Clay	Organic	Potatoes <sup>b,c,d,e</sup>	Kaliobia, Delta area,
5	NSC	NL	Sand	Conventional	Grass <sup>b</sup> , unplanted <sup>c,d</sup>	Marknesse, Northeast polder
6	NSO	NL	Sand	Organic	Grass <sup>b,c,d</sup>	Marknesse, Northeast polder
7	NCC	NL	Clay	Conventional	Winter wheat <sup>b</sup> , seed potatoes <sup>c</sup> , sugar beet <sup>d</sup>	Ens, Northeast polder
8	NCO	NL	Clay	Organic	Grains <sup>b</sup> , onions <sup>c</sup> , beans <sup>d</sup>	Ens, Northeast polder

<sup>a</sup> The first letter stands for the country of origin (Egypt or the Netherlands), the second for soil type (clay or sand), and the third for management type (conventional or organic)

<sup>b</sup> In 2003

<sup>c</sup> In 2004

<sup>d</sup> In 2005

<sup>e</sup> In the Egyptian fields, potatoes were still there or had just been harvested

soils were amended with compost (Compost 8.1S, made from 88% wood chips, 2.5% manure and 10% clay with 0.61% of total N content; 1.7 g compost-100 g<sup>-1</sup> soil) or manure (organic cow manure with 0.48% of total N content; 2.17 g·100 g<sup>-1</sup> soil) or left non-amended.

#### Soil characterization

For physical soil analysis, 100g samples of non-amended soils were air-dried at room temperature. Fractions of different soil particle sizes were determined with a Coulter LS230 particle size analyzer (Beckman Coulter, Inc., Fullerton CA, USA). The method used was obtained from the manual of the Coulter grain-sizer type of 230. Particle sizes of <2 µm were considered clay, 2–50 µm was considered silt and 50–2000 µm was considered sand (Table 2).

For chemical soil analysis, 100 g samples of non-amended and amended soils were air-dried at room temperature. N-NO<sub>3</sub> and N-NH<sub>4</sub> were extracted with 0.01 M CaCl<sub>2</sub> and total P was extracted with 0.01 M H<sub>2</sub>SO<sub>4</sub> before spectrophotometrical analysis with a Segmented Flow Analyzer (Skalar Analytical BV, Breda, the Netherlands). Total N and organic C contents were determined with a CHN1110 Element Analyzer (CE Instruments, Milan, Italy). Dissolved Na and K were determined after sample extraction with 0.01 M CaCl<sub>2</sub>, vapourized, and analyzed by flame emission spectrophotometer at a wavelength of 589.0 nm for Na and 766.5 nm for K. Since Ca can interfere with Na analysis, Ca was bound in Ca–Al complexes by adding aluminum salt (Houba et al. 1989). The pH was measured after 0.01 M CaCl<sub>2</sub> extraction using a pH/mV meter and combined electrode (Houba and Novozamsky 1998). Organic matter was determined by loss-on-ignition i.e. by dry combustion of the organic material in a furnace at 500–550°C and the loss in weight indicated the content of organic matter in the sample (Houba et al. 1997). Electric conductivity was determined according to ISO 7888-1985 (Water quality–Determination of electrical conductivity) and ISO 3696-1987 (Water for analytical laboratory use–Specification and test methods).

#### Microbial characterization-DGGE analysis

Total DNA was extracted from 0.5 g (wet weight) soil samples from bulk soil (at the beginning of

experiment 3) or rhizosphere soil (at the end of experiment 3) with the Bio101 FastDNA<sup>®</sup> SPIN Kit for soil according to the manufacturer's specifications (Bio101, Carlsbad, CA, USA); a 20 min incubation time at 65°C was added to enhance the elution. The DNA quality and quantity were checked (Sambrook et al. 1989) on 1.2% agarose gels with ethidium bromide and visualized by UV trans-illumination.

For DGGE analysis of the eubacterial soil population, PCR amplification of the V6 to V8 region of the 16S rRNA gene from total soil DNA was conducted as described previously (Hiddink et al. 2005). DNA extracts from rhizosphere were diluted ten times more than for bulk soil to have the same amount of extracted DNA (about 2 ng). Extracted DNA samples of different managements and amendments within the same soil type from the same country of origin were loaded on the same gel at least twice. DGGE was carried out using the Dcode system (BIOrad Laboratories, Hercules, CA, USA). The silver stained and scanned gels were analysed with Phoretix 1D (Non-Linear Dynamics Ltd., Newcastle upon Tyne, UK).

The bacterial diversity in the samples was estimated in two ways: as species richness *S*, and as the Shannon-Wiener index of bacterial diversity, *H'* as described previously (van Diepeningen et al. 2006).

#### Bacterial strains and inoculation

A mixture of equal amounts of three virulent *R. solanacearum* race 3 biovar 2 strains able to use nitrate as electron acceptor: PD5239, PD5240 and PD5241 (culture collection, Plant Protection Service, Wageningen, the Netherlands) were used in this study. These strains were of Egyptian origin, isolated from infected potato tubers. The cultures used for soil infestation were grown for 48 h on nutrient agar (NA) plates at 28°C. The bacterial culture was suspended in 0.01 M phosphate buffer (PB) and the bacterial density was adjusted using a spectrophotometer (OD<sub>600</sub> = 2.1 equals 5 × 10<sup>9</sup> CFU ml<sup>-1</sup>). The inoculum was mixed with the soil to have a final bacterial density of 10<sup>7</sup> CFU g<sup>-1</sup> dry soils. The moisture content varied for each soil, but was at about field capacity for all soils.

