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Investigation of the Effect of the Composting Process on Particular Plant, Animal and Human Pathogens known to be of Concern for High Quality End-Uses

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Abbreviations, Terms and Definitions

cfu colony forming units
DM dry matter
GW green waste
n.d. not detected
PDA potato dextrose agar
SMC spent mushroom compost
TMV *Tobacco Mosaic Virus*
propagule form or part of an organism by which it may disperse or reproduce

Summary

Consultations with end-users of composts indicated that potential pathogen content of green waste composts was a perception problem. The plant pathogens of greatest concern were: *Phytophthora* spp., *Pythium* spp., *Plasmodiophora brassicae, Rhizoctonia solani, Fusarium oxysporum* f.spp., and *Thielaviopsis basicola.* The animal/human pathogens *E. coli* and *Salmonella* spp. were of concern to retailers of composts. Eradication tests were conducted on all of the above plant and animal pathogens using bench-scale flask composting equipment, at controlled temperatures and times. Eradication tests were also conducted on the plant pathogens *Rhizoctonia solani, Verticllium dahliae, Microdochium nivale, Xanthomonas campestris* pv. *campestris,* and *Tobacco Mosaic Virus.* Tests were conducted on propagules of the pathogens and/or plant material affected with the pathogens. For *P. brassicae, F. oxysporum* f.sp. *lycopersici* and *V. dahliae*, eradication tests were also conducted in large-scale windrow and tunnel composting systems. Feedstocks in the tests consisted of green wastes and vegetable (onion) wastes.

Propagules of *Fusarium oxysporum* f.spp. *lycopersici* and *radicis-lycopersici*, *Pythium ultimum,* and *Thielaviopsis basicola,* and *Rhizoctonia solani, Fusarium oxysporum* f.sp. *lycopersici*, *Verticillium dahliae* and *Xanthomonas campestris* pv. *campestris* in affected plant material were eradicated by a compost temperature of 52˚C or less, held for 7 days. *Fusarium oxysporum* f.spp. *lycopersici* in affected tomato plant material and *Verticillium dahliae* in infected oat grains were also eradicated from compost that exceeded 50˚C for 4 days and peaked at 70˚C in a large-scale tunnel.

Propagules of *Phytophthora nicotianae* required a temperature of up to 58˚C for 7 days. These conditions were sufficient to eradicate all except the most temperature tolerant plant pathogens tested (*Microdochium nivale, Plasmodiophora brassicae* and *Tobacco Mosaic Virus).* Propagules of *Microdochium nivale* required a temperature of up 64˚C for 7 days for eradication from compost (initial moisture content at least 43% w/w). A duration of 1 day at this temperature may be adequate for eradication of this pathogen, but this requires further investigation.

Eradication of *Plasmodiophora brassicae* in infected Chinese cabbage plants depended on compost temperature, time and moisture content. A compost temperature of 60˚C for 1 day reduced the level of *Plasmodiophora brassicae* in affected Chinese cabbage plants to below the detection level of the test used for detecting the pathogen in compost, providing that the compost moisture was at least 59% w/w. In compost with a moisture content of 51% w/w, a temperature of 65˚C for 1 day was required for eradication of *P. brassicae*. Compost aeration had no significant effect on the temperature and time required to eradicate *P. brassicae*.

Tobacco Mosaic Virus in affected leaf material required a compost temperature of 80˚C for 7 days for eradication. The pathogen survived a compost temperature of 60˚C for 35 days.

Strains of Enterohaemorrhagic *E. coli, Salmonella typhimurium* and *S. enteriditis* were not detectable after 1 hour at 55˚C in green waste compost, even though introduced at much higher concentrations than would be

expected naturally. This indicates that green compost will not be a significant risk for the spread of most bacterial diseases.

Plant-based wastes in two large-scale composting systems exceeded 65°C for at least 1 day at a depth of 50 cm. At a depth of 10 cm, temperatures exceeded 64°C for at least 2 days. These conditions would be sufficient to eradicate all of the fungal and bacterial pathogens examined in this work.

1 Introduction

The composting process normally consists of three phases that can be more or less distinct: an initial mixing period with mesophilic growth, a high temperature thermophilic phase (or sanitization), and a longer and lower temperature mesophilic phase (maturation or stabilization) (Day & Shaw, 2001). The success of composting in eliminating pathogens is not solely a result of the heating process but also depends on the many and complex microbial interactions which may occur, as well as other compost parameters such as moisture content (Bollen, 1985). According to Bollen (1985), the eradication of pathogens from organic wastes during composting is primarily due to: (i) heat generated during the thermophilic phase of the composting process; (ii) the production of toxic compounds such as organic acids and ammonia; (iii) lytic activity of enzymes produced in the compost; (iv) microbial antagonism, including the production of antibiotics, and parasitism.

Other factors involved in eradication are: (v) competition for nutrients (Ryckeboer, 2001); (vi) natural loss of viability of the pathogen with time (Coventry *et al.*, 2002); (vii) compounds that stimulate the resting stages of pathogens into premature germination (Coventry *et al.*, 2002).

However, heat generated during the thermophilic high temperature phase of aerobic composting appears to be the most important factor for the elimination of plant pathogens (Bollen & Volker, 1996). Although pathogen numbers may continue to decline during compost maturation, the conditions are more difficult to define for sanitization standards, and are less likely to be conducive for reliable pathogen eradication than the high temperature phase.

Concerns about the potential presence of plant and human or animal pathogens are a major limitation to the increased up-take of composted waste by end-users of high-quality horticultural growing media. If compost producers were able to provide appropriate quality assurance (QA) data, it would develop increased confidence in the safety of composted materials and thereby increased uptake by end-users, particularly commercial growers. WRAP has commissioned this work to provide a detailed understanding of the fate of pathogens within the composting process in order to provide confidence in the process to end-users. The first stage of this work was to conduct literature reviews on the eradication conditions of plant pathogens and nematodes, and human and animal pathogens during composting (Noble & Roberts, 2003; Jones & Martin, 2003). This was followed by controlled tests to determine the conditions (composting temperature, time and other factors) required to eradicate particular pathogens. There are a vast number of fungal, bacterial, and viral plant pathogens and plant parasitic nematodes. Some of these are highly specific, infecting only a single plant species, others infect a broad range of hosts. End-users (commercial growers and amateur gardeners) want to be assured that there are no organisms present which will infect the particular plant species which they intend to grow. A selection procedure was therefore needed for including particular plant and animal/human pathogens into the tests. This was done on the following basis:

(i) of concern to end-users, based on the results of consultations
(ii) importance in high-quality growing media
(iii) insufficient reliable published information on eradication conditions
(iv) potential temperature tolerance from available literature.

The review of literature published by WRAP (Noble & Roberts, 2003) showed that for the majority of 60 plant pathogen and nematode species, a compost temperature of 55°C for 21 days was sufficient for ensuring eradication. However, in several of the references, the temperatures and times required for eradication were not precisely determined. Shorter periods and/or lower temperatures may be satisfactory, but they were not always examined. Most of the research has been conducted using composted organic wastes, although eradication data in other media was also obtained. For some organisms (e.g. *Pythium ultimum*) only temperature data using other, non-compost, media was available.

Results for *Plasmodiophora brassicae* and *Rhizoctonia solani* obtained from an EU funded project RECOVEG are included in this report. The inclusion of these and other pathogens followed a consultation with the end-users of composts, to ensure that the pathogens of greatest concern were included. The following fungal, bacterial and viral pathogens were included in the eradication tests for the reasons shown:

Fusarium oxysporum f.sp. *lycopersici*	causal agent of tomato wilt in non-resistant (i.e. some amateur) cultivars, potential temperature tolerance, only 1 previous reference in compost.
Fusarium oxysporum f.sp. radicis-lycopersici	tomato foot and root rot, potential temperature tolerance, no previous references in compost, importance in tomato growing media.
Microdochium nivale	no previous references in compost, potential temperature tolerance due to the production of chlamydospores, importance in turf grass (Fusarium patch disease).
Phytophthora nicotianae	root rot, basal stem rot, no previous references in compost, importance in tomato growing media.
Plasmodiophora brassicae	causes clubroot of Brassicas, variable results in previous references, potential temperature tolerance, longevity of resting spores and risk of infesting land.
Pythium ultimum	no previous references in compost, importance in growing media in causing damping-off, root rot.
Rhizoctonia solani	importance in growing media in causing damping-off, basal stem rot.
Verticillium dahliae	importance in growing media as causal agent of wilt diseases.
Thielaviopsis basicola	importance in growing media in causing black root rot, particularly in bedding plants 1 previous (unreliable) reference in compost.
Xanthomonas campestris pv campestris	importance in Brassica growing media in causing black rot no previous references in compost.
Tobacco Mosaic Virus	well-known high temperature tolerance, may be degraded by long composting?

Tests were conducted on propagules of the plant pathogens and/or plant material affected with the different pathogens for the following reasons:

(i) for some pathogens (obligate parasites, e.g. *P. brassicae*), only affected plant material is available as an inoculum
(ii) the plant material may provide some protection from the composting conditions
(iii) certain propagules (e.g. chlamydospores of *F. oxysporum*) may be more temperature tolerant than mycelium. Affected plant material may contain a range of growth stages of the pathogen.

A recent WRAP literature review on the effect of composting on animal/human pathogens (Jones and Martin, 2003) has revealed many papers that highlight the survival of various animal pathogens in the environment and also the potential for the environment to be contaminated by various pathogens. The review also focuses on many of the factors of composting that effect the survival of animal/human pathogens, such as temperature, moisture content, pH and duration of the process. It was important to ensure that the composting conditions used in the present project would also eradicate the main animal and human pathogens of concern, i.e. *E. coli* and *Salmonella* spp.

Sanitisation standards for compost have been developed in the US by the Composting Council of the US (Leege and Thompson 1997), in the UK by WRAP and the Composting Association (PAS100, Anon 2002) as well as in several other European countries (Stentiford 1996). These specify minimum compost temperatures of 55 - 65°C for periods of 3 - 14 days depending on the composting system (turned windrow, in-vessel, static aerated piles). However, it is not clear from the literature whether sufficiently high temperatures can be achieved using predominantly plant-based feedstocks such as green wastes that are destined to become high quality end products. Most references on temperatures during composting show mean or maximum temperatures achieved. However, of importance for pathogen eradication is the proportion of the compost which reaches the critical temperature and also the proportion which remains below the specified sanitisation standards. This will depend on the feedstocks used, the composting system and its management. In this project, the temperature profiles of large-scale composting systems (turned windrows and in-vessel tunnels) using plant-based wastes were determined. The conditions required to eradicate pathogens were than compared with the temperature profiles and the recommendations in PAS100.

2 Objectives

2.1 Overall project aim

The aim of the project was to undertake a testing regime to determine the composting conditions required to eradicate selected plant, animal and human pathogens and how this relates to commercial composting situations. The publication and dissemination of the results should improve the biological safety and reputation of composted materials in high quality end-uses.

2.2 Specific objectives

1. Determine pathogens of concern to high quality compost end-users, by consultations with end-users of different high quality end products.
2. Identification of the composting conditions required for eradicating temperature tolerant pathogens of concern from aerobic, thermophilic composting feedstocks for compost destined for high quality end users.
3. Confirmation that the above composting conditions will eradicate UK isolates of other plant, animal and human pathogens of concern in high quality end-uses, for which there is currently insufficient information.

4. Compare industrial-scale composting processes in terms of temperature profiles to determine current best practice.
5. Compare compost sanitisation conditions that are achievable with current best practice with standards in PAS100, for aerobic and thermophilic composting.
6. To recommend the exclusion of particular high-risk contaminated feedstocks, if the eradication of specific pathogens cannot be achieved using composting best practice.
7. Knowledge transfer through publication of results and dissemination to commercial compost producers and end-users.

