



Indicator organisms for assessing sanitization during composting of plant wastes

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ABSTRACT

The potential for using plant pathogens and seeds as indicator organisms for assessing sanitization of plant wastes during composting was tested in bench-scale flask and large-scale systems. *Plasmodiophora brassicae* was unsuitable due to high temperature tolerance in dry to moist composts, and detection of viable inoculum post-composting using bioassay plants not corresponding with that using TaqMan[®] PCR, possibly due to preservation of nucleic acids at elevated temperatures. Several other plant pathogens (*Sclerotinia sclerotiorum*, *Microdochium nivale*, *Phytophthora cinnamomi* and *Phytophthora nicotianae*) were unsuitable due their low temperature tolerance. *Fusarium oxysporum* f.sp. *cepae* and f.sp. *radicis-lycopersici* chlamydospores and tomato seeds were suitable indicators due to their moderate temperature tolerance and ease of viability testing post-composting. *Abutilon* seeds were more tolerant than tomato seeds of compost temperatures ≥ 52 °C but more prone to degradation at lower temperatures and therefore less suitable as indicators. Relationships between compost temperature during exposures of 2–10 days and subsequent viability of the above chlamydospores or seeds enabled the sanitizing effect of composting processes to be predicted within 2–6 days. Plant waste type (woody or vegetable) had a small but significant effect on the relationship for tomato seeds but not for *F. oxysporum* chlamydospores.

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1. Introduction

Composting is usually a phyto-hygienic means of ensuring plant pathogens are eradicated from organic wastes before they are recycled as composts into agricultural and horticultural landscapes, but plant pathogens can survive sub-optimal processes. Temperature and exposure time are usually the most important and easily verified factors in determining pathogen eradication during composting, but other factors such as moisture, gaseous conditions, and the presence of antagonistic microbes in the compost may also have an influence (Noble and Roberts, 2004; Noble et al., 2009). Reduction in the numbers of naturally occurring micro-organisms such as *Escherichia coli* in composting wastes has been used to augment time-temperature data (Christensen et al., 2002) although the results depend on the initial pathogen population in the waste. Testing for the eradication of known populations of indicator organisms inserted in the compost has therefore been used to verify sanitization (Bruns et al., 1993; Christensen et al., 2002). The following factors are important in selecting suitable indicator organisms: (a) survival responding to compost temperature, exposure time, and other properties such as moisture content in a similar manner to those of plant pathogens of concern, such as quarantine organisms, (b) ease of retrieval and viability testing

post-composting and (c) repeatability of results between different composts, composting systems, and sources of the organism.

There is a significant amount of data on the effect of compost temperature and exposure time on the eradication of different plant pathogens, some of which have been used as indicator organisms of sanitization (Noble et al., 2009). *Plasmodiophora brassicae*, the causal agent of clubroot of crucifers, and *Tobacco Mosaic Virus* have been used due to their heat tolerance (Idelmann, 2005; Anonymous, 2008) although *TMV* is resistant to temperatures above those which can usually be achieved during composting (Noble et al., 2009). A further disadvantage of using these pathogens as indicator organisms is the need for a lengthy bioassay with a susceptible host plant to obtain a test result. Other plant pathogens that have been used as indicator organisms for compost sanitization include *Fusarium oxysporum*, *Rhizoctonia solani*, and *Sclerotinia sclerotiorum* (Christensen et al., 2001; Idelmann, 2005). However, information on the effects of a full range of compost temperatures, exposure times, and other factors such as moisture content on the eradication of any particular organism is lacking (Noble et al., 2009). Here, the effect of a comprehensive range of temperatures and exposure times on isolates of *Plasmodiophora brassicae*, *F. oxysporum*, and *S. sclerotiorum* was studied. *Microdochium nivale* was also selected due to its potential heat tolerance, and *Phytophthora* species due to their importance in the subsequent use of composts and ease of retrieval on selective media (Noble et al., 2009).

Detection of viable inoculum post-composting has usually been with plating on selective media and/or using bait or bioassay test

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Table 1
Sources of plant pathogens used in the experiments.

Pathogen	Isolate	Source	Location	Inoculum
<i>Fusarium oxysporum</i> f.sp. <i>cepae</i>	–	Onion ^a	Lincolnshire	Chlamydo spores
<i>Fusarium oxysporum</i> f.sp. <i>radicis-lycopersici</i>	–	Tomato ^a	West Sussex	Chlamydo spores
<i>Microdochium nivale</i>	08263	Wheat ^b	Yorkshire	Chlamydo spores
<i>Microdochium nivale</i>	08251	Wheat ^b	Yorkshire	Chlamydo spores
<i>Microdochium nivale</i>	Caythorpe	Wheat ^b	Yorkshire	Chlamydo spores
<i>Microdochium nivale</i>	Morley	Wheat ^b	Yorkshire	Chlamydo spores
<i>Phytophthora cinnamomi</i>	P7	– ^c	Netherlands	Peat culture
<i>Phytophthora nicotianae</i>	PC3976	– ^b	UK	Peat culture
<i>Plasmiodiophora brassicae</i>	–	Cabbage ^b	UK	Chinese cabbage
<i>Plasmiodiophora brassicae</i>	–	Soil ^a	Warwickshire	Chinese cabbage
<i>Sclerotinia sclerotiorum</i>	13	Lettuce ^a	Cheshire	Sclerotia
<i>Sclerotinia sclerotiorum</i>	OSR 23	Oilseed rape ^a	Warwickshire	Sclerotia

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^b FERA York.

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plants. Although molecular biology tools such as TaqMan[®] PCR and nucleic acid microarrays have been used for detecting plant pathogens in compost (Franke-Whittle et al., 2009), they may not distinguish viable and dead cells (Noble et al., 2009). Here, plant bioassays and TaqMan[®] PCR were compared for assessing the post-composting viability of *Plasmiodiophora brassicae*.

Heat tolerant seeds such as tomato (*Lycopersicon esculentum*), white clover (*Trifolium repens*), wild oat (*Avena fatua*), and buckwheat (*Polygonum convolvulus*) have been used as indicators of compost sanitization; seed viability post-composting being determined by germination and/or tetrazolium stain tests (Pollmann and Steiner, 1994; Larney and Blackshaw, 2003; Anonymous, 2008; Stanford et al., 2009). To increase heat tolerance further, Idelmann (2005) used dried or partially dried tomato seeds, although the moisture content of composts will influence the moisture content of the seed and therefore the assessment of sanitization. Other heat tolerant seeds such as *Abutilon*, *Anoda*, and *Portulaca* species (Egley, 1990; Eghball and Lesoing, 2000) may also have potential as biological indicators of sanitization. Here, tomato seed was used a standard, together with *Abutilon*, which preliminary tests showed was more suitable as an indicator seed in compost than *Anoda* or *Portulaca*.