#### Experimental set-up

The experiments were conducted three times for differently managed soils (experiments 1, 2 and 3)

**Table 2** Physical and chemical composition, and rhizosphere biodiversity for organic and conventional sandy and clay soils with and without different amendments (one sample for soil texture, organic matter and pH in experiment 1; averages of experiments 2 and 3 (one sample each) for the other parameters)

Soil plus type	Soil composition <sup>a</sup> mg kg <sup>-1</sup> soil													Bulk soil biodiversity <sup>b</sup>		Rhizosphere biodiversity <sup>b</sup>	
	Clay%	Silt	Sand%	Org. mat. %	pH-KCl	Ca	Na	K	P	N	N-NO <sub>3</sub>	N-NH <sub>4</sub>	DOC <sup>c</sup>	S <sup>d</sup>	H <sup>e</sup>	S <sup>d</sup>	H <sup>e</sup>
ESC <sup>f</sup>	0.1	0.6	99.3	<0.2	7.9	15531	211	572	131	223	9.6	5	50	27	1.25	27.5	1.31
ESC + NPK						16536	326	755	166	251	36	11.9	47			27	1.3
ESO	0.1	0.6	99.3	0.3	8.2	22970	347	821	101	217	9.2	1.8	54	31	1.27	30	1.31
ESO + compost						27495	428	1069	127	300	17.1	1.6	80			29	1.33
ESO + manure						24782	354	861	106	228	9.2	1.9	53			18.5	1.19
ECC	9.4	54.5	36.1	4.9	7.6	4326	958	3925	786	1022	90.6	15.6	113	35	1.45	36.5	1.45
ECC + NPK						5512	1013	4076	796	1055	135.8	25.5	113			33.5	1.45
ECO	13.9	65.7	20.4	6.2	7.6	11292	1382	4848	1070	1242	36.8	15.6	89	30	1.43	31	1.32
ECO + compost						8029	1315	4961	1171	1313	40.6	15	96			31.5	1.36
ECO + manure						24034	879	5480	1224	1292	38.6	11.9	78			37.5	1.43
NSC	3.2	32.3	64.5	1.5	7.4	16419	1046	5035	744	1114	9.1	13.4	138	28	0.94	27.5	1.3
NSC + NPK						16105	1026	5147	763	1149	60.5	19.6	153			26.5	1.32
NSO	3.2	33.3	63.5	4.1	7.1	17092	973	4791	653	1260	18.3	12.2	170	28	0.99	32.5	1.39
NSO + compost						18418	993	4933	694	1381	23.4	12.2	196			28.5	1.3
NSO + manure						13462	1255	3632	708	1571	22.7	9.3	197			31	1.29
NCC	7.7	51.9	40.4	2.2	7.4	21556	653	3711	545	644	7.6	15.7	127	39	1.43	27	0.94
NCC + NPK						25377	878	6969	1086	1251	72.2	31.6	133			25.5	0.65
NCO	8.3	54.5	37.2	2.3	7.3	26108	751	3725	417	654	9.9	12.8	130	30	1.26	30.5	1.23
NCO + compost						23616	1191	6600	750	1161	19.3	16	135			29.5	1.22
NCO + manure						24401	963	6562	732	1210	9.9	15.6	152			24.5	1.52
LSD ( $\alpha = 0.05$ )						11076	598	3721	475	549	62.4	14.6	40			8	0.3

<sup>a</sup> Particle sizes: clay <2 µm, silt 2–50 µm, sand 50–2,000 µm

<sup>b</sup> Bacterial diversity determined by 16S rDNA PCR—DGGE, only in experiment 3 (two replicates)

<sup>c</sup> Total Organic Carbon

<sup>d</sup> Species richness

<sup>e</sup> Shannon-Wiener diversity index

<sup>f</sup> See Table 1 for soil codes

and two times (experiments 2 and 3) for differently amended soils in the quarantine greenhouse of the Plant Protection Service (PD) in Wageningen, The Netherlands. The susceptible potato variety Nicola was used in all experiments. In each experiment, six replicate pots were used per treatment, and two tuber eyepieces were planted per pot one day after inoculation of the soil. Two pots with non-inoculated soil per treatment were included as controls. The pots were placed on saucers, filled with water daily, keeping the soil moisture as constant as possible. The conditions of the greenhouse were adjusted to 25°C during the day and 20°C during the night, with a RH of 75–80% and a total of 14 h light d<sup>-1</sup>.

### Monitoring the disease

Disease development was monitored daily. Wilt was scored according to % wilted leaves relative to the total number of leaves per pot. Infection in the lower stem area (crown area) of each plant (with symptoms and latently infected) was checked at the end of the experiment (35 days). Infection was tested by plating the cut, weighed, and surface-sterilized plant tissues from the crown area (chopped in phosphate buffer saline PBS 0.01 M) on modified Selective Media South Africa (SMSA) plates (Anon. 1998). From all plant samples three ten-fold serial dilutions were plated (100 µl per plate) and the recovered *R. solanacearum* bacteria were counted after 5–7 days of incubation at 28°C.