3 Consultations with end-users of different high quality composts to determine the pathogens of concern

Consultations were conducted with 21 end-users of high quality composts:

Melcourt Industries Ltd (C. Dawson)
Newport City Council (S. Myrddin)
Double H Nurseries (M. Holmes)
National Trust (N. Cook)
Colleta & Tyson Nurseries(F. Richardson)
Petersfield Growing Media (C. Husband)
Bulrush Peat Co Ltd (N. Bragg)
Birmingham City Council (J. Shields)
Avoncrop Ltd (T. Horsman)
Marks&Spencer plc (J. Monaghan)
William Sinclair (C. Turner)
BIFFA (D. Morgan)
Vapogro (M. Taylor)
W.J. Findon (A. Fuller)
Greenvale plc (P. Coleman)
B&Q plc (I. Howell)
Soil Assocation (R. Haward)
Scotts (S. Cavanagh)
Bord na Mona (M. Prassad)
White Salads (P. Morley)
A.W. Jenkinson (D. Wood)

Table 1. Pathogens and associated diseases mentioned in the consultations and/or used in the eradication tests

Pathogen	**Disease(s)**	**Number of mentions**
Plant pathogens		
fungi, myxomycetes and phycomycetes		
Phytophthora spp.	root rot, basal stem rot	10
Pythium spp.	damping-off, root rot	8
Plasmodiophora brassicae	clubroot of Brassicas	6
Rhizoctonia solani	damping-off black scurf of potato brown patch of turf basal stem rot	6
Fusarium oxysporum fspp.	wilt	4
Thielaviopsis basicola	black root rot	3
Armillaria mellia	honey fungus	2
Pyrenochaeta lycopersici	tomato corky root	1
Verticillium dahliae	wilt	1
Microdochium nivale	Fusarium patch of turf	0
Bacteria		
Clavibacter michiganensis	ring rot of potato	1

Pathogen	Disease(s)	Number of mentions
ssp. *sepedonicus*		
Xanthomonas campestris pv. *campestris*	black rot of Brassicas	0
Viruses		
Pepino Mosaic Virus	Tomato pepino mosaic	1
Tobacco Mosaic Virus		0
Animal pathogens		
Escherichia coli		4
Salmonella spp		3

The following questions were asked to consultees:

1. Is pathogen content a concern in the acceptability or marketability of green waste compost in growing media?
2. If the answer to question 1 is yes, which pathogens are of concern?

Of the 21 consultees, 19 said that pathogen content of green waste compost was a concern, and of these, 17 were able to name at least one pathogen which was of particular concern. The pathogens that were of greatest concern in growing media are listed in Table 1.

The pathogens that were mentioned three or more times in the consultations were investigated in this study, as well as several other pathogens for the reasons previously stated in Section 1. Pathogen species not highlighted by end-users but which the literature review and/or plant pathologists suspected of being problematic were also included. *Plasmodiophora brassicae* and *Rhizoctonia solani* were examined by INRA Dijon as part of an EU funded project RECOVEG, the results of which are included in this report. Animal pathogens were of concern to some retailers of growing media whereas plant pathogens were of greater concern to commercial end-users of compost.

4 Eradication of plant pathogens in laboratory bench-scale composting system

4.1 Materials and methods

The plant pathogen eradication tests were conducted at two laboratories; HRI Wellesbourne, UK and INRA Dijon, France. An overall layout of the tests on pathogen propagules and affected plant material at the two laboratories is shown in Table 2.

4.1.1 Bench-scale composting equipment

Feedstock wastes were composted in 'Quickfit' multiadapter flasks (Fischer Scientific, Loughborough, UK) immersed in thermostatically controlled water baths, each holding three 2-L flasks (Noble et al, 1997). Samples of feedstock waste (700 g) were placed on a perforated stainless steel platform within each flask and the flasks immersed in the waterbaths such that the water level was above the level of the enclosed waste. Each flask was connected to ancillary equipment to aerate the waste for 2 min in every 30 min at a

flow rate of 250 ml min^{-1}. All of the airflow was vented from the flasks through an exhaust opening at the top. Flasks containing pathogen inoculum but no waste were used as a comparative treatment in order to separate the chemical and biological effects of the compost from the temperature effects. These 'empty' flasks were to partly simulate the scenario of pathogen inoculum being present in a dry pocket in a compost heap, and would provide information on the effect of heat on pathogens, independent from other compost variables. The temperature of the wastes or air in the flasks was monitored with Squirrel multipoint temperature loggers (Grant Instruments Ltd, Cambridge, UK). During the experiments, the relative humidity of the air in the empty flasks was 25 (±6) % and 97 (±3) % in the air in the flasks containing compost. Ammonia, carbon dioxide and oxygen concentrations in the wastes in the flasks were measured daily with a Dräger Accuro bellows pump with appropriate detector tubes (Drägerwerk, Lübeck, Germany). Previous measurements have shown that the microbial populations of the compost and gaseous conditions within flask-scale and large-scale composting systems are similar (Noble et al, 1997; Noble et al, 2002).

Table 2. Plant pathogen eradication tests at HRI Wellesbourne, UK and INRA Dijon, France and large-scale composting facilities at Hensby Composts and Organic Recycling

Pathogen	Propagules (flasks)	Affected plant material (flasks)	Affected plant material (large-scale)
Fusarium oxysporum f.sp. lycopersici	HRI	HRI	HRI, Hensby
Fusarium oxysporum f.sp. radicis-lycopersici	HRI	-	-
Microdochium nivale	HRI	-	-
Phytophthora nicotinae	HRI	-	-
Plasmodiophora brassicae	-	INRA	INRA, Hensby, ORL
Pythium ultimum	HRI & INRA	-	-
Rhizoctonia solani	INRA	-	-
Thielaviopsis basicola	HRI	-	-
Verticllium dahliae	HRI	HRI	HRI, Hensby
Xanthomonas campestris pv. *campestris*	-	HRI	-

4.1.2 Feedstock wastes

The following materials were used for the composting tests at HRI Wellesbourne:

(a) A 10:1 w/w mixture of wet onion waste (peelings and chopped whole bulbs) and dry onion waste (shale-skins and onion tops), obtained from Goldwood Ltd, Moulton, Lincs. (Coventry et al, 2002)
(b) Shredded green waste, obtained from Hensby Composts Ltd, Woodhurst, Cambs.

The following feedstock materials were used for the composting tests at the research partner INRA Dijon, France:

(a) Onion waste as above
(b) Spent mushroom compost, obtained from France Champignon, 49400 Chace, France
(c) Spent mushroom compost, obtained from HRI Wellesbourne
(d) Green waste compost, obtained from Hameau Fouchanges, 21 Arceau, France.

The feedstock wastes were analysed for moisture, pH, nitrogen and ash content before filling and after emptying the flasks according to methods in Anon 1986, 2000a,b.

4.1.3 Pathogen inocula and retrieval from composted wastes

The source of the pathogen isolates is shown in Table 3. Inocula, retrieval methods and detection limits for the pathogen propagules used are shown in Table 4. Samples of the prepared inocula were observed under a light microscope to determine the presence of the type(s) of propagules present. For tests organisms

incorporated on dry talc, the moisture content (water potential) of the talc equilibrated with that of the feedstocks during composting.

Plant pathogen inocula

Fusarium oxysporum f. spp*. lycopersici* and *radicis-lycopersici.* The organism was grown on potato dextrose agar (PDA) for *c.* 7-10 d at 20 °C. Cultures were flooded with 20 ml sterile distilled water and a spatula was used to dislodge the mycelium and conidia from the surface of the media. The mycelial/conidial suspension (3 ml) was added to sterilised talc (10 g) and kept at room temperature (*c.* 20 °C) for 6 weeks to allow chlamydospores to develop prior to testing.

Microdochium nivale. Propagules of this organism were prepared as described for *F. oxysporum* f.spp.
Pythium ultimum. The organism was grown on PDA for *c.* 7 d at 20 °C. Discs from the growing edge of a culture were removed using a cork borer and spatula and added to chopped potato soil medium (50 g fresh chopped potato + 500 ml sieved (2 mm) moist soil autoclaved for 2 x 1 h with 24 h between each autoclaving). The inoculated medium was incubated for *c.* 21 d at 25 °C to allow oospores to develop prior to use.

Phytophthora nicotianae. The organism was grown on PDA for *c.* 7-10 d at 20 °C. Discs from the growing edge of a culture were removed using a cork borer and spatula and added to sterilised Finnpeat containing Dolokal lime (8 gl^{-1}), PG-mix fertiliser (0.8 gl^{-1}) and ground oat flakes (2% dw/dw). The inoculated medium was incubated at 20 °C for *c.* 21 d to allow oospores to develop prior to use.

Verticillium dahliae. The organism was grown on PDA at 20 °C. Discs were cut from a 1 month old PDA culture and added to sterilised oat seed. The oat seed was soaked for 16 h then autoclaved at 121 °C for 1 h. The excess water was poured off and the oat seed incubated at room temperature for 24 h, and then autoclaved again for 1 h in flasks. The inoculated flasks were incubated for 4 weeks at room temperature to allow the microsclerotia to colonise the oat seed prior to use.

Thielaviopsis basicola. The organism was grown on PDA at 20 °C. Cultures were flooded with 20 ml sterile distilled water (SDW) and a spatula used to dislodge the mycelium and conidia from the surface of the media. The mycelial/conidial suspension (3 ml) was added to sterilised talc (10 g) and incubated at 20 °C for 2-3 weeks to allow conidia to develop prior to use.

Xanthomonas campestris pv. *campestris*. The plant debris consisted of infected leaves collected over period of several months from plants of *Brassica* spp. inoculated with a number of different isolates of Xcc. The leaves were stored in paper bags and allowed to air-dry at ambient temperature in the laboratory. Prior to the recovery experiments the dried leaves were coarsely crushed, combined and thoroughly mixed by hand.

The retrieval methods for the different pathogens are shown in Table 4. The detection limit for propagules was 100 cfu g^{-1}. Eradication is defined as a reduction in the numbers of the pathogen to levels below the detection limit of the detection method used.

Table 3. Source of plant pathogens used in the eradication tests

Pathogen	Source	Date	Collector/ Collection	Isolate number
Fusarium oxysporum f.sp. lycopersici	wilt of tomato Littlehampton, UK	1990	J. Carder	-
Fusarium oxysporum f.sp. radicis-lycopersici	root rot of tomato Littlehampton, UK		J. Carder	-
Microdochium nivale	Universite Louvain Belgium	-	-	31963
Phytophthora nicotinae	Benaki Phytopath. Institute, Greece		G. Zervakis NAGREF, Greece	IK51
Plasmodiophora brassicae	Brassica roots Brittany, France	2001	F. Rouxel	
Pythium ultimum	soil	2000	G. Petch	-

Pathogen	Source	Date	Collector/ Collection	Isolate number
Rhizoctonia solani	tomato, Dijon, France	2001	C. Alabouvette	Rh 1556
Thielaviopsis basicola	carrot	2004	J. Fletcher	
Verticillium dahliae	strawberry East Malling, UK	1998	D. Harris	12087
Xanthomonas campestris pv. *campestris*	Brassica leaves Wellesbourne, UK	2003	S.R. Roberts	mixed isolates

Table 4. Propagules (in addition to mycelium), inocula, retrieval methods and detection limits for pathogens. Detection limit: 100 cfu g^{-1}

Pathogen	Propagule(s)	Inoculum	Retrieval
Fusarium oxysporum *f.sp. lycopersici* *f.sp. radicis-lycopersici*	chlamydospores chlamydospores	propagules mixed with talc	Nash medium
Microchium nivale	chlamydospores	propagules mixed with talc	Nash medium, PDA + antibiotic
Phytophthora nicotianae	oospores	inoculated peat	PARPNH selective agar
Pythium ultimum	oospores	chopped potato/ soil media	*Pythium* selective medium
Thielaviopsis basicola	conidia	propagules mixed with talc	PDA + antibiotic

4.1.4 Eradication experiments at HRI

Tests with affected plant material are shown in Table 5. Pathogen inocula or infected plant material were enclosed within fine mesh polyester bags (prepared from 140 mm diameter circles and closed with a tie wrap). The bags were placed in the centre of the waste or suspended in the air in the flasks. The flasks were incubated in six water baths with set temperatures ranging from 40˚C to 70˚C (at 6˚C intervals) for up to 7 days. Other flasks were also kept at 18˚C. Flasks with *Tobacco Mosaic Virus* (TMV) were also incubated at 80 and 90˚C for 7 days and at 60˚C for up 6 weeks. The temperature and feedstock waste treatments are shown in Tables 9-16 in the Data Tables Section of the report.

Three replicate flasks were used for each temperature. These were arranged in three runs of the experiment, with one replicate of each treatment in each run. Bags containing different pathogens were placed in the same flasks. The allocation of temperatures to the six water baths to was changed between each run.

Table 5. Inocula, retrieval methods and detection limits for affected plant material.

Pathogen	Inoculum	Retrieval	Detection limit cfu g^{-1}
Fusarium oxysporum *f.sp. lycopersici*	tomato plants	selective agar	100
Phytophthora nicotianae	tomato plants	selective agar	100
Plasmodiophora brassicae	Chinese cabbage plants Brussels sprouts plants	Chinese cabbage plant bioassay	
Rhizoctonia solani	barley grains	malt agar + citric acid	100
Tobacco Mosaic Virus	tobacco leaves	tobacco plant bioassay	-
Verticillium dahliae	oat grains	selective agar	100

Pathogen	Inoculum	Retrieval	Detection limit cfu g^{-1}
Xanthomonas campestris pv. *campestris*	Brassica leaves	FS and MS media	150

4.1.5 Experiments with *Plasmodiophora brassicae* and *Rhizoctonia solani*

Tests with *P. brassicae* and *Rhizoctonia solani* were conducted at INRA Dijon, France using the same equipment used at HRI Wellesbourne but with different experimental layouts. Tests were also conducted on *Pythium ultimum* using the same isolate as in the HRI tests. Experimental treatments:

(a) Compost temperature 40, 50 and 65˚C for 1,3 and 7 days
Aerated, non-aerated
Spent mushroom compost (French)
(b) Compost temperature 40, 50 and 60˚C for 1, 3 and 7 days
Non-aerated
Green waste, Spent mushroom compost (French and UK), Onion waste
(c) Compost temperature 50 and 60˚C for 1, 3 and 7 days
Non-aerated
Green waste, Spent mushroom compost (French)
Moisture content 40, 50, 60 (± 3) % w/w at the start of the experiment.

