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Limited survival of *Ralstonia solanacearum* Race 3 in bulk soils and composts from Egypt

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Abstract Survival of *Ralstonia solanacearum* race 3 biovar 2 (phylotype II sequevar 1) in Egyptian soils and compost was studied under laboratory and field conditions. Survival of the pathogen under laboratory conditions varied with temperature, water potential and soil type, with temperature being the major determinant of survival of the pathogen. The effects of temperature and moisture content were variable between different experiments, but survival was generally longer at 15°C than at 4, 28 and 35°C respectively. Survival was also longer when moisture levels were constant compared with varying moisture levels at all temperatures. In experiments to compare the effects of progressive drying in sandy and clay soils there was a difference in survival times between the two soil types. In sandy soils, the pathogen died out more rapidly when soil was allowed to dry out than in controls where the soil was kept at constant water potential. In clay soils there was little difference between the two treatments, possibly due to the formation of a hard impermeable outer layer during the drying process, which retarded water loss from within. Survival in mature composts at 15°C was of the same order of magnitude as in soils but shorter at 28°C, possibly owing to increased biological activity at this temperature, or a resumption of the composting process, with concomitant higher temperatures within the compost itself. The maximum survival time recorded over all soil types and conditions during *in vitro* studies was around 200 days. In field studies, the maximum survival time in both bare sand and clay was around 85 days at depths up to 50 cm. The survival time was reduced in field experiments carried out in summer to less than 40 days and in one study when the ground was flooded for rice cultivation, the bacterium could not be detected 14 days after flooding. The maximum survival time of *R. solanacearum* in infected plant material or in infested soil samples incorporated into compost heaps was less than 2 weeks. At the culmination of field soil and compost experiments, no infection was detected in tomato seedlings up to 10 weeks after transplanting into the same soils or composts under glasshouse conditions at a temperature of 25°C.

Keywords Brown rot · Soil · Compost · Survival

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Introduction

R. solanacearum the causal agent of the disease bacterial wilt affects over 200 species of plants throughout the tropics and sub-tropics. It causes severe losses in a number of economically important crops including potato, tomato, banana, tobacco, groundnut and ginger (Kelman 1953).

R. solanacearum race 3 biovar 2 (phylotype II sequevar 1) as described by Fegan and Prior (2005) is a major factor in the potato trade between Egypt and the European Union. The disease is endemic in potatoes grown in the Nile Delta of Egypt and potato exports from this area to the EU have often been contaminated with the pathogen. As a result, all ware potatoes grown in Egypt for the EU market are required to be grown from pathogen-free seed imported from Europe at considerable expense to the Egyptian potato industry. Increasingly stringent requirements imposed on Egyptian exporters from the mid 1980s by a series of EU Decisions and Directives led to all potato production for the EU market being relocated from the traditional potato growing areas in the Nile Delta to pest free areas (PFAs) in, or on the fringes of, the desert which could be shown to have had no history of potato production or had been declared free from the pathogen after thorough sampling and testing of weeds, soil and surface and arterial waters. In addition, Egypt was required, with EU assistance, to establish efficient monitoring, inspection and testing procedures, applied throughout the potato production cycle, to minimise the possibility of infection of Egyptian export potatoes with *R. solanacearum*.

Additional EU financial and technical assistance was provided to the Egyptian Department of Agriculture to carry out both field and laboratory studies into the epidemiology of the disease and surveys to plot the distribution of the pathogen. This work provides information on some physical factors affecting survival of the pathogen in typical clay and sandy soils in Egypt.

Materials and methods

All sampling and testing methodology followed, as far as possible, protocols adopted by the EU (Anon 1998 and amendments).

Preparation of inoculum

Strains of *R. solanacearum* race 3 bv 2, isolated on SMSA medium (Englebrecht 1994 as modified by Elphinstone et al. 1996) from Egyptian soils and surface waters in the Nile delta of Egypt and identified by immunofluorescence antibody staining (IFAS; Janse 1988), real-time PCR, (Weller et al. 2000) and bioassay in tomato (Janse 1988), were used in all experiments. The biovar was confirmed by biochemical tests (Hayward 1964) and by real-time PCR (Weller et al. 2000). Isolates were stored either in sterile distilled water at room temperature or in 20% glycerol at −30°C. Strains were numbered according to the geographical locality (Monufiya or Garbiya), year of isolation and substrate (surface water (W) or soil (S)). Thus strain Mon102W represents isolate 1 from Monufia Province in the Nile delta, isolated in 2002 from surface water.

For *in vitro* studies, inoculum was prepared from 48 to 72 h cultures of the bacterium, grown on SMSA basal medium without addition of antibiotics. Bacterial colonies were suspended in a few millilitres of sterile phosphate buffer using a sterile spreader and cell numbers were adjusted to approximately 10^8 colony forming units per ml (cfu ml^{-1}) using a spectrophotometer calibrated such that a suspension with absorbance of 0.1 at 670 nm approximated to the required bacterial concentration.

Concentrations were checked by plating 100 µl volumes from serial dilutions of the suspension in 10 mM phosphate buffer, pH 7.2 ($NA_2HPO_4.12H_2O$: 2.7 g, $NaH_2PO_4.2H_2O$: 0.4 g, distilled water 1.0 l) onto plates of SMSA basal medium using a sterile plastic spreader. All plates were incubated at a temperature of 28°C in the dark and colonies with typical fluidal consistency and morphology with red colouration characteristic of *R. solanacearum* were counted after 48 and 72 h.

