Management of plant health risks associated with processing of plant-based wastes: a review

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Abstract

The rise in international trade of plants and plant products has increased the risk of introduction and spread of plant pathogens and pests. In addition, new risks are arising from the implementation of more environmentally friendly methods of biodegradable waste disposal, such as composting and anaerobic digestion. Because these disposal methods do not involve sterilisation, there is good evidence that certain plant pathogens and pests can survive these processes. The temperature/time profile of the disposal process is the most significant and easily defined factor in controlling plant pathogens and pests. In this review, the current evidence for temperature/time effects on plant pathogens and pests is summarised. The advantages and disadvantages of direct and indirect process validation for the verification of composting processes, to determine its efficacy in destroying plant pathogens and pests in biowaste, are discussed. The availability of detection technology and its appropriateness for assessing the survival of quarantine organisms is also reviewed.

Keywords: composting; agricultural wastes; fungi; bacteria; viruses; viroids; plant health; pests; anaerobic digestion; pathogens; quarantine organisms.

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1. Introduction

International trade in planting material (seeds and plants) has increased the risk of introduction of exotic plant pathogens and pests to horticulture, agriculture, forestry and public and private gardens. Jones and Baker (2007) recently reviewed introductions of plant pathogens into Great Britain between 1970 and 2004 and reported that the majority had been introduced on ornamental or horticultural plants moved by trade. Examples of the type of diseases which have been introduced to the UK or EU by such trade pathways are the A2 mating type of *Phytophthora infestans* found in imported potatoes from Egypt (Shaw et al., 1985), *Clavibacter michiganensis* subsp *sepedonicus* (Cms) also introduced on potato imports and various diseases linked with imported seed e.g. *Ascochyta imperfecta* (the causal agent of leaf spot of lucerne (*Medicago sativa*)), *Pseudomonas syringae* pv. *pisi* (a pathogen of peas (*Pisum sativum*)) and *Pepino mosaic virus* (PepMV). In addition, the importation of plant commodities for consumption also carries with it a risk of introduction of such organisms, an example of which is *Guignardia citricarpa*, the causal agent of black spot disease which has been intercepted in the USA on citrus fruit in trade (EPPO datasheet, Agostini et al., 2006). Disposal of waste from crops, gardens and from processing and handling of plant produce generates a risk of introduction or spread of plant pathogens and pests if this waste is not handled properly. New risks are now arising from the implementation of the European Union (EU) Landfill Directive (EC, 1999) for waste disposal to move away from landfill to more environmentally sensitive methods. All biodegradable waste, including that containing biological material ('biowaste'), will progressively be diverted towards composting, anaerobic digestion or other modes of waste processing. An additional incentive for the move toward these methods for disposal of waste is

provided by the introduction of constraints on burning plant waste (EU, 2006). However, there is evidence that certain plant pathogens and pests can survive composting or other waste treatment processes, sometimes through inadequate methods or failures in the process (Sansford, 2003; Noble and Roberts, 2004). The most likely organisms to survive, and therefore those which have the potential to infect crop and non-crop plant species, are micro-organisms with hardy resting spores, and heat resistant viruses that are mechanically transmitted.

Safe management procedures are needed for disposal of crop and, in some instances, non-crop plants (plants in public gardens, etc) and their associated wastes. Of particular concern are those plant residues produced after known introductions or outbreaks as the waste materials are infected with pathogens of quarantine or other regulatory importance or with other, heat-tolerant pathogens. It is important that the disposal methods for biowaste containing plant material are both environmentally sensitive and effective in preventing the introduction and further spread of plant pathogens.

Lethal temperatures and exposure times are key factors in the elimination of high-risk organisms during composting and anaerobic digestion but other factors are also involved. Heat generated during the thermophilic phase of the composting process is the most important factor for the elimination of plant pathogens (Ryckeboer, 2003). A sufficient and uniform compost moisture content is also important to ensure pathogen eradication.

Limited published information is available on particular lethal temperatures for different quarantine organisms and there are no examples in which the full range of temperatures and required exposure times are known for any particular organism. This

paper reviews the state of current knowledge on (a) the effects of temperature, exposure time and other factors on the eradication of some organisms of concern to plant health, (b) methods of phytosanitary risk assessment and reduction, and (c) the biology and risks posed by some current examples of quarantine organisms and technology for their detection.

2. Temperature-time effects on plant pathogens and pests

The pathogen and pest species covered in this review, together with their hosts and common names of the associated diseases are listed in Tables 1 and 2. The effects of temperature and time, either in compost or other systems, on pathogen and pest species not included in previous reviews (Sansford, 2003; Noble and Roberts, 2004), as well as new data on the same pathogens and pests are shown in Tables 3–8. Studies discussed in the text in which data was tabulated in Noble and Roberts (2004) are not repeated in Tables 3–8. Much of the previous data was obtained by exposing organisms to naturally self-heating compost heaps, so it is difficult to determine the precise eradication temperature/times. Conversely, all the data in Tables 3, 4, 5 and 7 and most of the data in Tables 6 and 8 have been obtained during controlled temperature composting or anaerobic digestion, in small systems. Heat was supplied from an external source such as a water bath or incubator to maintain a constant temperature. Where maximum or minimum temperatures were recorded in compost heaps, this is stated in Tables 6 and 8. Further data in Tables 4–8 have been obtained from several papers examining the thermal sensitivity of plant pathogens and pests in relation to the effect of soil solarisation (Sztejnberg et al., 1987; Juarez-Palacios et al., 1991; McGovern et al., 2000; Coelho et al., 2001; Gallo et al., 2007) and hot water treatment of seeds and plant material (Bega and Smith, 1962; Woodville, 1964; Lane, 1984; Fatmi et al., 1991; Qiu

et al., 1993; Jaehn, 1995; Gokte and Mathur, 1995). Significant differences in temperature tolerance reported for the same pathogen by different workers may be due to variability in the wastes or other conditions of the eradication tests, and/or differences between pathovars or condition of the pathogen and pest specimens used.

2.1. Fungi and oomycetes

New data on a number of fungal and oomycete pathogens confirm previous work (Hoitink et al., 1976; Bollen et al., 1989; Noble and Roberts, 2004) that composting for 7 days at 52°C is sufficient for eradication. These pathogens are *Fusarium culmorum, Pythium ultimum, Rhizoctonia solani, Sclerotinia sclerotiorum, Sclerotium rolfsii, Thielaviopsis basicola,* and *Verticillium dahliae.* Anaerobic digestion at 40°C for 21 days also eradicated *Sclerotium cepivorum.* Mycelium of *Rosellinia necatrix* was also highly sensitive to heat (Tables 3 and 4).

None of the *Fusarium oxysporum* formae speciales examined in compost in Table 3 or other systems in Table 4 survived a 7-day period at 52°C or a 5-day period at 56°C. Tolerance of *F. oxysporum* f. spp. *lycopersici* and *melongenae* to composting for 21 days, with peak temperatures of up to 74°C has been reported by Noble and Roberts (2004). This was either due to a short exposure to the peak temperature, or to significant differences in the formae speciales or isolates used. There is evidence that composts suppress *F. oxysporum* under ambient conditions after 30 days whereas peat or soil do not (Table 3). Chlamydospores of *Microdochium nivale* survived a compost temperature of 58°C for 7 days but were eradicated at 64°C for 7 days (Noble et al., 2004).

Data tabulated by Noble and Roberts (2004) from work by Lodha et al. (2002) showed that *Macrophomina phaseolina* was able to survive peak compost temperatures of 60–62°C and a composting duration of up to 21 days. However, results in Tables 4

indicate that it would not survive a constant temperature of 55°C for more than two days.

Teliospores of *Tilletia indica* were eradicated from water and moist soil at 55°C for 5 hours (Table 3). The susceptibility of teliospores in unruptured sori to heat has not been determined (Sansford, 2003). There are no data on survival of *T. indica* teliospores (free, or within sori) during composting.