Population densities of the pathogen were determined in rhizosphere soil of potato plants and in bulk soil at the end of each experiment. Ten-fold serial dilutions were made of 1 g of rhizosphere or 10 g of bulk soil in 0.05 M sterile phosphate buffer and after shaking for 2 h at 15°C plated on SMSA agar plates. Plates were incubated at 28°C for 5–7 days. Putative *R. solanacearum* colonies (one from each treatment) were randomly tested for identity using Immunofluorescence Antibody Staining (Janse 1988).

### Statistical analysis

Wilt severity was plotted over time and the Area Under the Disease Progress Curve (AUDPC) was calculated for each pot. AUDPC values and log transformed (log (x + 1)) densities of *R. solanacearum* in bulk soil, in rhizosphere soil and in plant tissue were

tested for normality. As these variables were generally not normally distributed, non-parametric analyses (NPar-Tests, Mann–Whitney Test) were conducted for AUDPC, log(CFU + 1) in soil, log(CFU + 1) in rhizosphere soil, and log(CFU + 1) in plant tissue using SPSS v 12 (SPSS Inc., Chicago, Illinois, USA) to test the effects of soil origin (countries), soil type, management, and amendment.  $\chi^2$  tests were conducted on contingency tables with two categories of wilted or infected plants per pot (wilted/infected or not) and soils from different origin, type, management, and amendment using Microsoft Excel 2003 (Microsoft Corporation, Seattle, WA, USA).

All soil characteristics were subjected to ANOVA (post-hoc, LSD) to test for differences between soil and management types and amendments, using SPSS v 12. Correlation analyses between AUDPCs (or population densities of *R. solanacearum* in soil, rhizosphere or plant tissues) and chemical characteristics of the soils were also conducted in SPSS v 12.

Cluster analysis was carried out on the DGGE band intensities of all lanes on individual gels, representing the bacterial composition for different managements or amendments within the same soil type from the same country of origin, using the Phoretix software (Nonlinear Dynamics Ltd, Newcastle upon Tyne, UK).

## Results

### Disease incidence and severity in organic and conventional non-amended soils

Pathogen behaviour was assessed using six indicators for each pot: % wilted and infected plants, AUDPC (based on disease severity), CFU in bulk soil, in rhizosphere, and inside plant tissue (lower stem).

Disease incidence and severity were not normally distributed, so that analysis of variance could not be carried out.  $\chi^2$  tests on incidence classes showed that more plants were wilted ( $\chi^2 = 24.33 > 16.27$ ,  $\alpha = 0.001$ ) and infected by *R. solanacearum* ( $\chi^2 = 22.38 > 16.27$ ,  $\alpha = 0.001$ ) when growing on Dutch sandy soils than plants growing on any of the other soils (Table 3). The number of wilted plants per pot was higher in organic than in conventional Dutch soils. This difference was just not significant for the sandy soil ( $\chi^2 = 3.00 < 3.84$ ,  $\alpha = 0.05$ ), but was

**Table 3** Contingency table for the effect of country of origin (Egypt or the Netherlands), soil type (clay or sand), and management (conventional or organic) on the number of pots distributed over three categories of bacterial wilt incidence on potato and number of plants from which *R. solanacearum* was isolated, 35 days after planting in three greenhouse experiments combined

Country and soil type	No. of wilted plants per pot			No. of infected plants per pot		
	0	1	>1 <sup>a</sup>	0	1	>1
ES <sup>b</sup>	26	5	5	20	6	10
EC	24	5	7	17	6	13
NS <sup>c</sup>	9	9	18	7	2	27
NC	26	4	6	21	4	11
$\chi^2$ <sup>d</sup>	24.3			22.4		
Management <sup>e</sup>	No. of wilted plants per pot			No. of infected plants per pot		
	0	1	>1	0	1	>1
ESC	12	3	3	7	4	7
ESO	14	2	2	13	2	3
$\chi^2$ <sup>f</sup>	0.35			3.40		
ECC	11	2	5	8	3	7
ECO	13	3	2	9	3	6
$\chi^2$	1.45			0.14		
NSC	6	6	6	5	2	11
NSO	3	3	12	2	0	16
$\chi^2$	3.00			2.21		
NCC	16	1	1	13	0	5
NCO	10	3	5	8	4	6
$\chi^2$	4.05			1.28		

<sup>a</sup> 2 or 3 wilted or infected plants per pot

<sup>b</sup> For soil codes see Table 1

<sup>c</sup> Significantly different from the other three soil types

<sup>d</sup> Significant when  $\chi^2 > 16.27$ ,  $\alpha = 0.001$

<sup>e</sup> Conventional (C) versus organic (O)

<sup>f</sup> Significant when  $\chi^2 > 3.84$ ,  $\alpha = 0.05$ , a trend when  $\chi^2 > 2.71$ ,  $\alpha = 0.10$

significant for the clay soil ( $\chi^2 = 4.05 > 3.84$ ,  $\alpha = 0.05$ ). The number of plants from which *R. solanacearum* was isolated was higher in the conventional Egyptian sandy soil than in its organic counterpart, while it was the reverse for the pair of Dutch sandy soils. However, the differences were only significant at  $\alpha = 0.1$  (Table 3). No management effects on disease incidence were observed for the other soil pairs.

