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Evidence Project Final Report

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Project identification

1. Defra Project code: PH0402

2. Project title:
   Management of plant health risks associated with processing of plant-based wastes.

3. Contractor organisation(s):
   The Food and Environment Research Agency
   Sand Hutton
   York
   YO41 1LZ

4. Total Defra project costs (agreed fixed price): £ 664,130

5. Project: start date ..... 01 July 2006

   end date ..... 28 February 2011

6. It is Defra's intention to publish this form.

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   Defra recognises that in a small minority of cases there may be information, such as intellectual property or commercially confidential data, used in or generated by the research project, which should not be disclosed. In these cases, such information should be detailed in a separate annex (not to be published) so that the Evidence Project Final Report can be placed in the public domain. Where it is impossible to complete the Final Report without including references to any sensitive or confidential data, the information should be included and section (b) completed. NB: only in exceptional circumstances will Defra expect contractors to give a "No" answer.

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   (b) If you have answered NO, please explain why the Final report should not be released into public domain

Executive Summary

7. The executive summary must not exceed 2 sides in total of A4 and should be understandable to the intelligent non-scientist. It should cover the main objectives, methods and findings of the research, together with any other significant events and options for new work.

Landfill sites for waste disposal are a finite and diminishing resource in the UK. There also strict targets for the reduction of biodegradable material going to landfill annually, for example those imposed by the European Union (EU) Landfill Directive (EC, 1999). Composting is a more environmentally friendly means of disposal of green wastes than landfill, with the further benefit that the resulting compost may be used on private, public and commercial premises so reducing the need for artificial alternatives. The composting process usually consists of three relatively distinct phases: an initial mixing period which favours the growth of mesophilic micro-organisms (species for which optimum growth temperatures are within the range 30 °C to 45 °C), a high temperature thermophilic (or sanitization) phase (from 45°C, up to about 70°C, or possibly 80°C), and a longer and lower temperature mesophilic (maturation or stabilization) phase. The reduction in the number of pathogens during composting is not entirely the result of temperature, but is due to the complex interaction of a number of physical and organic factors, however, it has been identified as an important factor.

The overall aims of the project were to:

1. Determine the specifications for safely composting plant materials to minimise the risk of carry-over of plant pathogens and seeds that could cause future problems for agricultural and horticultural crop production.
2. Assess the use of indicator organisms and materials for validating conditions during composting.
3. Demonstrate the practical use of composting in plant health protection and produce recommendations in support of industry guidance such as PAS100 and Defra policy decisions.

Organisms for study were chosen to represent important plant pathogens in four major taxonomic groups: bacteria -*Clavibacter michiganensis* subsp. *sepedonicus* (*Cms*) potato ring rot; protoctists-plasmodiophoromycetes *Plasmodiophora brassicae* (clubroot in brassicas) and *Polymyxa betae* (the vector for *Beet necrotic yellow vein virus* (*BNYVV*), the causal organism of rhizomania); fungi - *Phytophthora kernoviae* and *P. ramorum*, *Synchytrium endobioticum* (potato wart disease), *Phytophthora cinnamomi* and *P. nicotianae, Fusarium oxysporum* f.sp. *cepae* and f.sp. *radicis-lycopersici, Microdochium nivale, Sclerotinia sclerotiorum*; and viruses/viroids -*Potato spindle tuber viroid* (*PSTVd*). Five of the organisms studied are quarantine pathogens under the Plant Health Directive 2000/29/EC; *P betae* due to its association with *BNYVV* is regulated in some EU member state countries. The other organisms were chosen for their relevance to agriculture and horticulture in the UK.

The main outputs from the project are outlined below:

Review of information available for a range of organisms of interest

The main conclusions of the review on composting and anaerobic digestion were:

- Eradication conditions during composting for a number of pathogens of quarantine importance,

including *Phytophthora ramorum* and *P. kernoviae,* is lacking or unclear
- Detection methods used post-composting should assess pathogen viability
- Detecting viability of *P. ramorum* and *P. kernoviae* with mRNA is a possible option
- TMV is unsuitable for use as an indicator of compost sanitization.

Methods for assuring reliability of composting processes

Several plant pathogens were found to be either too temperature tolerant (*Plasmodiophora brassicae*) or too sensitive (*Phytophthora* species) to be useful as indicators of compost sanitisation. A relationship between compost temperature and subsequent tomato seed viability enabled the sanitizing effect of the composting process to be estimated. Chlamydospores of *Fusarium oxysporum* f.sp. *radicis-lycopersici* were also suitable indicator organisms due to their moderate temperature tolerance, and ease of retrieval on selective media post-composting. Discolouration of *Rhododendron ponticum* leaves in response to compost temperature was found to be a useful on-site method for checking the sanitisation of this type of plant waste.

Temperatures and times needed to eradicate specific pathogen propagules

Phytophthora cinnamomi* and *P. nicotianae. Mycelial peat-based inocula were reduced to below a detectable limit when exposed to a compost temperature of 40°C for 3 days (1 day for *P. cinnamomi*) or 45°C for 1 day (or higher and/or longer periods).***Fusarium oxysporum* f.sp. *cepae* and f.sp. *radicis-lycopersici.*** Chlamydospore inocula were reduced to below a detectable limit when exposed to a compost temperature of 40°C for 14 days, 7 days at 45°C, 3 days at 50°C, 1 day at 55°C (or higher and/or longer periods).***Microdochium nivale.*** Chlamydospores of four isolates were reduced to below a detectable limit when subjected to a compost temperature of 40°C for 3 days.***Sclerotinia sclerotiorum****.* No viable sclerotia of two isolates were detected after being exposed to a compost temperature of 45°C for 7 days or 50°C for 1 day or longer.

Pathogen eradication from infected plant material during composting

Plasmodiophora brassicae*.* Galled root material of two isolates was composted at up to 65°C for 7 days. All bioassay test plants grown with the composted material produced positive Taqman PCR results, and all the test plants produced galled root symptoms for one of the isolates. ***Phytophthora kernoviae* (*Pk*) and *P. ramorum* (*Pr*).** Isolates were grown on *Rhododendron* leaves, cellophane discs and in a peat-based medium. *Pr* inoculum contained hyphae, sporangia and chlamydospores; *Pk* inoculum contained hyphae and sporangia. There was no survival of *Pk* or *Pr* after exposure to compost at 40°C for 1 day. ***Polymyxa betae -*** *BNYVV*-free and *BNYVV*-positive *P. betae*-infected soil was treated in compost at ambient (17°C), 40°C, 55°C or 65°C for various times between one and 22 days inclusive. Survival of *P.betae* was confirmed by sugar beet seedling bait tests. Results suggested that the PAS100 recommended conditions of 65^{o}C for 7 days are probably suitable for removing viable *P.betae*, but preferred conditions would be 55^{o}C for 22 days or 65^{o}C for 15 days. ***S.endobioticum*** - spores from infected potato tubers were inserted into compost and subjected to a range of temperatures (ambient to 65^{o}C) for between 1 and 27 days. Following composting, the material was assayed for viable wart spores using bait potato plants. No viable wart disease was detected for composting temperatures over 50°C at any duration from one to 14 days and therefore the conditions recommended by PAS100 should be suitable. ***Clavibacter michiganensis* subsp. *sepedonicus* (*Cms*)** potato ring rot- Potato cores from infected tubers were composted in laboratory scale flask units. Two experiments were carried out in which flasks containing moist or dry compost were treated at 50°C, 60°C and 70°C or at ambient temperature (15^{o}C).To assess samples for the presence of viable ring rot cells they were inoculated into eggplants, then sap from the eggplants was tested using a DNA based PCR assay and also using an mRNA based PCR test. Taking into account the results of the RNA tests, the minimum treatments which eliminated *Cms* were 15^{o}C for 4 days, 50^{o}C for 2 days or 70^{o}C for 1 day. The PAS100 conditions of 65^{o}C for 7 days should therefore be more than adequate ***Potato spindle tuber viroid (PSTVd)*** - fresh *PSTVd*-infected tomato leaf was treated in moist and dry compost in flasks as described above at ambient temperature, 50°C and 60°C for between one and 56 days. The conditions for composting recommended by PAS100 are probably not good enough to ensure removal of *PSTVd* to below detectable levels reliably – therefore treatment at 65°C for a longer timescale such as 14 days would be preferred.

Pathogen survival risks in waste feedstocks in large-scale composting systems

Composts were prepared from shredded plant wastes in insulated composting bays at Warwick HRI and in turned windrows at three other commercial sites. Pathogen survival risks in different locations in the composts were assessed by measuring time-temperature profiles, and by inserting indicator organisms (*F. oxysporum* chlamydospores, *P. brassicae* inoculum, tomato seeds). The results showed that the predominant survival risk was in the 10 cm surface layer of the composts; at greater depths, compost temperatures exceeded 55°C for at least 7 days, and the viability of the indicator organisms, except *P. brassicae*, was not detectable.

In-situ composting system for sanitising *Pr* and *Pk* infected wastes

Insulated composting bays were constructed at Warwick HRI, and National Trust gardens Trengwainton, Cornwall and Biddulph Grange, Staffordshire. All the bays were filled with about 8 m^{3} shredded rhododendron and other shrub wastes on 2-6 occasions. Mean temperatures of at least 53°C were achieved in the all the batches of waste; maximum temperatures were over 70°C. The minimum mean compost temperatures were recorded in the lower corners of the bays (32–44°C) which was filled into the

centre on subsequent turns.

Establish eradication of *Pr* and *Pk* in composted infected plant wastes

Post-composting, leaves that tested positive for *Pk* or *Pr* using semi-selective plating were only retrieved from the corners of the composting bay at Trengwainton. There were strong negative overall effects of mean compost temperature on the proportions of leaves that tested positive for *Pk* or *Pr* post-composting. The fitted regressions have the following equation:

(1) $\ln(P/(1-P)) = bT + a + \ln(I_0)$

where P is the proportion of positive *Pk* or *Pr* leaves after 5 or 10 days in compost, T is the mean compost temperature, I_0 is the proportion of positive *Pk* or *Pr* leaves in the day 0 sample, and a and b are the model intercept and slope. No survival of *P. ramorum* was detected after exposure of infected leaves for 5 days to a mean temperature of ≥ 41.9°C (32.8 °C for *P. kernoviae*) or for 10 days at ≥ 31.8°C. Fitted survival probabilities of *P. ramorum* on infected leaves exposed for 5 days at 45°C or for 10 days at 35°C were less than 3%, for an average initial infection level of leaves of 59.2%. RNA quantification to measure viability was shown to be unreliable in environments that favour RNA preservation: high levels of ITS1 RNA were recovered from *Pk* and *Pr* infected leaves exposed to compost at >53°C, when all culture results were negative, possibly due to elevated temperatures preventing the enzymatic degradation of RNA.