Survival of the causal agent of potato wart, *Synchytrium endobioticum*, in water at 60°C for 2 hours, and eradication after 8 hours has been reported by Noble and Roberts (2004), but there is little information on its behaviour during composting. The pathogen was not recovered from waste from processing of infected potatoes, which had been saturated with ammonia and composted together with animal manure for 2 to 3 months (Efremko and Yakoleva, 1981). Zoosporangia were able to maintain viability for 2–3 months in untreated processing waste (sludge) at temperatures below 21ºC but were effectively killed by heat treatment.

Oospores of *Phytophthora nicotianae* were eradicated after 7 days at a compost temperature of 52–58°C depending on the waste feedstock used (Table 3). Tests with mycelium and chlamydospores on agar showed that heating for 3 hours at 50°C was sufficient for eradication (Table 4). Chlamydospores of *Phytophthora cinnamomi* and oospores of *Phytophthora cactorum* and *Phytophthora megasperma* were sensitive to heat (Table 4). Kable and Mackenzie (1980) reported that the survival of *Phytophthora infestans* in potato stem lesions, as measured by sporangia production from lesions, was not affected by treatment at 30^{o}C but progressively decreased with treatment at increasing temperatures above 32.5^{o}C, being reduced to 95% of the original level by treatment for 9 hours at 40^{o}C. Ostrofsky et al. (1977) detected *Phytophthora lateralis* in

soils within host debris using a bait method with branchlets or roots of Port Orford cedar. Results showed that sporangia in baits were not detected at below 15^{o}C or above 25^{o}C. This would indicate temperature sensitivity for growth but does not confirm lack of viability.

Studies on the efficacy of treatments for eradicating *P. ramorum* are affected by the difficulties of determining the viability of chlamydospores, which may be dormant rather than dead. In addition plant material may not contain chlamydospores when tested. For example, Tooley et al. (2008) showed that it took 2 to 3 weeks for chlamydospores to form in rhododendron leaves after inoculation with sporangia and incubation at 20ºC. Thus, the efficacy of a treatment may only pertain to other structures such as hyphae, mycelium or sporangia, which are less robust. For these reasons results should be treated with caution.

Harnik et al. (2004) were able to re-isolate *Phytophthora ramorum* from artificially inoculated California bay laurel (*Umbellularia californica*) leaves held at 55°C for up to 1 week. The pathogen was not recovered after 2 weeks at that temperature (Table 4). Linderman and Davis (2006) found that *P. ramorum* survived as chlamydospores for 12 months and as sporangia for 6 months in soil or a range of components of potting media at 20°C, but could not be detected in infected rhododendron leaf pieces after 1 month; this may reflect the difficulties of isolation from chlamydospores embedded in dried leaf tissue.

Tubajika et al. (2007) found that a treatment at 56°C for 30 minutes might not be adequate to kill *P. ramorum* in wood. However, the results were inconclusive, particularly because the detection of *P. ramorum* in the controls was low. Tooley et al. (2008) reported that *P. ramorum* as free chlamydospores, and in rhododendron tissue,

could survive a 4-day treatment at 35°C but not 40°C. The pathogen survived a 2-day treatment at 40°C as chlamydospores but not in infected leaf tissue. Chlamydospore viability in the controls for free chlamydospores was <50%. Mycelia of *Phytophthora kernoviae* and *P. ramorum* were more resilient to dry than to wet heat treatment (Turner et al., 2008). With a treatment time of 60 minutes for *P. ramorum* and 120 minutes for *P. kernoviae*, the lethal temperatures for dry heat were 50°C and 42.5°C respectively. Wet heat treatments were effective in killing mycelia at 40°C (after 15 minutes) or 37.5°C (after 90 minutes). A dry-heat temperature of 55°C was required to kill sporangia of both species after 15 minutes. Wet-heat treatment killed sporangia of both *Phytophthora* species at 42.5°C after 10 minutes. A 20 minute wet-heat treatment at 45°C was effective at eradicating both *P. kernoviae* and *P. ramorum* from detached leaves of viburnum, rhododendron and camellia inoculated with a sporangial suspension 24 hours previously (insufficient time for chlamydospores to have developed). Jennings (2008) reported that dry heat could kill mycelium and sporangia of *P. ramorum* and *P. kernoviae in vitro* at lower temperatures (42.5°C and 45°C) with extended (unspecified) exposure periods. Assays with detached leaves of viburnum, rhododendron and camellia inoculated with sporangia between 2 hours and 4 days prior to treatment (so prior to chlamydospore formation) showed that 45°C (dry heat) required 80 minutes exposure for eradication. Germinability of *P. ramorum* chlamydospores in the absence of plant material decreased with time at 40ºC and no spores could be germinated after 24 hours although it is not known whether they were dead or dormant (Anon., 2004). Sporangia were no longer viable after 1 hour at 40ºC.

Swain et al. (2006) indicated that appropriate composting can effectively eliminate *P. ramorum* from green-waste. In laboratory tests the pathogen could not be

isolated from infested leaves of *U. californica* and wood chips and cankered stems of Coast live oak (*Quercus agrifolia*) after a 24 hour exposure at 40°C or a 1-hour exposure at 55°C. In field composting trials the same type of material was considered free from *P. ramorum* after 2 weeks at 55–60°C. This was confirmed by isolation and by polymerase chain reaction (PCR) assay. The absence of *P. ramorum* DNA led the authors to conclude that the pathogen was absent and not merely suppressed or dormant.

There are no studies on the efficacy of composting in eradicating *P. kernoviae*.

2.2. Plasmodiophoromycetes

Results obtained by Idelmann (2005)and Fayolle et al. (2006) under constant compost or soil temperatures (Table 5) indicate that *Plasmodiophora brassicae* is less temperature tolerant than was indicated from exposures to peak compost temperatures of unknown duration in work previously tabulated by Noble and Roberts (2004). A compost temperature of 60°C for 1 day or 50°C for 7 days, with high compost moisture (60%) eradicated *P. brassicae*. A naturally infected cabbage inoculum of *P. brassicae* was less temperature tolerant than an artificially produced Chinese cabbage inoculum. Idelmann et al. (1998) have also shown differences in heat tolerance between *P. brassicae* isolates, with some being tolerant to 65°C for 1 day. Lopez-Real and Foster (1985) showed eradication of *P. brassicae* in a simulated compost after exposure at 55°C for 3–4 days or at 70°C for 1–2 days. Ylimäki et al. (1983) found that *P. brassicae* survived composting for 3 weeks in a windrow system that reached a maximum temperature of 60-65°C but was eradicated from compost that reached 70°C. Elphinstone (2005) reported that *P. brassicae* survived in infected plant material at 55–60°C for 21 days (Table 5). However, survival of *P. brassicae* in the above work may

have been due to the low compost moisture (30–50%), since adequate moisture may be required for pathogen eradication at critical compost temperatures (see Section 3).

Abe (1987) reported that a regime of 60°C for 10 minutes eradicated *Polymyxa betae* (the vector of *Beet necrotic yellow vein virus* (BNYVV), the cause of rhizomania of sugar beet) under moist conditions. Dickens et al. (1991) recommended a temperature of ≥70° C for 30 minutes to achieve kill for *P. betae* resting spores. However, complete eradication was not achieved, with some low-level survival being reported after treatment at 70 or 75°C for 30 minutes. Heterogeneity of inoculum and moisture levels appeared to be important factors affecting eradication. Nishinome et al. (1996) found that eradication temperatures for rhizomania infectivity of *P. betae* during composting of dewatered waste soil (80% soil) from sugar beet processing was 40ºC for 14 days or 60ºC for 1 day.

Van Rijn and Termorshuizen (2007) examined the survival of *P. betae* resting spores (cystosori) when treated with a range of temperature-time combinations under aerobic and anaerobic conditions in water or composting heap leachate (Table 5). Their findings suggested that anaerobic conditions enhanced the effects of heat, with 4 days at 40^{o}C significantly reducing viability compared with the aerobic treatment. Under aerobic conditions in water, the lethal temperatures were 60^{o}C for 30 minutes, 55^{o}C for 4 days or 40^{o}C for 21 days. Detection of *P. betae* was based on bait testing using a most probable number (MPN) quantification methodology similar to that of Tuitert (1990) followed by microscopic identification of cystosori.