The potential for using different seeds and plant pathogens as indicator organisms for assessing sanitization during composting was first tested in bench-scale heated flask equipment. The effects of a comprehensive range of temperatures and exposure times on indicator viability were examined using a controlled wetted and mature compost to avoid the effects of high levels of microbial activity and toxic gases such as ammonia, and variable moisture availability. The effects of a comprehensive range of compost temperatures and incubation times on the eradication of selected indicator organisms were then tested in larger-scale, microbiologically highly active composting systems, using wet (vegetable) and moist (woody green) wastes.

2. Materials and methods

2.1. Indicator organisms

Samples of seeds or pathogen inoculum were filled into polyester mesh bags, prepared from 50 mm discs and a tie-wrap, which were inserted for specified times into flask composts (controlled temperatures) or large-scale composts (measured temperatures), and then tested for viability. The initial viability of seeds or pathogen inocula was tested using the same methods as described for testing seeds or pathogen inocula after the composting tests. The initial viability tests were conducted on 15 replicate samples of

10 seeds, and except where stated, on 15 replicate samples of pathogen inoculum.

2.1.1. Seeds

The tomato cv. Ailsa Craig was used as a standard. Where mentioned, seeds of other cultivars of tomato and flowering maple (*Abutilon × hybridum*) were also used in a subset of the experiments. Each sample bag contained 15 seeds of each type. After each composting test, a tetrazolium stain test for viable cells was conducted on the seeds (Anonymous, 2006; Pollmann and Steiner, 1994). Staining of firm seeds was assessed as red (viable cells), pink (some viable cells remaining) or white (no viable cells). Soft, decaying seeds were also considered to have no remaining viable cells. A 'viability score' for each sample of seeds was based on the numbers of seeds with different coloured stains: Viability score = (2 × Red) + (1 × Pink) + (0 × White or Soft).

2.1.2. *Plasmiodiophora brassicae*

Infected 'galled' root material of Chinese cabbage (*Brassica oleracea pekinensis*) cv. Rocco, produced according to Fayolle et al. (2006), was stored before use for up to 6 months at 2 °C. Sample bags contained 3 g of galled roots and adhering growing medium produced from either of two *Plasmiodiophora brassicae* isolates obtained from FERA and WHRI (Table 1). After composting, the material retrieved from the bags was tested for *Plasmiodiophora brassicae* viability using a Chinese cabbage (cv. Rocco) seedling plant bioassay (Fayolle et al., 2006). Chinese cabbage seedlings were also potted in growing media containing non-composted gall inoculum of each isolate (15 positive controls) or without gall inoculum (15 negative controls). After assessment for clubroot symptoms expression on a 0–3 scale (Fayolle et al., 2006), the root material from the test plants was tested for the presence of *Plasmiodiophora brassicae* using a TaqMan[®] PCR detection method. The material was subjected to a dangling short bait test (Beemster and de Heij, 1987) using oilseed rape to ensure only live cells were detected. DNA was extracted from the bait plant roots using the Wizard[®] Magnetic DNA purification system (Promega, Southampton, UK) with the KingFisher system (Thermo Scientific, Waltham, MA, USA). Extracted DNA was tested by TaqMan[®] PCR using the following PCR recipe: 10 × ABI Taq Gold Buffer A (2.5 µl), 25 mM MgCl₂ (5.5 µl), 6.25 mM dNTPs (2.0 µl), 7.5 µM forward and reverse primers (1.0 µl), 5 µM TaqMan[®] probe (0.5 µl), AmpliTaq Gold DNA Polymerase (0.125 µl), sterile nuclease-free water (11.375 µl), DNA (1 µl). An internal control (COX) Weller et al. (2000) was also used to assess the quality of the DNA extract. Results from the TaqMan[®] PCR were categorised as strong positive ($C_T < 17$), positive ($C_T < 17-32$), weak positive ($C_T > 32$), and negative.

Table 2

Composition and analysis of feedstock waste materials before and after various exposure times of indicator organisms in large-scale composting systems. Analytical values are means followed by standard deviations.

Site ^a	Waste type	n ^b	Sacks ^c	Time (days)	Stage	Moisture (% w/w)	pH	EC (mS m ⁻¹)
A	Vegetable	2	20	2	Before	73 ± 1.6	5.8 ± 0.2	110 ± 6
					After	69 ± 5.7	6.1 ± 0.3	109 ± 7
A	Woody green	2	20	2	Before	60 ± 5.4	5.9 ± 0.2	59 ± 5
					After	60 ± 5.6	6.0 ± 0.2	63 ± 6
B	Woody green	4	16–18	2,5,10	Before	64 ± 3.8	6.1 ± 0.1	54 ± 9
					After	54 ± 3.3	6.5 ± 0.1	47 ± 12
C	Woody green	2	6	5,8	Before	47 ± 3.7	7.3 ± 0.3	65 ± 9
					After	48 ± 3.9	8.0 ± 0.6	176 ± 8
D	Vegetable	2	6	5,8	Before	78 ± 6.4	4.2 ± 0.3	60 ± 5
					After	75 ± 6.5	7.5 ± 0.4	87 ± 7
E	Woody green	2	6	8	Before	51 ± 4.1	7.9 ± 0.3	138 ± 10
					After	46 ± 4.0	8.7 ± 0.3	142 ± 9

^a A Warwick HRI; B Trengwainton Gardens, Penzance, Cornwall; C Organic Recycling; D G's Vegetables, Ely, Cambridgeshire; E Simpro Ltd., Gaydon, Warwickshire.

^b Number of composts.

^c Number of sacks with indicator organisms per compost.

2.1.3. *Phytophthora* species

Inocula of *Phytophthora cinnamomi* and *Phytophthora nicotianae* isolates (Table 1) were prepared in a peat-based medium containing lime (Dolokal, 8 g l⁻¹), fertiliser (PG mix 15 N-10P-20 K, 0.8 g l⁻¹) and oat flakes (40 g l⁻¹). The medium was moistened to 50% w/w moisture, sterilised and inoculated with the *Phytophthora* cultures grown on potato dextrose agar (PDA), and incubated at 20 °C for 21 days. Sample bags were filled with 3 g peat inoculum which contained hyphae and hyphal swellings. A serial dilution of 1 g of peat inoculum, from each of the retrieved bags after exposure to compost or each of the initial inoculum samples, was conducted on three replicate plates using PDA + PARPH agar (Mitchell et al., 1986). The plates were recorded for *Phytophthora* after three days incubation at 20 °C by counting colony forming units (cfu) with mycelium, hyphal swellings, and sporangia typical of *Phytophthora*. The detection limit of the test was 34 cfu g⁻¹ peat-based medium.