Soil source

For most experiments, clay soil randomly sampled from smallholdings in the Nile Delta and sandy soil obtained from commercial potato farms in desert PFA areas were used. Sampling sites were chosen to be representative of both the soils and farming methods of the delta region and desert respectively. For some experiments sandy and clay soils were sourced from

organically managed farms in the Delta and desert fringes respectively. Soils were generally collected 24 to 48 h before use and stored in the interim period at room temperature.

Chemical analysis of soils

Physical and chemical analysis of soils was carried out by staff of the Faculty of Agriculture, Ain Shams University, Cairo.

Sandy soils typically had a pH of around 7.8 and electrical conductivity of 1.82 dS/m. Composition was of the order of 95% sand, 4% silt and 1% clay. The concentration of elements (ppm) was: N, 5.00; P, 16.00; K, 316.00; Fe, 34.00; Mn, 23.00; Zn, 3.00; Cu, 2.00 and soluble ions (meq/l): Ca^{++}, 10.00; Mg^{++}, 2.24; Na^{+}, 5.00; K^{+}, 0.96; Cl^{-}, 8.10; HCO_3^{-}; 1.22; SO_4^{--}, 8.88.

Corresponding values for clay soils were: pH, 7.3; electrical conductivity, 3.60 dS/m; concentration of elements (ppm): N, 24.50; P, 70.00; K, 348.00; Fe, 47.00; Mn, 100.00; Zn, 6.00; Cu, 4.00 and concentration of soluble ions (meq/l): Ca^{++}, 19.20; Mg^{++}, 10.00; Na^{+}, 4.50; K^{+}, 2.28; Cl^{-}, 19.80; HCO_3^{-}; 2.20; SO_4^{--}, 14.00.

Estimation of water holding capacity

The water holding capacity of the soils was estimated by adding measured volumes of water to weighed quantities of oven dried soil which were then left to stand for 30 min to ensure complete absorption of the water by the soil. The excess water was then poured off and the volume of water absorbed by the soil estimated by subtraction. This amount of water was adjudged to give 100% water potential. For experiments using 50, 25 and 10% water potential, the relative volumes of water and bacterial suspension added to the soil was adjusted to give the desired water potential whilst maintaining constant bacterial populations in all treatments.

In vitro survival of *R. solanacearum* in soil at different temperatures

Experiments were carried out in triplicate. Sterile 50 ml capacity plastic screw cap centrifuge tubes using 20–30 g of soil were incubated in triplicate in controlled temperature cabinets at temperatures of 4, 15, 28 or 35°C. To study the effect of temperature fluctuation on survival, triplicate samples were switched between paired temperatures of 4 and 15°C, 15 and 28°C and 28 and 35°C at approximately 24 h intervals in three experiments.

Some experiments to investigate the effects of wetting and drying or continuous drying on survival were carried out using larger quantities of soil in 110 ml capacity sterile plastic screw cap containers with caps removed for the drying treatments and left in place for the controls held at constant water potential.

To study the effect of water fluctuation on the survival of *Ralstonia solanacearum*, weighed amounts of soil at an initial 100% water holding capacity were incubated in open 110 ml capacity containers at temperatures of either 15 or 28°C for a period of 7 days. After 7 days, the amount of evaporated water was calculated by reweighing the soil plus containers and replenished by adding a measured volume of sterile distilled water. Triplicate samples of soil held at each temperature were taken at 0, 24 and 48 h and thereafter at approximate weekly intervals. *R. solanacearum* was isolated from serial dilutions of 1.0 g samples of soil. 0.1 ml volumes of a range of soil dilutions were spread on plates of SMSA medium and colonies typical of *R. solanacearum* counted. Colony identity was confirmed periodically using lateral flow devices (Danks and Barker 2000), or by plating selected colonies onto SMSA basal medium without antibiotics, or sucrose peptone agar, and testing discrete colonies from these plates by immunofluorescence antibody staining (IFAS; Janse 1988), real-time PCR (Weller et al. 2000) and bioassay in tomato (Janse 1988).

In each experiment, maximum survival time in days was estimated to be the point mid-way between the time of the last positive isolation and the time of the first negative result when the bacterium was no longer recoverable. Where experiments terminated before a zero count was recorded, the maximum survival time was estimated by extrapolation from the population curve. Early termination was only necessary when the pathogen had remained below detectable levels in one or more treatments for at least three consecutive samplings but remained high in the remaining treatments.

Survival of *R. solanacearum* in compost

Compost was provided by a commercial compost producer in the Nile delta with the following characteristics:

1. Uncomposted mixture of shredded and homogenised plant materials
2. Cow manure, minerals, and plant materials: 100% composted
3. Cow manure, chicken manure and plant materials: 100% composted
4. Chicken manure, plant materials and minerals: 60% composted

The stage of composting specified above was based on information provided by the compost supplier

Both soil and compost experiments were carried out in triplicate. Sterile 50 ml disposable centrifuge tubes, containing 20–30 g of inoculated soil, compost or homogenised plant remains, were incubated at temperatures of 15 or 28°C to study the effect of temperature on survival.

Surviving bacteria from triplicate tubes in each temperature regime were isolated at zero, 1, 2 and 3 days after inoculation and thereafter at weekly intervals. The contents of each tube were suspended in 30 ml PB buffer by shaking for 1 h. Serial 10-fold dilutions of the suspension were then spread in 0.1 ml aliquots on duplicate plates of semi-selective SMSA agar and incubated at 28°C. Typical fluidal colonies with red colouration appearing after 48 to 72 h were counted. Identity of selected colonies was checked using specific monoclonal antibodies in lateral flow device (LFD) test kits, IF and PCR. Plates showing heavy growth of background organisms were ignored and means taken of colony counts from the remaining plates.