Pr and Pk eradication in Rhododendron and Vaccinium wastes in mobile composting units

Two types of mobile composting system were developed for composting and sanitising up to 20m^3 batches of shredded plant wastes: (a) system constructed of insulated refrigerated cargo containers (b) insulated composting bay. The costs of the systems were less than £2500. Mean compost temperatures (50-60^{o}C) and exposure times (>30 days) achieved in shredded rhododendron or chopped bilberry wastes in the majority of the compost in the insulated composting systems were above those needed to reduce *Pr* and *Pk* inocula to below detectable limits, and to eliminate any green colour in the indicator leaves. The exception was in the corners of the systems that contained >4 m^3 waste, and in the outer surfaces at one site where the volume of Vaccinium waste was only 2.9 m^3 (Tregonning Hill, Cornwall). Temperature-time profiles of the composts and positioned infected Vaccinium stems (*P. pseudosyringae*) and indicator leaves demonstrated that the main pathogen survival risk was in the corners of the insulated composting systems; pathogen survival risk could be minimised by positioning the corner material into the centre of the composting system during sequential refilling.

Project Report to Defra

8. As a guide this report should be no longer than 20 sides of A4. This report is to provide Defra with details of the outputs of the research project for internal purposes; to meet the terms of the contract; and to allow Defra to publish details of the outputs to meet Environmental Information Regulation or Freedom of Information obligations. This short report to Defra does not preclude contractors from also seeking to publish a full, formal scientific report/paper in an appropriate scientific or other journal/publication. Indeed, Defra actively encourages such publications as part of the contract terms. The report to Defra should include:
   - the objectives as set out in the contract;
   - the extent to which the objectives set out in the contract have been met;
   - details of methods used and the results obtained, including statistical analysis (if appropriate);
   - a discussion of the results and their reliability;
   - the main implications of the findings;
   - possible future work; and
   - any action resulting from the research (e.g. IP, Knowledge Exchange).

Project Objectives.

1. Identify the most suitable (the most resistant to composting and relevant to plant health) organisms for use in composting experiments.

2. Develop methods than can reliably and accurately detect viable plant pathogens in plant-based waste before and after composting/digestion (with an aim to detect <102 propagules/g substrate for the range of plant pathogens to be tested), with the aim of achieving adequate detection methods for use in Objectives 3 & 4. Develop methods for monitoring temperature for quality assurance.

3. Determine the temperatures and times, and other composting and digestion conditions, that are required to eradicate known populations of pathogen propagules to below minimum detection limits.

4. Compare the conditions needed to eradicate pathogens from naturally-infected plant material during composting and anaerobic digestion with those obtained for specific propagules in (1). Laboratory experiments will be used to model conditions required to eradicate pathogens.

5. Identify, and test at the commercial scale, specifications (based on 3 and 4) for large-scale industrial composting of plant waste that minimise survival, re-growth and risks of escape of plant pathogens.

6. Disseminate the research findings and explore opportunities for commercial take-up of the results.

Feasibility study for composting SOD waste:

7. Develop a testing procedure for detecting Phytophthora ramorum and P. kernoviae in SOD infected plant materials and composts, capable of detecting <102 propagules g-1 substrate.

8. Optimise the mechanical pre-treatment and composting of comparable non-infected plant material in terms of cost, composting efficiency and with the minimum of other input materials.

9. Develop an in-situ composting system that uniformly achieves sufficiently high temperatures (>50°C for 1 week) with the prevalent types of infected material and during different seasons.

10. Establish the eradication of Phytophthora ramorum and P. kernoviae in the composting systems in (8) and (9) using the method developed in (7), to produce a defined testing methodology to ensure composted material is free of P. ramorum and P. kernoviae.

11. Report and disseminate the research findings.

SOD composting at low temperatures:

12, Identify the most suitable Fusarium oxysporum formae speciales and isolates for use as compost sanitisation indicator organisms.

13. Test and improve the sanitisation efficiency of the developed composting system for SOD infected wastes during periods of low ambient temperature.

14. Dissemination of the research findings.

Mobile composting extension:

15. Develop a mobile in-vessel composting system using non-infected host plant material.

16. Test Pr/Pk eradication conditions in Rhododendron and Vaccinium wastes in mobile and insulated bay composting units.

17. Estimate operational costs of the composting methods.

Detailed report by objectives and milestones:

Objective 1. Identify the most suitable (the most resistant to composting and relevant to plant health) organisms for use in composting experiments.

A review of current knowledge on the sanitisation of plant wastes by composting was completed in the first year of the project and published as a paper in Bioresource Technology (see Noble *et al*, 2009 and Annex 1). The review identified the most important organisms to investigate in the current project. The choice of organisms included both quarantine and non-quarantine organisms: organisms with hardy resisting spores; *Polymyxa betae* (*P.betae*), *Plasmodiophora brassicae (P.brassicae)*, *Synchytrium endobioticum* (*S.endobioticum*): heat tolerant viruses and viroids ;*Potato spindle tuber viroid* (*PSTVd)*; other quarantine organisms of topical concern: *Phytophthora ramorum* and *P. kernoviae*, *Clavibacter michiganensis* subsp. *sepedonicus (Cms)*, and other plant pathogens of current commercial concern: *Rhizoctonia solani*, *Fusarium oxysporum* f. spp., *Phytophthora* spp.and *Sclerotinia sclerotiorum*. Plant seed were also included.

Milestone 1. Review information available for a range of organisms of interest
The main conclusions of the review were:

- There is no information on the eradication conditions during composting for a number of pathogens of quarantine importance. These include fungi (*S. endobioticum*, *Guignardia citricarpa*, *Tilletia indica*), oomycetes (*P. kernoviae*), bacteria (*C. michiganensis* subsp. *sepedonicus*), and viroids (*PSTVd*).
- Organisms with temperature tolerance include Plasmodiophoromycetes producing resting spores (*P. brassicae* and *P. betae*), fungi that produce chlamydospores (*Fusarium oxysporum* and *Microdochium nivale*) or sclerotia (*Sclerotinia sclerotiorum*, *Macrophomina phaseolina*), and viruses with protein coats (*Tobacco mosaic virus*).
- *P. brassicae* and *TMV* are currently recommended by EPPO for use as indicator organisms of compost sanitisation but their high temperature tolerance and/or variability could mean they are unsuitable for this purpose.
- There is conflicting data on the temperatures and exposure times needed to eradicate *P. ramorum*, and the minimum requirements to eradicate this pathogen and *P. kernoviae* during composting have not been precisely defined.
- Detection methods exist for the quarantine organisms under consideration although methods for detecting *G. citricarpa* are poor. Detecting viability of *P. ramorum* chlamydospores is also problematic but the use of mRNA detection is a possible option.
- It is important that that the detection methods used post-composting should be able to assess viability as well as presence of the pathogen. Validated bioassay tests exist for *Cms*, *PSTVd*, *S. endobioticum*, and *Pepino mosaic virus*. Although detection methods exist for *Polymyxa* spp., *P. ramorum* and *P. kernoviae*, they require further validation.

Objective 2. Develop methods than can reliably and accurately detect viable plant pathogens in plant-based waste before and after composting/digestion (with an aim to detect <10^2 propagules/g substrate for the range of plant pathogens to be tested), with the aim of achieving adequate detection methods for use in Objectives 3 & 4. Develop methods for monitoring temperature for quality assurance.

In order to evaluate the survival of the chosen organisms through the composting process it was important to use inocula containing appropriate survival structures for the organisms concerned, for example, chlamydospores in the case of *Fusarium* spp. Methods for the production of these inocula are detailed below:

Milestone 2. Develop production methods for fungal propagules
Fusarium oxysporum formae speciales and *Microdochium nivale.* Petri dish cultures of *F. oxysporum* f.sp. *cepae* and f.sp. *radicis-lycopersici* and *M. nivale* were grown on potato dextrose agar (PDA) which were used to produce mycelial and conidial suspensions for inoculating sterilised talc. The talc was kept for 6 weeks at 20°C to allow chlamydospores to develop.
Sclerotinia sclerotiorum. Sclerotia were produced in moist sterile wheat grain, inoculated with *S. sclerotiorum* cultures grown on PDA.
Phytophthora species. Inocula of *Phytophthora cinnamomi* and *P. nicotianae* isolates were prepared in a peat-based medium . The medium was moistened to 50 % w/w moisture, sterilised and inoculated with the *Phytophthora* cultures grown on PDA. The peat-based media were incubated at 20°C for 21 days, after which they contained hyphae and hyphal swellings.

Further details are provided in Annex 2.

Milestone 3. Develop methods for producing test plant materials
P. brassicae. Infected 'galled' root material was produced by adding infected roots or soil to a peat-based growing medium in pots into which Chinese cabbage (*Brassica oleracea pekinensis*) seedlings were transplanted. The galls

were harvested after 6 weeks and stored before use for up to 6 months at 2°C.

P. kernoviae and *P. ramorum*. Each isolate was grown on *Rhododendron* leaves, cellophane discs, and in a peat-based medium.

P. betae and *Beet necrotic yellow vein virus* (BNYVV). *P.betae* is an obligate biotroph and is present in soils as resting spores (cystosori). These resting spores are likely to be the most resilient to high temperatures. Soil containing BNYVV-free *P. betae* cystosori (Rothamsted Research, Broom's Barn, Bury St. Edmunds, UK) was used as inoculum for flasks.

PSTVd. Stock tomato leaf material (cultivar Rutgers, infected with PSTVd reference culture AJ583449, Food and Environment Research Agency) was confirmed as being infected with PSTVd using a PCR-based assay for PSTVd RNA (Boonham *et al.* 2004).