2.1.4. *F. oxysporum* formae specialis and *Microdochium nivale*

PDA cultures of *F. oxysporum* f.sp. *cepae* and f.sp. *radicis-lycopersici* and four isolates of *M. nivale* (Table 1) were flooded with 20 ml sterile distilled water to produce mycelial and conidial suspensions for inoculating 66 g sterilised talc. The talc was kept for 6 weeks at 20 °C to allow chlamydospores to develop. Sample bags contained 1.25 g talc inoculum. Talc (1 g), from each of the retrieved bags after exposure to compost or each of the initial inoculum samples, was added to 9 ml of sterile distilled water. The suspension (0.1 ml) was plated on to each of three replicate plates of PDA + chlorotetracycline which were then incubated at 22 °C for 6 days. Viable cfu with mycelium and conidia typical of *F. oxysporum* or *M. nivale* were detected with a similar threshold to the *Phytophthora* method.

2.1.5. *Sclerotinia sclerotiorum*

Sclerotia were produced in moist sterile wheat grain, inoculated with either of two *S. sclerotiorum* isolates (Table 1) and incubated for 5 weeks at 20 °C. Sample bags contained 40 sclerotia (2–5 mm). After composting, sclerotia were surface sterilised with 50:50 sodium hypochlorite solution: 100% ethanol, halved and incubated at 20 °C on PDA + chlorotetracycline. Viability was assessed by growth of mycelium and new sclerotia. The same testing procedure was used on 30 sclerotia of each of the two isolates before composting.

2.2. Bench-scale flask composts

Bench-scale composting equipment consisted of thermostatically controlled water baths holding four 2-litre multiadapter flasks (Fayolle et al., 2006). Flasks contained green waste compost (1200 g, moisture 41 ± 1% w/w) prepared in turned windrows using wastes from parks and gardens and fruit and vegetables for 9 months (Organic Recycling Ltd., Peterborough, UK).

A series of separate experiments were conducted for each indicator organism; the compost temperature and exposure time treatments within each experiment varied depending on the likely tolerance range of each organism, determined from preliminary tests. Three replicate flasks of each compost temperature treatment were prepared for each experiment, each flask containing 1–5 sample bags of the specified indicator organisms (seed cultivar or pathogen isolate), one bag for each of the exposure times specified below.

2.2.1. Comparison of *Abutilon* and tomato cultivars as indicators

The effect of four compost temperatures × three exposure times (50, 55, 60 and 65 °C for 1, 3 and 7 days) on the viability of *Abutilon*

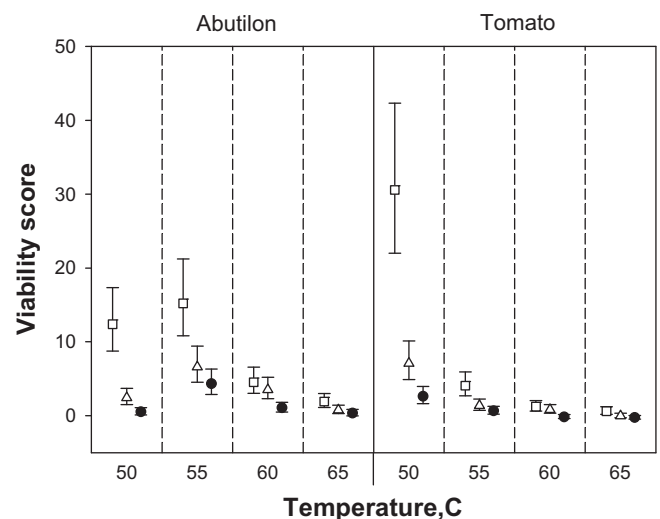


Fig. 1. Back-transformed means with 95% confidence intervals for fitted viability scores from the linear regression, of tomato and *Abutilon* seeds after exposure to flask composts for □ 1 day, △ 3 days, or ● 7 days. Initial mean viability scores of 10 samples of 15 seeds of each type were 29.8 ± 0.1 for tomato and 28.7 ± 0.3 for *Abutilon* (maximum score for 15 seeds = 30).

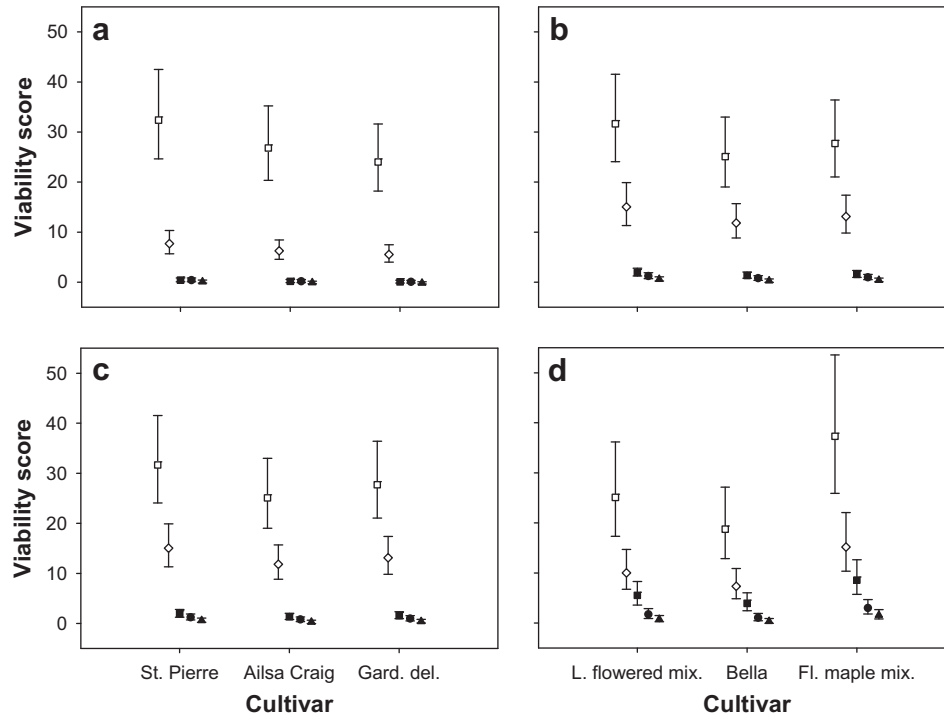


Fig. 2. Back-transformed means and 95% confidence intervals for fitted viability scores from the linear regression, of seeds after 2-day exposure to compost in an insulated bay. (a) Tomato cultivar and (b) *Abutilon* cultivar seeds in vegetable waste (c) tomato cultivar and (d) *Abutilon* cultivar seeds in woody green waste. Average compost temperature: □40 °C, ◇ 52 °C, ■ 56 °C, ● 59 °C, ▲ 63.5 °C. Each value is the mean of 8 (vegetable wastes) or 4 (woody green wastes) samples. Initial mean viability scores of 10 samples of 15 seeds of each type were 29.8 ± 0.2 for tomato and 28.6 ± 0.4 for *Abutilon* (maximum score for 15 seeds = 30).