Survival of *R. solanacearum* in the field

For field experiments sandy and clay soils were collected from the field and uniformly mixed with a suspension of approximately 10^8 cfu ml^{-1} of *R. solanacearum*. Excess suspension was drained off and the soils were then packed into duplicate 55 cm plastic pipes (diameter 10 cm) with three 1.5 cm holes drilled at equal distances round the circumference 5 cm from the top and bottom and in the mid-section to facilitate collection of samples from different soil depths. The soil columns were buried vertically at two field sites in the Nile delta and sampled at fortnightly intervals until the pathogen could no longer be isolated. The soils were then distributed into 5 cm diameter pots and planted with 2-week-old tomato seedlings (var Moneymaker). Seedlings were grown on in a quarantine glasshouse at 25°C and monitored daily to detect signs of wilting. After around 10 weeks, samples were collected from remaining plants and rhizosphere soil and plated on SMSA medium to check for the presence of the pathogen.

For field compost experiments, farmers were asked to produce traditional compost heaps from harvest waste, weeds, soil and livestock manure. The compost was thoroughly mixed and artificially inoculated *R. solanacearum*-infected, chopped-up, tomato seedlings (approximately 100 g per replicate), potato tubers and pathogen infested soil samples (200 g per replicate), in muslin bags, were placed in the compost at different depths and sampled at fortnightly intervals. Volumes/weights of introduced infected material varied depending on availability. The concentration of *R. solanacearum* in this material also varied but was typically of the order of 10^8–10^9 cfu/g wet weight. The temperature within the compost was continuously measured using a data logger. When *R. solanacearum* could no longer be detected, the compost was planted with tomato seedlings and the seedlings were monitored in the same way as for the soils above.

The dry weights of all soils used in experiments were calculated by drying replicate samples of soil to constant weight in an oven at a temperature of 80°C. Results of all experiments were expressed as cfu g^{-1} dry weight of soil.

Statistical analysis

Data from all experiments was transformed logarithmically ($\log_{10}$ x+1) to stabilise the variance. Data points on all graphs represent the mean of at least three observations with the relevant standard error bars. The area under the population curves (AUPC) was calculated using the trapezoidal method from time zero to the time when bacterial populations fell to non-detectable levels in one or more treatments. AUPCs were compared in all experiments by conducting an ANOVAR using Genstat 8.1 (VSN

International Ltd) on the AUPC data. Data from all experiments over a 2-year period on estimated survival time of *R. solanacearum* in clay and sand at 4, 15, 28 and 35°C was pooled to give overall estimates of the mean and maximum survival times at these temperatures.

Results

Factors affecting survival of *R. solanacearum* in soil

Effect of temperature

Experiments carried out at temperatures of 4, 15, 28 and 35°C in clay and sandy soils, respectively, produced broadly consistent results, although the number of experiments carried out at temperatures of 4 and 35°C was low compared with those carried out at 15 and 28°C (see Table 1). In most cases, *R. solanacearum* was not recovered from soils after 180 days, irrespective of temperature or soil type. In one exception, the bacterium was recovered from clay soil after 180 days at a constant temperature of 15°C. Survival was generally longest at 15°C and shortest at 35°C. Survival at 28°C was generally between that at 15 and 35°C whilst survival at 4°C was variable between experiments. Typical plots at each of the above temperatures are shown in Fig. 1

Results between experiments were variable even using soils from the same source in a number of experiments. Nevertheless, clear differences in the effect of temperature on survival in both clay and sandy soils were observed. In almost all experiments, survival at 15°C was longer than at 28°C, in both clay and sandy soils, irrespective of their origin. Survival at 35°C was always shorter than at either 15 or 28°C in clay or sand, but in comparisons between the two soil types, survival at 35°C in clay was occasionally longer than at 28°C in sand. At 4°C in clay soils, survival was shorter than at 15, 28 or 35°C, whilst in sandy soils mean survival at 4°C was usually shorter than at 15°C, but longer than at temperatures of 28 and 35°C. This variability may be a consequence of the low number of experiments carried out at 4°C (Table 1).

Data on estimated days to extinction of *R. solanacearum* in all *in vitro* experiments (Table 1) indicated that survival was slightly longer in clay soils than sand and that survival was longer at 15°C than at 28°C or 35°C. Survival was also longer at 28°C than at 35°C in both sandy and clay soils although the difference in survival times at these two temperatures was small. In clay soils, survival at 4°C was shorter than at the other three temperatures but in sandy soils it was longer than at temperatures of 28 and 35°C. Because of the low number of experiments carried out at temperatures of 4 and 35°C data on survival times at these temperatures will be less reliable than data from experiments carried out at temperatures of 15 and 28°C respectively. In all cases, extinction of recoverable populations was expected within 200 days irrespective of temperature or soil type.

The population profiles and maximum survival times (not shown) of *R. solanacearum* cultures incubated under fluctuating temperatures (approximately 24 h cycle) tended to be intermediate

Table 1 Mean maximum survival times (d) and estimated time to extinction of *R. solanacearum* in sandy and clay soils at temperatures of 4, 15, 28 and 35°C

	Survival (d)[a]			
Temperature (°C)	4	15	28	35
Soil Type				
Clay	64 (68)[b] SE=3.670; *n*=3	126 (200) SE=9.321; *n*=14	107 (152) SE=7.131; *n*=19	101 (123) SE=12.441; *n*=3
Sand	85 (112) SE=27.50; *n*=2	102 (167) SE=11.957; *n*=10	78 (114) SE=8.782; *n*=12	67 (85) SE=7.941; *n*=4

[a] Mean maximum time in days rounded to nearest whole number after which *R. solanacearum* could no longer be detected.

[b] Maximum estimated survival time (d) observed by extrapolation from population curve.