S. endobioticum Harvested gall tissue of potato wart disease (Pathotype 1; source Kelvin Hughes,FERA) was air-dried in order to produce winter sporangia (the most resistant type of spore),

Potato ring rot *(Cms).*Potato tubers (cultivars Estima, Maris Piper, Cara and Saxon) were confirmed as being infected with the ring rot organism using a polymerase chain reaction (PCR) DNA-based assay (Schaad *et al.* 1999) on a small sample of each tuber. Using a small cork borer, cores (approximately 0.1g each) were taken from each potato tuber, taking care to ensure each core included material from the vascular ring where infection normally proliferates. After thoroughly mixing all cores, 10 cores were drawn at random and wrapped in nylon mesh as detailed above.

Further details are provided in Annex 2 and 6.

Milestone 4. Detecting pathogens using bioassays, serological and molecular methods

The key issue in the choice of methods for the detection of the organisms concerned was the requirement to determine that the organisms were still viable and therefore capable of causing infection. A second requirement was that the method should be sufficiently sensitive to detect levels of the organism that could cause a viable infection. Adequate methods existed for detection of a number of the organisms used in the project, however, these often proved unsuitable for use in detection of viable organisms in compost because they had been designed for detection in plant material. The methods used in the project are summarised below:

Fusarium oxysporum formae speciales and *Microdochium nivale.* After exposure to compost, talc containing chlamydospores was obtained from the retrieved bags and added to sterile distilled water. The suspension was plated on to plates of PDA + chlorotetracycline which were then incubated at 22°C for 6 days. Viable chlamydospores were detected by counting colony forming units (cfu) with mycelium and conidia typical of *F. oxysporum,* with a similar detection threshold to the method used for *Phytophthora.* Both formae speciales had pinkish mycelium facilitating differentiation from other moulds on the plates.

Sclerotinia sclerotiorum. The sclerotia were surface sterilised with 50:50 sodium hypochlorite solution: 100% ethanol. Viability was tested by assessing mycelial growth from halved sclerotia on PDA + chlorotetracyline at 20°C.

P. brassicae. Gall material was tested for viability using a Chinese cabbage seedling bioassay. Gall inoculum was mixed with peat-based growing medium and filled into pots. After six weeks, the plants were uprooted and assessed for clubroot symptoms expression on a 0-3 score scale. After assessment, the material from the test plants was tested for the presence of *P. brassicae* using a Taqman PCR detection method. The material was subjected to a Beemster & de Heij 'dangling' short bait test using oil seed rape to ensure only live cells were detected by the Taqman PCR.

Phytophthora cactorum, P. cinnamomi and *P. nicotianae.* A serial dilution of the material in the bags retrieved from compost was conducted on plates using PDA + PARPH agar. The plates were recorded for *Phytophthora* after three days incubation at 20°C by counting cfus with mycelium, hyphal swellings, and sporangia typical of *Phytophthora*.

Phytophthora infestans. Inoculum was produced on potato slices and retrieved from compost using PDA + PARPH agar.

P. kernoviae and *P. ramorum*. Inoculum was tested for viability by plate culture on PDA + PARPH agar. Plates from cellophane and leaf inocula were classed as positive if mycelia or spores characteristic of *Phytophthora* were present; plates from peat samples were classed positive if germinating spores were detected. RNA quantification by real-time polymerase chain reaction (RT-PCR) was also used as a method to measure viability.

S. endobioticum. Detection was achieved using a bioassay scored for presence of warts. Following composting,

samples were placed in large test tubes and vigorously mixed with sterile distilled water. After steeping in the water for 24 hours samples were applied (one sample per pot) to chitted potato tubers (var. King Edward) in plastic pots with coarse sand and Plantacote (slow-release nutrient capsules) at 4g/kg of sand (see figure 2 below). Two positive control pots were also set up using a sub-sample of the wart/talc mix that had not been composted. Negative controls (two pots) were also produced by simple omitting wart mix from the set up.

Potato ring rot(Cms). The eggplant bioassay of Lelliot and Sellar (1976) and EPPO (1990) was used. The limit of detection for this is quoted as10^3 cells /ml. This was combined with and RNA detection using RTPCR developed within the project.

P. betae. A sugar beet seedling bioassay broadly similar to that of Tuitert (1990) was used. Confimation of infection by identification of cystosori using light microscopy. The limit of detection for this method is 50–100 cystosori.

PSTVd. A bioassay using indicator tomato plants was used for detection of viable PSTVd as reported by Grasmich and Slack (1985),Spence (2004), Singh et al.(1990). This assay is reported to be able to detect 5.6 x 10^2 propagules/ ml.

Milestone 5. Pathogen detection thresholds for the methods developed for wastes and composts
The colonised substrates containing the pathogens in M4 were immersed in flasks of green waste compost for up to 14 days at temperatures between 18 and 55°C.
Phytophthora cinnamomi and *P. nicotianae.* The detection limit of the test following retrieval of inoculum from compost was 34 cfu g^{-1} peat-based medium.
Phytophthora cactorum and *P. infestans*. No inoculum of either species could be detected after retrieval from the compost flasks in the tests due to severe competition from other moulds. It was therefore decided to use *P. cinnamomi* and *P. nicotianae* for further tests due to the ease of retrieval from composts.
P. kernoviae and *P. ramorum*. The detection limit in the peat-based inoculum was 33 cfu g^{-1} for both *P. kernoviae* and *P. ramorum*.
P. brassicae. Viable inoculum was detected using both the plant bioassay and Taqman PCR detection methods after exposure of infected gall material to 60°C for 7 days.
Sclerotinia sclerotiorum. Viable sclerotia were detected after exposure to compost at 45°C for 3 days.

Further details are provided in Annexes 2 and 3.

Objective 3. Determine the temperatures and times, and other composting and digestion conditions, that are required to eradicate known populations of pathogen propagules to below minimum detection limits.

Objective 4. Compare the conditions needed to eradicate pathogens from naturally-infected plant material during composting and anaerobic digestion with those obtained for specific propagules in (1). Laboratory experiments will be used to model conditions required to eradicate pathogens.

Milestone 6. Investigate methods for assuring reliability of composting processes
The use of chemical temperature indicators (Omega Engineering Ltd) to determine composting temperatures proved impractical as they reacted instantly at the target temperature and gave no indication of exposure time or 'day degrees'. Experimentation was carried out to determine the usefulness of pathogens, seeds, and waxy leaves as indicator organisms.
Plasmodiophora brassicae was an unsuitable indicator due to very high temperature tolerance in dry to moist composts and difficulty in detecting viable inoculum post-composting, whereas several other plant pathogens (*Phytophthora cinnamomi, P. nicotianae, Microdochium nivale, Sclerotinia sclerotiorum*) were unsuitable due their low temperature tolerance in compost. A relationship between compost temperature and subsequent tomato seed viability determined from a tetrazolium test enabled the sanitizing effect of the composting process to be estimated in both the flask and large-scale systems. Plant waste type (woody or vegetable) had a small but significant effect on this relationship. *Abutilon* × *hybridum* seeds were more tolerant of compost temperatures >55 °C, but more prone to degradation at lower temperatures than tomato seeds. Chlamydospores of *Fusarium oxysporum* f.sp. *radicis-lycopersici* were also a suitable indicator organism due to their moderate temperature tolerance in compost, indifference to various plant waste types, and ease of retrieval on selective media post-composting.
Discolouration of *Rhododendron ponticum* leaves subjected to compost temperatures above 35 °C was found to be a useful on-site method for checking the sanitisation of this type of plant waste.

Further details are provided in Annexes 1, 2 and 4.

Milestone 7. Design experiments for laboratory and large-scale composting systems to account for inherent sources of variability
Statistical designs for flask and large-scale composting experiments were prepared by S. Pietravalle, FERA to take account of positional variability, both within bench-scale composting equipment or composting systems, and within incubators for viability testing. Factorial experiments were designed to determine the effects of temperature × time ×

isolate or seed species interactions on the viability of indicator organisms (*Phytophthora* spp., *Fusarium oxysporum* chlamydospores and tomato seeds). Due to the non-normality of the population of surviving propagules of some of the test species following compost treatments (*Phytophthora* spp., *F. oxysporum*) non-parametric statistics (Steel's many-one-rank test) were used for analysing some of the data. All analyses were done using Genstat 10.2.

Milestone 8. Determine temperatures and times needed to eradicate specific pathogen propagules under controlled composting conditions

Composting was conducted in bench-scale equipment consisting of 'Quickfit' multiadapter flasks immersed in thermostatically controlled water baths, each holding up to four 2-L flasks . Each flask contained green waste compost and was connected to equipment to aerate the compost. A series of separate experiments were conducted for each pathogen; the compost temperature and exposure time treatments within each experiment depending on the likely tolerance range of each organism, determined from preliminary tests. In each experiment three replicate flasks of each compost temperature treatment were prepared, each flask containing sufficient numbers of sample bags of the pathogen isolates for each of the specified exposure times.

(a) *Phytophthora cinnamomi* and *P. nicotianae*: A peat-based inoculum was composted at 18, 35, 40, 45, 50 and 55°C for 1, 3 and 7 days. Steel's many-one-rank test was used for comparing treatments with two controls: the day 0 inoculum propagule count, and the most extreme combination of temperature and time (55°C for 7 days) which was sufficient to eradicate all propagules of both species in all replicates (eradication control). For *P. cinnamomi*, the inoculum was reduced to below a detectable limit when exposed to a compost temperature of 40°C for 1 day (or higher and/or longer periods). For *P. nicotianae,* the inoculum was reduced to below a detectable limit when exposed to a compost temperature of 40°C for 3 days or 45°C for 1 day (or higher and/or longer periods).

(b) *Phytophthora cactorum* and *P. infestans*: Cultures were incubated at 18, 35, and 40°C for 3 days. Due to difficulties in retrieving uncontaminated inoculum from compost, the effect of temperature on plate cultures of *Phytophthora cactorum* and *P. infestans* was examined. Both species survived after exposure to 35°C but were eradicated at 40°C.