and tomato seeds was examined, using three cultivars of each species: *Abutilon* 'Bella', 'Flowering Maple Mixture', and 'Large Flowered Mixed'; tomato 'Ailsa Craig', 'Gardener's Delight' and 'St. Pierre'. Nearly all the tomato and *Abutilon* seeds in the non-composted, day 0 control groups produced a red stain after testing with tetrazolium (mean = 99.6% for tomato and 94.1% for *Abutilon*). Seeds from the day 0 groups were discarded from the rest of the analyses. The viability scores were first \log_{10} transformed to adjust for non-constant variability, and were then analysed using a linear regression, with compost temperature and exposure time as the independent variables.

2.2.2. Effect of compost incubation on plant pathogen indicators

The following compost temperature \times exposure time treatments were examined for the isolates of each plant pathogen (Table 1):

- Plasmodiophora brassicae*: 18, 50 and 60 °C for 1, 3 and 7 days, 65 °C for 7 days
- Phytophthora* species: 18, 35, 40 and 45 °C for 1, 3 and 7 days
- F. oxysporum*: 18, 40, 45, 50 and 55 °C for 1, 3, 4, 7 and 14 days
- M. nivale*: 18, 40 and 50 °C for 3 days
- S. sclerotiorum*: 18, 45, 50 and 55 °C for 1, 3 and 7 days.

2.3. Large-scale composts

Composts were prepared in insulated composting bays at two sites and in turned windrows at three sites. At each site, up to four composts were prepared from vegetable wastes (potato:straw 10:1 w/w or waste onions) or from wetted stems of woody shrubs (Table 2). Waste feedstocks were mechanically crushed and shredded to produce pieces of about 50 mm length before being filled into the bays or formed into windrows. The temperature of the

composting wastes was then allowed to rise for about 7 days. Nylon mesh sacks (0.5 \times 0.3 m) containing waste, sample bags of indicator organisms, and a temperature probe were inserted into 6–20 different locations of the large-scale systems (centre, corners, sides), from the surface to depths of up to 0.7 m, to obtain a range of exposure temperatures (Table 2). The sacks contained tomato cv. Ailsa Craig seeds and talc inoculum of *F. oxysporum* f.sp. *cepae* and/or *F. oxysporum* f.sp. *radicis-lycopersici*. Site A sacks also contained seeds of the other tomato and *Abutilon* cultivars and inoculum of the two *Plasmodiophora brassicae* isolates used in the flask

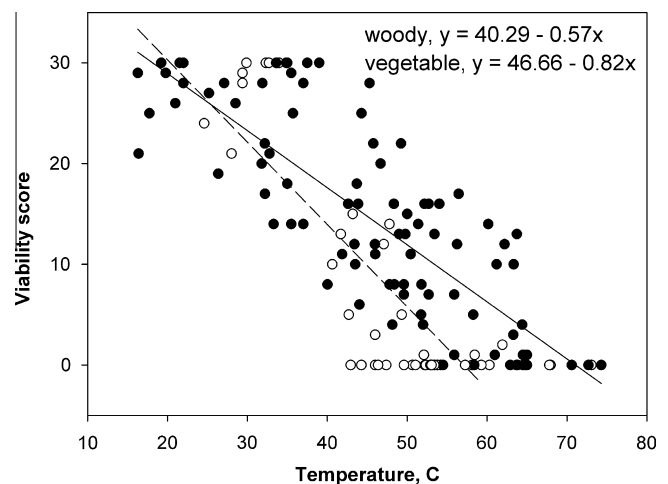


Fig. 3. Relationship between compost temperature and viability scores for tomato seeds in ● (solid line) vegetable wastes and ○ (dashed line) woody green wastes after 2–10 day exposure in large-scale composting systems. Adjusted R^2 for multiple regression = 0.702. The average initial mean viability score of 10 samples of 15 seeds after was 29.8 ± 0.1 (maximum score for 15 seeds = 30).

Table 3

Chinese cabbage pot bioassay results for *Plasmodiophora brassicae* inoculum viability following different combinations of temperature and exposure time treatments in flask composts. Each value is the number of positive flasks (at least one plant out of three showing gall symptoms) from three replicate flasks, followed by the TaqMan[®] PCR result^a conducted on a combined sample.

Compost temp. °C	<i>Plasmodiophora brassicae</i> inoculum					
	FERA			WHRI		
	1 day	3 days	7 days	1 day	3 days	7 days
18	3 +++	3 ++	3 +	3 ++	3 +	3 +
50	3 +++	3 +	0 +	3 +++	3 +	3 +
60	2 +++	0 +	0 +	3 +++	3 +	2 +
65	n.t.	n.t.	0 +	n.t.	n.t.	2 +

^a +++ strong positive ($C_T < 17$), ++ positive ($C_T < 17-32$), + weak positive ($C_T > 32$), n.t., not tested.

experiments. The sample bags were retrieved from the sacks after 2–10 days (Table 2) and viability of the indicator organisms determined as previously described.

The viability data obtained from the five replicate composting sites were analysed using multiple linear regression, using a forward stepwise regression. In this approach, each of the four

Table 4

Chinese cabbage pot bioassay results for *Plasmodiophora brassicae* inoculum viability following 2-day exposure to different temperatures in vegetable waste and woody green waste composts produced in an insulated bay. Each value is the number of plants out of four showing gall symptoms, followed by the TaqMan[®] PCR result^a conducted on a combined sample.