2.3. Bacteria

All the bacterial plant pathogens in Table 6, as well as those previously reviewed by Noble and Roberts (2004), were eradicated by a constant temperature of 60°C for

1 hour, in plant material or other moist systems. Cms was eradicated at 82°C for 5 minutes; the effect of lower temperatures for longer periods was not investigated (Secor et al., 1987). The effect of temperature in compost on eradication of this pathogen has not been examined. Turner et al. (1983) concluded that the related pathogen *Clavibacter michiganensis* subsp. *michiganensis* (Cmm) was effectively reduced during anaerobic digestion at 35ºC. Cmm was also shown to be eradicated from naturally infected tomato seeds that were soaked in water at 52ºC for 20 minutes or at 56ºC for 30 minutes (Shoemaker and Echandi, 1976; Fatmi et al., 1991). Zanon and Jorda (2008) found that Cmm required 4 weeks at 45ºC for eradication from tomato debris in soil (Table 6). It has been reported to survive for 63 months in potato stems (Nelson, 1984). Treatment at 51ºC for 1 hour was shown to control the related pathogen *Clavibacter xyli* subsp. *xyli* in infected sugarcane seed (Ramallo and Ramallo, 2001).

Xanthomonas campestris pv. *campestris* was eradicated from compost after 7 days at 40°C but survived in dry heat at 64°C for 7 days (Noble et al., 2004), but this is unlikely to occur in a composting system. Elorrieta et al. (2003) found that *Pectobacterium carotovorum, Pseudomonas syringae* pv. *syringae* and *Xanthomonas campestris* pv. *vesicatoria* were eradicated in 60 hours or less from composting plant residues that reached a maximum of 65°C. The pathogens were eradicated from plant residues incubated at a constant 50°C for 15 hours or 60°C for 2 hours (Table 6). Ryckeboer et al. (2002) demonstrated that *R. solanacearum* could be destroyed to below detectable limits within one day during anaerobic digestion at 52°C of source separated household wastes. Similarly, Termorshuizen et al. (2003) showed that *R. solanacearum* could be reduced below detection levels following 6 weeks mesophilic (maximum temperature 40°C) anaerobic digestion of vegetable, fruit and garden waste.

2.4. Viruses and viroids

Previous work reported in Noble and Roberts (2004) showed *Tobacco mosaic virus* (TMV) and *Tobacco rattle virus* to be highly temperature tolerant, both surviving maximum compost temperatures of at least 64^{o}C and composting durations of at least 6 days. This is confirmed by more recent work on TMV in compost and anaerobic digestate (Table 6). *Potato mop-top furovirus* was eradicated at 90°C for 15 minutes; the effect of lower temperatures and/or different durations was not examined (Nielsen and Mølgaard, 1997) (Table 7).

There are no data on eradication conditions of viroids during the composting process e.g. *Potato spindle tuber viroid* (PSTVd). Spence (2004) reported that PSTVd can survive in both dried and moist leaf material for at least 6 months. PSTVd can also survive for at least 4 weeks at 30^{o}C on aluminium (in the light and dark) and on glass (in the light). There is also some evidence that viroids can bind to plastics and survive for long periods in this state (Spence, 2004).

2.5. Pests

Bøen et al. (2006) showed that although a small proportion of cysts of the golden potato cyst nematode (*Globodera rostochiensis*) hatched after treatment with a compost temperature of 50°C for 7 days, none of the hatched juveniles developed into nematodes capable of reproduction (Table 8). Results reported in Noble and Roberts (2004) and further experiments in Table 8 (Noble, 2005) show a lower temperature tolerance for the white and golden potato cyst nematodes, *Globodera pallida* and *G. rostochiensis*. This may be due to the use of fresh cysts, whereas Bøen et al. (2006) used one year old cysts.

The other nematode species in Table 8 were eradicated by a temperature of 48–50°C held for 90 minutes or less.

Larvae of carrot and onion flies were eradicated in composting onion waste at 50°C. The low temperature tolerance of insect larvae is confirmed in the effectiveness of hot water treatment in eradicating narcissus fly larvae (*Merodon equestris* and *Eumerus strigatus*) (Table 8). Fletcher et al. (1986) report that 49°C for 20 minutes in compost is lethal to tarsonemid mites. Treatment of narcissus bulbs in water at 46.7°C for 3 hours has been found to be effective in eradicating the mites *Ditylenchus dipsaci* and *Steneotarsonemus laticeps* (Table 8).

3. Other compost factors involved in pathogen eradication

Noble and Roberts (2004) reviewed factors other than temperature and time that may influence pathogen eradication from composts. Of these factors, compost moisture is most likely to determine pathogen eradication although compost feedstocks (type, particle size and mixing regime), toxic compounds and volatiles, and microbial antagonism may also have an influence. *X. campestris* pv. *campestris* survived in dry brassica trash at 64°C for 7 days but was eradicated after 7 days at 40°C in moist compost (Noble et al., 2004). Survival of *P. nicotianae* chlamydospores following heat treatment was lowest in saturated soil, and increased as soil moisture decreased (Coelho et al., 2001) (Table 4). The importance of compost moisture in determining eradication was confirmed with *P. brassicae* by Fayolle et al. (2006) who showed that the survival rate decreased with increasing compost moisture at the same temperature. Idelmann (2005) showed that dried galls of *P. brassicae* were more temperature tolerant than moist galls. Eradication temperatures and times obtained in hot water treatment may also be lower than those obtained in compost.

As discussed previously, feedstocks influenced the eradication temperature of *Phytophthora nicotianae*. Feedstocks used in compost (green waste, onion waste, spent mushroom compost at equivalent water potentials) had only minor effects on the eradication of *P. brassicae,* in spite of significant differences in pH (Fayolle et al., 2006). The effect of pH on the eradication of plant pathogens under normal composting conditions may be minimal. Smilanick et al. (1985) found that the germination of teliospores of *T. indica* on water agar was optimum between pH 6–9.5; however inhibition occurred below pH 4.5 and above pH 10. Due to their high C:N ratio, plant-based wastes are unlikely to produce ammonia in concentrations significant for eradication of pathogens (Noble et al., 2004). However, addition of nitrogen in composts may result in sufficient ammonia being released for this to be a factor in enhancing pathogen eradication (Chun and Lockwood, 1985).

Composts are known to suppress populations of soil-borne plant pathogens through microbial antagonism, by mechanisms including parasitism and the production of enzymes with lytic activity, antibiotics, and other toxic compounds such as organic acids and ammonia (Noble and Coventry, 2005). However, these suppressive effects are secondary in importance and more difficult to define or monitor for sanitisation standards than temperature-time profiles of composting (Noble and Roberts, 2004). The suppressive effects of microbial antagonism on plant pathogens are more likely to result in an under-estimate than an over-estimate of the sanitizing effect of a composting treatment based on temperature-time measurements alone.

The time taken for a pathogen population to be eradicated from compost depends on the initial concentration of the pathogen. When interpreting the results of eradication tests, the absolute time to extinction is less important than the rate at which

the concentration declines. This has been demonstrated for salmonellas in cattle slurry (Jones and Martin, 2003). However, many studies on eradication of pathogens from compost and other organic wastes report only the time to extinction, without calculating the rate of decay or reporting the initial pathogen concentration. Carrington et al. (1982) obtained decay rates of 3.4 log to 4.4 log for *Salmonella duesseldorf* in anaerobic digesters at 48°C. Godfree and Farrell (2005) reported reduction rates of *Salmonella entiriditis* and *Salmonella dublin* in compost of 6.18 log. However, Idelmann (2005) found that the starting inoculum concentration in the range of 10^5 to 10^8 spores L^{-1} substrate had no influence on the measured eradication time and temperature of *P. brassicae* (50°C for 3 days for all spore concentrations).