(c) *Fusarium oxysporum* f.sp. *cepae* and f.sp. *radicis-lycopersici*: Chlamydospores in talc were composted at 18, 40, 45, 50 and 55°C for 1, 3, 4, 7 and 14 days. Non-parametric statistics were again used for analysing the effects of compost temperature and exposure time on the surviving propagule population using Steel's many one rank test, in the same way for analysing *Phytophthora* propagule populations. Again two controls were used: the day 0 inoculum propagule count, and the most extreme combination of temperature and time (55 °C for 14 days) which was sufficient to eradicate all propagules of both formae speciales in all replicates (eradication control). For f.sp. *cepae*, the inoculum was reduced to below a detectable limit when exposed to a compost temperature of 40°C for 14 days, 3 days at 45°C, 1 day at 50 and 55°C, or longer periods of time at each of these temperatures respectively. For f.sp. *radicis-lycopersici,* the inoculum was reduced to below a detectable limit when exposed to a compost temperature of 40°C for 14 days, 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.

(d) *Microdochium nivale*: Chlamydospores in talc were composted at 18, 40 and 50°C for 3 days. The chlamydospores of four isolates were eradicated when subjected to a compost temperature of 40°C for 3 days.

(e) *Sclerotinia sclerotiorum*: Sclerotia were composted at 18, 45, 50 and 55°C for 1, 3 and 7 days. No viable sclerotia of two isolates were detected after being exposed to a compost temperature of 45°C for 7 days or 50°C for 1 day or longer.

Further details are provided in Annex 2.

Milestone 9. Determine the effect of waste composition and gaseous environment using laboratory systems

F. oxysporum f.sp. *cepae* and f.sp. *lycopersici*. Chlamydospores were subjected to temperatures of 18, 40 and 50°C for 4 days in the following media: green waste compost (moist and dry), anaerobic compost (zero detectable oxygen), vegetable waste, moist sand, and dry talc. Survival of both *F. oxysporum* ff.sp. was detected in all the media at 18°C. Survival of *F. oxysporum* f.sp. *cepae* was also detected in moist and dry composts, moist sand, and dry talc at 40°C but not in anaerobic compost or vegetable waste at 40°C or in any of the media after 4 days at 50°C. The results indicate that pathogen survival is more likely in dry to moist substrates than in wet or anaerobic substrates at the same temperatures.

Milestone 10. Compare pathogen eradication (temperatures-times, composition, etc) using infected plant material

(a) *Plasmodiophora brassicae*: galled root material was composted at 18, 50, 60 and 65°C for 1, 3 and 7 days. Although the intention was to investigate the presence of a relationship between compost temperature, exposure time and the probability of survival two *P. brassicae* isolates, the data were not seen to be suitable to investigate such a relationship. This was because the shift from positive to negative results for the for the FERA isolate, based on the appearance of gall symptoms in the test plants, was sudden and occurred in the same temperature × time treatment for almost all flask replicates, which prevented any model to be fitted to the score data. Inoculum of the FERA isolate composted at 50°C for 3 days, or at 60°C for 3 or 7 days did not produce gall symptoms in bioassay plants. Inoculum of the WHRI isolate produced positive gall symptoms in all the flask replicates following all the compost temperature × exposure time treatments, i.e. at least one out of three test plants grown with the inoculum from each replicate flask produced positive gall symptoms. All bioassay test 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.
(b) *Phytophthora kernoviae* and *P. ramorum* were used for bench-scale flask composting experiments. Each isolate was grown on *Rhododendron* leaves, cellophane discs by placing the discs over agar cultures, and in a peat-based medium. The *P. ramorum* inoculum contained hyphae, sporangia and chlamydospores; the *P. kernoviae* inoculum contained hyphae and sporangia. The following compost temperature × exposure time treatments were examined: 18 and 35°C for 1, 3, 5 and 10 days; 40 and 45°C for 1 and 3 days. There were between 2 and 6 replicate flasks of each temperature treatment. *P. kernoviae* survived as peat-based inoculum exposed to compost for 1 day at 35°C but did not survive this treatment as cellophane or leaf inoculum. There was no survival of *P. kernoviae* after exposure to compost for 3 days at 35°C or for 1 day at 40°C. *P. ramorum*, as cellophane, leaf and peat-based inocula, survived in compost at 35°C after 5 days but was eradicated after 10 days. There was no survival of *P. ramorum* after exposure to compost at 40°C for 1 day. After incubation at composting temperatures, sample bags containing leaf inoculum was also kept for a further 14 days at 20˚C in the compost to detect viability after potential 'dormancy'. No viability was detected in these inoculum samples.

(c) *P.betae*

BNYVV-free and BNYVV-positive (Brooms Barn) *P. betae*-infected soil was treated in compost flasks (between two and seven replicate flasks at each treatment) held at ambient (17°C; SD 0.62°C), 40°C, 55°C or 65°C for various times between one and 22 days inclusive.

Ambient temperatures did not limit the viability of *P betae*, with all time points up to 22 days showing 100% response. The same was also the case with 40°C except that only one out of three replicates still showed viable *P betae* after composting for 22 days. Results were more variable at temperatures above 40°C than below, with no clear dose/response relationship between temperature/time and viability of *P betae*. For example, following composting for four days at 55°C *P betae* was not detectable, however, composting for seven days at 55°C produced one positive replicate out of three. Similarly, at 65°C, 0/3 replicates were positive after two days composting whilst 1/3 replicates were positive after three days. Despite the lack of a definable dose/response trend, higher temperatures and longer composting times did, on the whole, result in fewer replicates with viable *P betae*. As there were no differences between results for *P betae* with and without *Beet necrotic yellow-vein virus*, all results were pooled to estimate the likelihood of the observed distribution of zero viabilities occurring by chance. The results for the treatments were explored using Bayesian simulations and the beta distribution. In 100,000 simulations there were no cases generated in which all of the results for the treatments were negative. Hence the likelihood of the observed results occurring by chance is less than 0.001%.

Therefore, in summary, the PAS100 recommended conditions of 65^{o}C for 7 days are probably suitable for removing *P.betae*, but preferred conditions would be 55^{o}C for 22 days or 65^{o}C for 15 days.

(d) *PSTVd*

Bench-scale composting in moist and dry compost was carried out as described above at ambient, 50°C and 60°C using approximately 0.8g of fresh *PSTVd*-infected tomato leaf per compost 'parcel' with either two or four replicates per treatment for between one and 56 days.

The relationships between temperature, composting duration and survivorship, the data were analysed using probit analysis. Visual consideration of the data suggested that duration of composting (beyond 24 hours - no data were generated for less than 24 hours) was not a significant factor in wart survival and that temperature was the more important factor. This hypothesis was investigated by constructing three temperature/mortality probit curves: all data combined; data up to seven days composting; and data for composting for more than seven days. There was found to be no difference between data from up to seven and less than seven days (p = 0.945). This was confirmed using accumulated analysis of deviance, which showed no significant additive effect of composting duration over the effect of temperature on mortality (p = 0.30). The probit curve constructed using all data predicts that the upper 95% confidence limit of the composting temperature required to kill 99% of wart organisms was 60.7°C and that the upper 95% confidence limit of mortality if composting was conducted at 65°C for at least 24 hours is 99.9%.

It is disappointing that the data from composting *PSTVd* were not susceptible to any of the usual statistical analyses. This is indicative of considerable variability in the susceptibility of the organism to control during the composting process. The survival of the viroid for seven days at 65°C in dry compost whilst it was absent after composting for 2, 3 and 4 days at this temperature is of particular concern. Whilst the PAS100 standard calls for 7 days composting at ≥65°C with >50% moisture content and the moisture content of the dry compost was only around 20% water, it nevertheless calls into question the safety of using composting to sanitise waste containing *PSTVd*. Although it may be tempting to consider an absence of *PSTVd* after 14, 28 and 56 days composting at 65°C as some assurance that longer composting periods are efficacious for control, the absence of a fitted model to calculate a probability of successful sanitisation under these treatments cautions against this.

In summary, the PAS100 recommended conditions are probably not enough to be certain that *PSTVd* will be

eliminated. Treatment at 65°C for a longer timescale such as 14 days would be preferred.

(e) *Cms*

Potato cores from infected tubers were composted in laboratory scale flask units. Two experiments were carried out in which four flasks (two moist and two dry compost) were placed in a water bath at 50°C, four in a water bath at 60°C and four at 70°C or at ambient temperature (15^{o}C). To assess samples for the presence of viable ring rot cells they were inoculated into eggplants, then sap from the eggplants was tested using a DNA based PCR assay and also using an mRNA based PCR test.

Using PCR-based DNA tests for *Cms* produced positive results for potato cores at the highest temperature/longest composting time combinations for both dry and moist compost. Similarly, positive DNA results were found for sap from eggplants inoculated with one of the two core samples that had been composted in moist compost for four days at 70°C. Although neither sample from dry composting at 70°C resulted in *Cms* DNA being detected in corresponding sap samples, both core samples from dry composting at 70°C for three days did result in positive DNA tests in sap samples. In contrast, none of the bioassay eggplants from the treatment groups and only one of two positive control plants showed symptoms of ring rot.

RNA testing of cores from composting in experiment 2 also showed a significant number of positive results at the higher temperatures (50°C and 70°C), although no positive results were recorded at 11 days regardless of temperature. Conversely, composting at ambient temperatures returned a positive result in only one of two cores after one day's composting, with all other time periods being negative. The RNA tests on bait plants from experiment 2 produced a different pattern of results from both the core and sap results of experiment 1 and the core results in experiment 2, with *Cms* RNA only being detected following ambient composting for three days or less and from composting at 50°C for just one day.

It is our belief that heating compost (particularly the well-matured compost heated artificially in these experiments) has the effect of destroying natural dexoyribonucleases (DNases) and RNases in the compost, therefore allowing the DNA and RNA from dead *Cms* cells to persist in the composted potato cores. This "sterilising" effect on enzymes of degradation explains why more potato cores were positive for *Cms* RNA at 50°C and 70°C than at ambient temperature. "Surviving" DNA from dead cells seems also to persist throughout the bait test bioassay and produces false positives in eggplant sap samples. RNA from dead cells, being more labile than DNA, cannot survive the bait test and we conclude that the tests of *Cms* RNA on sap from eggplants inoculated with composted CMS-infected potato cores gave the best indication of the compost conditions that can control *Cms* in infected plant wastes.