Mean compost temp., °C	<i>Plasmodiophora brassicae</i> inoculum			
	FERA		WHRI	
	Vegetable	Woody green	Vegetable	Woody green
40	4 +++	4 +++	4 +++	4 +++
52	4 –	4 ++	4 +	2 ++
56	4 ++	3 ++	4 ++	3 –
59	4 +	2 ++	4 +	3 +
64	0 +	0 +	0 –	0 +

^a +++ strong positive ($C_T < 17$), ++ positive ($C_T < 17-32$), + weak positive ($C_T > 32$), – negative.

variables (compost temperature, exposure time, moisture content and waste type) was tested and the one explaining most of the variability of the data was added to the model, and the process repeated with the remaining variables and interactions, when appropriate. *F. oxysporum* chlamydospore data was analysed as binary (survival or no survival), through a generalised linear model, because where survival occurred, generally very large numbers of spores survived and only in a very limited number of treatments was 'intermediate' survival observed.

Ammonia, O₂ and CO₂ concentrations in the substrates during composting were measured with a Dräger Gas Detector (Drägerwerk, Lübeck, Germany) with appropriate sample tubes (CH20501, 6728081, and CH31401). Dry matter (dm), organic matter, and nitrogen contents and pH and electrical conductivity of samples of the materials before and after composting were determined according to Anonymous (1986). Statistical analyses of the surviving populations of seeds and pathogen propagules in the flask and large-scale composting systems were conducted using Genstat 12.1. Unless stated, results were significant at $P < 0.05$.

3. Results

3.1. Environmental conditions in composts

The green waste compost used in the flask experiments had an electrical conductivity of 215 mS m⁻¹ and a pH 7.6. Mean O₂ and CO₂ concentrations in the flasks during composting were 17.1 ± 2.2% and 2.9 ± 1.8% v/v respectively. Ammonia concentrations were 0.7 ± 0.6 mg m⁻³. After the composting experiments, the compost had a mean moisture content of 36.8 ± 3.0% w/w.

Compost temperatures in insulated bays ranged from 23 ± 7 °C in the front bottom corners to 65 ± 3 °C in the centre at a depth of 0.7 m. Temperatures in windrows of woody waste composts ranged from 36 ± 6 °C on the surface to 72 ± 2 °C at depths of 0.3 to 0.5 m. In windrow vegetable waste composts at site D, temperatures ranged from 33 ± 7 °C on the surface to 46 ± 2 °C at a depth of 0.3–0.5 m. Mean O₂ and CO₂ concentrations in the composts were 13.2 ± 3.9% and 6.5 ± 3.7% v/v respectively. Mean ammonia concentrations in woody green waste composts at sites C and E (2.2 ± 1.0 mg m⁻³) were higher than those in the wastes at the

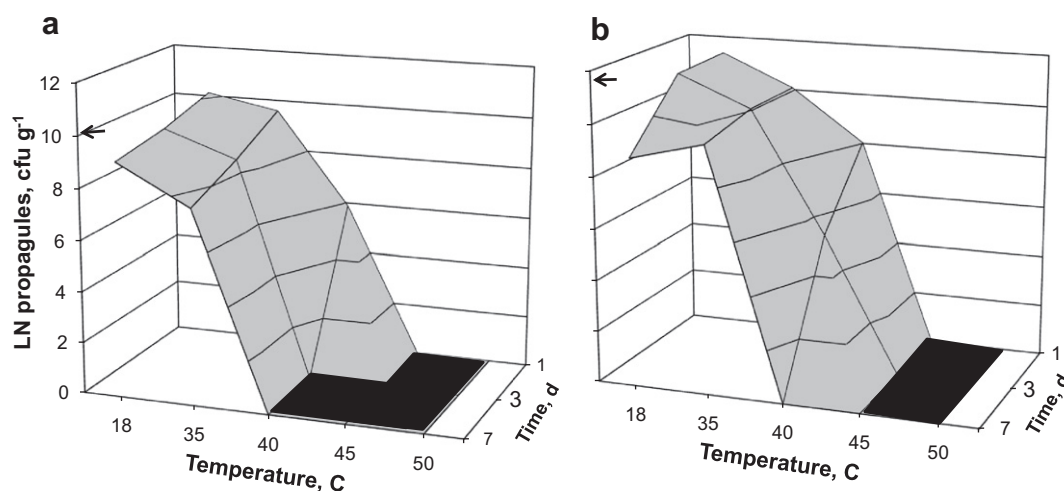


Fig. 4. Number of viable propagules (\log_{10}) in peat-based inoculum of (a) *Phytophthora cinnamomi* and (b) *Phytophthora nicotianae* after various combinations of compost temperature and exposure time in a flask system. The black zone indicates treatment combinations that were not significantly different from the eradication control (45 °C for 7 days) using Steel's many-one-rank test. Each value is the mean of two experiments and three replicate flasks. The average initial viable propagule population of the inoculum is indicated by the arrows.

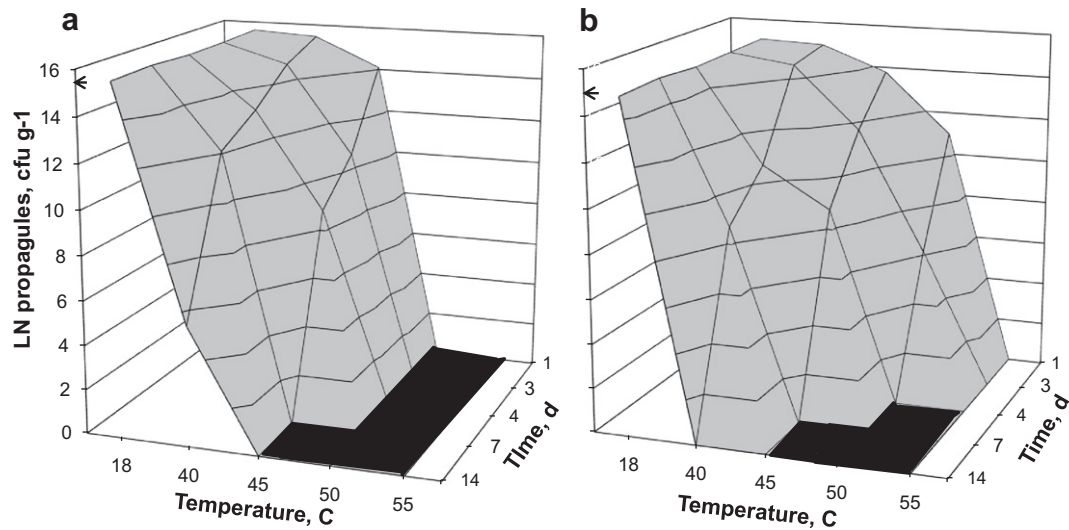


Fig. 5. Number of viable propagules (\log_e) in talc inoculum of *Fusarium oxysporum* (a) f.sp. *cepae* and (b) f.sp. *radialis-lycopersici* after various combinations of compost temperature and exposure time in a flask system. The black zone indicates treatment combinations that were not significantly different from the eradication control (55 °C for 14 days) using Steel's many-one-rank test. Each value is the mean of three replicate flasks. The average initial viable chlamydo-spore population of the inoculum is indicated by the arrows.

other sites (mean $0.4 \pm 0.3 \text{ mg m}^{-3}$). The moisture contents of the vegetable wastes were higher than those of the woody wastes (Table 2).