4. Phytosanitary risk assessment and reduction

Termorshuizen et al. (2005) considered the following to be key parameters in assessing the phytosanitary risk of composts: (a) the proportion of host biomass relative to the total quantity of biowaste, (b) the proportion of host infected with a pathogen, (c) the density of infected host material, (d) the proportion of propagules of a pathogen that survived the process, and (e) the threshold density of a pathogen in soil above which disease of the host is expected to develop. This latter threshold for soil-borne pathogens will depend on the conditions such as soil temperature, moisture, pH, soil type, cropping patterns, and time of year at which a pathogen is introduced, so this is not necessarily relevant when assessing the risks of using composted waste that may contain viable plant pathogens. Methods have been developed for testing the phytosanitary safety of compost, based either on measuring the conditions within the compost or testing the effects of the composting process on indicator organisms. The terminology that is used for these methods varies between publications with ‘direct’ and ‘indirect’ methods

being used for biological monitoring as well as physical monitoring of the process. Christensen et al. (2002) refer to 'indirect' validation as monitoring the naturally occurring population of a micro-organism (or organisms) or viable seeds in the compost (see Section 4.2). Indirect process validation (or supervision) has also been referred to as the recording of temperatures at regular intervals throughout the composting process (EPPO, 2006b, 2008) (see Section 4.3).

4.1. Direct validation

The use of indicator organisms for testing the sanitation of the composting process has been referred to as 'direct' validation (EPPO, 2006b, 2008). This involves subjecting known samples of test organisms to a composting process, followed by retrieval and testing for viability. Plant pathogens such as *P. brassicae* and TMV as well as tomato seeds have been recommended for direct validation of the composting process (Idelmann, 2005; EPPO; 2008). Due to the high temperature tolerance of TMV, eradication would not normally be expected in a short composting process. A significant disadvantage of using pathogens is that indicator organisms may themselves compromise the phytosanitary safety of the compost depending upon how they are introduced and retrieved from the material being composted. Also, the natural variability in temperature tolerance between different isolates (e.g. *P. brassicae*, see Section 2.2) may lead to variable test results.

The use of non-pathogenic fungi as indicator organisms excludes their use in bioassays since no disease symptoms would be produced in the test plants used at the end of the process to test for infectivity, although the presence of viable propagules can be tested for using plating or PCR methodology (Tables 3–5). Tomato seeds and reproductive plant parts are also used as direct validation organisms with a zero

tolerance for viability during the period of the test (Pollmann and Steiner, 1994; EPPO, 2006b, 2008). It is unlikely that the effect of composting conditions (moisture, pH, microbial community etc) on the viability of seeds and seed tissue would be the same as the effect on plant pathogens. However, the development of a reliable direct validation process that did not involve the use of pathogenic indicators would provide additional information on the sanitation of composts.

4.2. Indirect validation using compost micro-organisms and seeds

The changes in microbial community in compost resulting from heating to different temperatures for different durations were studied by Termorshuizen (2006) using denaturing gradient gel electrophoresis (DGGE) analysis. Although significant differences in microbial communities between different heating treatments were detected, the method was not sufficiently developed for it to be used in practice.

Test methods using *E. coli* and *Salmonella* spp. as well as a number of other fungal and bacterial animal pathogens as indicator organisms in compost are described by Leege and Thompson (1997). The UK compost standard, PAS 100:2005 (Anon., 2005) specifies *Salmonella* spp. and *Eschericha coli* as indirect indicator species. *Salmonella* spp. must be absent in a 25 g compost sample and the upper limit of *E. coli* is 1000 colony forming unit (cfu) g^{-1} compost. Germinating weed seeds or weed propagule regrowth must also be absent from the tested compost. Christiensen et al. (2002) used *E. coli* and *Enterococcus faecalis* as indicator organisms for compost sanitisation. These would not be good indicators for the more resilient plant pathogens and there is consequently a need to identify indicator organisms similar to the organisms being eradicated.

The use of direct and indirect indicator organisms provides information on the sanitising effect of composting, which may not be fully evaluated by measurement of temperatures alone. This includes factors such as compost moisture and volatiles, and microbial antagonism. However, biological variability of indicator organisms means that continuous temperature monitoring is required in validating the sanitising effect of a composting process.

4.3. Indirect validation by temperature monitoring

Monitoring can be achieved by data logging the temperatures at different depths during the composting process. Generation and conductance of heat during composting appears to be the most important factor for the elimination of plant pathogens (Sansford, 2003; Noble and Roberts, 2004).

The European Plant Protection Organisation (EPPO) phytosanitary procedure recommends that the entire quantity of material being composted should be exposed to temperatures of at least 55°C for an uninterrupted period of at least two weeks, or, alternatively to a temperature of 65°C (or, in the case of enclosed facilities 60°C) over a continuous period of one week (EPPO, 2006b; 2008). The EPPO procedure further requires that material suspected of containing quarantine pathogens or heat-resistant pathogens should receive a separate treatment of 74°C for 4 hours (wet heat) before or after processing; the revised procedure (EPPO, 2008) gives alternatives as 80°C for 2 hours or 90°C for 1 hour.

In the UK, PAS 100:2005 recommends a minimum compost temperature of 65°C for 7 days, with at least two mixes (Anon., 2005). The temperatures must be recorded daily with at least one monitoring point in every 250 m^3 of compost. The EPPO and PAS 100:2005 procedures specify minimum compost moisture contents of

40 and 50% respectively (Anon, 2005; EPPO, 2006b, 2008). Similar compost sanitisation procedures have been developed in other countries (Noble and Roberts, 2004).

5. Quarantine organisms and vectors, inoculum potential and available detection technology

When performing experimentation to determine the survival of organisms through composting treatments it is important to ensure that (a) suitable levels and types of inoculum are used and (b) the detection techniques used are able to identify live organisms rather than the dead remnants of the inoculum used, to a high degree of sensitivity and specificity. Organisms included in this review were selected on the basis of their importance to plant health quarantine legislation in the EU and their likely survival through the composting process. However, they do provide a wide spectrum of examples illustrating the problems commonly encountered in assessing eradication in compost. Tables 9 and 10 summarise the available data on inoculum type and levels considered to be relevant to the assessment of composting risk and the currently available detection methodologies with their sensitivity and suitability for assessing viability.

5.1 Guignardia citricarpa

The fungal pathogen *G. citricarpa* is an ascomycete. Its principal hosts are *Citrus* species, including lemons (*C. limon*), limes (*C. aurantifolia*), grapefruit (*C. paradisi*) and oranges (*C. sinensis*), although a range of other species are also hosts, or can harbour the fungus (EPPO data sheet). This fungus may be considered to be a risk to *Citrus* spp. if compost is produced from infected citrus material, although, there is no

published evidence that it can cause infections via compost. The fungal stage likely to be of most risk in waste disposal is the asexual pycnidiospore found on the surface of infected fruit lesions or internally in pycnidia. The ascocarp/ascospore stage does not occur on mature fruit although it is present on infected leaves (Van Gent Pelzer, 2007). The spores appear to have a fairly short lifespan, ranging from 1 month to 1 year depending on the inoculum source and storage conditions (EPPO data sheet: *Guignardia citricarpa*).

Germination of both ascospores and conidia occurs quite readily (EPPO data sheet). The EPPO protocol for detection of *G. citricarpa* (EPPO, 2003) recommends plating on potato dextrose agar, oatmeal agar and cherry decoction agar followed by either morphological examination or PCR for detection. Agostini et al. (2006) used visual examination of fruit pieces to look for signs of the disease and cultured the fungus on potato dextrose agar. Both PCR (Bonants et al, 2003) and real time PCR (Van Gent Pelzer et al, 2007) methodologies have been developed for *G. citricarpa* and can be used in conjunction with plating to confirm the identity of *G. citricarpa* and confirm viability. There is little information available on the sensitivity of these methods although Peres et al. (2007) have reported that all PCR methods tested are effective for the use in conjunction with cultures.

5.2. Synchytrium endobioticum

S. endobioticum is a member of the Chytridiomycota, and causes wart disease in potato (*Solanum tuberosum*). It can survive in soil as winter sporangia (thick walled spores) for 30 years in the absence of the host (EPPO data sheet: ***Synchytrium endobioticum***). Spread is by means of zoospores produced initially from the winter sporangia and by the short-lived summer sporangia during the growing season. The main risk for compost

therefore comes from survival of winter sporangia from infected tubers or soil through the composting process, which would then be able to re-infect potato plants. There are reports that aging of spores contributes to the variability of viability of spores and that pathotypes exist. Hampson and Wood (1997) reported that a spore density of 10 g^{-1} soil gave 100% infection of bait plants, whereas a density of 5 g^{-1} soil gave 60% infection of potato bait plants (cv. Purple Chief). To be of practical use, any detection methodology would have to be able to detect viable spores down to a level of c. 0.1 spores g^{-1} soil.