Log-transformed AUDPC and CFUs were still not normally distributed, so that Mann–Whitney non-parametric tests were carried out. The median AUDPC for the plants growing on Egyptian soils and for those on Dutch conventional clay soils was 0

although disease did occur in some of the plants (Table 5). Most disease occurred in plants growing on Dutch sandy soils, irrespective of their type of management. AUDPC of plants growing on infested Dutch sandy soils were significantly higher than of those growing on infested Dutch clay soils or Egyptian soils ( $P < 0.001$ ). Similarly, CFUs were higher in soil, rhizosphere, and potato stems for Dutch sandy soils ( $P = 0.001$ , in all cases). This is in agreement with the higher percentages of wilted and infected plants in Dutch sandy soils, as described above. Disease severity (AUDPC) and population densities of *R. solanacearum* in soil, rhizosphere and stem were slightly lower in the organic than in the

conventional Egyptian sandy soil (not significant), while CFUs  $g^{-1}$  soil were higher in the organic Dutch sandy and clay soils ( $P = 0.05$ ). Thus, there was a tendency that the pathogen and disease development were reduced in organic Egyptian sandy soil and increased in Dutch organic and conventional sandy soils (Tables 3 and 4).

#### Physical and chemical soil characteristics for non-amended soils

The clay soils from Egypt and the Netherlands did not differ substantially in their clay, silt, and sand content, while the sandy soils from the two countries had clearly different textures. The Egyptian sand was 99% pure sand while the Dutch sandy soils contained 32–33% of silt (Table 2). The Egyptian soils (especially the sandy soils) had a slightly higher pH than the comparable Dutch soils ( $P < 0.001$ ), but all soils were alkaline. The electric conductivity (EC) was between 0.12 and 0.31  $mS\ cm^{-1}$  for all differently managed soils except the Egyptian clay soils where it was 0.43  $mS\ cm^{-1}$ . Dutch sandy soils had generally higher nutrient (except Ca) and DOC contents ( $P \leq 0.001$ ) than Egyptian sandy soils. Dutch clay soils had a higher Ca and DOC content ( $P < 0.001$  and 0.04, respectively), but lower OM and nitrate content than Egyptian clay soils ( $P < 0.001$  and 0.006, respectively). Egyptian clay soils had higher

OM, DOC and general nutrient contents ( $P \leq 0.001$ ) than Egyptian sandy soils, while Egyptian sandy soils contained more Ca ( $P = 0.008$ ) than Egyptian clay soils. The Dutch clay soil had a higher Ca content ( $P = 0.018$ ) compared to the Dutch sandy soil. Organic soils from Egypt had higher Ca, Na and K contents ( $P < 0.05$  to  $< 0.001$ ) than their conventional counterparts, while the conventional sandy soil contained more ammonium ( $P = 0.012$ ) than its organic counterpart. For Dutch soils, the nitrate content was significantly higher in the organic than in the conventional sandy soil ( $P = 0.002$ ).

Overall, there were significant positive correlations between DOC and AUDPC, final disease severity and % wilted and infected plants ( $P = 0.009$  to 0.034). There were negative correlations between pH and AUDPC, disease severity, wilt incidence, number of infected plants and *R. solanacearum* density in soil at the end of the experiment ( $P = 0.004$  to 0.043).

For the Dutch soils, where the disease was lower in the clay than in the sandy soils (Table 3), availability of Ca and K in non-amended soils was negatively correlated with the AUDPC ( $r = -0.51$ ,  $P = 0.045$  and  $r = -0.50$ ,  $P = 0.051$ , respectively). There were significant ( $P < 0.05$ ) positive correlations between Na and % infected plants, CFU in soil and CFU in rhizosphere, and negative ( $P < 0.05$ ) correlations between ammonium and AUDPC, disease severity, and incidence of wilt and infected plants. On the

**Table 4** Effect of organic versus conventional management on potato brown rot, AUDPC and CFU  $g^{-1}$  of soil, rhizosphere soil and per plant (log transformed + 1), 35 days after planting of potato in three greenhouse experiments (data combined)

Soil type	AUDPC		CFU $g^{-1}$ soil			CFU $g^{-1}$ rhizosphere			CFU $g^{-1}$ plant			
	Median	5% Trimmed mean	Mean rank	Median	5% Trimmed mean	Mean rank	Median	5% Trimmed mean	Mean rank	Median	5% Trimmed mean	Mean rank
ESC <sup>c</sup>	0	83.06	67.28	4.14	2.83	67.64	5.97	4.65	64.58	2.79	3.13	70.64
ESO	0	17.78	55.03	0.39	1.78	52.83	2.78	3.12	49.81	0	1.57	53.44
ECC	0	63.98	68.08	4.05	2.93	70.81	6.18	4.65	71.47	3.46	3.26	73.03
ECO	0	59.07	62.94	1.38	2.02	55.22	5.61	4.94	71.58	3.12	3.22	74.11
NSC	133.5	177.25	91.17	4.84	3.67	86.36 <sup>a</sup>	6.81	6.33	88.56	4.92	4.43	88.81
NSO	348.5	375.56	109.92	5.45	5.08	111.94 <sup>a</sup>	7.67	7	105.11	7.06	5.48	100.11
NCC	0	11.79	50.22	2.55	2.30	55.50 <sup>b</sup>	5.31	4.08	55.67	0	1.41	52.83
NCO	12.25	91.16	75.36	4.30	3.68	79.69 <sup>b</sup>	5.95	5.32	73.22	0	2.57	67.03

<sup>a</sup> Significant difference between organic and conventional soils at  $\alpha = 0.05$