3.2. Effect of compost incubation on seed indicators

The fitted means from the linear regression and confidence intervals presented in the figures are back-transformed values. Depending on the model and the significance of interactions (and therefore their inclusion or not in the model), these fitted means are slightly different to the actual means.

Increasing flask compost temperature and exposure time reduced seed viability score ($P < 0.001$), although for *Abutilon*, there was no significant difference in viability score between seeds exposed to 50 or 55 °C (Fig. 1). There was a significant ($P < 0.001$) interaction between the effects of compost temperature and species on seed viability. After exposure to 55, 60 or 65 °C *Abutilon* seeds had a slightly higher viability score than tomato seeds, but after exposure to 50 °C this trend was reversed. This was probably due to the degradation of some *Abutilon* seeds at 50 °C.

Tomato and *Abutilon* seeds exposed to large-scale composts at 56–63.5 °C for 2 days had viability scores near to zero, except *Abutilon* seeds in woody waste compost at 56 °C (Fig. 2). *Abutilon* seeds also had higher viability scores than tomato seeds after exposure to vegetable waste compost at 52 °C for 2 days. Differences in viability score between cultivars of *Abutilon* or tomato were small or not significant (Fig. 2).

There was a strong negative effect of temperature ($P < 0.001$) on the viability scores of tomato seeds (Fig. 3). Overall, seeds in vegetable wastes had higher viability scores than those in woody wastes ($P < 0.001$). However, there was a significant interaction between temperature and waste type ($P = 0.004$), with viability score decreasing more rapidly with increasing temperature for vegetable wastes than for woody wastes. The effect of exposure time within the range 2–10 days on seed viability score was not significant.

Although the effect of compost temperature on tomato and *Abutilon* seeds was similar in both the flask and large-scale systems, the influence of exposure time, within the range 2–10 days, was only significant in the flask system, possibly due a difference in the compost such as lower moisture or organic

matter content, or the larger variability in conditions inside large-scale compost within the exposure time.

3.3. Effect of compost incubation on plant pathogen indicators

Although the intention was to investigate the presence of a relationship between compost temperature, exposure time and the probability of survival of *Plasmodiophora brassicae*, in the same way as was conducted for seed viability scores, the data were not suitable to investigate such a relationship. This was because the shift from positive to negative results for the FERA isolate (Table 1), based on the appearance of gall symptoms in the test plants, was sudden and occurred in the same temperature \times time treatment for almost all flask replicates, which prevented any model to be fitted to the score data (Table 3). All of the positive control samples and all the 18 °C treatments of both isolates from the flask composting experiment produced clear, score 3 gall symptoms in the bioassay plants. Of the plants bioassayed with *Plasmodiophora brassicae* inoculum samples exposed to 50 °C for one day, 72% had score 2 gall symptoms, but none had score 3 gall symptoms. Inoculum of the FERA isolate composted at 50 °C for 7 days, or at 60 °C for 3 or 7 days did not produce gall symptoms in the bioassay plants. Inoculum of the WHRI isolate produced positive gall symptoms in all the flask replicates following all the compost temperature \times exposure time treatments (Table 3). The inoculum exposed to 50 °C for at least 3 days or to 60 °C for 1 day or longer resulted in 42% of bioassay plants with score 1 gall symptoms and 22% with score 2. Inoculum exposed to 65 °C for 7 days produced score 1 gall symptoms in 22% of test plants, but no score 2 and 3 gall symptoms. None of the negative control bioassay plants produced gall symptoms. All of the bioassay plant samples, except the negative controls, produced positive TaqMan[®] PCR results, although there was some evidence that increasing compost exposure time of the inoculum, irrespective of temperature, reduced the strength of the positive result (Table 3).

The influence of compost temperature and exposure time on *Plasmodiophora brassicae* inoculum was similar in flask and large-scale systems. Results for exposure of *Plasmodiophora brassicae* inoculum in the large-scale composting system were also not statistically analysed due to the lack of variability between replicate bioassay plants, i.e. almost all of the bioassay plants for the same

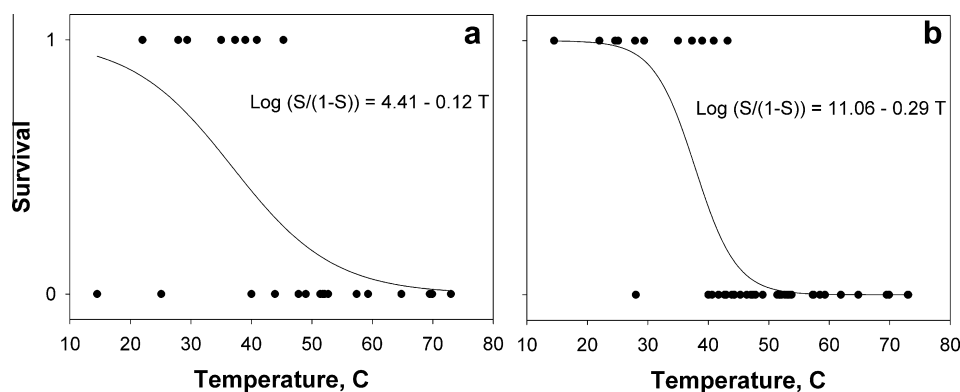


Fig. 6. Effect of compost temperature on the viability of *Fusarium oxysporum* (a) *f.sp. cepae* and (b) *f.sp. radicis-lycopersici* chlamydospore inocula following exposure to large-scale composts for 2–10 days.

temperature treatment produced the same positive or negative gall symptom result (Table 4). All of the positive control samples and all the 40 °C treated samples of both isolates produced score 3 gall symptoms in the bioassay plants. All the *Plasmodiophora brassicae* inoculum samples exposed to mean temperatures of 52–59 °C for 2 days in vegetable wastes produced score 1 or 2 gall symptoms in bioassay plants, and inoculum exposed to the same temperatures in the woody wastes resulted in at least 50% of bioassay plants having score 1 or 2 gall symptoms. None of the negative controls or inoculum samples exposed to a mean temperature of 64 °C for 2 days resulted in positive gall symptoms. Increasing compost temperature generally reduced the positive strength of the subsequent TaqMan[®] PCR result on bioassay plants, although two negative results were obtained from samples of bioassay plant that had gall symptoms (Table 4). Positive TaqMan[®] PCR results were obtained from all the bioassay plant samples, except the negative controls, and plants grown with the WHRI isolate inoculum exposed for 2 days at 64 °C in vegetable wastes or at 56 °C in woody wastes, or the FERA isolate inoculum exposed for 2 days at 52 °C in woody wastes.