Two main methods exist for the detection of potato wart disease in soil. The first is based on a direct analysis of the soil (EPPO, 1999) and involves a modification of a wet sieving method (Pratt, 1976) followed by chloroform centrifugation. Laidlaw (1985) also developed a similar floatation method and estimated that over 90% of sporangia could be recovered from sand. Determination of spore viability is difficult and is carried out mainly by microscopy or inoculation on to plants. Microscopic assessment of spores can be used to determine if spores are viable.

The second method is based on a bioassay using susceptible potato cultivars (EPPO, 2004), which is reported to be able to detect c. 1–5 sporangia g^{-1} soil. The assay requires a 70-100 day growth period after which the tubers are examined for the presence of the characteristic warts.

Van den Boogert et al. (2005) have developed a PCR test that is reported to have a detection limit of 10 sporangia per PCR assay. A conventional PCR method (Niepold and Stachewicz, 2004) and a microarray method (Abdullahi et al., 2005) have not been properly validated to date.

5.3. Tilletia indica

T. indica causes Karnal bunt of wheat and survives in the form of teliospores which are protected within hardy resting structures (sori). Although cereal waste would not normally be composted, the teliospores of *Tilletia* spp. are likely to survive ingestion by animals (Smilanick et al., 1986) so manure produced up to 5 days after feeding infected grain would be a contamination risk. Also, teliospores survived for at least 3 years in field soils under containment in the UK, Italy and Norway and this may provide an additional route for contamination of plant waste.

There is little experimental evidence for the inoculum thresholds required to cause infection, although modelling estimates have proposed that 15 germinating teliospores per square metre leads to 1 bunted grain per square metre (Murray et al., 2005).

Teliospores are known to have a period of dormancy before germination, which may complicate the detection procedure (Smilanick et al., 1985). Methods exist for the estimation of teliospore numbers in soils. Inman et al. (2008) assessed the survival of teliospores in soil by extracting spores using sieving and sucrose centrifugation, based on a method developed by Babadoost and Mathre (1998). Teliospores were then counted microscopically and viability assessed by germination on 2% water agar containing ampicillin and streptomycin.

5.4. Phytophthora ramorum and P. kernoviae

Phytophthora ramorum and *P. kernoviae* are oomycetes. *P. ramorum* has been shown to produce long-lived oospores and chlamydospores in culture but oospores have not been found to occur in nature. *P. kernoviae* has been shown to produce oospores in culture and occasionally in artificially-inoculated plant material; however, it has not been shown to produce chlamydospores. Because of this, it may be more-easily eradicated

than *P. ramorum* during a well-managed composting process. In the UK they can both survive at least 2 years in soil with *P. ramorum* surviving at least 3 years (Sansford and Woodhall, 2007; Sansford, 2008).

Although the species was not formally described at the time, *P. ramorum* was first found on symptomatic Rhododendron species in Germany and the Netherlands in 1993 (Werres et al., 2001) and has since been confirmed as the cause of Sudden Oak Death in California and Oregon (Rizzo et al., 2002). The pathogen has a wide natural host range in trees and ornamentals (Sansford and Woodhall, 2007).

P. kernoviae was first found in south-west England affecting beech (*Fagus sylvatica*) and rhododendron in October 2003 (Sansford and Woodhall, 2007). It has also been recently reported from New Zealand on *Annona cherimola* (cherimoya) and has been present since at least the mid-1950s when it was detected as an unknown *Phytophthora* under stands of symptomless radiate pine (*Pinus radiata*) (Ramsfield et al., 2007). Its host-range is relatively wide but is less than that of *P. ramorum*.

Baiting, serological and DNA based detection methods for *P. ramorum* and *P. kernoviae* are available but data on detection limits are scant (Schena et al., 2006). Serological methods (Lane et al., 2007) are used for pre-screening infected plant material for *Phytophthora* spp. but are not specific enough for species identification. Molecular methods for identification of *P. ramorum* include conventional PCR (Kox et al., 2002; Wagner and Werres, 2003; Lane et al., 2003; Guglielmo et al., 2007) and Real-Time PCR (Hughes et al., 2006; Hayden et al., 2004; Belbahri et al., 2007; Bilodeau et al., 2007). PCR methods are generally more specific for *P. ramorum*. In order to confirm viability of the fungal pathogen it is necessary to carry out some form of isolation of the pathogens from composted material, which may be difficult. Plant

material can either be incubated to produce sporulation or the organisms can be reisolated on to a selective medium (EPPO 2006a; Fichtner et al 2007). A difficulty arises because of the presence of chlamydospores for *P. ramorum,* which may be dormant and not detected by reisolation. Conventional PCR methods can detect DNA but cannot determine spore viability. Swain et al. (2006) used re-isolation on to a semi selective medium containing pimaricin, ampicillin, rifamycin, PCNB and hymexazol (PARP) medium (Jeffers and Martin, 1986) and a pear baiting methodology to determine viability of *P. ramorum*. Molecular diagnostic methods for *P. kernoviae* are similar to those for *P. ramorum* but different primers are used in molecular tests.

Chimento and Garbelotto (2007) have developed a reverse transcriptase-PCR method, which detects mRNA as a viability marker. The use of this test should exclude the possibility of false positive PCR tests arising from the detection of dead cells. This method has shown that after 9 days, RNA of freeze-dried killed *P. ramorum* in leaves of *U. californica* was undetectable while DNA gave a positive signal. This method would be usefully deployed to validate the earlier work of Swain et al. (2006), in order to determine whether mRNA remained viable after composting infected plant material when DNA-testing is negative.

5.5. Polymyxa species

Polymyxa species are plasmodiophoromyctes and are obligate biotrophs which means they cannot grow in the absence of a host plant. *Polymyxa graminis* (Ledingham, 1939) and *P. betae* (Keskin, 1964) are examples of species which can act as vectors of significant pathogenic soil-borne viruses, for example, *Soil borne cereal mosaic virus* (Brakke, 1971) and BNYVV (Fujisawa and Sugimoto, 1976). Although these particular viruses are no longer of statutory concern to the UK, their vectors are still likely to be of

quarantine concern in the future as resistance-breaking strains of current viruses or new viruses appear. In-season spread of *Polymyxa* spp. is carried out by zoospores produced from sporangia in plant roots. However, the resting spores of this group of organisms are particularly long-lived and contribute to the difficulties encountered when attempting to eradicate these pathogens in soil. Spores are generally produced in clusters of varying size, which may also contain spores of varying degrees of dormancy, whereby only a proportion of the spores in the cluster will germinate on contact with a plant root at any one time. In addition to this, only a proportion of the spores will be viruliferous (Tuitert, 1990). This makes the use of bait testing to detect viable soil inoculum less reliable.

Fujisawa and Sugimoto (1976) reported that ≤50 spore clusters of *P. betae* viruliferous for BNYVV generally did not cause infection of beet plants by either virus or vector, whereas, 100-150 cystosori per plant did initiate infection by both. These figures need to be treated with caution as they will depend on age of the spores and the proportion of viruliferous spores in the original inoculum, which was not measured. Giunchedi and Langenberg (1982) reported that infection of sugar beet roots with both virus and vector was obtained after inoculation using a rate of 60 zoospores per plant in glasshouse experiments. Gerik and Duffus (1988) reported differences in the aggressiveness of isolates of *P. betae,* which were viruliferous for BNYVV, although it is difficult to interpret these results as the number of spores used was not reported. Any detection method would therefore have to be capable of detecting c. 50-100 cystosori g^{-1} soil and be able to distinguish viable from dead cystosori.

Tuitert (1990) estimated levels of soil inoculum using bait seedlings combined with an MPN method where the infective unit was considered to be 50–100 resting

spore clusters, each of the spore clusters containing an average of 37 spores (Tuitert and Bollen, 1993).