Therefore taking into account the results of the RNA tests, the minimum treatments which eliminated *Cms* were 15^{o}C for 4 days, 50^{o}C for 2 days or 70^{o}C for 1 day. The PAS100 conditions of 65^{o}C for 7 days should therefore be more than adequate.

(f) *S.endobioticum*

Mesh bags containing *S.endobioticum* spore mixture were inserted into the bench-scale compost flasks and subjected to one of a range of temperatures (ambient to 65^{o}C) for between one and 27 days. Two to eight replicates were constructed for each temperature/time combination (in three separate experiments).Following composting, the wart/talc material was assayed for viable wart spores by a method based on the EPPO bioassay (EPPO, 2004) using bait plants.

Although it seems reasonable to suppose that there is an inverse relationship between temperature/exposure time and the viability of wart spores statistical analysis of the data did not bear this out as there was no effect of composting duration on mortality over and above that caused by temperature. It is possible that time of exposure to the compost is important over time scales less than 24 hours, but this could not be investigated due to the nature of the flask system. Regardless of the time/mortality relationship up to 24 hours, it appears that for any given temperature the susceptible individuals have been killed during the first day and the remaining spores can endure that temperature for a much greater time. It is possible that these resistant spores would be killed by exposures greater than those used in this study (i.e. >27 days), but this would be very problematic on a practical basis in commercial composting operations. The current UK compost standard, PAS 100:2011 (Anon, 2011) specifies a temperature of 65°C for seven days: the results predict that 65^{o}C would control 99.9% of spores with 95% confidence, and it is reassuring to note that no viable wart was detected for composting temperatures over 50°C at any duration from one to 14 days (72 replicates).

One interesting feature of this work is the appearance of warts well beyond the recommended 100-day observation period up to 180 days . It is not known whether warts may have started to appear in negative treatment pots if the observation had been carried on for longer. However, by 180 days the assay plants were starting senesce. If the inoculum potential can be reduced by composting to the point where the disease is not able to establish over the

course of a growing season, which is unlikely to be more than 180 days, then in a field situation, unless there was some external addition of viable spores, it would be unlikely to establish in subsequent seasons.

Further details are given in Annex 3 and 6.

Milestone 11. Determine the survival of pathogens in compost leachate and bioaerosols in laboratory-scale composting system

Fusarium oxysporum f.sp. *cepae* and f.sp. *radicis-lycopersici*. Chlamydospores of both formae speciales were exposed to compost leachate and heated moist air above compost in flask experiments. Chlamydospores of f.sp. *cepae* survived at 40˚C for at least 4 days in bioaerosol but were eradicated after 14 days at this temperature or after 1 day at 50˚C. Chlamydospores also survived in compost leachate for 4 days at 18˚C but no survival was detected after 4 days at 40˚C. Chlamydospores of f.sp. *radicis-lycopersici* did not survive after 4 days in either leachate at 18˚C or bioaerosols at 40˚C.

The results indicate that the eradication conditions for *F. oxysporum* are similar in moist air than they are in moist green waste compost. The probability of survival in compost leachate is lower than that in moist green waste compost at the same temperature.

Objective 5. Identify, and test at the commercial scale, specifications (based on 3 and 4) for large-scale industrial composting of plant waste that minimise survival, re-growth and risks of escape of plant pathogens.

Milestone 12. Establish pathogen survival risks in waste feedstocks in large-scale composting systems

Composts were prepared in insulated composting bays at (A) Warwick HRI, Wellesbourne, and in turned windrows at three other sites: (B) Organic Recycling Ltd, Peterborough (C) G's Vegetables, Ely, Cambridgeshire, and (D) Simpro Ltd, Gaydon, Warwickshire. Waste feedstocks were first mechanically crushed and shredded to produce pieces of about 50 mm length before being filled into the bays or formed into windrows. Windrows at the three sites were about 50 m long and 4.5 m wide at the base, with sloping sides reaching an apex about 3 m from the concrete base. Composts in the insulated bays were prepared from potato waste or shredded rhododendron. The composts at sites A and C were prepared from green wastes, predominantly stems and leaves of woody shrubs from parks and gardens, and the compost at site B was produced from waste onions. The temperature of the composting wastes was then allowed to rise for about 7 days. Sample bags of indicator organisms were prepared as for flask experiments and enclosed in nylon mesh sacks containing the same compost that was in the large-scale systems and a temperature probe connected to a data logger . The sacks were inserted into different locations of the large-scale systems (centre, corners, sides), from the surface to depths of up to 0.7 m, and at the base, to obtain a range in exposure temperatures. Sample bags of inoculum of each of two *F. oxysporum* and *P. brassicae* isolates and tomato and Abutilon seeds were inserted in each of the sacks in the composts. All sample bags were retrieved from the composts after two to ten days and the viability of the indicator organisms determined as previously described. Compost temperatures in the 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 the windrow green waste composts at sites B and D ranged from 36 ± 6 °C on the surface to 72 ± 2 °C at depths of 0.3 to 0.5 m. In the windrow onion waste composts at site C, temperatures ranged from 33 ±7 °C on the surface to 46 ±2 °C at a depth of 0.3 to 0.5 m.

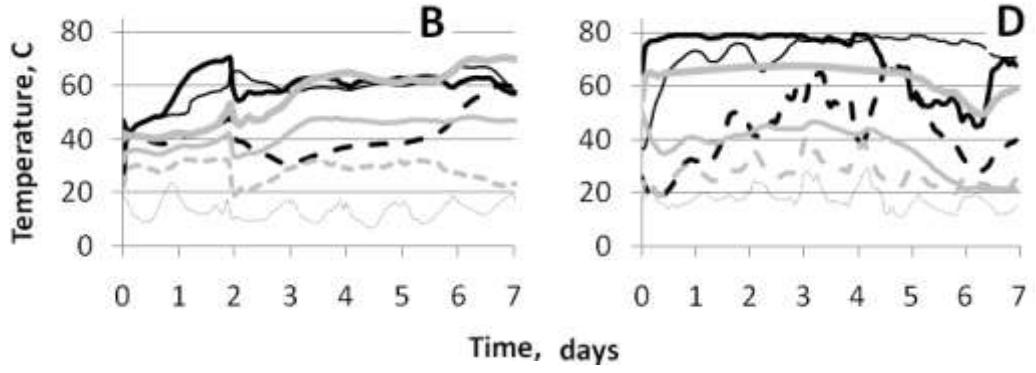


Fig. 1. Compost temperatures at two large-scale windrow composting sites; top of windrows, black lines; side of windrows, grey lines; thick solid lines 30, cm depth; thin solid lines, 10 cm depth; dashed lines, surface; dotted lines, air temperature.

The seed viability scores data obtained from the composting sites was analysed using multiple linear regression, using a forward stepwise regression. In this approach, each of the four 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 was repeated with the remaining variables and interactions, when appropriate. There was strong evidence of a negative effect of temperature ($P < 0.001$) on the viability scores of tomato seeds. Waste type was also shown to have a significant effect ($P < 0.001$), with, overall, larger viability scores for potato and vegetable wastes than for woody wastes. However, there was also a significant interaction between

temperature and waste type (P = 0.004), with viability score decreasing more rapidly with increasing temperature for potato and vegetable wastes than for woody wastes. The effect of exposure time within the range 2–10 days on seed viability score was not significant.
Results for exposure of *P. brassicae* inoculum in the large-scale composting system were not statistically analysed due the lack of variability between replicate test plants, i.e. almost all of the test plants for the same temperature treatment produced the same positive or negative gall symptom result. All of the positive control samples and all the 40°C treated samples of both isolates produced score 3 gall symptoms in the bioassay tests plants. All the *P. brassicae* inoculum samples exposed to mean temperatures of 52 to 59°C for 2 days in potato compost produced score 1 or 2 gall symptoms in test plants, and inoculum exposed to the same temperatures in the rhododendron compost resulted in at least one out of four test 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 test plants, although two negative results were obtained from samples of test plant that had gall symptoms. Positive Taqman PCR results were obtained from all the test plant samples, except the negative controls, and plants grown with the WHRI isolate inoculum exposed for 2 days at 64°C in potato compost or at 56°C in rhododendron compost, or the FERA isolate inoculum exposed for 2 days at 52°C in rhododendron compost.
A similar multiple regression approach was taken for the *F. oxysporum* chlamydospore data from the composting sites as for the tomato seed viability data, although it was decided to only look at the data as binary (survival or no survival), instead of looking at the actual number of surviving spores. The reason for choosing this approach was the range of surviving spores observed: for many combinations of compost treatments, no survival was observed, whereas, when survival was observed, very large numbers of spores survived and in only a very limited number of cases was 'intermediate' survival observed. For *F. oxysporum* f.sp. *cepae*, there was a rapidly decreasing probability of survival with increasing temperature(P < 0.001). There was also a significant effect of exposure time (P = 0.04) on survival. There were no significant effects of compost moisture or waste type on survival. For *F. oxysporum* f.sp. *radicis-lycopersici* there was strong evidence (P < 0.001) of a temperature effect on the survival of the spores, with a decreasing probability of survival with increasing temperature. However, there were no significant effects of compost exposure time, moisture content, or waste type on spore survival.
Compost temperature data was also obtained from a fifth site, a large-scale in-vessel composting system, Vital Earth, Market Drayton, Shropshire. The vessel contained mixed green and food wastes. Temperature probes were positioned 15 mm from the surface and side walls, and 40 mm from the base. All the probes reached 70 °C for at least 6 hours; it was therefore considered that the plant pathogen survival risk in the compost was small.
Further details are given in Annex 2.

Milestone 13. Determine risks in large-scale composting systems of pathogen re-growth and escape through leachate and bioaerosol
Samples of leachate were obtained from heaps of immature composts from composting sites A to D described above on two separate occasions. The samples were tested for the presence of *Fusarium oxysporum* and *Phytophthora* species, but none were detected. Compost leachate was therefore not considered to be a major source of contamination of these plant pathogens on commercial composting sites.