The initial concentrations of *M. nivale* propagules in the talc inoculum ranged from 1.01×10^5 cfu g⁻¹ talc for isolate Caythorpe to 8.4×10^5 cfu g⁻¹ talc for isolate 08251. There were no significant changes in population of viable propagules in the *M. nivale* inocula following incubation at 18 °C in flask composts. Chlamydospores of all four *M. nivale* isolates were eradicated when subjected to a temperature of 40 °C for 3 days.

For both *S. sclerotiorum* isolates, at least 29 out of the initial 30 sclerotia germinated in the day 0 samples and in the samples exposed to 18 °C for up to 7 days in flask composts. The mean numbers of sclerotia germinating after exposure at 45 °C for 3 days were 4 (SD ± 1.7) and 6 (SD ± 2.6) for isolates 13 and OSR, but none germinated after exposure at 45 °C for 7 days. No viable sclerotia were detected after being exposed to a temperature of 50 or 55 °C for 1 day or longer.

The initial viable *Phytophthora* propagule concentrations in the peat-based inoculum were 2.2×10^4 cfu g⁻¹ for *Phytophthora cinnamomi* and 1.5×10^5 cfu g⁻¹ for *Phytophthora nicotianae*. The initial viable propagule counts in the talc inoculum were 5.8×10^4 cfu g⁻¹ for *F. oxysporum f.sp. cepae* and 3.5×10^4 cfu g⁻¹ for *F. oxysporum f.sp. radicis-lycopersici*. Because of the range of data observed for both *Phytophthora* species and *F. oxysporum*, from zero or a few tens of surviving propagules after the higher temperature treatments to over 3×10^4 propagules after the 18 °C treatments in flasks (Figs. 4 and 5), and the consequent non-normality of the residuals for any regression, even after attempting to transform the data, or the very large over-dispersion when using Poisson generalised linear models, it was decided to

use non-parametric statistics. Steel's many-one-rank test (Steel, 1959) was used for comparing treatments with two controls: the day 0 inoculum propagule count, and the most extreme combination of temperature and time (45 °C for 7 days for *Phytophthora* spp. and 55 °C for 14 days for *F. oxysporum*) which was sufficient to eradicate all propagules of both species in all replicates (eradication controls).

The numbers of viable *Phytophthora cinnamomi* propagules were significantly lower after all the compost treatments than the day 0 control, except after 1 day at 18 °C (Fig. 4a). When comparing the numbers of viable propagules to those of the eradication control, there were significantly greater numbers after 1, 3 or 7 days at 18 °C or 35 °C. However, the numbers of viable propagules after exposure to 40 °C for 1, 3 and 7 days or 45 °C for 1 and 3 days were not significantly different from the eradication control. The number of viable *Phytophthora nicotianae* propagules after 1, 3 and 7 days at 40 °C and 45 °C were significantly lower than that in the day 0 control. However, there was no difference in number from the day 0 control after 1, 3 and 7 days at 18 °C and 35 °C (Fig. 4b). The numbers of viable propagules after exposure to 40 °C for 3 or 7 days or at 45 °C for 1 or 3 days were not significantly different from the eradication control.

Numbers of viable *F. oxysporum f.sp. cepae* and *f.sp. radicis-lycopersici* propagules were significantly lower after 3 and 4 days respectively, and any longer period, at 40 °C, compared with those of the day 0 control (Fig. 5a and b). When comparing the number of viable propagules of *f.sp. cepae* after composting with those of the eradication control, the numbers were not significantly different from zero after 14 days at 40 °C, 3 days at 45 °C, 1 day at 50 and 55 °C, or longer periods of time at each of these temperatures respectively. When comparing the number of viable propagules of *f.sp. radicis-lycopersici* after composting with those of the eradication control, the numbers were not significantly different from zero after 14 days at 40 °C, 7 days at 45 °C, 3 days at 50 °C, 1 day at 55 °C, or longer periods of time at each of these temperatures respectively.

There was a rapidly decreasing probability of survival of *F. oxysporum f.sp. cepae* in large-scale systems with increasing temperature ($P < 0.001$) (Fig. 6a) but there was no significant effect of moisture content on survival. For *F. oxysporum f.sp. radicis-lycopersici* there was a decreasing probability of survival with increasing temperature ($P < 0.001$) (Fig. 6b) but there were no significant effects of compost exposure time, moisture content, or waste type on survival.

There was a similar effect of compost temperature on the chlamydospores of *F. oxysporum f.sp. cepae* and *f.sp. radicis-lycopersici* in both the flask and large-scale systems. As with seed viability score, the influence of exposure time was only significant

in the flask system, possibly due to the different composts used, or greater variability in conditions during an exposure time in the large-scale systems.

4. Discussion

4.1. Seeds as indicator organisms

The effects of compost temperature and exposure time on the subsequent viability of tomato seeds are comparable with results reported elsewhere. Hermann et al. (1994) and Idelmann (2005) showed that non-dried tomato seeds could germinate after a maximum of 1 day at 60 °C, 3–5 days at 50 °C, and 9 days at 45 °C. Christensen et al. (2001) found no germination of tomato seeds after they were exposed for 14 days to a peak compost temperature of 58 °C and mean of 43 °C, and Ryckeboer et al. (2001) found that germination of tomato seed was reduced to 0–0.2% of untreated seeds after 20–24 h of anaerobic digestion at 52 °C. Pollmann and Steiner (1994) found that although no tomato seeds germinated after exposure to compost for 6 weeks, some produced red stained tissue following the tetrazolium test. Hermann et al. (1994) also found that tomato seeds produced a positive tetrazolium test result after 3–18 days in compost windrows that reached peak temperatures of 73–81 °C, although they were no longer capable of germination. The greater compost temperature range producing positive test viability scores, together with the 21-day period needed for the germination test, means that the tetrazolium test is more suitable than the germination test for use on seed indicators of compost sanitization.