Sample bags containing pathogen propagule were retrieved after 1, 3 and 7 days of incubation from the same flasks. Three replicate flasks for each treatment.

R. solani and *Pythium ultimum* were only examined in experiment (b).

Rhizoctonia solani. An inoculum was prepared on barley grains using the same procedure used for preparing an inoculum of *V. dahliae* on oat grains.

For *Plasmodiophora brassicae* inoculum. Chinese cabbage plants (susceptible cultivar Granaat) were cultivated in a growth chamber for 6 weeks. Plants were then harvested, roots washed, and the whole clubroot galls were harvested and stored at –20°C. Bags containing 3 to 5 g of galls were introduced into the feedstock waste placed in the flasks and incubated at the above temperatures with or without aeration.

The galls were ground to obtain a suspension of resting spores, then diluted to obtain a concentration of $1x10^5$ resting spores ml^{-1}. Steam treated soil, filled into 300ml pots, was infested with 20 ml of the resting spore suspension. There were 9 pots of 5 plants per treatment. After 6 weeks, the plants were up-rooted, the roots were washed and examined. A symptom index was given according to the following scale (Fig.1):
0 = healthy plant, no gall
1 = one or two galls
2 = many galls but some roots were still healthy
3 = the entire root system was attacked.
A pathological index (I) was calculated whereby n0, n1, n2 and n3 were the numbers of plants in each category:
I = {([n0 x 0] + [n1 x 0.25] + [n2 x 0.5] + [n3])/5} x 100.
In each experiment, plants inoculated with the resting spore suspension were used as positive controls, and uninoculated plants were used as negative controls.

4.2 Results

Data Tables are at the end of the report on page 22 onwards. Results Figures are on page 29 onwards.

4.2.1 Analysis of feedstocks

Of the three feedstocks used, green waste (GW) was the driest and onion waste the wettest, with spent mushroom compost (SMC) intermediate in moisture content (Table 7). The onion waste was acidic and the GW and SMC were neutral to slightly alkaline. The Hensby GW had the lowest N content; the other feedstocks had N contents of about 2% of DM. All the measured compost parameters are shown in Table 7.

Gas analysis of the flasks during composting is shown in Table 8. Aeration of the feedstocks increased oxygen and reduced carbon dioxide concentration. With the exception of French GW and HRI SMC, ammonia was only detectable in the aerated flasks. The highest level of ammonia was in the aerated HRI SMC.

4.2.2 Eradication of pathogen propagules

Pythium ultimum. Oospores and mycelium were present in the inoculum. At INRA Dijon in tests without aeration, the pathogen was eradicated after 3 days at 50˚C or higher in all four composts, but survived in 3 composts at 40˚C for 7 days (Fig.2). At HRI in tests with aeration, the pathogen was eradicated in both of two composts at 40˚C or higher for 7 days but it survived in empty flasks at 46˚C for 7 days (eradicated at 52˚C)(Table 9).

Phytophthora nicotianae. Oospores and mycelium were present in the inoculum. The number of surviving propagules declined as temperature increased from 18˚C to 52˚C. In onion waste and empty flasks, propagules of the pathogen were eradicated at 58˚C for 7 days, and in GW at 52˚C for 7 days (Table 10).

Fusarium oxysporum f.sp. *radicis-lycopersici.* Chlamydospores and mycelium were present in the inoculum. Propagules of the pathogen were eradicated at 46˚C for 7 days in onion waste and empty flasks. In GW, the pathogen was eradicated at 40˚C for 7 days (Table 11).

Fusarium oxysporum f.sp. *lycopersici.* Chlamydospores and mycelium were present in the inoculum. The number of surviving propagules declined as compost temperature was increased from 18˚C to 46˚C. Propagules of the pathogen were eradicated in GW at 46˚C for 7 days, and in onion waste and empty flasks at 52˚C for 7 days (Table 12).

Microdochium nivale. Chlamydospores and mycelium were present in the inoculum. There was some variability in the eradication results; survival being detected at 58˚C in both onion waste and green waste, but not at 46˚C. Propagules of the pathogen were eradicated from the onion waste and GW at 64˚C for 7 days. In empty flasks, a temperature 52˚C for 7 days eradicated the pathogen (Table 13).

Thielaviopsis basicola. Propagules were eradicated at 40˚C for 7 days in GW, onion waste and empty flasks (Table 14).

4.2.3 Eradication of plant pathogens using affected plant material

Rhizoctonia solani (INRA Dijon). The pathogen was not detected in any of the composts at 50˚C for 1 day or longer, or at 40˚C or higher for 7 days (Fig.3).

Plasmodiophora brassicae (INRA Dijon). Three separate experiments were completed using clubroot affected Chinese cabbage plants. In experiment (a) Section 4.1.5, the pathogen was eradicated by composting at a temperature of 65˚C for 1 day or longer, or by composting for 7 days at 40˚C or higher (Fig.4). There was no significant effect of aeration on eradication. However, in experiment (b), the pathogen survived in four composts at 40˚C for 7 days (Fig. 5). Survival at 50 and 60˚C was better in the two drier composts A and B than in the two wetter composts C and D. In the latter composts, which had moisture contents of 59 and 74.5 % w/w at the start of the test, the pathogen was reduced to below the detection limit of the bioassay by composting at 60˚C for 1 day (Fig. 5). The effect of moisture content on eradication was also shown in experiment (c) (Fig. 6). There was no survival of *P. brassicae* after 7 days at 50 or 60˚C, or after 3 days at 60˚C. Survival of *P. brassicae* generally declined at increasing moisture content of both SMC and GW.

Fusarium oxysporum f.sp. *lycopersici*. The pathogen was eradicated in infected plant material in green waste at 46˚C (Table 12).

Verticillium dahliae. The pathogen was eradicated at 46˚C for 7 days in onion waste and in empty flasks, and at 40˚C in green waste compost (Table 15).

Xanthomonas campestris pv. *campestris.* The pathogen survived at 64˚C for 7 days in the empty flasks but was eradicated in both composts at 40˚C or higher for 7 days (Table 16). There was no significant effect of temperature in the range 18-64˚C in the empty flasks on the pathogen (Table 16).

Tobacco Mosaic Virus. TMV symptoms of leaf curling and mottling were present on tobacco plants inoculated with infected leaf material composted (or heated) at 70˚C and below (Table 17 and Fig.7). The pathogen was eradicated at 80˚C for 7 days. After 5 weeks at 60˚C, the tobacco leaf material was still infectious.

5 Eradication of plant pathogens in large-scale composting systems

5.1 Materials and methods

5.1.1 Large-scale composting systems and feedstocks

The pathogen inocula used in the large-scale eradication tests and their location are shown in Table 3. Two large-scale composting systems with two different feedstock wastes were used for the tests:

(1) Insulated aerated tunnels, each containing 100 tonnes of shredded green waste (Hensby Composts Ltd, Woodhurst, Cambridgeshire). The dimensions of the tunnels were 4 x 30 x 4 (high) m. The green waste was source-separated green waste from local authorities. The composting duration in the tunnels was 8 days and there was no mixing during this period. Fan speed and proportion of fresh and recirculated air depended on the activity of the feedstocks (initial values were 50%, declining to about 30% by the end of the 8 days).

(2) Turned windrows (100 tonnes) of vegetable and fruit waste (45% w/w), green waste and straw (Organic Recycling Ltd, Crowland, Lincs). Windrows were 4 m high at the centre and 30 m long. The ratio of green waste and straw vary during the year depending on the availability of green waste. The green waste is source-separated and collected by local authorities. The windrows were covered with a 15 cm layer of straw for odour control. The straw was mixed into the compost during the subsequent turn and fresh straw applied to the surface. The windrows were turned at 5-10 day intervals (depending on wind direction) but were not turned during the pathogen eradication tests (7 days).

The inoculum bags were positioned 50 cm from the top surface in the centre of the tunnels or windrows. Temperatures were monitored hourly from probes positioned next to the inoculum bags using multipoint data loggers. Moisture content of composting feedstocks was determined at the start and end of the test. Two replicate tests were conducted in each composting system.

5.1.2 Pathogen inocula and retrieval from composted wastes

Affected plant material with the following pathogens was used (Tables 3 and 5):
Plasmodiophora brassicae, Fusarium oxysporum f.sp. *lycopersici, Verticillium dahliae.*
Plant material affected with different pathogens used in the tests are shown in Table 5. Inocula were enclosed in the same type of bags used for the bench-scale tests, except that these were then enclosed in a further red nylon mesh bag filled with the same compost feedstock. The sample bags were retrieved after 1 week in the composting batch.

5.2 Results

Plasmodiophora brassicae. Resting spores were observed in the galls on clubroot affected roots before composting (Fig.8). After composting for 3 – 7 days in tunnels or windrows, the root material and galls were very degraded and no resting spores were observed under the microscope (Fig.8). Temperatures in the tunnel and windrow exceeded 65˚C for at least 1 day (Figs.9 and 10). Tests were also conducted in the tunnel in which temperatures exceeded 65˚C for 1 day and the galls were removed after 3 days. Although the galls on the uncomposted roots produced galls in the Chinese cabbage test plants in the subsequent bioassay, no galls were produced from the composted roots from any of the tests in the subsequent bioassay.

Fusarium oxysporum f.sp. *lycopersici*. Temperatures during the composting test at Hensby Composts are shown in Fig.11. The peak temperature was 70˚C, and temperatures exceeded 60˚C for 2 days. The pathogen was retrieved from the uncomposted tomato plant material but was not recovered from the composted plant material.

Verticillium dahliae. The same tunnel compost was used for *V. dahliae* as for *F. oxysporum.* The pathogen was retrieved from the uncomposted oat grains but not from the composted material.

6 Eradication of animal/human pathogens in laboratory bench-scale composting system

6.1 Materials and methods

A short study was carried out to assess the effect of composting green waste on several different species of bacteria that are pathogens of both humans and animals. Strains of enterohaemorrhagic *Escherichia coli (EHEC)*, *Salmonella typhimurium* and *S. enteritidis* that are known pathogens were selected according to their pathogenicity for either humans or animals (Table 6). For safety reasons a strain of EHEC O157:H7 was selected which was isogenic (genetically-identical) to pathogenic strains found in cases of disease but had the gene that codes for toxin induction removed. A strain of EHEC known to cause disease in cattle and two strains of *Salmonella* were used as 'wild-type' without deletion of genes involved in pathogenicity. Strains were constructed for resistance to nalidixic acid to facilitate their isolation and enumeration in the event of competition from naturally-occurring coliforms or salmonellas. In practice this was not a problem.

A known amount of sterile sand was mixed with overnight Brain Heart Infusion broth cultures (approximately 10^8 cfu/ml) of *Escherichia coli* or *Salmonella*. An appropriate number of 2 g samples of each isolate were then packaged in small permeable nylon bags as described previously (Section 4.1.1). Flasks simulating composting conditions were filled with approximately 700 g of shredded green waste (supplied by Hensby Composts). Each then had several of the small bags of sand containing bacteria buried within. Separate flasks were used for each different bacterial species. The flasks were connected to the aeration equipment (Section 4.1.1) and a thermometer inserted into the green waste. The flasks were then incubated in a water-bath set at 55°C. A single bag was removed at each time interval after the compost had reached the correct temperature and a total viable count performed to assess viability of the organisms. The time taken for a significant reduction in count was determined from these samples. pH and moisture content analysis was also performed on the green waste. To measure pH, 5 g of green waste compost were diluted in 20 ml sterile water (pH 7.0) and pH read using an electronic probe. To determine the moisture content at the end of the composting period 100 g was removed and dried to constant weight at 42°C.