<sup>b</sup> Same as under<sup>a</sup>

<sup>c</sup> For soil codes see Table 1



**Table 5** Contingency table for the effect of amendment on the number of pots distributed over three categories of bacterial wilt incidence on potato and number of plants from which *R. solanacearum* was isolated, 35 days after planting in two greenhouse experiments (data of experiments 2 and 3 combined)

Country and soil type <sup>a</sup>	No. of wilted plants per pot			No. of infected plants per pot		
	0	1	>1 <sup>c</sup>	0	1	>1
ESC	8	1	3	5	0	7
ESCN	12	0	0	9	0	3
$\chi^2$ , <sup>d</sup>	3.8			2.74		
ESO	10	1	1	10	0	2
ESOM	11	0	1	10	0	2
$\chi^2$	0.05			0		
ECC	9	2	1	6	0	6
ECCN	12	0	0	11	0	1
$\chi^2$	1.43			5.04		
ECO	7	4	1	7	1	4
ECOM	10	2	0	10	0	2
$\chi^2$	1.53			1.2		
NSC	6	3	3	5	0	7
NSCN	7	1	4	9	0	3
$\chi^2$	0.22			2.74		
NSO	3	3	6	2	0	10
NSOM	8	0	4	8	0	4
$\chi^2$	2.67			6.17		
NCC	11	0	1	8	0	4
NCCN	7	2	3	7	2	3
$\chi^2$	14.19			0.44		
NCO	8	4	0	7	1	4
NCOM	11	0	1	10	0	2
$\chi^2$	1.47			1.2		

<sup>a</sup> For soil codes see Table 1

<sup>b</sup> Amendment with NPK (N) or manure (M): the last letter of the soil code

<sup>b</sup> 2 or 3 wilted or infected plants per pot

<sup>c</sup>  $\chi^2 < 3.84$  not significant,  $\chi^2 > 2.71$  is a trend  $\alpha = 0.10$

other hand, there were positive correlations of nitrate with AUDPC, disease severity, and incidence of wilt and infected plants ( $P = 0.003$  to  $0.018$ ).

For Egyptian soils, fewer correlations were found, e.g. a positive correlation between nitrate and CFU in plant tissue ( $r = 0.53$ ,  $P = 0.037$ ) and a positive correlation between DOC and disease severity ( $r = 0.50$ ,  $P = 0.047$ ).

#### Biological characteristics of non-amended soils

One bulk soil sample of each of the different soils from each country was included in duplicate in the

DGGE analysis. In general, biodiversity [S] and Shannon Wiener Index [ $H'$ ] had higher values in clay than in sandy soils in both countries (Table 2). The organic Egyptian sandy soil had a higher diversity compared to the conventional counterpart, while the organic Egyptian clay soil had lower diversity than its conventional counterpart. There were no clear differences between organic and conventional Dutch sandy soils, while the organic Dutch clay soil had also less biodiversity compared to the conventional counterpart. These results were in agreement with the biodiversity data for the same soils used in experiments on survival of *R. solanacearum* (Messiha

2006), except that in the latter study the Dutch organic clay soil had higher diversity compared to the conventional counterpart.

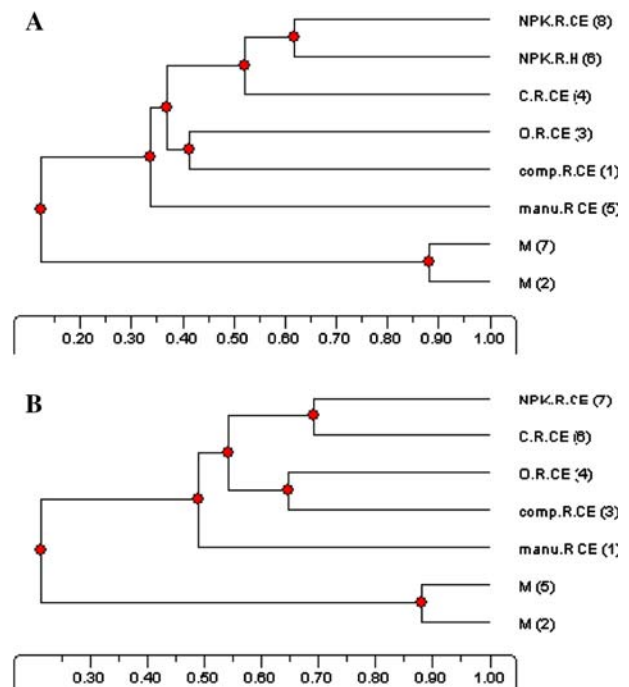
Cluster analysis of DGGE banding patterns of 16S-rDNA extracted from the rhizosphere of potatoes grown in the different soils resulted in a clustering according to organic or conventional management (Messiha 2006). There were no significant differences in microbial community composition between rhizosphere and bulk soil for any of the soils as detected with DGGE (data not shown). There was a clear shift in microbial biodiversity after cow manure amendment especially for sandy soils from both countries (Fig. 1A and B).