Figures represent combined dated from *in vitro* studies carried out in soils at water holding capacity (WP) between 50 and 100% (SE = standard error; n = number of experiments used for estimation of survival times).

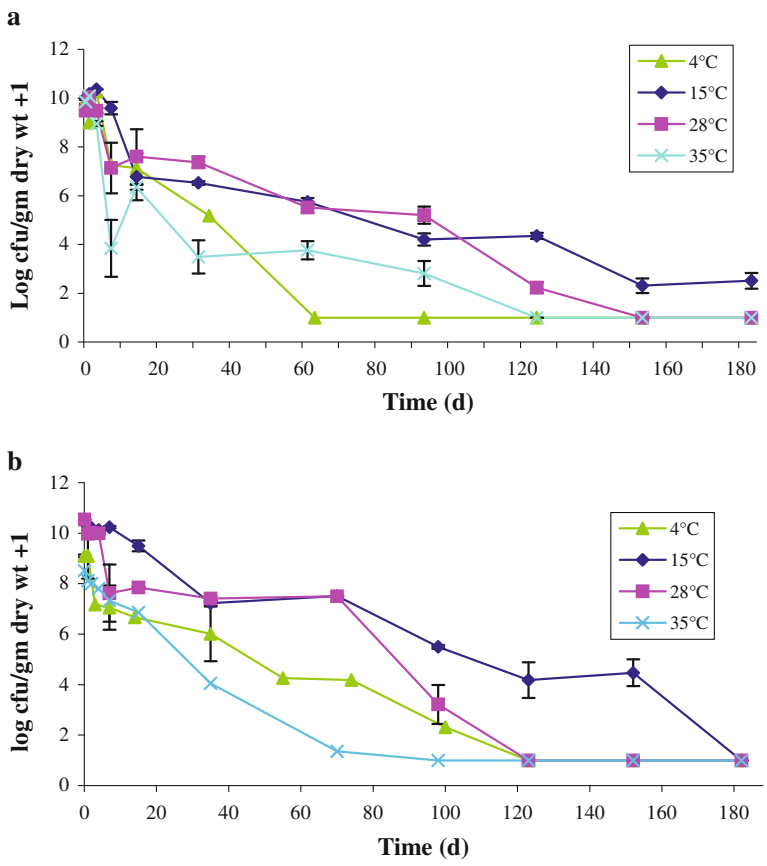

Fig. 1 Survival of *R. solanacearum* strain Mon102W in clay (**a**) and sandy (**b**) soils (100% WP) at temperatures of 4, 15, 28 and 35°C. Differences in the AUPC between temperature (F=516,630; df=3, 47; P<0.001) and soil type (F=340,431; df=1, 47; P<0.001) were highly significant

between the corresponding population profiles and survival times of cultures incubated at constant temperatures.

Effect of water potential (WP)

There was no consistent effect of reduced WP on survival of *R. solanacearum* relative to survival at 100% WP except when WP was reduced as low as 10%. At 10% WP, survival in clay and sandy soils was around 150 days at 15°C and 90 days at 28°C, compared with around 180 days (15°C) and 120–150 days (28°C), respectively, at 100%WP.

Populations of *R. solanacearum* at an initial WP of 100% declined more rapidly when incubated on a 7 day cycle of wetting and drying than populations held at a constant 100% WP at temperatures of 4, 15 and 28°C respectively in both clay and sandy soils (Fig. 2a–f).

The most marked differences in survival times between fluctuating and constant water potentials were observed at a temperature of 28°C. In clay soils survival was 30–40 days under fluctuating WP and 108–120 days under constant WP whilst in sand, the corresponding figures were 40–64 days and 110–120 days, respectively.

In a separate experiment, the effect of continuous drying versus constant WP (100%) was compared (results not shown). In these experiments, *R. solanacearum* appeared to die out in clay soil between 35 and 75 days at both constant WP and with progressive drying. In sandy soil survival was 35–75 days at constant WP but only 20–35 days in soil subject to drying.

Survival of *R. solanacearum* in soils under field conditions

Triplicate clay and sandy soil cores were buried in field soil at two sites in the Nile Delta potato-growing region. Experiments were repeated to cover all seasons.

Data for experiments carried out at a site in Monofiya Province in spring (Fig. 3a, b), summer