The total viable count was carried out by removing 1 g of the sand/bacteria mixture and diluting this serially 1 in 10 in phosphate-buffered saline. A 100μl volume of appropriate dilutions were spread on agar plates (see below) in triplicate and incubated as described below. After incubation colonies growing on the plates were recorded and the total viable count calculated. *Salmonella* strains were enumerated on brilliant green agar (Oxoid) containing 20μl/ml nalidixic acid after aerobic incubation for 18 hours at 37°C. They were isolated by enrichment in 10 ml volumes of Rappaport broth (RAPP) (Rappaport, Konforti and Navon, 1956) or selenite brilliant green broth (SBG) (Jones and Matthews, 1975). The RAPP cultures were incubated at 37°C and the SBG at 43°C and plated onto brilliant green agar containing 20μg/ml nalidixic acid (as above). Plates incubated at 37°C were examined after 24h hours and non-sucrose-fermenting bacteria, resembling salmonellas in colony morphology, were identified serologically according to the method of Kauffmann (1972). Strains of *E. coli* were enumerated on Sorbitol MacConkey Agar containing 20μg/ml nalidixic acid (O103:H2) or on Sorbitol MacConkey Agar containing 20μg/ml nalidixic acid and 2.5μg/ml sodium tellurite (O157:H7) after aerobic incubation for 18 hours at 37°C as above (Stevens *et al.*, 2002a). They were isolated by enrichment in 10 ml volumes of MacConkey broth (O103:12) or MacConkey broth containing 20μg/ml sodium tellurite at 37°C for 24 hours.

Table 6 Isolates of animal/human pathogens used

Isolate	Details
E. coli O157:H7 85 – 170 Nal^r	Tzipori *et al*, 1987
E. coli O103:H2 PMKS Nal^r Δ*stx*1	Stevens *et al*, 2001
S. enteritidis P125109 Nal^r	B. Rowe, PHLS, Colindale
S. typhimurium PJ 4/74 ST Nal^r	Rankin and Taylor 1966

6.2 Results

An initial investigation of reduction of *E. coli* and *Salmonella* indicated that the test pathogens were unlikely to survive at 55°C for much more than 30 minutes. Time points were selected in order to achieve a survival curve of the pathogens. Table 20 shows total viable counts of the pathogens at time points up to one hour and Fig.16 illustrates the survival curve. All isolates were not detectable either directly, or by enrichment, after 1 hour (Table 21). The pH values of the green waste did not seem to change significantly throughout the composting time of 60 minutes (Table 22) and the moisture content of the green waste did not vary greatly (Table 23). These results were confirmed in further experiments (Table 26 and 27). Survival did not appear to be related to the pH of the compost at extinction of the organisms.

This data suggested that the cause of inactivation may have been heat and not a composting-related effect. The composting conditions were therefore repeated on the *S. typhimurium* strain using green waste that had been sterilised by autoclaving. *S. typhimurium* was able to survive in significant numbers for over an hour at 55°C (Table 24) in contrast to less than an hour in normal green waste. The pH of the green waste pre- and post-autoclaving was not significantly changed (Table 22) thus suggesting that a reduced pH is not significantly affecting *Salmonella* survival.

A room temperature control (Table 25) was also included and showed that, at 24 - 28°C, in green waste, *S. typhimurium* survived in significant numbers for several days thus demonstrating that temperature is a major contributing factor in the deactivation of pathogens during composting.

These results may merit further investigation since, although temperature appears to be the principal determinant of survival the composting process also exerts an effect, even over a short period (10 minutes). This could be related, for example, to the production of fatty acids which are known to be inhibitory to salmonellas and E. coli (Jones, 1976) or to the production of inhibitory products of the indigenous microflora such as natural antibiotics. Whatever was responsible the period for their production/accumulation was very short.

The lack of survival of both salmonellas and pathogenic *E. coli* in composted green waste is encouraging for the safe use of this material. Experiments were carried out using levels of pathogens far in excess of those

which would normally be found in green waste (Epstein, 2002; from E and A Consultants Inc, 1994). Both *E. coli* and salmonellas survived for shorter periods than those previously reported for equivalent wastes (green waste has not previously been reported) (Day and Shaw, 2000; Stern, 1974; Golueke, 1982). Small numbers (<10 cells) of both enterohaemorrhagic *E. coli* and *Salmonella* may occasionally cause disease (Tarr, 1995; anon, 1997; Stevens *et al*, 2002b; Blaser and Newman,1982; Gillet *et al*, 1983; Lipson, 1976; Craven *et al*, 1975; Jones *et al*, 1982). However, infections normally result from much larger doses. The main source of these organisms for the human population is the consumption of contaminated animal products – green waste compost is unlikely to make a significant contribution.

7 Determination of time-temperature profiles of industrial scale composting processes

7.1 Materials and methods

7.1.1 Large-scale composting systems and feedstocks

Four large-scale composting systems with three different feedstock wastes were used for the tests.
(1) & (2) Two of these facilities are listed under Section 5.1.1 on eradication of plant pathogens.
(3) Outdoor windrow of composting of vegetable wastes, straw and poultry manure at HRI Wellesbourne. The windrow measured 2.5 x 15 x 2 (high) m and consisted on 20 tonnes of feedstocks wastes. The windrow was turned at 10-15 day intervals over a period of 48 days.
(4) Aerated tunnel (in-vessel) with insulated walls containing 20 tonnes of onion waste over a period of 12 days. Temperatures were recorded with data loggers; oxygen, carbon dioxide and ammonia were recorded with gas detector tubes, and feedstocks were analysed for moisture, pH, N, and ash content before, during and after composting as previously described.
Temperatures were measured at depths of 10, 20, 40 and 50 cm from the top surface of each windrow/ composting batch.

7.2 Results

7.2.1 Analysis of feedstocks and gases during composting

The GW from Hensby Composts was the driest feedstock and the vegetable wastes at HRI were the wettest (Table 17). The pH, N and ash contents of all the feedstocks increased during composting. Oxygen concentrations were higher and carbon dioxide levels lower in the two tunnel systems than in the turned windrows (Table 18). Ammonia concentrations were higher in the composts containing GW than in the composts containing only vegetable wastes.

7.2.2 Temperature profiles

Temperatures during the 7-9 day process in tunnels at Hensby Composts are shown Fig.12. Temperatures at a depth of 50 cm exceeded 65˚C for at least 1 day and exceeded 55˚C for at least 4 days. Temperatures 10 cm from the side walls were up to 10˚C cooler than at 50 cm depth (Fig.12) but still exceeded 64˚C for 2 days and 55˚C for 4 days.

Temperatures in composting onion waste in tunnels at depths of 20-40 cm were 45˚C or higher for the entire 10 day composting period. Temperatures 10 cm from the sides were cooler and only exceeded 45˚C for 3 days (Fig.15).

Measurements at Organic Recycling showed that temperatures at 50 cm depth exceeded 65˚C for at least 7 days and at 10 cm depth were above 50˚C for at least 6 days (Fig.13). Temperatures in the smaller windrows of composting vegetable waste at HRI were lower and only exceeded 40˚C for 5-10 days (Fig.14).

8 Discussion

8.1 Eradication conditions for fungal plant pathogens

Comparison of the results obtained under controlled temperatures with previous work in compost as detailed in the literature review (Noble & Robert, 2003) is in some cases difficult because the temperatures and times required for eradication were often not precisely defined in the literature. Usually a peak temperature during composting was quoted, but it is not clear whether a lower temperature and/or shorter composting duration would have resulted in eradication of the test pathogen.

Christensen et al (2001) showed that a peak temperature of 65°C and composting duration of 21 days eradicated *Fusarium oxysporum* f.sp. *lycopersici* in a large-scale composting system. Although there was survival after a peak temperature of 62°C and duration of 21 days, the effects of lower constant temperatures and/or shorter durations on eradication were not studied in detail. Results from several researchers indicate that other *F. oxysporum* f.spp. are less temperature tolerant than *F. oxysporum* f.sp. *lycopersici* (Noble & Roberts, 2003). Results in this study indicate that *F. oxysporum* f.sp. *radicis-lycopersici* is less temperature tolerant than f.sp. *lycopersici* but neither pathogen would be difficult to eradicate from composting material, i.e. a compost temperature of 52°C for 7 days is sufficient for eradication.

The results for *Thielaviopsis basicola* and *Verticillium dahliae* agree with previous results by other workers which showed that neither of these pathogens could survive at a temperature of 56°C, either in compost or water (Noble & Roberts, 2003).

The results obtained for *Pythium ultimum* were similar to those obtained in compost for *Pythium irregulare* by Hoitink et al (1976) who showed that it was eradicated at 40°C for 7 days. The results obtained for *Phytophthora nicotianae* are similar to those obtained previously for other *Phytophthora* species. Bollen et al (1989) showed that peak compost temperatures of 55 and 60°C and composting for 21 days eradicated *P. infestans* and *P. cryptogea* respectively. Garbelotto (2003) eradicated *P. ramorum* in compost at 55°C for 14 days, and Hoitink et al (1976) eradicated *P. cinnamomi* in compost at 40°C for 7 days.

P. nicotianae and *P. ultimum* were more temperature tolerant in the empty flasks than in those containing composting material, unlike *Microdochium nivale*, which survived at least as well in composting material as in the empty flasks. Of the fungal pathogens tested, *M. nivale* and *Plasmodiophora brassicae* were the most temperature tolerant in compost. Since temperature effects were examined in 6°C increments, an exact temperature of eradication in this experiment for *M. nivale* between 59 and 64°C cannot be stated. The eradication of this pathogen would be of particular importance if green waste compost, which may include grass clippings in the feedstock, were applied as turf top-dressing (Dawson, 1970; Nelson & Boehm, 2002).

For the pathogens in Tables 9 to 17, the difference in eradication conditions between tests conducted in GW and onion waste were generally 6°C. Since these materials had very different moisture contents (43 and 75% w/w), it can be assumed that for these pathogens, the effect of compost moisture content within this range on eradication temperature is small. The effect of compost moisture content on the temperature tolerance of *P. brassicae* may at least partly explain the variability in previously reported results for this pathogen (Noble & Roberts, 2003). Bollen & Volker (1996) also state that the survival of a pathogen at a normally lethal temperature may be due to dry pockets in the compost.

There was no effect of forced aeration on the eradication conditions of *P. brassicae* in SMC, although gas measurements showed that the SMC remained aerobic, even without forced aeration. Ryckeboer et al (2002) found that *P. brassicae* was less temperature tolerant in anaerobic digester liquid than in aerobic compost.

8.2 Eradication conditions for bacterial and viral plant pathogens

The low temperature tolerance in composting material of *Xanthomonas campestris* pv. *campestris* is similar to previous results obtained for other bacterial plant pathogens (Noble & Roberts, 2003). The survival of the pathogen in dry Brassica trash at 64°C for 7 days indicates the importance of ensuring moist conditions during composting.

The high temperature tolerance of TMV (>70°C) agrees with previous results of other researchers (Noble & Roberts, 2003). Although Ryckeboer et al (2002) found that TMV did not survive after 184 days of composting at low temperatures (30-50°C), here, a period of 35 days at 60°C did not eradicate TMV or completely degrade the host leaf material. It is possible that degradation of the leaf material is required to eradicate TMV. However, TMV is not considered to be of importance in the UK since tomato cultivars are now bred with TMV resistance.

8.3 Temperature profiles of large scale systems

Temperatures measured in the large-scale tunnel and windrow systems using plant-based wastes were sufficient for the eradication conditions of *P. brassicae*, i.e. a compost temperature of at least 65°C was maintained for at least 1 day. This was demonstrated by the eradication of *P. brassicae*, *F. oxysporum* f.sp. *lycopersici* and *Verticillium dahliae* from the affected plant material inserted in the compost. The uncomposted, ground woody gall material of clubroot infected Brussels sprouts plants gave a positive test result in the subsequent plant bioassay.

In both composting systems, the outer part of the compost was insulated, either by the insulated walls of the tunnel or by a layer of straw on the windrows. Reviews of previous temperature profiling studies of composting systems show that there may be significant cool zones in static or non-insulated systems (Noble & Roberts, 2003). Based on survival probabilities, a report by Gale (2002) recommends that windrows should be turned at least three times and the composting process should last at least 14 days. This is to ensure that the entire composting mass reaches a temperature of 60°C for at least two days. Christensen et al (2002) recommend 5 turns in windrow systems, the same as in PAS100 which also recommends a minimum composting duration of 14 days. The windrows at ORL are turned weekly during a 5 week period, which meets this standard.

8.4 Compost sanitization standards

PAS100 (Anon 2002) contains information on monitoring the composting process and recommendations for sanitization processes. PAS100 states that:
'Particle size, temperature, moisture, pH, ammonia concentration, mixing of composting materials and oxygen supply affect sanitization performance. Temperature should be maintained within the optimal range of 55 °C to 70 °C for a duration of at least 7 days (in-vessel) or 14 days (windrows). Moisture should be maintained within the optimal range of 40% to 60% mass/mass. If moisture content moves out of this range and is allowed to remain significantly below the recommended minimum or above the recommended maximum, sanitization performance may be unsatisfactory'.