#### Disease incidence and severity in amended soils

Fertilization of conventional soils with NPK suppressed the disease in some cases. NPK-amendment to Egyptian sandy soil reduced wilt severity at the end of the experiment by 100% (from 18.8% to 0%;

$\chi^2 = 3.8$ ,  $\alpha = 0.05$ ) (Table 5), *R. solanacearum* density in stem tissues by 67.6% and AUDPC by 100% ( $P = 0.02$  and  $P = 0.033$ , respectively) (Table 6). NPK-amendment to Egyptian clay soil reduced % infected plants by 84% ( $\chi^2 = 5.04 > 4.02$ ,  $\alpha = 0.025$ ) (Table 6) and CFU of *R. solanacearum* in bulk soil by 93%, in the rhizosphere by 80%, and in stem tissues by 90.1% ( $P < 0.05$ ), but not AUDPC ( $P = 0.07$ ) (Table 6). On the other hand, NPK amendment to the Dutch sandy soils significantly increased the % wilted plants from 8.3% to 28.4% ( $\chi^2 = 14.19 > 10.83$ ,  $\alpha = 0.001$ ) (Table 5) and final wilt severity from 4.3% to 27.1% (data not shown) in Dutch clay soils.

Amendment of organic soils with cow manure also suppressed brown rot in some cases. Manure amendment reduced soil CFU counts by 100% ( $P = 0.031$ ) in Egyptian sandy soil and rhizosphere CFU counts by 62% ( $P = 0.045$ ) in Egyptian clay soil (Table 6). Amendment of Dutch sandy soil with manure decreased the % infected plants from 83.3% to 33.3% ( $\chi^2 = 6.17 > 5.02$ ,  $\alpha = 0.025$ ) (Table 5) as well



**Fig. 1** Cluster analysis (Phoretix 1D, NonLinear Dynamics Ltd., Newcastle upon Tyne, UK) for PCR-DGGE products from the rhizosphere of different amended sandy soils from Egypt (**A**) or the Netherlands (**B**). The scale on the X-axis depicts similarity. The first set of letters indicate amendment (NPK = fertilizer, comp = compost, manu = cow manure)

O = organic and C = conventional) or marker (M), the second letters stand for rhizosphere (R) or bulk soil (B), the third letters for treatment (H = inoculated but healthy and C = non-inoculated control), and the fourth letters for sampling time (B = beginning and E = end). The numbers between brackets indicate the lane number on the gel

**Table 6** Effect of amendment with NPK to conventional soils and manure to organic soils on AUDPC, CFUs in bulk soil, rhizosphere soil and inside plant tissues (stem base), 35 days after planting potatoes in two greenhouse experiments (data of experiments 2 and 3 combined)

Soil type	AUDPC			CFU g <sup>-1</sup> soil			CFU g <sup>-1</sup> rhizosphere			CFU g <sup>-1</sup> plant		
	Median	5% Trimmed mean	Mean rank	Median	5% Trimmed mean	Mean rank	Median	5% Trimmed mean	Mean rank	Median	5% Trimmed mean	Mean rank
ESC <sup>a</sup>	0	134.7	14.50* <sup>b</sup>	14.14	3.03	14.58	6.44	4.97	15.08	2.79	3.21	14.92*
ESCN <sup>d</sup>	0	0.0	10.50*	0.00	1.06	10.42	1.51	2.71	9.92	0.00	1.04	10.08*
ESO	0	11.6	12.92	0.00	1.85	15.00*	2.78	3.07	14.17	0.00	0.91	12.67
ESOM	0	19.6	12.08	0.00	0.00	10.00*	0.00	1.11	10.83	0.00	0.64	12.33
ECC	0	55.9	14.00	2.54	2.57	14.92*	3.08	3.50	14.92*	1.71	2.42	15.00*
ECCN	0	0.0	11.00	0.00	0.19	10.08*	0.00	0.70	10.08*	0.00	0.22	10.00*
ECO	0	59.1	13.00	0.00	1.69	13.58	5.61	4.57	15.25*	0.00	2.06	14.00
ECOM	0	28.2	12.00	0.00	0.72	11.42	0.00	1.76	9.75*	0.00	0.62	11.00
NSC	15	169.0	11.13	3.56	2.74	13.17	5.52	5.39	12.79	2.58	3.14	13.17
NSCN	0	142.7	13.88	3.17	3.50	11.83	5.61	4.25	12.21	0.00	2.65	11.83
NSO	495	386.8	15.17*	5.17	4.60	10.08	7.18	6.03	13.25	4.56	4.33	13.21
NSOM	0	144.9	9.83*	5.95	5.72	14.92	5.05	4.74	11.75	3.60	3.44	11.79
NCC	0	10.7	10.46	3.81	2.99	12.00	5.63	4.75	12.33	0.00	1.64	10.46
NCCN	0	247.1	14.54	3.60	3.69	13.00	5.83	4.02	12.67	1.63	2.69	14.54
NCO	0	114.0	13.92	3.96	3.38	13.92	5.60	4.61	13.75	0.00	2.57	13.79
NCOM	0	11.5	11.08	3.01	2.56	11.08	3.97	3.83	11.25	0.00	0.94	11.21

<sup>a</sup> For soil type see Table 1

<sup>b</sup> \*Asymptotic significant difference between amended and unamended soils (2-tailed): ( $P \leq 0.05$ )

as the AUDPC by 52% ( $P = 0.05$ ) (Tables 5 and 6) and final wilt severity from 47% to 20% (data not shown). Amendment of organic soils with compost had no effects on CFU of *R. solanacearum* or on brown rot incidence or severity (data not shown).