The use of PCR methodology to detect *Polymyxa* spp. in plant tissue is common e.g. Mutasa-Gottingens et al. (1993, 1995, 1996, 2000), Ward et al. (2004). However, this methodology is prone to false positive results due to the detection of small amounts of DNA in dead target material. A rapid method for the detection of *Polymyxa* DNA directly from soil reported by Ward et al. (2004) was capable of detecting as few as 2.78×10^3 cysts of *P. betae* g^{-1} soil, but was unable to distinguish viable from non-viable cystosori. Kingsnorth et al. (2003) developed a recombinant antibody ELISA assay for the *P. betae* glutathione-S-transferase, which is expressed during infection. Outputs from this assay produced a linear response for zoospores. It is likely that the best assay for *Polymyxa* spore viability after composting will be based on a plant bait test method followed by microscopy or testing for glutathione-S-transferase using the ELISA methodology.

5.6. Clavibacter michiganensis subsp. *sepedonicus*

The ring rot bacterial pathogen Cms infects potatoes naturally. Cms is a short, motile, Gram-positive rod, which does not produce any resting spores. Spread is generally through infected potato seed. The main risk to compost is from disposal of infected potato tubers. There is very little information on the inoculum level required to cause potato ring rot disease, but information accumulated on the inoculum levels required to infect aubergine plants in the bioassay test suggest that levels of <10 cfu may cause infection in plants when artificially inoculated (Lelliott and Sellar, 1976).

Most available detection methods are designed to test potato tubers or stolons for Cms infection. Currently, methods able to establish the presence of viable Cms

include a bioassay on aubergine, which in practice is reliably able to detect down to 10^3cells ml^{-1} potato extract (EPPO, 1993). The disadvantage of this test is its relatively long timescale of 15-21 days.

Other available tests for Cms include serological tests using immunofluorescence antibody staining (IFAS) (De Boer and Naughton, 1986) with a reported sensitivity of 5×10^4 cells ml^{-1}(in practice more usually 5×10^3) of extract and ELISA methods (De Boer et al., 1988). Detection limits refer mainly to detection of Cms in cultures of bacteria isolated from infected plants. Both multiplex and Real Time PCR methods have a similar level of sensitivity as the serological methods (Pastrik, 2000; Schaad et al., 1999). The use of a pre-incubation on growth media has the additional advantage of confirming bacterial viability. The EPPO diagnostic protocol (EPPO 2006) recommends the use of either MTNA medium (Jansing and Rudolph, 1998) or NCP88 medium (de la Cruz et al., 1992). Detection limits of Taqman BIOPCR (where bacteria are isolated onto media and then tested using Taqman real time PCR) have been quoted as 2-3 cells ml^{-1} (Schaad et al., 1999).

5.7. Pepino mosaic virus

PepMV is a member of the genus *Potexvirus* and was originally described from pepino in Peru. However, the virus is also capable of infecting potato and tomato (*Lycopersicon esculentum*). The RNA virus genome has been completely sequenced (Aguilar et al., 2002; Cotillon et al., 2002). Tomato is the crop most at risk in Europe and therefore the material most likely to be encountered for disposal is infected tomato plants and fruit. Tomato seed has been reported to carry the virus and infection of the emerging seedlings is reported to occur (Krinkels, 2001). Spread through the seed trade is thought

to account for international spread of the virus. The virus has been shown to be highly contagious when introduced into crops (Spence et al., 2006).

Detection methods for PepMV include both serological and molecular methods. Serological methods include DAS ELISA- DSMZ antisera, lateral flow kits which can detect 1: 2049 dilution of infected leaf (Danks and Barker, 2000) and TAS ELISA using monoclonal which can detect 1:256 dilution of infected leaf (Salome and Roggero, 2002). Molecular methods include an Immunocapture Real Time PCR assay (no data on sensitivity) (Mansilla et al., 2003) and a Real Time PCR assay (detected 0.1–0.5 ug ul^{-1} RNA). The use of Real Time PCR has detected 1:100,000 dilution of infected leaf in uninfected leaf (Martinez-Culebras et al., 2002). Traditional indicator plants are still used to detect viable virus and can be combined with one of the above tests to determine viability.

5.8. Potato spindle tuber viroid (PSTVd)

PSTVd is a small viroid of 359 nucleotides and has been completely sequenced (Gross et al., 1978). It is becoming clear that a range of viroids with slightly different sequences cause different diseases so that PSTVd is probably part of a continuum of similar viroids. The main host for PSTVd is potatoes but it is also reported on tomatoes and sweet potatoes, as well as other *Solanum* spp. Transmission is mainly from infected plant material, which would be the main risk for composted wastes. Seed transmission is also reported, for example, through potato seed at levels of 0-100% and tomato seed at levels of 0.3–23% (Spence, 2004).

Bioassays can be used to detect the viroid using tomato or potato as indicator plants. Grasmich and Slack (1985) reported the use of tomato as an indicator plant could detect 5.6×10^{-2} propagules ml^{-1} inoculum when indexed by polyacrylamide gel

electrophoresis (PAGE). Combinations of a bioassay and sensitive confirmation using molecular tests are likely to increase this detection limit.

Jefferies and James (2005) detected the following amounts of infected tissue (mg) using the following methods: hybridisation using digoxigenin-labelled RNA probes (0.25–0.5), Real Time PCR (0.062), fluorescent Real Time PCR (0.0155), Return PAGE (10-20). Boonham et al. (2004) report a real time (Taqman) PCR assay with a detection level 1000 times more sensitive than a chemi luminescent hybridisation system. There is little information on how this relates to inoculum levels.

Spence (2004) developed a bioassay to assess the viability of PSTVd using 9 day old tomato seedlings, which were inoculated with material by gently rubbing the leaves with a cotton bud, soaked in water and celite (an abrasive powder). They were tested by TaqMan PCR assay after 3 weeks.

6. Conclusions

- Composting at constant temperatures in externally heated, small-scale systems has resulted in more precisely defined (and usually shorter) durations needed for eradicating pathogens such as *Fusarium oxysporum* ff.sp. and *Plasmodiophora brassicae*, than exposure in compost heaps, where temperatures are uncontrolled.
- There is no information on the eradication conditions from compost for a number of pathogens of quarantine importance. These include fungi (*G. citricarpa*, *S. endobioticum, T. indica*), oomycetes (*P. kernoviae*), bacteria (Cms) and viroids (PSTVd).
- Contradictory results for the temperature tolerance of the same pathogen reported by different workers may be due to variability in the wastes or other conditions of

the eradication tests, and/or differences between pathovars or condition of the pathogen and pest specimens used.

- A constant temperature of 60°C for 1 hour was sufficient to eradicate all the bacterial plant pathogens that were studied in plant material or other moist systems.
- A temperature of 50°C for 7 days was sufficient to destroy the reproductive capability of all the nematode and insect pests studied.
- Chlamydospores of *Microdochium nivale* survived a compost temperature of 58°C for 7 days (eradicated at 64°C).
- A compost temperature of 60°C for 1 day or 50°C for 7 days, with high compost moisture (60%) eradicated *P. brassicae*, but there is evidence that this organism can withstand higher temperatures with lower compost moisture (30-50%).
- Studies have examined the time to extinction of plant pathogens (which depends on the initial pathogen concentration) rather than the rate of decline, which is more important when defining conditions for eradication of pathogens of phytosanitary concern or which are heat-tolerant.
- Direct validation of compost hygiene using indicator organisms provides information on the likelihood of phytopathogen destruction.
- Highly temperature tolerant or variable plant pathogens such as TMV and *P. brassicae* may be unsuitable as indicator organisms and may, in some circumstances, compromise the safety of the compost.
- Detection methods exist for the quarantine organisms under consideration. However, methods for detection of *G. citricarpa* are poor. Detection of the

viability of chlamydospores of *P. ramorum* is also problematic but the use of mRNA detection methodology may be a possible option.

- It is particularly important that the detection methods used should be able to assess viability as well as presence of the pathogen. A validated bioassay test exists for Cms, PSTVd, *S. endobioticum* and *PepMV*. Cms can also be cultured on selective media to demonstrate viability although this can be unreliable because of competition from other bacteria. Although detection methods exist for *Polymyxa* spp., *P. ramorum* and *P. kernoviae*, they either are not suitable for viability testing or require further validation.

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Table 1. Plant pathogens covered in this review, hosts and common name of diseases caused.