Information obtained in this project and in the literature review (Noble & Roberts, 2003) indicates that the independent effects of pH, ammonia concentration and oxygen supply (within the range found in composting) are small or not significant compared with the effects of temperature and moisture content. A moisture content in excess of 50% w/w was found to improve the eradication of *P. brassicae* and there was no evidence that moisture content in excess of 60% w/w would reduce sanitization performance. However, excessively high moisture content, as well as a restricted oxygen supply are both likely to impede the maintenance of high compost temperatures. The moisture content of the feedstocks used in the large-scale

systems at Hensby Composts and Organic Recycling (43 -59% w/w) fell with the recommended limits specified in PAS100.

For composting systems where the batch will not be mixed or turned during the santization phase, monitoring should occur 'near surface' as well as in the core zone. Results here indicate that the 'near surface' measurements should not be deeper than 10 cm, otherwise sanitization performance may be overestimated. If the temperatures near the surface or walls of in-vessel systems are below those recommended, the insulation of the system needs to be improved and/or the batch should be turned during the process.

Results obtained in this project show that the recommended minimum temperatures in PAS100 (Table C.1) were exceeded in the two large-scale composting systems. The conditions that were achieved (at least 65˚C for at least 1 day) would result in the eradication of all of the pathogens (except TMV and possibly *Microdochium nivale*) examined. These temperatures were achieved using plant-based wastes using insulated windrows or in-vessel (tunnel) composting systems. For *P. brassicae* a compost temperature of 65˚C for 1 day was required (compost moisture content of at least 51% w/w). For *M. nivale*, at least 58˚C, and up to 64˚C, for 7 days was required for eradication (compost moisture content 43-75 % w/w). However, further tests are needed to determine if a shorter duration can be used (see recommendations for further work).

9 Conclusions

(a) Consultations with end-users of composts indicated that pathogen content of green waste composts was a concern.

(b) The plant pathogens of greatest concern were: *Phytophthora* spp., *Pythium* spp., *Plasmodiophora brassicae, Rhizoctonia solani, Fusarium oxysporum* f.spp., and *Thielaviopsis basicola.*

(c) The animal/human pathogens *E. coli* and *Salmonella* spp. were of concern to retailers of composts.

(d) Propagules of *Fusarium oxysporum* f.spp. *lycopersici* and *radicis-lycopersici*, *Pythium ultimum,* and *Thielaviopsis basicola,* and *Rhizoctonia solani, Fusarium oxysporum* f.sp. *lycopersici*, *Verticillium dahliae* and *Xanthomonas campestris* pv. *campestris* in affected plant material were eradicated in laboratory tests by a compost temperature of 52˚C or less, held for 7 days.

(e) *Fusarium oxysporum* f.spp. *lycopersici* in affected tomato plant material and *Verticillium dahliae* in infected oat grains were eradicated from compost that exceeded 50˚C for 4 days and peaked at 70˚C in a large-scale tunnel.

(f) Propagules of *Phytophthora nicotianae* required a temperature of up to 58˚C for 7 days for eradication. These conditions were sufficient to eradicate all except the most temperature tolerant plant pathogens tested (*Microdochium nivale, Plasmodiophora brassicae* and *Tobacco Mosaic Virus).* Propagules of *M. nivale* required a temperature of up 64˚C for 7 days for eradication from composting material.

(g) For the above pathogens, the difference in eradication temperatures between tests conducted with GW (moisture content 43% w/w) and onion waste (75% w/w) were small (6˚C or less). It can therefore be assumed that the effect of compost moisture within this range on eradication temperature for the above pathogens (unlike *P. brassicae*) is small.

(h) Eradication of *Plasmodiophora brassicae* in infected Chinese cabbage plants depended on compost temperature, time and moisture content. A composting batch temperature of 60˚C for 1 day reduced the level of *Plasmodiophora brassicae* in affected Chinese cabbage plants to below the detection level of the test used for detecting the pathogen in compost, providing that the composting material moisture was at least 59% w/w. In compost with an initial moisture content of 51% w/w, a temperature of 65˚C for 1 day was required for eradication of *P. brassicae*.

(i) Conclusions (f) and (h) indicate that a minimum composting temperature of 65˚C for 7 days, with a minimum compost moisture content of 51% w/w at the start, is required to eradicate all the pathogens examined, with the exception of TMV. However, a temperature of 65˚C for only 1 day may be adequate if this can be shown to eradicate *M. nivale* (see recommendations for further work).

TMV is not considered to be of major significance in the UK since crops that were previously susceptible (e.g. tomato) are now bred with TMV resistance. Previous work has also shown that degradation of the host leaf material, which occurs during maturation, is effective in eliminating TMV.

(j) Composting batch aeration had no significant effect on the temperature and time required to eradicate *P. brassicae*.

(k) *Tobacco Mosaic Virus* in affected leaf material required a composting temperature of 80˚C for 7 days for eradication. The pathogen survived a composting temperature of 60˚C for 35 days, although the host plant material was not completely degraded. However, this pathogen is not considered to be important in the UK since commercial tomato cultivars are now bred with resistance to TMV.

(l) Strains of *E. coli, Salmonella typhimurium* and *S. enteriditis* were not detectable after 1 hour at 55˚C in green waste compost.

(m) Plant-based wastes in two large-scale composting systems exceeded 65˚C for at least 1 day at a depth of 50 cm. At a depth of 10 cm, temperatures exceeded 64˚C for at least 2 days.

10 Recommendations for future research

(a) The effect of moisture content using different composting feedstocks on the eradication of *Plasmodiophora brassicae* using woody gall material should be examined (currently in progress). The effect of moisture status of the pathogen propagule on eradication should also be examined.

(b) The effect of composting temperatures in the range 58 to 65˚C and times in the range 1 to 7 days on the eradication of *Microdochium nivale* from composting feedstocks should be examined.

(c) The best methods for measuring and controlling composting batch moisture content in large-scale systems should be established. The effects of using different compost feedstocks (including seasonal variation) on composting batch moisture content should be established.

11 References

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12 Data Tables

Table 7. Analysis of feedstocks used in laboratory bench-scale composting tests (mean of 3 values)

Feedstock	Source	Moisture % w/w	pH	N % of DM	Ash % of DM
Green waste	Hensby Composts Ltd Woodhurst, Cambs	43.3	6.8	0.68	49
Green waste	France	43.1	7.9	1.71	54
Spent mushroom compost	Warwick HRI, Wellesbourne	59.0	6.9	2.08	36
Spent mushroom compost	France	51.3	7.8	1.73	51
Onion waste	Goldwood Ltd Moulton, Lincs.	74.5	4.3	1.84	8

Table 8. Gas analysis during bench-scale composting (mean of 3 values)

Feedstock	Source	Aeration	O_2 %v/v	CO_2 %v/v	NH_3 ppm
Green waste	Hensby Composts Ltd Woodhurst, Cambs	No Yes	3.0 17.3	17.0 1.8	0 1
Green waste	France	No Yes	5.0 17.0	15.0 2.0	1 1
Spent mushroom compost	Warwick HRI, Wellesbourne	No Yes	7.0 19.5	13.0 0.5	2 25
Spent mushroom compost	France	No Yes	5.0 18.9	15.0 1.0	0 1
Onion waste	Golwood Ltd Moulton, Lincs.	No Yes	1.0 19.5	20 0.5	0 2

Table 9. Effect of temperature after 7 days on eradication of *Pythium ultimum* propagules in compost and empty flasks.

Temperature (°C)	Environment		
	Empty	Onion waste	Green waste
18	2.0×10^3	3.5×10^4	3.5×10^4
40	1.1×10^3	n.d.	n.d.
46	1.0×10^2	n.d.	n.d.
52	n.d.	n.d.	n.d.
58	n.d.	n.d.	n.d.
64	n.d.	n.d.	n.d.
70	-	n.d.	n.d.

- test not conducted, n.d. not detected in any of 3 replicates

Table 10. Effect of temperature after 7 days on eradication of *Phytophthora nicotianae* propagules in compost and empty flasks

Temperature (°C)	Environment		
	Empty	Onion waste	Green waste
18	1.4×10^7	7.1×10^5	2.1×10^6
40	6.5×10^5	8.0×10^4	1.0×10^2
46	6.7×10^4	n.d.	3.0×10^1
52	6.6×10^3	1.3×10^3	n.d.
58	n.d.	n.d.	n.d.
60	n.d.	n.d.	n.d.
64	n.d.	n.d.	n.d
70	-	n.d.	n.d

- test not conducted, n.d. not detected in any of 3 replicates

Table 11. Effect of temperature after 7 days on eradication of *Fusarium oxysporum* f.sp. *radicis-lycopersici* propagules in compost and empty flasks

Temperature (°C)	Environment		
	Empty	Onion waste	Green waste
18	1.8×10^4	1.5×10^5	9.1×10^4
40	1.0×10^4	6.3×10^4	n.d.
46	n.d.	n.d.	n.d.
52	n.d.	n.d.	n.d.
58	n.d.	n.d.	n.d.
64	n.d.	n.d.	n.d.
70	-	n.d.	n.d.

- test not conducted, n.d. not detected in any of 3 replicates

Table 12. Effect of temperature after 7 days on eradication of *Fusarium oxysporum* f.sp. *lycopersici* propagules in compost and empty flasks

Temperature (°C)	Propagules Environment			Affected plant material
	Empty	Onion waste	Green waste	Green waste
18	1.1×10^6	2.0×10^6	3.4×10^5	+
40	8.0×10^3	2.6×10^4	5.3×10^3	+
46	6.9×10^2	3.3×10^2	n.d.	n.d.
52	n.d.	n.d.	n.d.	n.d.
58	n.d.	n.d.	n.d.	n.d.
64	n.d.	n.d.	n.d.	-
70	-	n.d.	n.d.	-

+ detected in retrieved plant material in at least one replicate, - test not conducted,
n.d. not detected in any of 3 replicates

Table 13. Effect of temperature after 7 days on eradication of *Microdochium nivale* propagules in compost and empty flasks

Temperature (°C)	Environment		
	Empty	Onion waste	Green waste
18	4.5×10^4	1.0×10^6	5.1×10^5
40	3.7×10^3	2.5×10^5	1.5×10^3
46	1.0×10^3	n.d.	n.d.
52	n.d.	1.6×10^5	n.d.
58	n.d.	1.5×10^3	1.2×10^3
64	n.d.	n.d.	n.d.
70	-	n.d.	n.d.

- test not conducted, n.d. not detected in any of 3 replicates

Table 14. Effect of temperature after 7 days on eradication of *Thielaviopsis basicola* propagules in compost and empty flasks

Temperature (°C)	Environment		
	Empty	Onion waste	Green waste
18	1.5×10^4	4.7×10^3	7.2×10^4
40	n.d.	n.d.	n.d.
46	n.d.	n.d.	n.d.
52	n.d.	n.d.	n.d.
58	n.d.	n.d.	n.d.
64	n.d.	n.d.	n.d.
70	-	n.d.	n.d.

- test not conducted, n.d. not detected in any of 3 replicates

Table 15. Effect of temperature after 7 days on eradication of *Verticillium dahliae* on infected oat grains in compost and empty flasks

Temperature (°C)	Environment		
	Empty	Onion waste	Green waste
18	3.7×10^4	4.1×10^4	2.3×10^4
40	2.5×10^4	3.2×10^4	n.d.
46	n.d.	n.d.	n.d.
52	n.d.	n.d.	n.d.
58	n.d.	n.d.	n.d.
64	n.d.	n.d.	n.d.
70	-	n.d.	n.d.

- test not conducted, n.d. not detected in any of 3 replicates

Table 16. Effect of temperature after 7 days on eradication of *Xanthomonas campestris* pv. *campestris* in compost and empty flasks

Temperature (°C)	Environment		
	Empty	Onion waste	Green waste
18	3.7×10^8	7.0×10^7	3.8×10^8
40	8.0×10^7	n.d.	n.d.
46	9.0×10^7	n.d.	n.d.
52	1.1×10^8	n.d.	n.d.
58	3.6×10^7	n.d.	n.d.
64	3.2×10^7	n.d.	n.d.
70	-	n.d.	n.d.

- test not conducted, n.d. not detected in any of 3 replicates

Table 17. Effect of temperature after 7 days on eradication of *Tobacco Mosaic Virus* in compost and empty flasks. + detected in at least one replicate, n.d. not detected in any of 3 replicates

Temperature (°C)	Environment		
	Empty	Onion waste	Green waste
18	+	+	+
40	+	+	+
46	+	+	+
52	+	+	+
58	+	+	+
64	+	+	+
70	+	+	+
80	n.d.	n.d.	n.d.
90	n.d.	n.d.	n.d.