#### Physical and chemical soil characteristics of amended soils

Amendment of Egyptian conventional sandy soil with NPK increased the EC and significantly increased both the nitrate and the ammonium contents ( $P = 0.028$  and  $P = 0.019$ , respectively, Table 2). Amendment of Egyptian organic sandy soil with compost and cow manure significantly increased the DOC contents ( $P = 0.035$  and  $P = 0.023$  respectively). Amendment of Dutch organic clay soil with cow manure significantly increased the DOC content ( $P = 0.015$ ). Amendments of the other soils did not affect the studied chemical characteristics of those soils. Correlations between soil characteristics and

disease measurements or pathogen populations were similar for the data sets with and without amendments.

#### Biological characteristics of amended soils

Amendment of conventional soils with NPK and organic soils with compost did not affect the bacterial diversity in the rhizosphere (Table 2). On the other hand, amendment of organic soils with manure affected bacterial diversity in various ways. In the poor Egyptian sandy soils, rhizosphere bacterial diversity [S] decreased from  $30.0 \pm 1.00$  to  $18.5 \pm 0.50$  ( $P = 0.001$ ), indicating that some species grew at the cost of others thanks to the substrate added with the manure. In the Dutch clay soils, manure amendment decreased ( $P = 0.003$ ) the rhizosphere [S] from  $30.5 \pm 0.50$  to  $24.5 \pm 0.50$ , and increased rhizosphere [ $H'$ ] from  $1.23 \pm 0.3$  to  $1.52 \pm 0.02$  ( $P = 0.035$ ). In Dutch sandy soils, the amendments did not affect bacterial diversity in the

rhizosphere as determined with DGGE (data not shown).

## Discussion

The main objective of this study was to investigate if organic management could reduce infection of potato by *R. solanacearum* and potato brown rot development, since root diseases caused by fungi are generally suppressed in organically-managed soils (van Bruggen and Termorshuizen 2003), and various bacterial wilt diseases can be suppressed by organic amendments (Gorissen et al. 2004; Islam and Toyota 2004; Satoh and Toyota 2004). In this study, there was only a tendency for reduced infection by *R. solanacearum* in plants grown on organic sandy soil from the Egyptian desert. The biodiversity in these poor desert soils was higher in the organically-managed soil, possibly leading to more competition for the limited substrate compared to the conventional soil (Messiha 2006). Availability of nutrients in potato root exudates may have overcome this competition, so that the difference between organically and conventionally-managed soils was just not significant any more in the rhizosphere.

Similar to our previous results on survival of the pathogen, both density in bulk soil and disease incidence (proportion of wilted plants) were higher in organic than in conventional sandy and clay soils from the Netherlands. This may be related to the high availability of substrate in the Dutch soils, especially the organically-managed soils, where large quantities of manure have commonly been used (van Diepeningen et al. 2006). Indeed, in this study the DOC content in soil was positively correlated with AU-DPC, and was higher in the organic than in the conventional Dutch sandy soil. Cluster analysis for the DGGE bands of DNA samples from Dutch soils indicated that there was a rhizosphere effect in organic clay soil and not in conventional clay soil. This is the reverse of previous results, where a larger rhizosphere effect in terms of microbial communities was observed in soil from a conventional farm than in that from an organic farm (van Diepeningen et al. 2005). The lower disease incidence and severity in the conventional than in the organic soils in the current experiments are in agreement with the notion that a lower rhizosphere effect (as found in the

conventional soil here), called ‘root camouflage’, would lead to less pathogen attraction and root disease (Gilbert et al. 1994).

A second objective was to determine if *R. solanacearum* and potato brown rot could be suppressed by various organic amendments. Amendment of cow manure to organically-managed soil gave significant disease suppression in the sandy soil from the Netherlands and a significant reduction in density of *R. solanacearum* in the organic sandy soil from Egypt. No significant effects were found for the clay soils. Satoh and Toyota (2004) also found that the effect of repeated manure amendments on bacterial wilt development (on tomato) varied from soil to soil. The suppressive effects of manure added to organic sandy soils from the Netherlands and Egypt could have been due to the immediate release of ammonia, which is known to be toxic to microorganisms (Michel and Mew 1998) or to a shift in the microbial community (Gorissen et al. 2004). The bacterial diversity in the rhizosphere was not changed by manure amendment to Dutch sandy soil, but bacterial composition did change (Fig. 3b). Effects of bacterial composition rather than diversity on root disease suppression has been documented earlier (Hoitink and Boehm 1999). In the poor Egyptian sandy soils, rhizosphere bacterial composition also changed after manure amendment (Fig. 1b), and diversity decreased, indicating that some, likely fast-growing species, increased at the cost of others thanks to the substrate added with the manure. In clay soils both from Egypt and the Netherlands, the Shannon-Wiener index increased after manure amendment, and no significant effects on potato brown rot were found. Thus, the effects of manure on *R. solanacearum* may be caused by effects on survival (reduced CFU in Egyptian sandy soil), ammonia volatilisation or higher microbial activity by a limited number of fast-growing bacteria (Islam and Toyota 2004), or effects on pathogen growth in the rhizosphere and infection (reduced incidence and severity in Dutch sandy soil), again caused by ammonia production or specific inhibition by rhizosphere organisms.