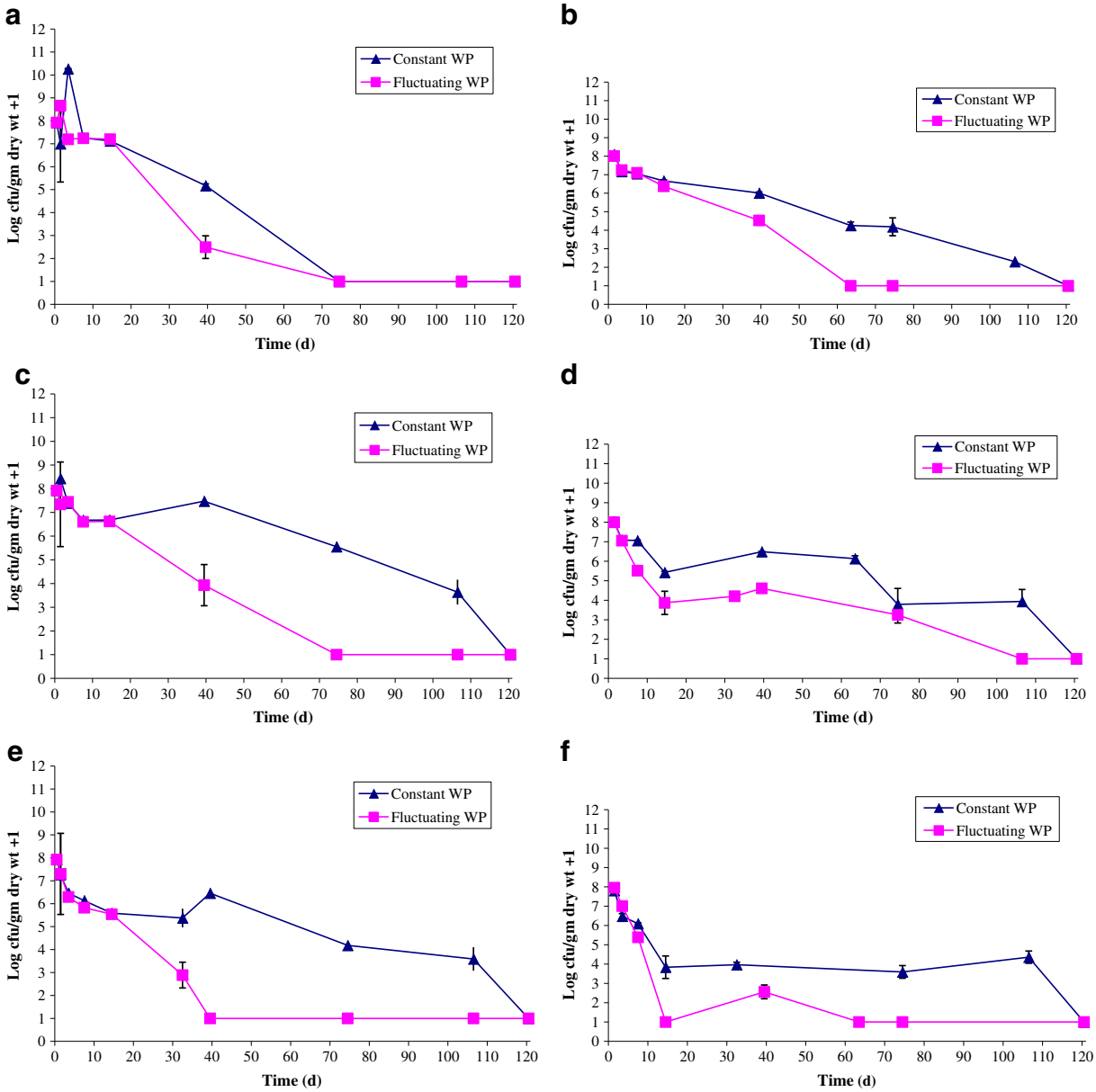

Fig. 2 Survival of *R. solanacearum* strain Mon102W in clay (**a**, **c**, **d**) and sandy (**b**, **d**, **f**) soils at 4°C (**a**, **b**), 15°C (**c**, **d**) and 28°C (**e**, **f**) under constant and fluctuating WP. Differences in the AUPC between temperature (F=37,184; df=2, 71; P=0.009) and fluctuating vs constant WP (F=385,034; df=1, 71; P<0.001) were highly significant. Interaction between temperature and WP (F=92,777; df=2, 71; P<0.001) was also highly significant. The difference in survival in sand and clay respectively was not significant

(Fig. 3c, d) and winter (Fig. 3e, f) show a difference in maximum survival time in different seasons. In spring, survival was between 48 and 60 days in sand and clay irrespective of sampling depth down to 50 cm. Corresponding figures for summer were 15–37 days for both soil types, again with no differences apparent at different sampling depths and for winter, 63–87 days in clay and sandy soils for all samples except samples collected at 50 cm depth in sandy soil, when viable populations of the bacterium could still be detected at 87 days but not at 95 days. Seasonal differences in survival were highly significant, primarily due to the longer survival in the cooler period between September and December). There were no