Eghball and Lesoing (2000) found that seeds of *Abutilon theophrasti* were more resistant to composting than the seeds of seven other weed species. After composting for 100 days with a peak temperature of 55 °C, 14% of the seeds remained viable. During a 52-day exposure, Stanford et al. (2009) found linear losses in seed viability of three weed species as compost temperature increased from 10 to 45 °C. Larney and Blackshaw (2003) found that exposure times and temperatures, expressed as cumulative degree day, accounted for only 17–29% of the loss in seed viability of nine weed species during windrow composting of cow manure.

4.2. Plant pathogens as indicator organisms

The lack of variability between Chinese cabbage bioassay plants grown with *Plasmiodiophora brassicae* inoculum from the same compost temperature treatments indicates that the bioassay is not sufficiently sensitive or the scoring system does not capture subtle differences in gall symptoms. The difficulty in detecting viable *Plasmiodiophora brassicae* inoculum using bioassay plants was also found by Idelmann (2005) who noted that the gall symptoms became very indistinct, even after the inoculum had been exposed for 1 day at 50 °C. Bruns et al. (1993), Idelmann (2005) and Fayolle et al. (2006) also noted significant differences in temperature tolerance of isolates from different sources. The ability of *Plasmiodiophora brassicae* to survive at 60–65 °C for at least 1 week in substrates with moisture contents of less than 50% w/w confirms earlier work by Ylimaki et al. (1983), Bruns et al. (1993) and Fayolle et al. (2006). The present and earlier work has also shown that in substrates with moisture contents above 50% w/w, eradication of *Plasmiodiophora brassicae*, based on the results of bioassay plants, occurred in 3–98 days at temperatures of 40–60 °C (Bruns et al., 1993; Christensen et al., 2001; Ryckeboer et al., 2001; Termorshuizen et al., 2003; Idelmann, 2005; Fayolle et al., 2006). However, the positive TaqMan[®] PCR test results from inoculum exposed to an average temperature of 64 °C for 2 days in the vegetable and woody waste composts indicates that *Plasmiodiophora brassicae*

may survive at low levels under these conditions even in substrates with >60% w/w moisture. The greater sensitivity of a PCR-based diagnostic test than a plant bioassay test is confirmed by results of Staniaszek et al. (2001) but may be due to detection of nucleic acids in dead cells. Juvonen et al. (2010) observed that elevated temperatures can prevent the enzymatic degradation of RNA after heat treatment of *Lactobacillus*.

Chlamydo-spores of *M. nivale*, sclerotia of *S. sclerotiorum* and peat-based inocula of *Phytophthora cinnamomi* or *Phytophthora nicotianae* were eradicated when exposed to a compost temperature of 45 °C for 7 days or less. The isolates of these pathogens would therefore be of limited value as indicator organisms of compost sanitization since several plant pathogens are known to have higher temperature tolerances in compost (Noble et al., 2009). The results for *M. nivale* contrast with those of Noble et al. (2009) who found that chlamydo-spores of a different isolate survived in compost at 58 °C for 7 days.

The results for *Phytophthora cinnamomi* are in agreement with those showing eradication on infected plant material after incubation using a water bath for 7 days at 40 °C (Hoitink et al., 1976) or for 20 min at 45 °C (Juarez-Palacios et al., 1991). Downer et al. (2009) found that inoculum of *Phytophthora cinnamomi* containing hyphae and chlamydo-spores survived for up to 1 week in compost that reached 60 °C. The results obtained here for *Phytophthora nicotianae* are similar to incubator studies showing eradication of mycelium on agar after 3 h at 50 °C (McGovern et al., 2000) and chlamydo-spores after 3 h in moist soil at 47 °C (Coelho et al., 2001). However, Noble et al. (2009) found that oospores of a different isolate of *Phytophthora nicotianae* survived in onion waste compost at 52 °C for 7 days. Differences in temperatures and times reported to achieve eradication to those required here for the same pathogens may be due to differences in moisture availability, the isolates of pathogens used, and their physiological stage or condition.

Results for *S. sclerotiorum* sclerotia agree with those obtained by Idelmann (2005) showing eradication after 36 h at 44 °C and by van Loenen et al. (2003) showing eradication after 3 min in steam–air at 60 °C, although Hermann et al. (1994) and Downer et al. (2009) indicated a greater temperature tolerance of *S. sclerotiorum* sclerotia.

Combinations of temperature and exposure time required to eradicate chlamydo-spores of *F. oxysporum* f.sp. *cepa* and f.sp. *radicis-lycopersici* are similar to those previously reported for various formae speciales in moist systems (Noble et al., 2009; Idelmann, 2005). The results of this work have shown that chlamydo-spores of *F. oxysporum* f.sp. *radicis-lycopersici* are more temperature tolerant in compost than those of f.sp. *cepa* and of the propagules of most other plant pathogens (Noble et al., 2009).

For most of the indicator organisms examined, exposure to compost at ambient temperature (18 °C) did not reduce survival compared with the initial day 0 inoculum. *Phytophthora nicotianae* inoculum was adversely affected by compost at ambient temperature, possibly due to other suppressive characteristics of the compost (Noble and Coventry, 2005).

5. Conclusions

S. sclerotiorum sclerotia, *M. nivale*, *Phytophthora cinnamomi* and *Phytophthora nicotianae* were unsuitable indicator organisms due to limited temperature tolerance in compost. *Plasmiodiophora brassicae* was unsuitable for testing compost sanitization because of high temperature tolerance in dry to moist composts, and variability of survival characteristics of different isolates. *Plasmiodiophora brassicae* inoculum viability assessed post-composting with bioassay test plants did not correspond with that obtained using

TaqMan® PCR, possibly due to the preservation of nucleic acids in dead cells. Relationships between compost temperature during exposures of 2–10 days and subsequent viability of *F. oxysporum* f.sp. *cepae* and f.sp. *radicis-lycopersici* chlamydospores or tomato seeds enabled the sanitizing effect of a composting process to be predicted within 2–6 days. Although *Abutilon* seeds were more tolerant than tomato seeds of compost temperatures ≥ 52 °C, they were more prone to degradation at lower temperatures and therefore less suitable than tomato seeds as indicators.

The indicator organisms identified in this work, and the methods used for testing their viability following exposure to a composting process could be used by the composting industry to test the sanitizing efficacy of new and existing composting systems in response to changing ambient temperatures and waste composition. The information obtained could be used in decisions concerning the subsequent use, disposal, or further treatment requirements of composted plant wastes, in order to minimise the risk of contamination of soils or growing media substrates with plant pathogens.

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