Amendment of soils with wood chip compost was not effective in potato brown rot suppression (data not shown). Similarly, compost from tree bark or coffee residues did not suppress, but even enhanced bacterial wilt of tomato, while compost from farm-

yard manure plus rice straw and of poultry manure suppressed *R. solanacearum* in soil and reduced disease development (Islam and Toyota 2004). The suppressive effects of composted farmyard manure and poultry manure was attributed to high substrate availability with high N content (high DOC content, high available N content, and a low C/N ratio), and high microbial activity (Islam and Toyota 2004). Others also showed that the disease-suppressive or enhancing effects of compost depend on their chemical and biological composition (Litterick et al. 2004), as well as on the pathogen involved (Termorshuizen et al. 2006). In all these studies, compost was added to conventional field soil or peat substrate, while we added compost to organic field soil, which was already relatively suppressive in the case of organic sandy soil from Egypt. Also, relatively small amounts of compost were added, in accordance with the stringent Dutch regulations, compared to other studies (Islam and Toyota 2004; Termorshuizen et al. 2006).

Amendment of conventional soils with NPK gave remarkable disease suppression only in Egyptian sandy soil and a reduction in *R. solanacearum* populations in soil, rhizosphere and potato plants in Egyptian clay soil. The pH of Egyptian soils was slightly, but significantly higher, especially for sandy soils (7.9–8.2), than that of Dutch soils (7.4). The observed disease suppression may be a result of higher ammonia concentrations at these pH levels (Kissel et al. 1985), which are toxic to *R. solanacearum* (Michel and Mew 1998). The effect of NPK amendment may be a combination between the effect of ammonia and potassium (K). Fertilization of potato plants with superphosphate (15%  $P_2O_5$ ) or potassium sulphate (48–58%  $K_2O$ ) decreased incidence of brown rot disease (Fahmy and Mohamed 1990). Mixing NPK fertilizers with compost was most effective in suppressing bacterial wilt of banana (Roy et al. 1999) and brown rot of potato (Lemaga et al. 2005). On the other hand, amendment of conventional clay soil from the Netherlands with NPK increased % wilted potato plants. It is not clear what the reason for this increase could be. The available nutrients and DOC were already high in the non-amended Dutch conventional clay soil, but this was also the case for the Egyptian conventional clay soil.

Another objective of this study was to relate brown rot development to various characteristics of different

soil types from different locations, to find factors that might be associated with disease suppression. Disease severity was highest in Dutch sandy soils compared to all other soils. Chemical analysis revealed that the dissolved organic carbon (DOC) was the highest in these soils and was positively correlated with disease incidence, suggesting high substrate availability and better survival chances for *R. solanacearum* (Messiha 2006).

Compared to Dutch sandy soils, the disease was suppressed in Dutch clay soils, where the DOC was also relatively high, but both the Ca and K contents were remarkably higher in the Dutch clay compared to the sandy soils and were negatively correlated with disease incidence. Both K and Ca are known for enhancing plant defences (Flego et al. 1997). As mentioned above, fertilization with potassium sulphate reduced the incidence of brown rot on potato (Fahmy and Mohamed 1990). In our study, the Ca and K contents were also higher in organic than in conventional Egyptian sandy soil, and the proportion of *R. solanacearum*-infected plants was reduced in the organic soil, supporting previous findings about enhanced resistance in plants high in Ca and/or K.

The Na contents were significantly higher in the Egyptian clay (especially the organic clay soil) and Dutch soils than in the sandy soils from Egypt, which also had low *R. solanacearum* populations and infection, (particularly in the organic sandy soil from Egypt). Thus, a positive correlation was found between Na availability and % infected plants, CFU in the rhizosphere and CFU in the plant, which may explain the absence of disease suppression in the organic clay soil from Egypt even when both Ca and K concentrations were high. It is well known that root exudation is increased in sodic soils, enhancing the attraction for and susceptibility to various root diseases (Snapp et al. 1991).

The EC was highest in the Egyptian clay soils as compared to all other amended soils. In a previous study (Messiha 2006), the survival of *R. solanacearum* was higher in Egyptian clay soils compared to the sandy ones while in this study the disease expression was similar in both soil types. Also NPK fertilization showed noticeable increase of the EC, which was correlated with disease suppression.

In conclusion, contrary to our original expectation of disease suppression in organically-managed soils (van Bruggen and Termorshuizen 2003), populations

of *R. solanacearum* were higher in the bulk soil and rhizosphere of potato plants grown in organic soils from the Netherlands, in particular organic sandy soil. Also disease incidence was higher in these organically-managed soils. This was probably related to the availability of substrate for growth of the pathogen in soil and the rhizosphere. Lack of substrate in the organically-managed Egyptian sandy soil due to high competition may have been responsible for the suppression in that soil compared to its conventional counterpart. When Ca and K contents of soils were high, potato brown rot incidence or severity was generally low, both in soils naturally high in these elements and in soils amended with manure. Application of NPK fertilizer reduced the disease, especially at high soil pH in Egyptian soils, probably due to ammonia volatilization. However, high fertilizer applications cannot be recommended for control of brown rot, as these may enhance susceptibility to other diseases, and would not be sustainable in the long run. Farmers could apply moderate levels of cow manure to reduce the disease, but to such a level that DOC content does not stimulate the pathogen. Application of composted poultry manure would be an interesting option to be explored for the Nile Delta of Egypt (Islam and Toyota 2004), but for the Egyptian desert and the Netherlands, prevention of spread and quarantine remain important tools to combat potato brown rot. This study shows that mechanisms of disease suppression of soil-borne plant pathogens may vary strongly according to the soil type, especially if quite different types of soil are under study.

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