Milestone 14. Devise methods to minimise pathogen survival, re-growth and escape risks in large-scale composting systems
The following methods were devised for increasing compost temperatures and their uniformity in composting systems to reduce the risk of pathogen survival:

- Insulation of the composting system with materials such as tarpaulin or used carpet waste (tested at sites A and C above)
- optimising available nitrogen by addition of poultry, dairy or horse manures (5-10% w/w) in the plant-based wastes (tested at sites A)
- optimising compost moisture content to around 55-60% w/w (tested at sites and C)

All the above methods were found to be practical and would not significantly increase the cost of the composting process.

Feasibility study for composting SOD waste:

Objective 7. Develop a testing procedure for detecting Phytophthora ramorum and P. kernoviae in SOD infected plant materials and composts, capable of detecting <102 propagules g-1 substrate.

Milestone 16. Develop a testing procedure for detecting *Phytophthora ramorum* and *P. kernoviae* in SOD infected plant materials and composts
Naturally *Pr* and *Pk* infected leaves, predominantly rhododendron and viburnum, were obtained from several sites in Cornwall by PHSI staff at Polwhel, and inserted in sacks of compost in the tests at Trengwainton. Samples of *Pr* and *Pk* infected leaves were retrieved from composts after the specified exposure periods (M10) and the inoculum tested for viability by plate culture on PDA + PARPH agar. Leaf inocula were classed as positive if mycelia or spores characteristic of *Phytophthora* were present. Samples of leaf samples were also used for reverse transcriptase (RT)

real-time PCR (RT-Taqman) assays, to relatively quantify RNA and hence live *Phytophthora* cells.
Further details are given in Annex 3.

Objective 8. Optimise the mechanical pre-treatment and composting of comparable non-infected plant material in terms of cost, composting efficiency and with the minimum of other input materials.

Milestone 17. Establish optimum mechanical pre-treatment and composting formulation and system for non-infected material

Rhododendron waste was shredded with the following types of equipment: GreenMech-Arborist shredder (b) Wessex type (c) Timberwolf (d) Seko waste shredder/crusher powered by a tractor. All these types of shredding equipment were effective and required 7-9 litres of fuel to shred 1 tonne of waste. 1000 m^3 of rhododendron required 3 man days to produce 1.5 tonnes of shredded waste. Shredded rhododendron waste was found to compost readily without other additives except water (about 100-150L water per tonne of waste were required). Further details are given in Annex 4.

Objective 9. Develop an in-situ composting system that uniformly achieves sufficiently high temperatures (>50°C for 1 week) with the prevalent types of infected material and during different seasons.

Milestone 18. Develop an in-situ composting system that uniformly achieves compost sanitisation using SOD infected wastes

A method was developed for composting of *Rhododendron ponticum* waste using a local supply of non-infected plant material. A composting bay with a capacity of 8 m^3 at Warwick HRI was insulated with a double layer of waste wool/hessian carpet. The waste was either coarse or fine shredded and wetted to achieve a moisture content of 55-60%, filled into the composting bay and covered with a double layer of waste carpet. The composting process was monitored by a multipoint data logger and temperature probes, and by inserting indicator organisms (seeds, clubroot infected *Brassica* plants, *Fusarium oxysporum* chlamydospores) in compost filled nylon bags into the compost. The wastes were emptied, re-wetted, mixed and refilled into the bay at 2-week intervals on two occasions.

The bulk of the fine shredded waste (10 cm or more from the side walls and below the surface) reached a temperature of 60-65°C after 6 days, and maintained a temperature above 50°C for 26 days after the bay was filled (i.e. 12 days after the first remixing). The surface of the compost and sides of the composting bay, in contact with the carpet waste, maintained a temperature of 28-30°C for 24 days after filling.

The bulk of the coarse shredded waste reached a temperature of 45-60°C after 6 days, and maintained a temperature above 40°C for 10 days after the bay was filled. The surface and sides of this compost were also lower than of the fine shredded waste and remained at 15-30°C throughout the composting period.

The viability of seeds and *Fusarium* chlamydospores were both useful indicators of compost temperatures in the range 35-60°C, whereas the clubroot inoculum remained viable even at the highest temperatures obtained.

Two existing composting facilities at National Trust gardens (Trengwainton in Cornwall and Biddulph Grange in Staffordshire) were modified to replicate the results achieved at Warwick HRI. At each site, a dividing wall was constructed in a bay to produce two smaller bays with a capacity of about 8 m^3. At Trengwainton, the walls of two composting bays were insulated with a double layer of waste wool/Hessian carpet. At Biddulph, both bays were lined with expanded polystyrene and wood, and further insulated with a double layer of waste carpet.

All the bays were filled with fine shredded rhododendron and other shrub waste, mixed with water or wet leaf waste to increase moisture content. The wastes were covered with a double layer of waste carpet and a plastic sheet. The wastes were emptied, mixed and refilled into the bays at about 14 day intervals, with additional water added at remixing if required.

Bags containing waste, indicator seeds, and leaves infected with either *Phytophthora kernoviae* or *P. ramorum* were inserted in the corners, sides, top, base and centre of the compost, 9 days after filling at Trengwainton. The bags were retrieved after 5 days, and seeds and some of the leaves removed for viability testing. Bags in the corner locations were reinserted in the compost, after emptying and remixing, for a further 5 day period.

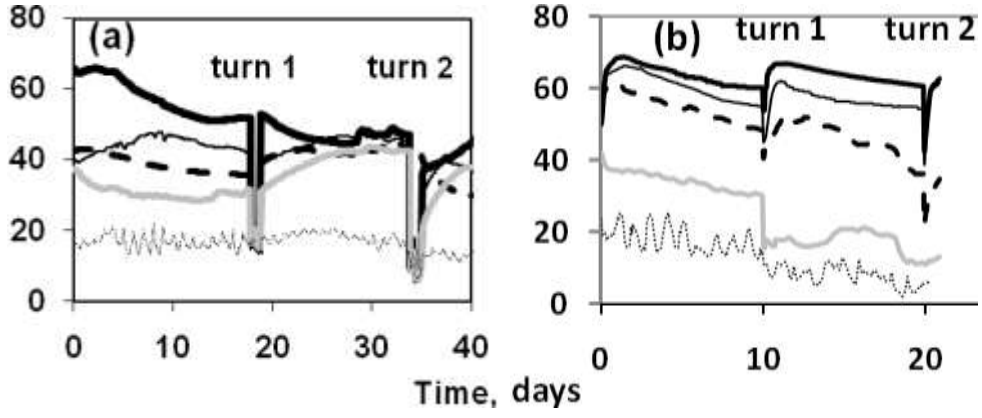


Fig.2 Compost temperatures (°C) in rhododendron waste at (a) Biddulph Grange and (b) Trengwainton Gardens. Centre, thick black line; surface, thin black line; side, dashed black line; bottom corner, thick grey line; air temperature, dotted thin line.

Mean and maximum temperatures of at least 53 and 65°C were achieved in the centre of each of the four batches of shredded plant wastes at Trengwainton (Fig. 2b). The minimum mean compost temperatures were recorded in the lower corners of the bay (31–47°C). Mean ambient temperatures ranged from 5.6 °C during the fourth compost batch to 17.7 °C during the second compost batch.

At Biddulph Grange, mean and maximum temperatures of at least 47 and 59°C were achieved in the centre of each of the four batches of shredded plant wastes (Fig. 2a). The minimum mean compost temperatures were recorded in the lower corners of the bay (32–44°C). Mean ambient temperatures ranged from 9°C during the sixth compost batch to 18 °C during the fourth compost batch.

Mean compost temperatures at the three sites declined by about 5°C during each of two consecutive turns of the compost, made at about 14 day intervals (Fig. 2).

Further details are given in Annex 2.

Objective 10. Establish the eradication of Phytophthora ramorum and P. kernoviae in the composting systems in (8) and (9) using the method developed in (7), to produce a defined testing methodology to ensure composted material is free of P. ramorum and P. kernoviae.

Milestone 19. Establish eradication of *P. ramorum* and *P. kernoviae* in composted SOD infected plant wastes

Post-composting, leaves that tested positive for *P. kernoviae* or *P. ramorum* were only retrieved from the corners of the composting bay. All leaves retrieved from bags positioned in the surface, base, sides, back, front and centre of the bay, where mean compost temperatures were ≥41.9°C over a 5-day period, tested negative for both pathogens.

There were strong negative overall effects of mean compost temperature ($P < 0.001$) on the proportions of leaves that tested positive for *P. kernoviae* or *P. ramorum* post-composting. Fitted regressions have the equation:

$$\ln(P/(1-P)) = bT + a + \ln(I_0) \quad (1)$$

where P is the proportion of positive *P. kernoviae* or *P. ramorum* leaves after composting (after exposure times of 5 or 10 days), T is the mean compost temperature, I_0 is the proportion of positive *P. kernoviae* or *P. ramorum* leaves in the day 0 sample, and a and b are the values of the model parameters (intercept and slope).

There was an overall strong negative effect of compost exposure time ($P = 0.003$) on the proportion of leaves that tested positive for *P. kernoviae*, although there was no evidence that the effect of temperature was different between the two composting times. However, only six observations (out of 46) showed some survival of *P. kernoviae* after composting (four after 5 days and only two after 10 days). After 5-days exposure to mean compost temperatures exceeding 32.8 °C, there were no leaves that tested positive for *P. kernoviae*.

Unlike for *P. kernoviae*, there was no overall difference between the two compost exposure times, 5 and 10 days, in the proportion of leaves that tested positive for *P. ramorum* post-composting in the large-scale system. However, there was a significant interaction ($P = 0.002$) between the effects of compost temperature and exposure time, with a more rapidly decreasing proportion of *P. ramorum* survival with increasing temperature for leaves exposed for 10 days than for those exposed for 5 days. After exposure to compost for 5 days, there were no leaves that tested positive for *P. ramorum* at compost temperatures of ≥ 41.9°C; the corresponding temperature for a 10-day exposure was 31.8°C. The fitted survival probabilities of *P. kernoviae* and *P. ramorum* on leaves, based on all the test data, indicate that survival is still a small possibility, even at higher temperatures. The probabilities of obtaining a positive result for *P. kernoviae* or *P. ramorum* on leaves exposed to compost for 5 days at 45°C or for 10 days at 35°C were less than 3% .