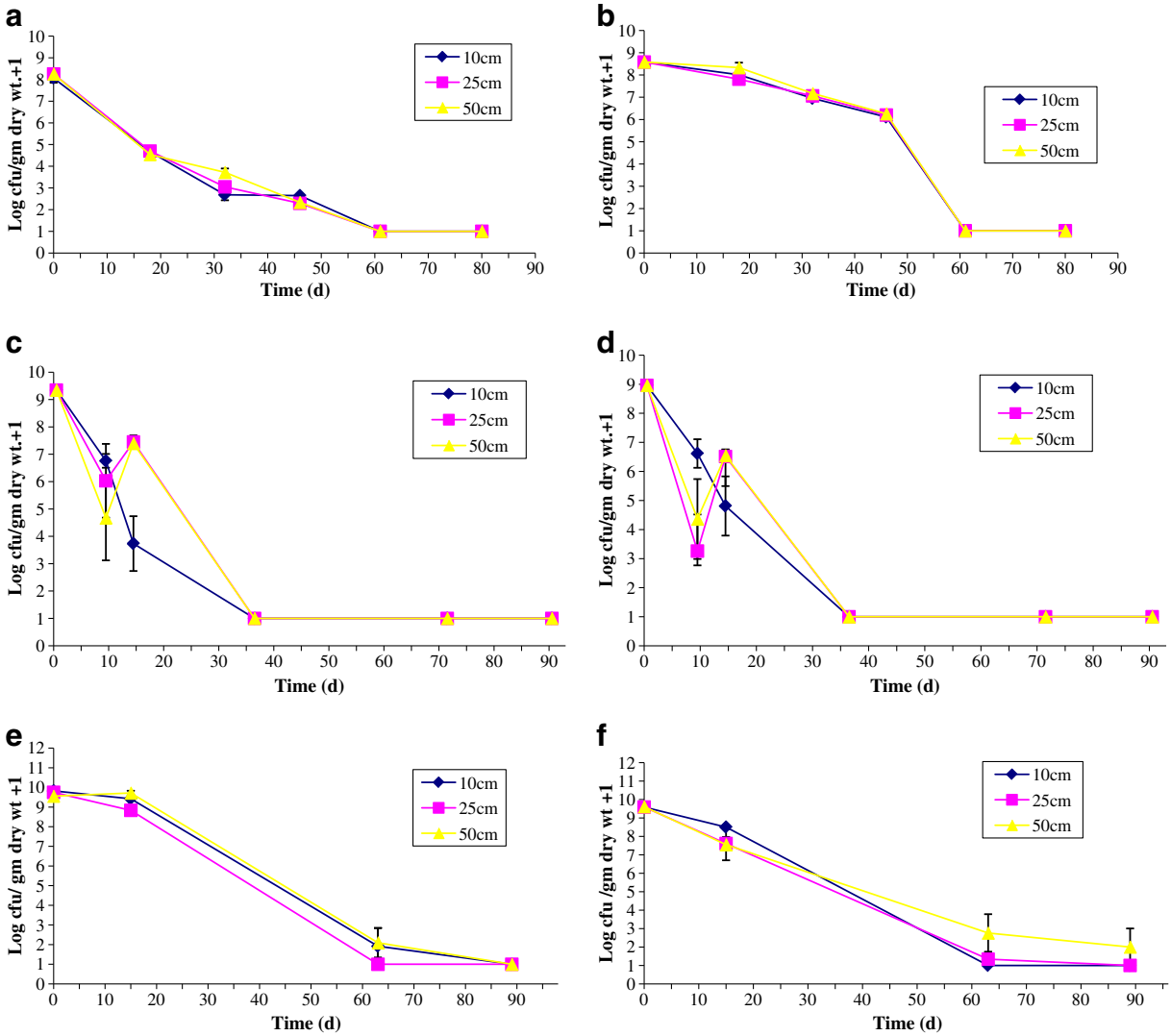

Fig. 3 Survival of *R. solanacearum* strain Mon 302S in clay (**a**, **c**, **e**) and sandy (**b**, **d**, **f**) soils under field conditions March-June (**a**, **b**), June-August (**c**, **d**) and September-December (**e**, **f**) in Monufiya Province. Differences in the AUPC between seasons were highly significant (F=13,934.55; df=2, 71; P< 0.001). There was no significant difference in AUPC between sand and soil or at different soil depths, respectively

significant differences in survival time between sand and clay or with depth.

Effect of temperature on survival of *R. solanacearum* in composts

In *in vitro* experiments to monitor the survival of *R. solanacearum* in composts produced from a) homogenised plant remains; b) mixtures of 100% composted cow manure plant debris with added minerals; c) cow manure, chicken manure and plant debris and d) 60% composted chicken manure, plant debris with added minerals (Fig. 4a–d), survival at 15°C was comparable to survival times in soil. In contrast, survival of the pathogen at 28°C was much shorter than either survival at 15°C in composts or survival at 28°C in soil. Survival of *R. solanacearum* at this temperature in homogenised plant materials (Fig. 4a), fully composted mixtures of cow manure, plant debris and minerals (Fig. 4b), fully composted cow manure, chicken manure and plant waste (Fig. 4c) and partially composted chicken