+ detected in at least one replicate, - test not conducted, n.d. not detected in any of 3 replicates

Table 18. Analysis of feedstocks used in large-scale composting tests (mean of 3 values)

Location	System	Feedstocks	Stage	Moisture % w/w	pH	N % of DM	Ash % of DM
Hensby Comp Ltd	Tunnel	Green waste	before after	43.5 24.9	6.8 8.1	0.65 1.60	49 59
Org Recyc Ltd	Turned windrow	Green waste + Veg. waste	before after	59.8 52.5	7.3 8.1	1.33 1.65	37 44
Warwick HRI	Tunnel	Onion waste	before after	75.0 74.5	4.3 7.1	0.86 1.84	7 8
Warwick HRI	Turned windrow	Veg. waste + straw	before after	66.5 69.0	7.5 7.7	1.57 2.12	24 28

Table 19. Gas analysis during large-scale composting (mean of 3 values)

Location	System	Feedstocks	O_2 %v/v	CO_2 %v/v	NH_3 ppm
Hensby Comp Ltd	Tunnel	Green waste	15.9	3.0	26
Org Rec Ltd	Turned windrow	Veg. + Green waste	13.5	5.5	7
Warwick HRI	Tunnel	Onion waste	14.5	0.5	1
Warwick HRI	Turned windrow	Veg. waste + straw	12.5	9.0	1

Table 20. Survival of *E. coli* and *Salmonella* spp. in green waste compost at 55°C

Isolate	Inoculum Titre (CFU/g)	Total Viable Count (CFU/g)			
		0 mins	10 mins	30 mins	60 mins
E. coli O157:H7	1.1×10^9	2.9×10^4	$<1 \times 10^2$	$<1 \times 10^2$	$<1 \times 10^2$
E. coli O103:H2	1.3×10^8	2.1×10^7	$<1 \times 10^2$	$<1 \times 10^2$	$<1 \times 10^2$
S. enteritidis	8.4×10^8	3.0×10^6	7.2×10^6	1.6×10^4	$<1 \times 10^2$
S. typhimurium	1.2×10^8	2.8×10^5	2.0×10^7	4.5×10^3	$<1 \times 10^2$

Table 21. Isolation of *E. coli* and *Salmonella* spp. by enrichment from green waste compost at 55°C from samples shown in Table 20

Isolate	Enrichment			
	0 mins	10 mins	30 mins	60 mins
E. coli O157:H7	+	-	-	-
E. coli O103:H2	+	+	+	-
S. enteritidis	+	+	+	-
S. typhimurium	+	+	+	-

Table 22. pH of green waste composts used to determine the survival of *E. coli* and *Salmonella* spp. shown in Tables 21 and 24

Material	pH
Green waste – Pre-composting	7.9 – 8.4
E. coli O157:H7– Post-composting in waterbath-immersed flask	8.2
E. coli O103:H2– Post-composting in waterbath-immersed flask	8.0
S. enteritidis – Post-composting in waterbath-immersed flask	8.4
S. typhimurium – Post-composting	8.4
Autoclaved green waste – pre-composting	8.0
Autoclaved green waste – post composting in waterbath-immersed flask	8.0
Normal green waste – post composting at room temperature	8.7

Table 23. Moisture content values of green waste composts used to determine the survival of *E. coli* and *Salmonella* spp. shown in Tables 21 and 24

Flask contents	Moisture Content (%)
E. coli O157:H7– Post-composting in waterbath-immersed flask	59.4
E. coli O103:H2– Post-composting in waterbath-immersed flask	55.3
S. enteritidis – Post-composting in waterbath-immersed flask	59.2
S. typhimurium – Post-composting in waterbath-immersed flask	65.1
Autoclaved green waste – post-composting	65.4

Table 24. Effect of using autoclaved green waste

Isolate and conditions	Inoculum Titre (CFU/g)	Total Viable Count (CFU/g)			
		0 mins	10 mins	30 mins	60 mins
S. typhimurium Normal Green Waste 55°C pH 8.4, moisture content 65.1%	1.2×10^8	2.8×10^5	2.0×10^7	4.5×10^3	$<1 \times 10^2$
S. typhimurium Autoclaved green waste 55°C, pH 65.4, moisture content 65.4%		7.3×10^6	4.6×10^5	2.9×10^5	1.4×10^4

Table 25. Survival of *S. typhimurium* in green waste at ambient temperature

Isolate and Conditions	Inoculum Titre (CFU/g)	Total Viable Count (CFU/g)		
		0 mins	72 hours	96 hours
S. typhimurium green waste 25-28°C, pH 8.4, moisture content 64.4%	1.2×10^8	5.2×10^7	5.1×10^7	NA
E. coli O157:H7 green waste 24-26°C, pH 8.5, moisture content 61.2%	1.6×10^8	1.5×10^8	8.8×10^8	3.4×10^7

Table 26. Survival of *E. coli* and *Salmonella* spp in green waste at 55°C (repeat of experiment shown in Table 21)

Isolate and conditions	Inoculum titre (cfu/g)	Total viable count (cfu/g)			
		0 mins	10 mins	30 mins	60 mins
E. coli O157:H7; pH 6.3, moisture content 55.7%	1.6×10^8	2.5×10^7	$<1.0 \times 10^2$	$<1.0 \times 10^2$	$<1.0 \times 10^2$
E. coli O103:H2; pH 6.2, moisture content 54.9%	1.0×10^8	4.1×10^7	3.0×10^4	$<1.0 \times 10^2$	$<1.0 \times 10^2$
S. enteritidis; pH 6.1, moisture content 59.5%	9.5×10^7	2.7×10^7	4.9×10^6	4.0×10^4	$<1.0 \times 10^2$
S. typhimurium; pH 6.1, moisture content 56.4%	1.2×10^8	8.4×10^7	2.7×10^6	1.2×10^4	$<1.0 \times 10^2$

Table 27. Isolation of *E. coli* and *Salmonella* spp. by enrichment from green waste compost at 55°C from samples shown in Table 26

Isolate	Enrichment			
	0 mins	10 mins	30 mins	60 mins
E. coli O157:H7	+	-	-	-
E. coli O103:H2	+	+	+	-
S. enteritidis	+	+	+	-
S. typhimurium	+	+	+	-

13 Figures

Fig.1 Chinese cabbage plants, healthy (category 1) and infected with clubroot (*Plasmodiophora brassicae*)(category 3) (INRA Dijon)

Fig.2 Effect of temperature and time on eradication of *Pythium ultimum* in composting green waste, spent mushroom compost (F and GB) and composting onion waste (INRA Dijon)

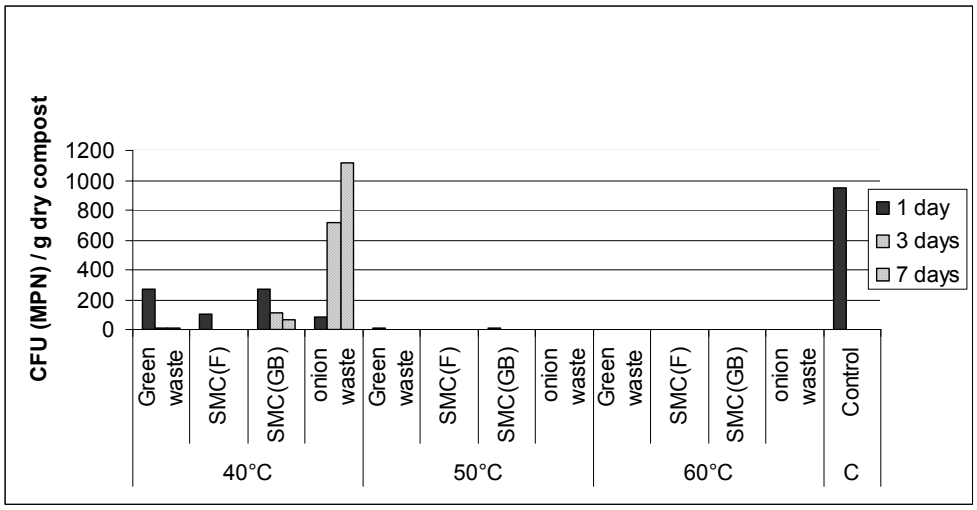


Fig. 3 Effect of temperature and time on eradication of *Rhizoctonia solani* in green waste (GW), spent mushroom compost (SMC) and onion waste compost. There was no survival after 7 d at any of the temperatures in any of the wastes (INRA Dijon)

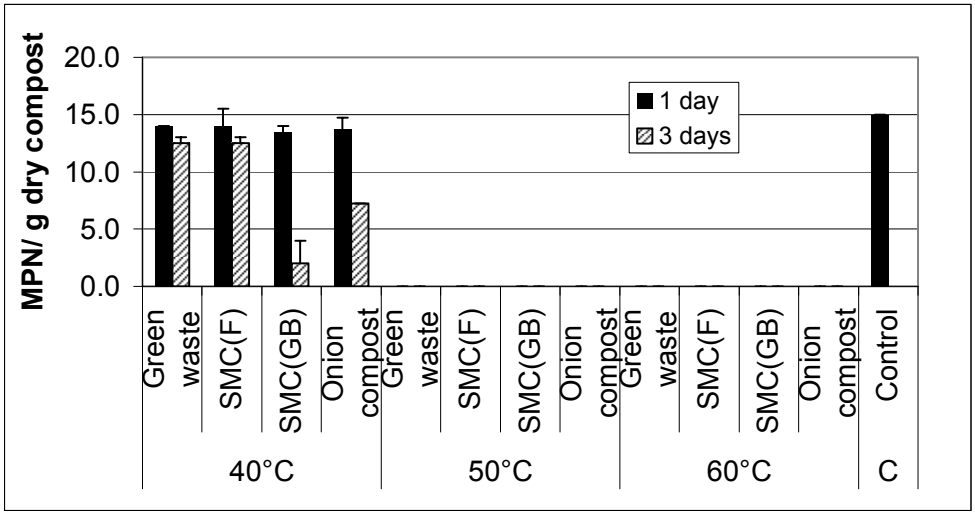


Fig. 4 Effect of temperature, time and compost aeration on eradication of *Plasmodiophora brassicae* in spent mushroom compost (F). There was no survival at 65˚C after 1,3 or 7 d (o values for these treatments are not shown). T+ and T- are the results for test plants with and without untreated *P. brassicae* inoculum (INRA Dijon).

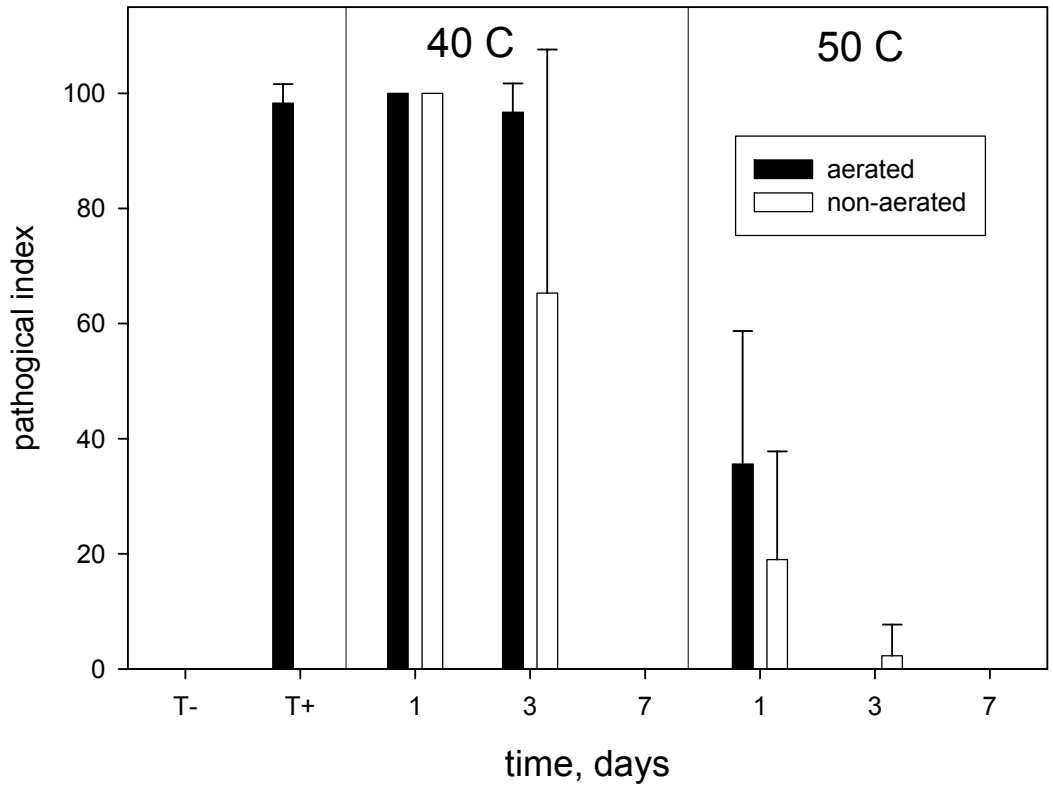


Fig. 5 Effect of temperature and time on eradication of *Plasmodiophora brassicae* in composting green waste (A), spent mushroom compost, France (B) and UK (C) and composting onion waste (D). T+ and T- are the results for test plants with and without untreated *P. brassicae* inoculum (INRA Dijon).