Pathogen	Host(s)	Disease common name
Fungi		
Fusarium culmorum	cereals	foot rot
Fusarium oxysporum f. sp. *asparagi*	asparagus	wilt
F. oxysporum f.sp. *basilici*	sweet basil	wilt
F. oxysporum f. sp. *lycopersici*	tomato	wilt
F. oxysporum f.sp. *melongenae*	eggplant	wilt
F. oxysporum f. sp. *narcissi*	narcissus	basal rot
F. oxysporum f. sp. *radicis-lycopersici*	tomato	foot and root rot
Macrophomina phaseolina	various	dry root rot
Microdochium nivale	*Fusarium* patch	turf grass
Rhizoctonia solani	various	damping-off
Rosellinia necatrix	fruit crops	white root rot
Sclerotium cepivorum	*Allium* spp.	white rot
Sclerotinia sclerotiorum	various	soft rot
Sclerotium rolfsii	various	southern blight
Synchytrium endobioticum	potato	wart
Thielaviopsis basicola	various	black root rot
Tilletia indica	wheat	Karnal bunt
Verticillium dahliae	various	wilt
Oomycetes		
Phytophthora cactorum	various	crown and root rot
Phytophthora cinnamomi	various	root rot
Phytophthora citricola	various	stem and root rots
Phytophthora citrophthora	various	collar, crown, root rots
Phytophthora infestans	potato	blight
Phytophthora kernoviae	various	sudden oak death
Phytophthora lateralis	cypress	root rot
Phytophthora megasperma	various	stem and root rot
Phytophthora nicotiana	various	crown and root rot
Phytophthora ramorum	various	sudden oak death
Pythium ultimum	various	damping-off
Plasmodiophoromycetes		
Plasmodiophora brassicae	Brassicaceae	clubroot
Polymyxa betae	sugar beet	BNYVV vector
Polymyxa graminis	cereals	cereal virus vector
Bacteria		
Clavibacter michiganensis ssp. *michiganensis*	tomato	canker
Clavibacter michiganensis ssp. *sepedonicus*	potato	ring rot
Pectobacterium carotovorum	various	soft rot
Ralstonia solanacearum	various	bacterial wilt
Pseudomonas syringae pv. *syringae*	various	various
Xanthomonas campestris		
pv. *campestris*	Brassicaceae	black root rot
pv. *vesicatoria*	tomato, pepper	spot, scab
Viruses and viroids		
Pepino mosaic virus	tomato, potato	
Potato mop-top furovirus	potato	
Potato spindle tuber viroid	Solanaceae	
Tobacco mosaic virus	various	
Tobacco rattle virus	various	

Table 2. Insects, mites and plant parasitic nematodes covered in this review, hosts and common name of pests.

Pest	Host(s)	Common name of pest
Nematodes		
Aphelenchoides subtenuis	narcissus	narcissus bulb and leaf
Ditylenchus dipsaci	narcissus	narcissus stem
Globodera pallida	potato	white potato cyst
Globodera rostochiensis	potato	golden potato cyst
Meloidogyne incognita	beet	southern root-knot
Meloidogyne javanica		Javanese root-knot
Insects		
Delia antiqua	onion	onion fly
Eumerus strigatus	narcissus	small narcissus fly
Merodon equestris	narcissus	large narcissus fly
Psila rosae	carrot	carrot fly
Mites		
Rhizoglyphus echinops	narcissus	bulb mite
Steneotarsonemus laticeps	narcissus	bulb scale mite
Tarsonenemus myceliophagus	mushroom	tarsonemid mite

Table 3. Constant temperature-time eradication conditions of plant pathogenic fungi and oomycetes in compost.

Pathogen	Inoculum or infested plants	Feedstock	Temp. °C	Time days	Detection method	Medium	Reference
Fungi							
Fusarium oxysporum							
f. sp. *basilici*	macroconidia	straw, manure, fruit	ambient	30	plating	Nash medium	Noble et al. (2004)
f. sp. *lycopersici*	chlamydospores	onion waste	52	7	plating	Nash medium	Noble et al. (2004)
f. sp. *lycopersici*	tomato plants	green waste	46	7	plating	Nash medium	Noble et al. (2004)
f. sp. *narcissi*	chlamydospores	onion waste	50	7	plating	Nash medium	Noble (2005)
f. sp. *radicis-lycopersici*	chlamydospores	onion waste	46	7	plating	Nash medium	Noble et al. (2004)
f. sp. *radicis-lycopersici*	macroconidia	straw, manure, fruit	ambient	35	plating	Nash medium	Noble et al. (2004)
Microdochium nivale	chlamydospores	green waste	64	7	plating	Nash medium	Noble et al. (2004)
Rhizoctonia solani	mycelium	green waste	50	1	plating	malt agar + citric acid	Noble et al. (2004)
Rhizoctonia solani	mycelium	green waste	40	7	plating	malt agar + citric acid	Noble et al. (2004)
Sclerotium rolfsii	sclerotia	grape marc	50	1	plating	PDA	Noble (2005)
Thielaviopsis basicola	conidia/mycelium	green waste	40	7	plating	PDA + antibiotic	Noble et al. (2004)
Verticillium dahliae	microsclerotia	onion waste	46	7	plating	selective medium	Noble et al. (2004)
Verticillium dahliae	barley grains	onion waste	46	7	plating	selective medium	Noble et al. (2004)
Oomycetes							
Phytophthora nicotianae	oospores	onion waste	58	7	plating	PARPNH agar	Noble et al. (2004)
Phytophthora nicotianae	oospores	green waste	52	7	plating	PARPNH agar	Noble et al. (2004)
Phytophthora ramorum	bay leaves, oak wood and stems	green waste	55	14	plating, PCR, bioassay	PARP pear	Swain et al. (2006)
Pythium ultimum	oospores	potato/soil	52	7	plating	selective medium	Noble et al. (2004)
Pythium ultimum	oospores	green waste	50	3	plating	selective medium	Noble et al. (2004)

Table 4. Constant temperature-time conditions for eradication of plant pathogenic fungi and oomycetes in other systems.

Pathogen	Inoculum or infested plants	Medium	Temp. °C	Time h	System	Detection method	Medium	Reference
Fungi								
Fusarium oxysporum								
f.sp. *asparagi*	chlamydospores	green wastes	40	504	an. digester	plating	Komada's	Termorshuizen et al. (2003)
f.sp. *narcissi*	chlamydospores	soil	56	108	water bath	plating	PDA	Idelmann (2005)
Fusarium culmorum	wheat grains	soil	44	36	water bath	plating	PDA	Idelmann (2005)
Macrophomina phaseolina	tomato stem	water	55	0.2	water bath	plating	water agar	Bega and Smith (1962)
Macrophomina phaseolina	sclerotia	water	50	1.7	water bath	plating	PDA	Bega and Smith (1962)
Macrophomina phaseolina	mycelium	PDA	50	24	incubator	plating	PDA	Mihail and Alcorn (1984)
Macrophomina phaseolina	mycelium	PDA	55	48	incubator	plating	PDA	Mihail and Alcorn (1984)
Rosellinia necatrix	mycelium	soil	38	< 24	water bath	plating	MEA	Sztejnberg et al. (1987)
Sclerotinia sclerotiorum	sclerotia	soil	44	36	water bath	plating	MEA	Idelmann (2005)
Sclerotium cepivorum	sclerotia	green wastes	40	504	an. digester	bioassay	onion	Termorshuizen et al. (2003)
Sclerotium rolfsii	mycelium	PDA	50	6	incubator	plating	PDA	Mihail and Alcorn (1984)
Sclerotium rolfsii	mycelium	PDA	55	3	incubator	plating	PDA	Mihail and Alcorn (1984)
Tilletia indica	teliospores	water, soil	55	5	incubator	plating	agar	Smilanick et al. (1986)
Oomycetes								
Phytophthora cactorum	oospores	soil or twigs	45	0.5	water bath	plating	selective agar	Juarez-Palacios et al. (1991)
Phytophthora cinnamomi	chlamydospores	soil or twigs	45	0.3	water bath	plating	selective agar	Juarez-Palacios et al. (1991)
Phytophthora kernoviae	sporangia	leaves	45	0.3	water bath	plating	selective agar	Turner et al. (2008)
Phytophthora megasperma	oospores	soil or twigs	42	0.3	water bath	plating	selective agar	Juarez-Palacios et al. (1991)
Phytophthora nicotianae	mycelium	CMA	50	3	water bath	plating	CMA	McGovern et al. (2000)
Phytophthora nicotianae	chlamydospores	moist soil	47	3	water bath	plating	selective agar	Coelho et al. (2001)
Phytophthora ramorum	chlamydospores	moist sand	40	96	incubator	plating	PARPH + V8	Tooley et al. (2008)
Phytophthora ramorum	rhodod. leaves	moist sand	40	48	incubator	plating	PARPH + V8	Tooley et al. (2008)
Phytophthora ramorum	bay leaves	moist paper	55	336	incubator	plating	PARP	Harnik et al. (2004)
Phytophthora ramorum	bay leaves, oak wood and stems	moist paper	40	24	incubator	plating bioassay	PARP pear	Swain et al. (2006)
Phytophthora ramorum	sporangia	leaves	45	0.3	water bath	plating	selective agar	Turner et al. (2008)