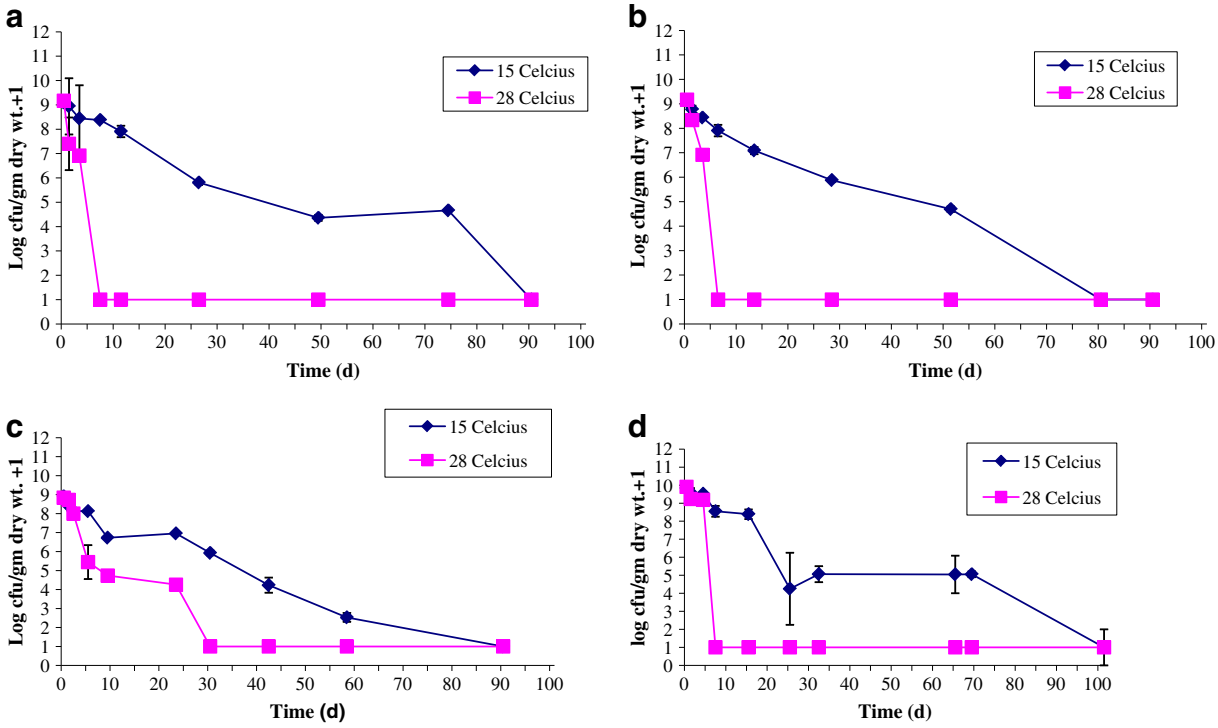

Fig. 4 Survival of *R. solanacearum* Mon 202S in **a** uncomposted plant waste, **b** a mixture of fully composted cow manure (40%), plants (50%) and minerals (10%), **c** a mixture of fully composted cow manure (25%), chicken manure (25%) and plant waste (50%) and **d** around 60% composted chicken manure (33%), plant waste (50%) and minerals (17%). Effects of temperature were highly significant in treatments a (F=260608; df=1, 23; P<0.001), b (F=155750; df=1, 23; P<0.001) and d (F=254872; df=1, 15; P<0.001) but not for treatment c

manure, plant debris and minerals (Fig. 4d) respectively was less than 10 days. Survival in a mixture of 100% composted cow manure, chicken manure and plant debris at 28°C was longer at around 30 days.

R. solanacearum did not survive for more than 3 weeks in infected tomato seedlings, potato tubers or artificially infested soil when placed at different depths in compost heaps during traditional composting of farm waste comprising residues from harvested potato crops mixed with animal manure, at two sites adjacent to smallholder potato farms in the Nile delta. This is despite the fact that in neither compost heap did temperatures rise above 40°C internally or above 35–40°C at the surface, probably due to incomplete mixing during the composting process. Complete elimination of the pathogen was confirmed by the absence of infection of tomato seedlings planted in a consolidated mixture of composted waste from throughout the compost heap at the conclusion of the experiment.

Discussion

R. solanacearum race 3 bv 2 does not appear to survive indefinitely in Egyptian soils in the absence of host plants.

Previous studies on the survival of *R. solanacearum* in soil have followed three approaches. Firstly, experiments have been carried out in artificially infested soil microcosms in the laboratory under controlled conditions (Moffet et al. 1983; Granada and Sequeira 1983a, b; Shekhawat and Perombelon 1991; van Elsas et al. 2000). Secondly, soil from wilt-infested fields has been collected and survival of the pathogen has been assessed in the laboratory or glasshouse by planting susceptible tomato seedlings in batches of the soil at regular intervals and monitoring infection by the pathogen (Nesmith and Jenkins 1979; McCarter et al. 1969; Graham and Lloyd 1979). Thirdly, and more commonly, direct observations have been made in the field after harvest

of an infected potato crop after various periods of fallow or rotation with non-host crops (Kelman 1953; Shamsuddin et al. 1978; Pradhanang et al. 1998; van Elsas et al. 2000).

Survival appears to depend upon a number of interacting factors including temperature, water potential and the physical characteristics of the soil, as well as the race and strain of *R. solanacearum.*

There have been comparatively few *in vitro* studies on the effects of temperature on the survival of *R. solanacearum* in soil. Those that have been done are in broad agreement with values reported here. Shekhawat and Perombelon (1991) in a study on artificially infested clay and sandy soils at temperatures of 5, 10, 15, 30 and 35°C reported maximum survival of between 161 and 203 days for both races 1 and 3 of the pathogen in heavy, aerated soils at temperatures between 10 and 30°C, soil moisture between 20 and 60%, and pH between 5.5 and 8.5; conditions which they considered optimum for survival of *R. solanacearum*. Survival was less at 35°C (56–70 days) and 5°C (84 days), but longer in clay than sandy soil, particularly under conditions of low soil moisture. They concluded from their studies that "the innate capacity of the bacterium to survive as a free-living soil organism is rather poor and is affected by temperature, moisture, oxygen status and soil type".

Temperature was also shown to be a determinant of survival of *R. solanacearum* by Van Elsas et al. (2000). In *in vitro* studies at temperatures of 4, 12 and 20°C in a loamy sand l and two silt loam soils, they could still detect the pathogen after 210 days in one of the silt loam soils at both 12 and 20°C but not in the loamy sand soil after around 90–100 days. In all soils, there was a rapid fall in numbers to below detectable levels at 4°C in around 15 days in the loamy sand soil and 20–30 days in the silt loam soils. An explanation for this difference might be the lower initial inoculum level used in the loamy sand soil (4.4 versus~6.0 log units).

Granada and Sequeira (1983a) studying *in vitro* survival of *R. solanacearum* race 3 in a sand-soil mixture at a temperature of 28°C found that bacterial numbers declined from a starting value of 1.57×10^{7} cfu g^{-1} dry wt soil to just above detectable levels at 1.16×10^{2} cfu g^{-1} dry wt soil within 56 days and fell to below detectable levels between 56 and 84 days, leading them to conclude that the pathogen is not capable of long term survival in the soil in the absence of host plants. However, these same workers (Granada and Sequeira 1983b) reported that race 3 of the pathogen survived for 673 days in soil sealed in plastic bags at a temperature of 4°C suggesting that some strains of race 3 might be better adapted for prolonged survival in soil at low temperature. McCarter et al. (1969) recorded survival times of *R. solanacearum* (race not established, probably bv. 1) in soils from infected potato fields in Georgia, USA for up to 4 months with longer survival of the pathogen in the upper soil layer (0–30 cm). Conversely, Graham and Lloyd (1979) found that race 3 of the pathogen could be detected in the deeper soil layers (55–65 cm) for more than 82 days but died out within 13 days in the surface layer (10–15 cm).