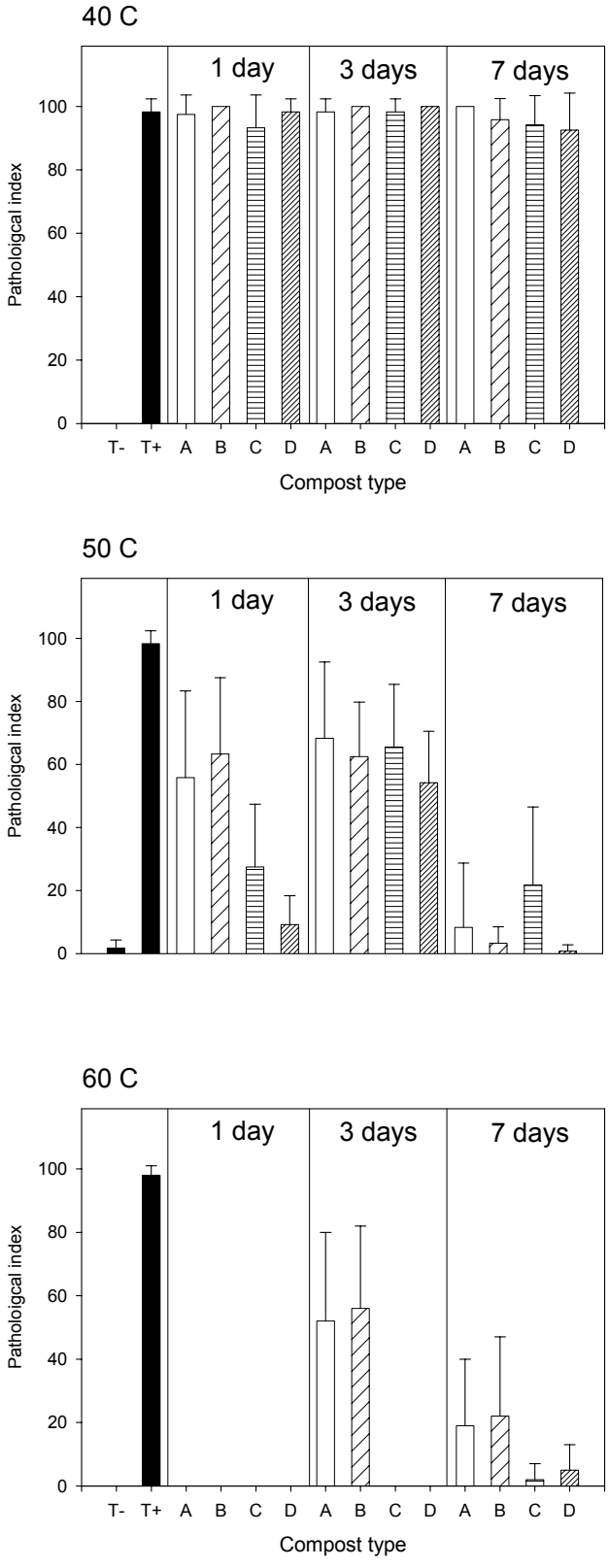
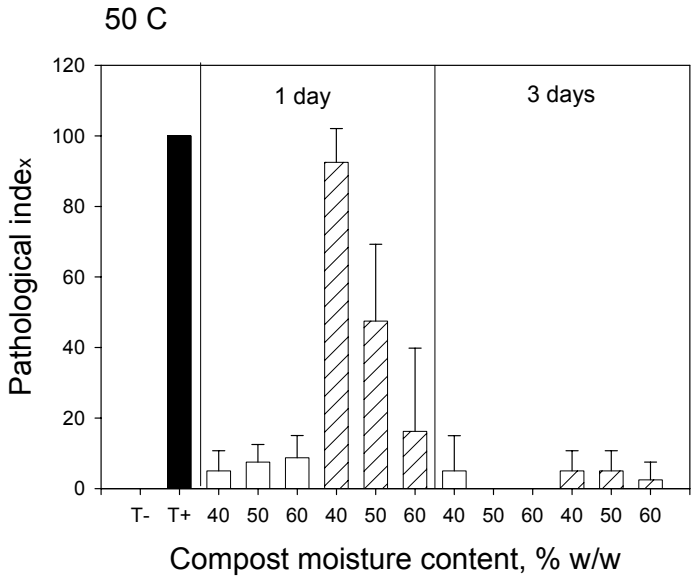


Fig. 6 Effect of temperature and time on eradication of *Plasmodiophora brassicae* from green waste (white bars) and spent mushroom compost (shaded bars) at different moisture contents. There was no survival of *P. brassicae* after 7 d at 50 C or 60 C (o values for these treatments are not shown). T+ and T- are the results of plants inoculated with and without untreated *P. brassicae* inoculum (INRA Dijon).



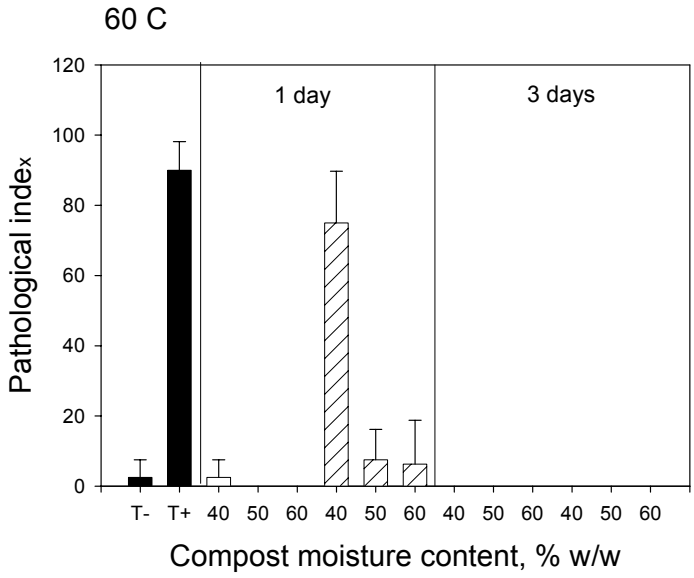


Fig. 7 Effect of compost temperature on the infectivity of tobacco leaves in tobacco plant bioassay

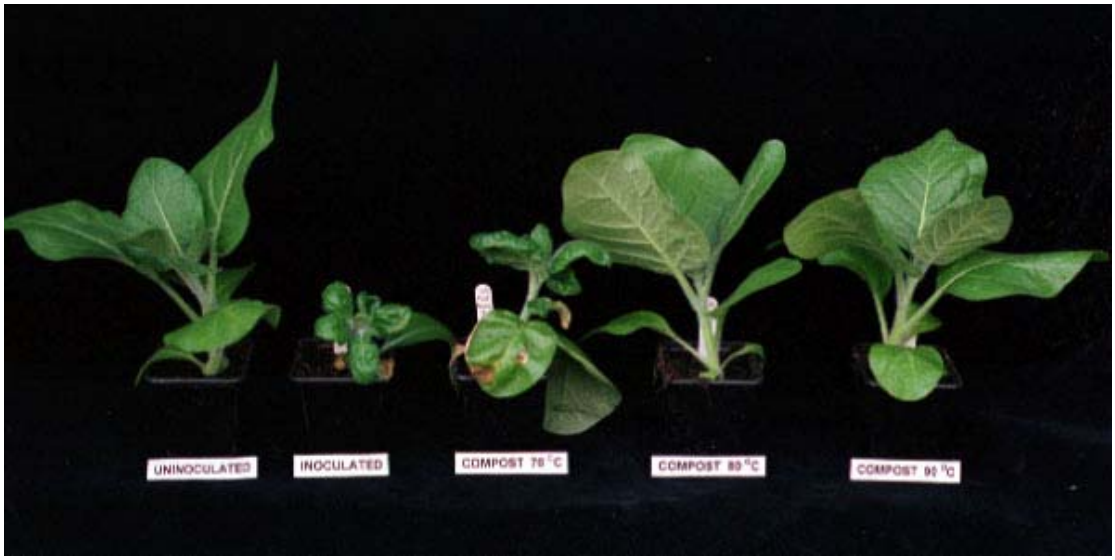


Fig.8 Brussels sprouts affected with clubroot (*Plasmodiophora brassicae*) before and after composting

Fig.9 Temperatures in tunnel (50 cm from the surface) at Hensby Composts during *Plasmodiophora brassicae* eradication test. Initial green waste moisture content was 61% w/w

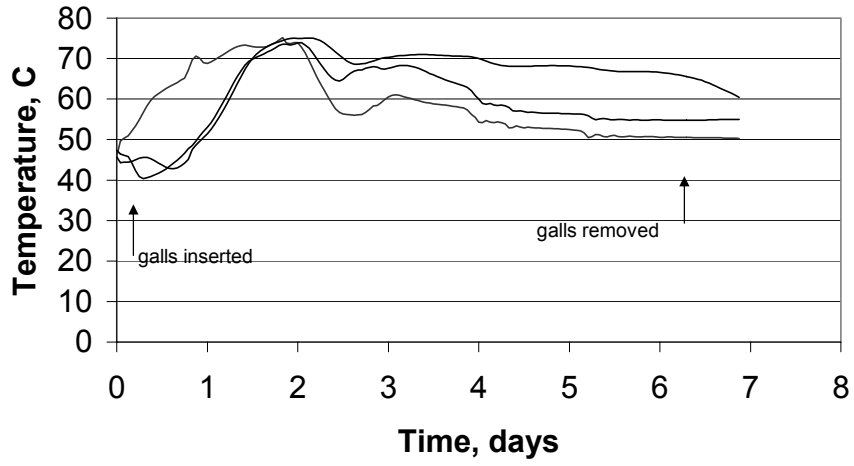
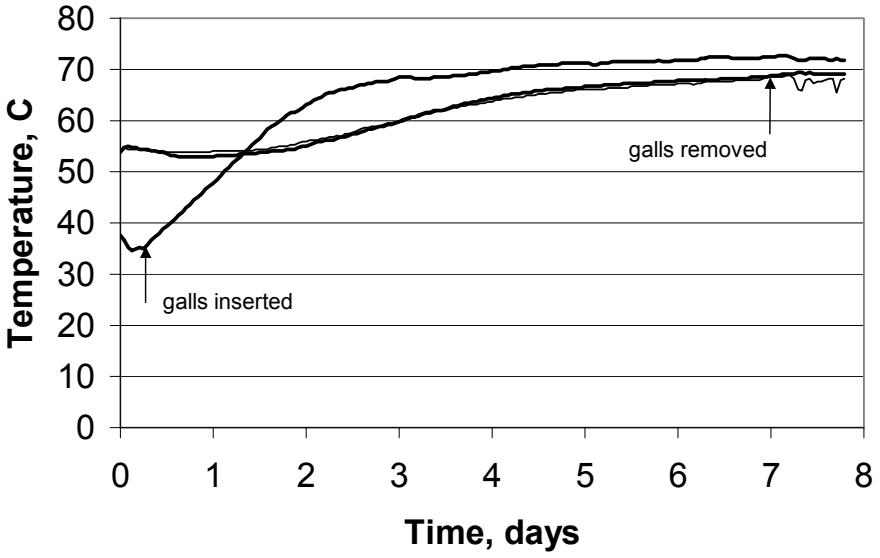


Fig.10 Temperatures in windrow at Organic Recycling (50 cm from the surface) during *Plasmodiophora brassicae* eradication test. Feedstock moisture content was 64% w/w in the upper graph and 57 % w/w in the lower graph.



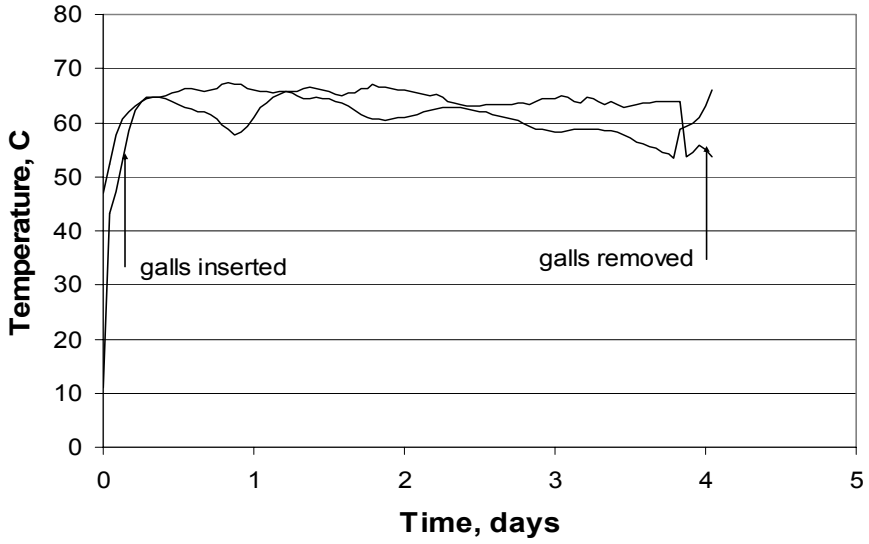


Fig.11 Temperatures in tunnel at Hensby Composts during *Fusarium oxysporum* f.sp. *lycopersici* eradication test. Feedstock moisture content was 62% w/w.