RT-Taqman results from the large scale composting experiment showed the same trend as culture results, with a sharp decline in ITS1 RNA in both species correlated with increasing temperature (also a log relationship). However, there were two important differences between ITS1 RNA measurements and viability as assessed by culturing in both species. First, in the range 41-45°C no viable cultures were found, but RNA was still detected at approximately 5% of the level observed at 16-20°C; second, at temperatures > 53°C there was an apparently much higher level of ITS1 RNA recovered from composted leaf samples. RNA quantification to measure viability was therefore shown to be unreliable in environments that favour RNA preservation.

Further details are given in Annexes 3 and 4.

Milestone 20. Identify optimum *F. oxysporum* isolates for use as compost sanitisation in bench-scale composting experiments

Talc inoculum of chlamydospores of the following *F. oxysporum* formae specials were prepared: *cepae* (4 isolates), *narcissi* (3 isolates), *lycopersici* (race 1, 2 isolates), *lycopersici* (race 2, 2 isolates), *pisi* (race 1), *pisi* (race 2). Bags containing the talc inoculum were inserted in flasks of green waste compost for 1 day at 46 °C and 1.5 days at 50 °C. All of the isolates survived the 1 day at 46 °C treatment except the two f.sp. *lycopersici* (race 2) isolates. Only one isolate (an f.sp. *cepae*) survived the 1.5 days at 50 °C treatment. This isolate was therefore the most useful compost sanitisation indicator and was used in further large scale tests.

Milestone 21. Optimised compost sanitisation indicators tested in large-scale composting systems

The following sanitisation indicators were tested in three large scale systems (Warwick HRI, Coombe Abbey and Cannock Chase):

- *Fusarium oxysporum* f.sp. *cepae* chlamydospores
- Tomato cv Alisa Craig seeds
- Leaves of *Rhododendron ponticum* and Portugal laurel (*Prunus lusitanica*)

The indicator organisms were inserted in composts for 10 days, after which viability was tested and the mean compost temperature determined. There were good correlations between the mean compost temperature and the viabilities of Fusarium chlamydospores and tomato seeds, (Fig. 3) and the discoloration of rhododendron and laurel leaves (Fig. 4) during composting.

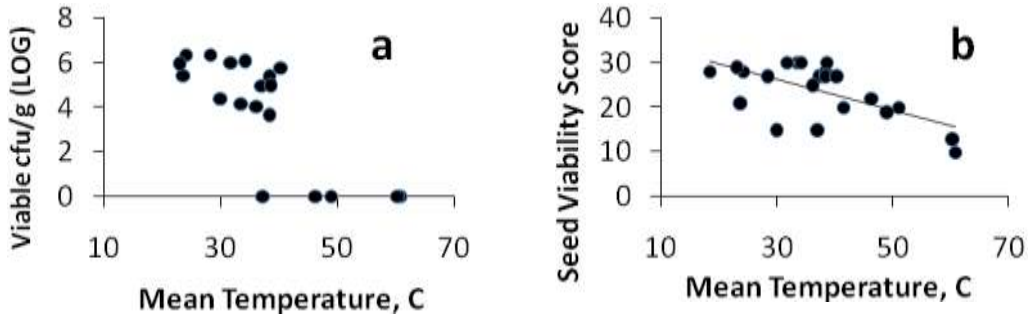


Fig. 3. Relationship betweeen mean compost temperature and viability of (a) Fusarium oxysporum chlamydospores and (b) tomato seeds.

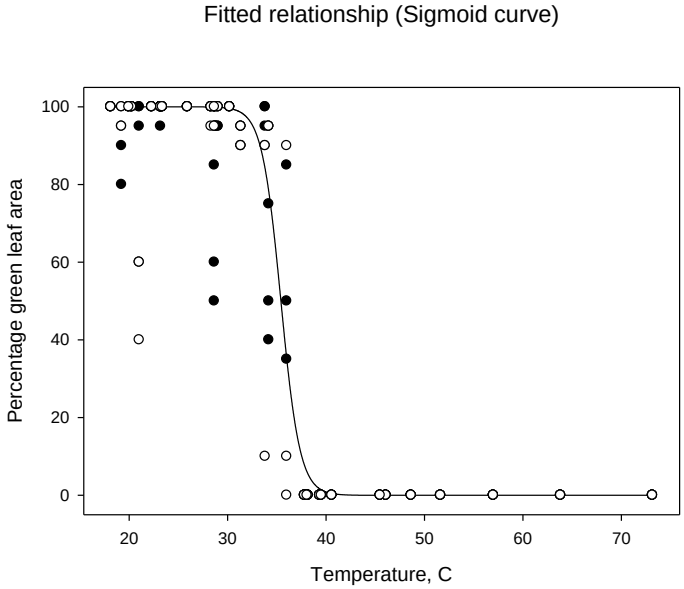


Fig. 4 Relationship between compost temperature and leaf discoloration after 10 days. Rhododendron, solid circles; Laurel, open circles.

There was a strong negative effect of mean compost temperature (*T*) on the percentage of green leaf area (*G*) in the large-scale systems , with a sharp decline in *G* between *T* values of 35 and 40°C over a 10-day period (Fig. 4). The fitted relationship between *T* and *G* was:

$$G = \frac{100}{1+e^{-B(T-M)}} \quad (2)$$

where *M* is the inflection point and *B* is the slope of the curve at the inflection point. Temperature-time profiles of the composts and positioned indicator leaves demonstrated that the main pathogen survival risk was in the corners of the insulated composting systems; pathogen survival risk could be minimised by positioning the corner material into the centre of the composting system during sequential refilling.

The composted rhododendron and vaccinium wastes were also spread as a 50 mm layer mulch to test for regrowth. There was no regrowth from any of the composts.

Further details are given in Annex 4.

Mobile composting extension:

Objective 15. Develop a mobile in-vessel composting system using non-infected host plant material.

Objective 16. Test Pr/Pk eradication conditions in Rhododendron and Vaccinium wastes in mobile and insulated bay composting units.

Milestones 22 & 23. Composting of SOD infected plant wastes tested at low ambient temperatures and Methods developed for increasing compost temperatures and improving compost sanitisation at low ambient temperatures

Composts were prepared from shredded rhododendron waste at Trengwainton Gardens and Biddulph Grange as described in section M18 during periods of low ambient temperature (3 – 9°C). The following methods developed in M14 were used to increase compost temperatures:

- addition of 20 kg poultry manure/tonne waste or 100 kg dairy or horse manure/ tonne waste
- addition of water (100L/tonne waste) to increase compost moisture content to 55% w/w
- insulation of the sides and surface of the composting systems with a double layer of wool/hessian carpet waste.

At both sites, compost temperatures of 50–60°C were achieved in the composts during three turns; these are similar to those achieved during periods of higher ambient temperatures (15 – 20°C) without addition of animal manures.

Milestone 25. Test pathogen eradication in non-infected Rhododendron and Vaccinium waste in mobile composting units at WHRI

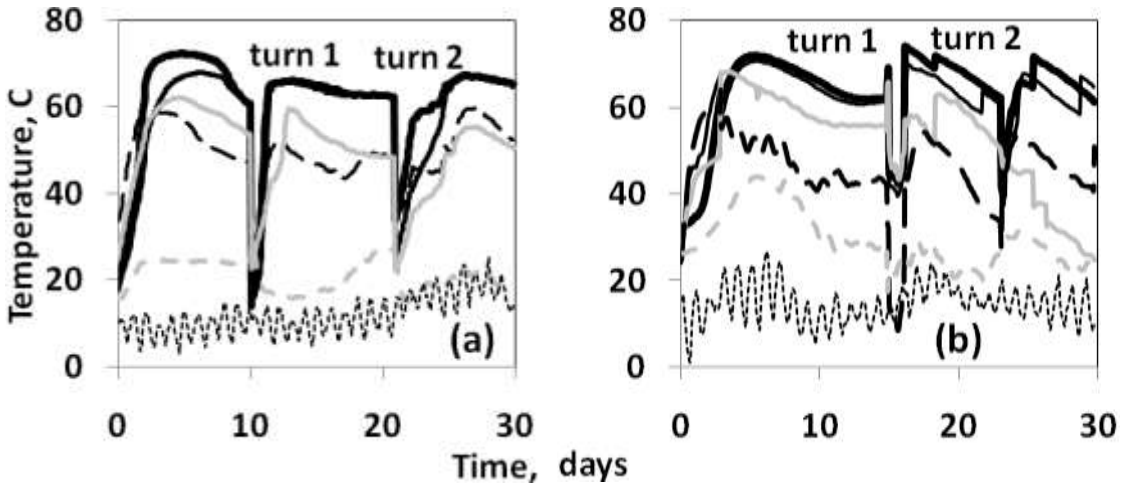
Two types of mobile composting system were developed for composting and sanitising 8 to 20m^3 batches of shredded plant wastes: (a) system constructed of insulated refrigerated cargo containers (b) insulated composting bay. Compost was prepared from non-infected rhododendron waste in both systems at Warwick HRI, Wellesbourne. The composting systems were found to maintain temperatures of 50–60°C for at least 30 days after filling, in the entire mass except the lower corners. These conditions are well above those previously found necessary to reduce Pk/Pr inoculum levels to below detectable limits. Sanitisation indicators (*F. oxysporum*, tomato seeds, leaves) showed that the corners were the only survival risk. Pathogen survival risk was minimised by refilling the lower corner material into the centre of systems after each of two turns. No re-growth of rhododendron from any of the composts has been detected.

Further details are given in Annex 4.

Milestones 26 & 28. Test Pr/Pk eradication conditions in Rhododendron and Vaccinium wastes in mobile and insulated bay composting units

Composts were prepared in insulated bays at Coombe Abbey Country Park, Coventry and Tregonning Hill, Helston, Cornwall, and in an insulated cargo container at Cannock Chase, Staffordshire. The insulated bays were constructed of insulated wooden walls on three sides mounted on a double layer of thick polythene.

A mobile insulated container composting system has been used at Cannock Chase to compost Vaccinium waste infected with *Phytophthora pseudosyringae*. Cutting and removal of infected Vaccinium from the field sites, and filling and emptying of the container system with the waste was fully mechanised using a tractor mounted flail mower.