In vitro studies (Shekhawat and Perombelon 1991; van Elsas et al. 2000) have been consistent in showing that *R. solanacearum* survives for longer in heavier clay soils than sandy soils. Shekhawat and Perombelon (1991) observed a slower rate of decline in populations of the pathogen in wet (60% water holding capacity) clay than wet sand at 30°C. Survival in both clay and sand was greater than 84 days. In dry (0% water holding capacity) clay and sand at 35°C, the pathogen could no longer be detected after around 28 days in clay and 21 days in sand. The corresponding figures in experiments of Van Elsas et al. (2000) were in excess of 210 days at both 12 and 20°C in a silt loam soil and around 90 days for a loamy sand soil. These figures are comparable with the estimated maximum survival times in this study of 200 days for an Egyptian clay soil and 167 days for an Egyptian sandy soil at 15°C and 100% water holding capacity.

Other workers also provide supporting evidence for our findings of shorter survival in soils with lower moisture levels. *S*hekhawat and Perombelon (1991) in a study using moisture levels of 0 to 100% of water holding capacity (WHC) observed the most rapid decline in populations of *R. solanacearum* in dry soil at temperatures of 4 and 35°C. At a temperature of 30°C the pathogen could not be detected after 21 days in dry sand and 28–42 days in dry clay. The slowest rate of decline in population numbers was at 60% WHC followed by 20–40% and 80–100% WHC. The decline in clay was slower than in sandy soil. At 60% WHC in clay at temperatures between 15 and 30°C the bacterium survived for between 160 and 200 days.

Van Elsas et al. (2000) also observed an effect of soil moisture on survival of *R. solanacearum*. Their

data indicated that the optimum WHC for survival of the pathogen in three soils was around 70%. Survival at 50 and 90% WHC was shorter whilst drying the soils to 10–20% WHC led to a decline in population numbers to below detectable levels within 15 days.

Whilst we observed a more rapid decline in bacterial numbers at 10% WHC in sand and clay compared with bacterial populations held at 100% WHC, the effect was variable at all other WHCs used and dependent to some extent on the incubation temperature. Decline rates were normally higher at 28°C than at 15°C in both sand and clay soils.

Experiments carried out at constant water potential do not accurately reflect conditions occurring in the field where the water potential in the surface layers is likely to vary on a daily and seasonal basis. Experiments reported here show that *R. solanacearum* appears to be susceptible to fluctuating water potential. The bacterial populations declined more rapidly in both sand and clay soils at temperatures of 4, 15 and 28°C with fluctuating WP than in soils at constant WP. Surprisingly, the pathogen declined more rapidly in clay than sandy soils at these temperatures. Field studies in clay and sandy soils at two sites in the Nile Delta over three seasons indicated that *R. solanacearum* numbers fell to below detectable limits in around 90–100 days down to a depth of 50 cm.

Other observations on survival of *R. solanacearum* race 3 in field soil have reported survival times of more than 233 days (Graham et al. 1979) and 2.5 years (Shamsuddin et al. 1978) on the Northern Tablelands soils of New South Wales, Australia; 168 days (Akiew 1985), also in Australia and 10–12 months (van Elsas et al. 2000) in the Netherlands. In all these cases, the observations were made after harvesting an infected potato crop and the presence of crop debris, unharvested tubers and susceptible weeds, providing protective niches for the pathogen, could not be discounted.

A number of workers (Graham and Lloyd 1979; McCarter et al. 1969) have proposed that *R. solanacearum* may survive longer in the deeper soil layers where soil temperature and moisture may be less variable and where the populations of potential antagonists may be lower. Our results did not confirm these observations. This was possibly because the soils used in our experiments had been thoroughly mixed with a suspension of the pathogen before packing into the columns, thereby creating homogeneous conditions throughout the columns.

Amendment of soil with compost (Schonfeld et al. 2003), organic material (Messiha et al. 2007a) or antagonistic organisms (Messiha et al. 2007b) have been shown to adversely affect survival of *R. solanacearum* and increase disease suppression in some soils. This is possibly caused by an overall stimulation of microbial growth by the nutrients in the added compost or a shift in the population dynamics and incidental enhancement of microbes antagonistic towards *R. solanacearum*. The *in vitro* studies reported here tend to support the notion that microbial competition is involved in accelerating the decline of the pathogen in a range of composted and partially composted substrates. This is the most likely explanation for the more rapid decline in pathogen numbers at 28°C, a temperature close to optimum for many soil microorganisms, compared with 15°C at which microbial activity might be expected to be lower.

The rapid population decline of *R. solanacearum* during on-farm composting in the field is likely to be due to both the growth of competitive organisms in the compost heap, the temperature attained during the composting process (40°C) as well as other factors such as ammonia production.

The reported ability of *R. solanacearum* to survive in a viable but non-culturable state in the environment (Grey and Steck 2001; van Elsas et al. 2005) is obviously an important consideration in production of potato for the domestic and international markets. In a previous paper (Tomlinson et al. 2009) reporting on the survival of *R. solanacearum* in Egyptian waterways, no evidence of survival of the pathogen in a viable but non-culturable state was found. In this study also, the pathogen could not subsequently be detected in soils or composts after bacterial counts had fallen to below detectable levels, either directly, by attempted isolation from the soil or indirectly, by growing tomato seedlings in such soils.

Further evidence that the potato race of *R. solanacearum* (race 3, bv 2) does not survive long term, at least in temperate soils, is the reported eradication from the Northern Tablelands of New South Wales, Australia (French 1994), Sweden (Persson 1998), the Netherlands (Schans and Steeghs 1998), France (De Guenin 1998) and UK potato fields (Elphinstone et al. 1998), despite its continued presence in the semiaquatic weed *Solanum dulcamara*

in some of these countries (Elphinstone et al. 1998; Janse et al. 1998).

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