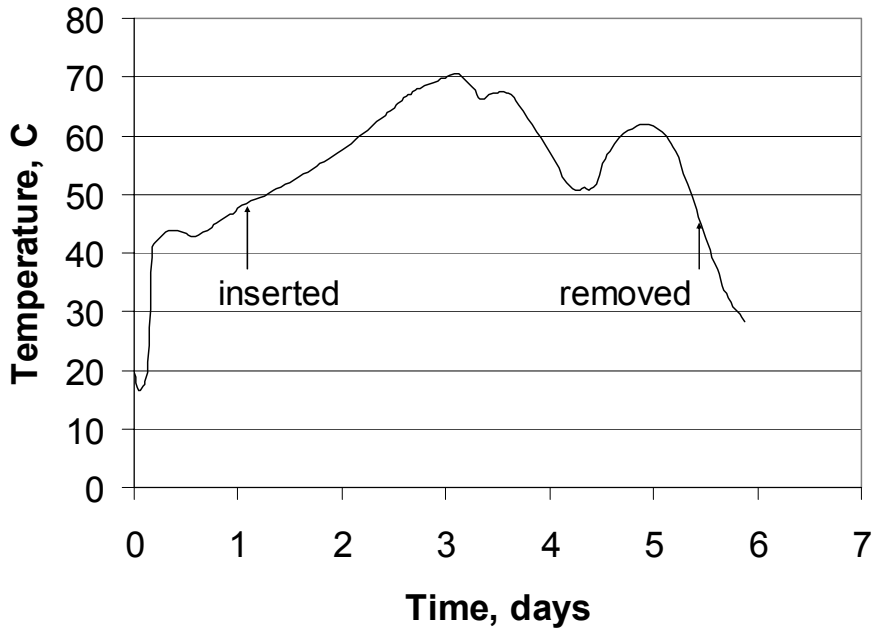
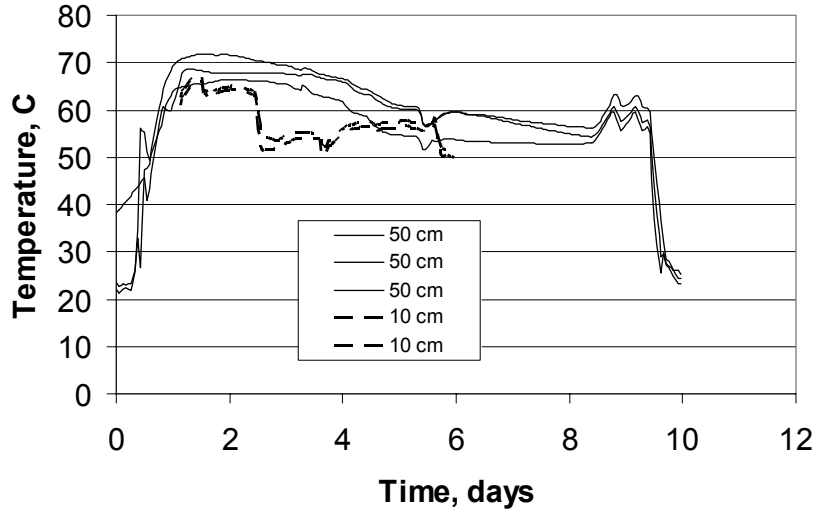
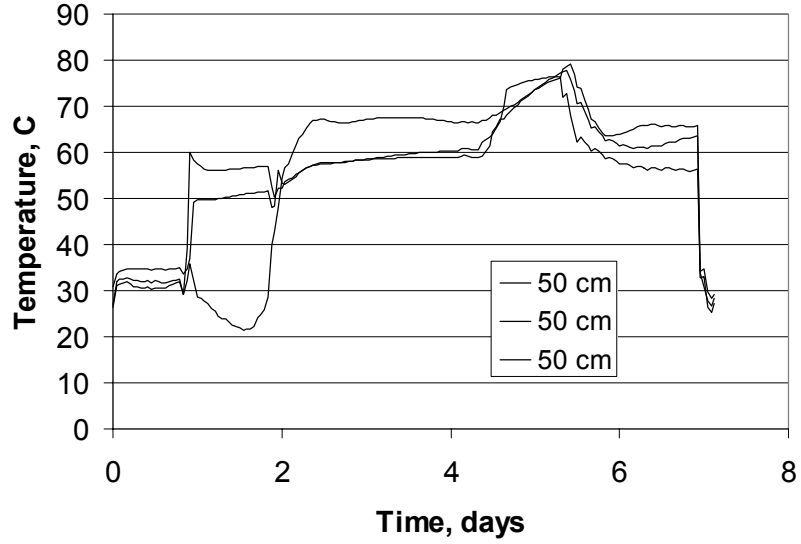


Fig. 12 Temperatures in composting green waste in tunnels at Hensby Composts.




Temperature, C
90
80
70
60
50
40
30
20
10
0
0
2
4
6
8
Time, days
50 cm
50 cm
50 cm

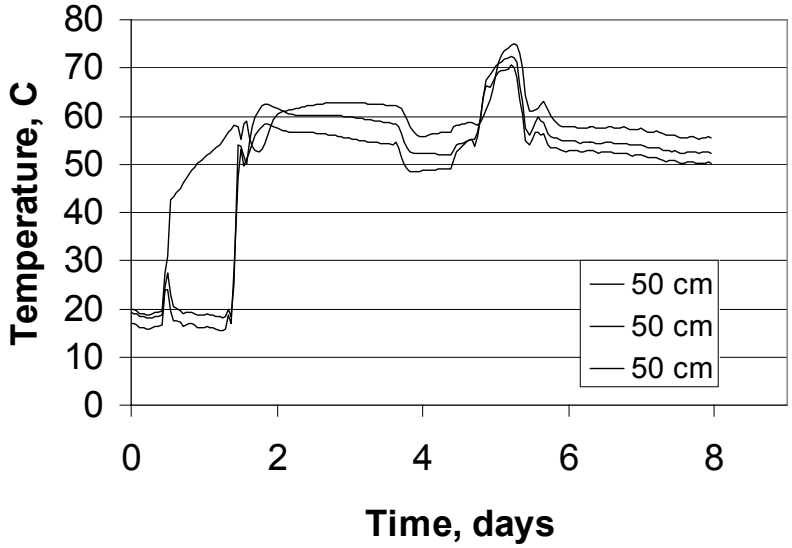
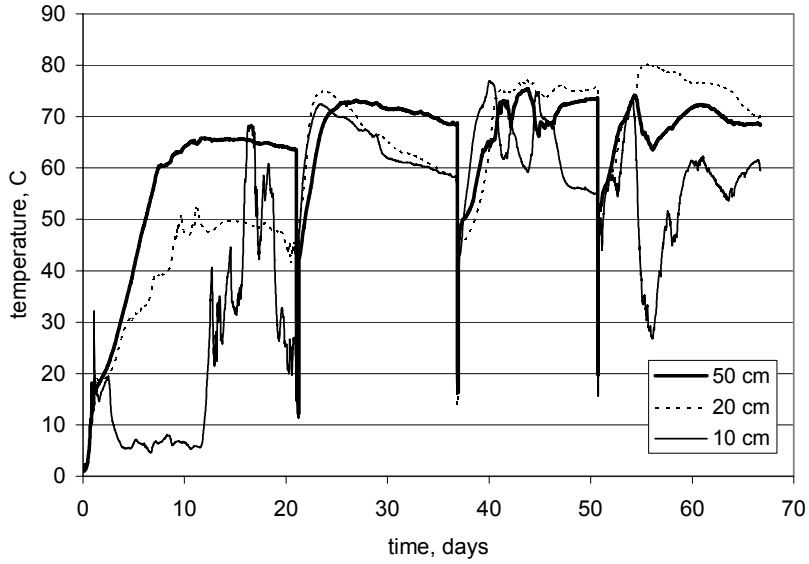

Temperature, C
80
70
60
50
40
30
20
10
0
0
2
4
6
8
Time, days
50 cm
50 cm
50 cm

Fig. 13 Temperatures in windrows of green waste, vegetable waste and straw at Organic Recycling



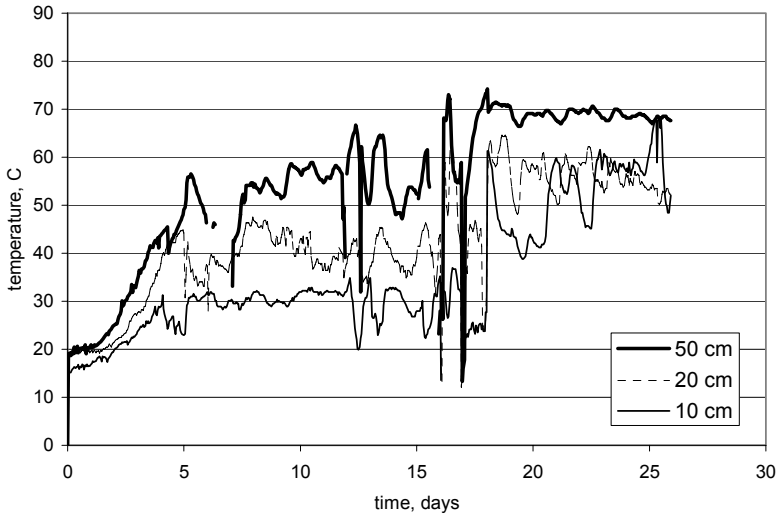


Fig. 14 Temperatures in windrow of vegetable waste, poultry manure and straw at HRI

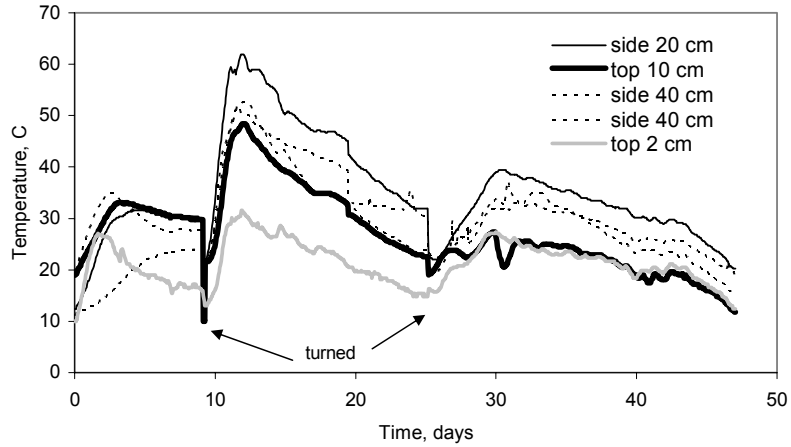


Fig. 15 Temperatures in tunnel of composting onion waste at HRI

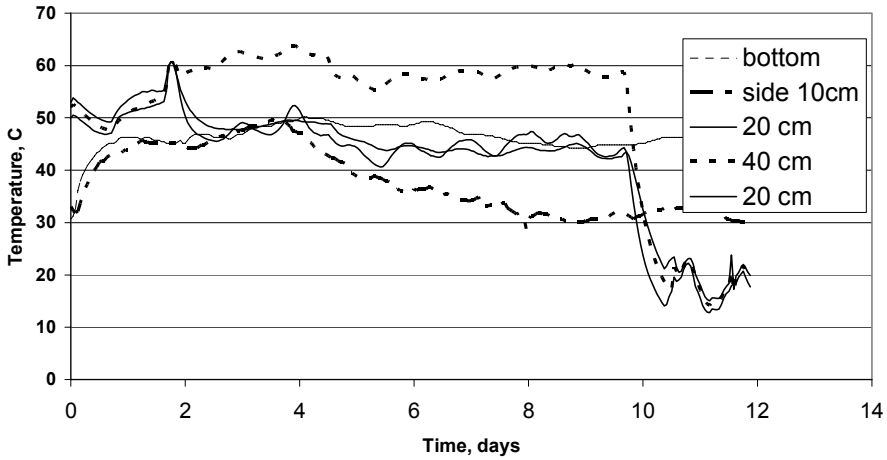


Fig. 16 Survival of animal/human pathogens during composting