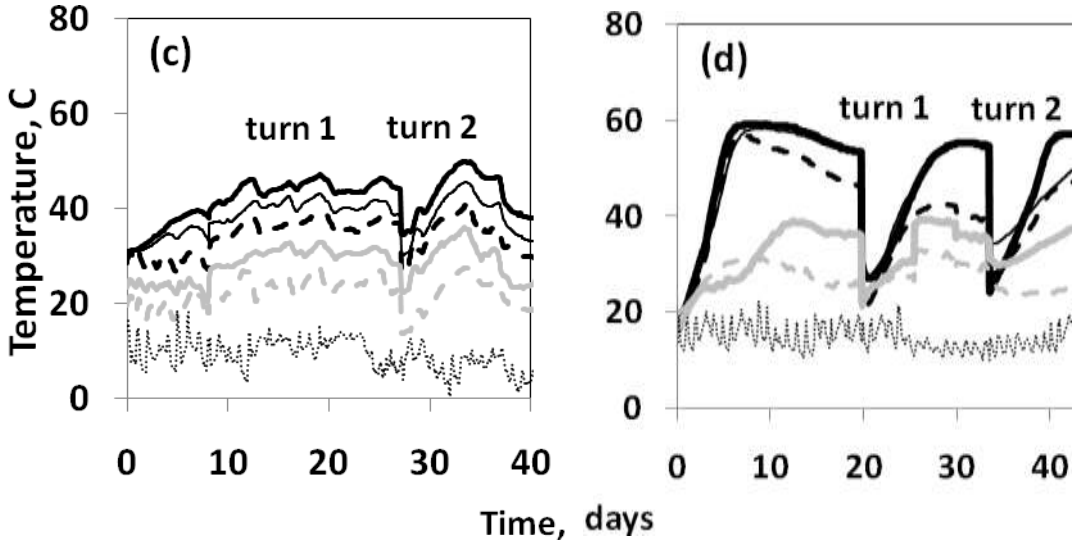


Fig. 5. Compost temperatures in rhododendron waste in (a) insulated bay at Coombe Abbey and (b) cargo container at Warwick HRI and in Vaccinium waste in (c) insulated bay at Tregonning Hiil and (d) cargo container at Cannock Chase. Centre, thick black line; surface, thin black line; side, dashed black line; top corner, thick grey line; bottom corner, dashed grey line; air temperature, dotted thin line.

There was a strong negative effect of mean compost temperature on the proportion of Vaccinium stems that tested positive for *P. pseudosyringae* post-composting. No survival of *P. pseudosyringae* was detected after exposure of infected stems for 10 days at ≥ 25.9°C. Vaccinium stems were also inserted in the waste at Tregonning Hill, but none of these (or the day 0 samples) tested positive for *Phytophthora* species. Mean compost temperatures and exposure times achieved in shredded rhododendron or chopped bilberry wastes in the majority of the compost in the insulated composting systems were above those needed to reduce inoculums of *Pr* and *Pk* to below detectable limits, and to eliminate any green colour in the indicator leaves (Fig. 5). The exception was in the corners of the systems that contained >4 m^3 waste, and in the outer surfaces at Tregonning Hill where the volume of Vaccinium waste was only 2.9 m^3 (Fig. 5c). Temperature-time profiles of the composts and positioned sanitisation indicators (*F. oxysporum*, tomato seeds, leaves) demonstrated that the main pathogen survival risk was in the corners of the insulated composting systems; pathogen survival risk could be minimised by positioning the corner material into the centre of the composting system during sequential refilling.

Further details are given in Annex 4.

Objective 17. Estimate operational costs of the composting methods

Milestones 27 & 29. Estimates of operational costs of the composting methods

Insulated bay system: Cost of materials and construction: £1000

Converted refrigerated cargo container: Cost of container, inc delivery: £2500

Harvesting and shredding of rhododendron waste

1000 m^3 of rhododendron required 3 man days (@£150/day) to harvest; 1 man day is needed to produce 1.5 tonnes of shredded waste

Shredder hire inc fuel (12 litres) £100 per day

1000 m^3 of rhododendron costs £700 to harvest and shred manually

Harvesting of Vaccinium waste

1000 m^3 of Vaccinium requires 3 man days (@£150/day) to produce 0.5 tonne of shredded waste, strimmer hire£20/day inc fuel (6 litres)

or 0.5 man days with tractor mower, hire cost inc fuel £300 per day

1000 m^3 of Vaccinium costs £510 to harvest manually or £225 to harvest with a tractor mower.

Composting costs: Per 3 tonne load of waste

Bob-cat hire for emptying and filling waste, inc. fuel £150 per day, 4 days

Emptying and filling wastes, 4 x 0.25 man days = 1 man day

Each 1 tonne of waste costs £250 to compost

Total Costs (harvesting and composting, excluding capital cost of composting system)

Per 1000 m^3 of cleared area

- Rhododendron, harvested manually, composted £1075
- Vaccinium, harvested with tractor mower, composted £350
- Vaccinium, harvested manually, £635

Per 1 tonne of shredded waste

- Rhododendron, harvested manually, £717
- Vaccinium, harvested with tractor mower, £700

- Vaccinium, harvested manually, £1270.

Objectives 6,11 & 14. Report and disseminate the research findings.

M15, M24, M30. Final Report, dissemination of results; incorporate the results into Quality Assurance schemes

Publications

R. Noble, J. Elphinstone, C.E. Sansford, G.E. Budge, C.M. Henry (2009) Management of plant health risks associated with processing of plant-based wastes: a review. *Bioresource Technology* 100: 3431-3446.
R. Noble, C. Henry (2008) Reducing the plant health risks associated with composting. Composting News 12(2): 32–33.
R. Noble, A. Dobrovin-Pennington, S. Pietravelle, C.M. Henry (2010) Indicator organisms for assessing sanitization of composting plant wastes. submitted to *Waste Management*.
R. Noble, J. Blackburn, G. Thorpe, A. Dobrovin-Pennington, S. Pietravelle, G. Kerins, C.M. Henry (2010) Eradication of the exotic plant pathogens *Phytophthora kernoviae* and *Phytophthora ramorum* during composting. To be submitted to *Plant Pathology*.
R. Noble, A. Dobrovin-Pennington, S. Pietravalle, C.M. Henry. Composting of rhododendron and bilberry wastes to contain the spread of exotic plant pathogens *Phytophthora kernoviae* and *Phytophthora ramorum*. To be submitted to *Compost Science & Utilization.*
R Noble (2010) Risks and benefits of soil amendment with composts in relation to plant pathogens. *Australasian Plant Pathology* (in press).

Presentations

R. Noble, February 2010. Presentation on composting of SOD infected plant material to National Trust head gardeners at Warwick HRI.
R. Noble, March 2010. Presentation on eradication of plant pathogens during composting to Plant Network meeting, Sheffield University.
R. Noble, August 2010. Risks and benefits of using composts as soil amendments. Australasian Plant Pathology Conference, Queensland, Australia.
R. Noble, December 2010. Managing the risks of plant pathogens in composting. AFOR PAS100 Workshop, Northampton University.

Other dissemination activities

Standard Operating Procedures for composting of SOD infected plant wastes prepared.
Meeting with Ann Payne, Jon Brown and Paul Mettson, FERA Polwhel and Jan Hoyland of the National Trust on 19 May 2008 to present results on composting of SOD infected wastes.
Meeting with J. Jacobs, E. Nichols and Kiara Zennaro of the Composting Association on 17 March 2008 to discuss pathogen eradication results and large-scale composting experiments.
Meeting with J. Hawkins, Staffs. County Council, July 2009, to discuss composting of plant wastes at Shugborough Hall Estate.
Meeting with FERA staff and staff of Westonbirt Arboretum to discuss composting of SOD infected plant wastes, March 2010.

Annex 1
R. Noble, J. G. Elphinstone, C.E. Sansford, G.E. Budge, C.M. Henry (2009) Management of plant health risks associated with processing of plant-based wastes: a review. *Bioresource Technology* 100: 3431-3446.

Annex 2
Noble R, Dobrovin-Pennington A, Pietravalle S, Weekes R, Henry CM. (2011). Indicator organisms for assessing sanitization during composting of plant wastes. *Waste Management* **31:** 1711-1719.

Annex 3
R. Noble, J. Blackburn, G. Thorp, A. Dobrovin-Pennington, S. Pietravalle, G. Kerins, T. R. Allnutt, C.M. Henry (2011). Control of the exotic plant pathogens *Phytophthora kernoviae* and *Phytophthora ramorum* during composting. *Plant Pathology* 60, 1077-1085.

Annex 4
R. Noble, A. Dobrovin-Pennington, S. Pietravalle, C.M. Henry (2011). Composting of rhododendron and bilberry wastes to contain the spread of the exotic plant pathogens *Phytophthora kernoviae* and *Phytophthora ramorum*. *Compost Science & Utilization 19(4), 219-225*.

Annex 5 SOD standard operating procedure.

Annex 6

G. Kerins, J. Blackburn, T. Allnutt, S. Weller, T. Nixon, M. Daly, H. Stanford, S. Pietravalle & C.M. Henry. Composting to sanitise plant-based waste infected with organisms of plant health importance.

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References to published material

9. This section should be used to record links (hypertext links where possible) or references to other published material generated by, or relating to this project.

R. Noble, C. Henry (2008) Reducing the plant health risks associated with composting. Composting News 12(2): 32–33.

R. Noble, J. G. Elphinstone, C.E. Sansford, G.E. Budge, C.M. Henry (2009) Management of plant health risks associated with processing of plant-based wastes: a review. *Bioresource Technology* 100: 3431-3446.

R. Noble, A. Dobrovin-Pennington, S. Pietravalle, R. Weekes, C.M. Henry (2011) Indicator organisms for assessing sanitization of composting plant wastes. *Waste Management* 31, 1711-1719.

R. Noble, J. Blackburn, G. Thorp, A. Dobrovin-Pennington, S. Pietravalle, G. Kerins, T. R. Allnutt, C.M. Henry (2011). Control of the exotic plant pathogens *Phytophthora kernoviae* and *Phytophthora ramorum* during composting. *Plant Pathology* 60, 1077-1085.

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