Table 5. Constant temperature-time conditions for eradication and survival of plant pathogenic plasmodiophoromycetes in compost and other systems.

Eradication or survival, Pathogen	Inoculum or infested plant	Medium	Temp. °C	Time h	System	Bioassay	Reference
Eradication in compost							
Plasmodiophora brassicae	galls	green wastes	50	168	flasks	Chinese cabbage	Fayolle et al. (2006)
Plasmodiophora brassicae	galls	green wastes	60	24	flasks	Chinese cabbage	Fayolle et al. (2006)
Survival in compost							
Plasmodiophora brassicae	galls, roots	green wastes	50	24	flasks	Chinese cabbage	Fayolle et al. (2006)
Eradication other systems							
Plasmodiophora brassicae	brassica roots	plant wastes	40	504	an. digester	Chinese cabbage	Termorshuizen et al. (2003)
Plasmodiophora brassicae	galls	soil	50	96	water bath	mustard	Idelmann (2005)
Polymyxa betae	?	?	60	0.2	?	?	Abe (1987)
Polymyxa betae	infested soil	leachate	55	96	an. digester	sugar beet	Van Rijn and Termorshuizen (2007)
Polymyxa betae	infested soil	leachate	55	96	shaken flask	sugar beet	Van Rijn and Termorshuizen (2007)
Survival in other systems							
Plasmodiophora brassicae	brassica roots	plant wastes soil/water	55-60	504	flasks effluent heat	mustard	Elphinstone (2005)
Polymyxa betae	infested soil	suspensions	55	96	treatment	sugar beet	Dickens et al. (1991)
Polymyxa betae	resting spores	soil/water suspensions	70-75	96	effluent heat treatment	sugar beet	Dickens et al. (1991)

Table 6. Constant temperature-time conditions for eradication of bacterial plant pathogens in compost and other systems.

Bacterium	Inoculum or infested plants	Feedstock/ Medium	Temp. °C [a]	Time	System	Detection method	Medium	Reference
In compost								
Pectobacterium carotovorum	suspension	pepper wastes	65 max	60 h	heap	plating	Rose Bengal agar	Elorrieta et al. (2003)
Pseudomonas syringae pv. *syringae*	suspension	pepper wastes	65 max	12 h	heap	plating	Rose Bengal agar	Elorrieta et al. (2003)
Xanthomonas campestris pv. *campestris*	brassica leaves	green waste	40	7 days	flasks	plating	FS & MS media	Noble et al. (2004)
Xanthomonas campestris pv. *vesicatoria*	suspension	pepper wastes	65 max	36 h	heap	plating	Rose Bengal agar	Elorrieta et al. (2003)
In other systems								
Clavibacter michiganensis ssp. *michiganensis*	tomato seed	water	52	0.4 h	water bath	plating	agar	Fatmi et al. (1991)
ssp. *michiganensis*	tomato seed	water	56	0.5 h	water bath	bioassay	tomato	Shoemaker and Echandi (1976)
ssp. *michiganensis*	tomato debris	peat, sand	45	28 days	incubator	bioassay	tomato	Zanon and Jorda (2008)
ssp. *sepedonicus*	suspension	water	82	5 min	water bath	plating	non-selective	Secor et al. (1987)
Clavibacter xyli subsp. *xyli*	sugar cane seed	?	51	1 h	?	?	?	Ramallo and Ramallo (2001)
Pectobacterium carotovorum	suspension	pepper wastes	50	15 h	incubator	plating	Rose Bengal agar	Elorrieta et al. (2003)
Pectobacterium carotovorum	suspension	pepper wastes	60	1 h	incubator	plating	Rose Bengal agar	Elorrieta et al. (2003)
Pseudomonas syringae	suspension	pepper wastes	50	15 h	incubator	plating	Rose Bengal agar	Elorrieta et al. (2003)
pv. *syringae*	suspension	pepper wastes	60	2 h	incubator	plating	Rose Bengal agar	Elorrieta et al. (2003)
Ralstonia solanacearum	plant residues	inoculum	45	12 h	incubator	?	?	Date et al. (1993)
Ralstonia solanacearum	ginger roots	inoculum	50	30 min	incubator	PCR/plating	M9 agar	Tsang and Shintaku (1998)
Ralstonia solanacearum	potato tubers	plant wastes	40	21 days	an. digester	plating	SMSA medium	Termorshuizen et al. (2003)
Ralstonia solanacearum	?	water	45	2 h	water bath	plating	SMSA medium	Termorshuizen (2006)
Ralstonia solanacearum	?	water	55	0.1 h	water bath	plating	SMSA medium	Termorshuizen (2006)
Xanthomonas campestris pv. *vesicatoria*	suspension	pepper wastes	50	15 h	incubator		Rose Bengal agar	Elorrieta et al. (2003)
	suspension	pepper wastes	60	1 h	incubator		Rose Bengal agar	Elorrieta et al. (2003)

[a] Compost temperatures were constant unless stated as maximum or minimum values.

Table 7. Constant temperature-time survival and eradication conditions for viral plant pathogens in compost and other systems.

Virus	Inoculum or infected plants	Feedstock/ medium	Temp. °C	Time	System	Detection method	Medium	Reference
Compost - survival								
Tobacco mosaic virus	tobacco leaves	green waste	60	35 days	flask	bioassay	*Nicotiana*	Noble et al. (2004)
Compost - eradication								
Tobacco mosaic virus	tobacco leaves	green waste	80	7 days	flask	bioassay	*Nicotiana*	Noble et al. (2004)
Tobacco mosaic virus	tobacco leaves	biowastes	50	35 days	incubator	bioassay	*Nicotiana*	Idelmann (2005)
Other systems - survival								
Tobacco mosaic virus	tobacco leaves	digestate	36	74 days	anaerobic digester	bioassay	*Nicotiana*	Termorshuizen (2006)
Tobacco mosaic virus	tobacco leaves	soil	70	21 days	water bath	bioassay	*Nicotiana*	Idelmann (2005)
Other systems - eradication								
Potato mop-top furovirus	*Spongospora subterranea* spores	soil	90	15 min	oven	bioassay/ ELISA	*Nicotiana*	Nielsen and Molgaard (1997)

Table 8. Temperature-time eradication conditions for insects, mites and plant parasitic nematodes in compost and other systems.

Pest	Inoculum or infested plants	Feedstock/ medium	Temp. °C [a]	Time h	System	Detection method	Medium	Reference
Compost - Insect								
Delia antiqua	larvae	onion waste	50	168	compost	microscopy	-	Noble (2005)
Psila rosae	larvae	onion waste	50	168	compost	microscopy	-	Noble (2005)
Other systems - Insect								
Merodon equestris	larvae	water	44	1	water bath	?	-	Lane (1984)
Eumerus strigatus	larvae	water	44	1	water bath	?	-	Lane (1984)
Compost - Mite								
Tarsonemus myceiophagus	adult	compost	49	0.33	compost	?	-	Fletcher et al. (1986)
Other systems - Mite								
Rhizoglyphus echinops	*Narcissus* bulbs	water	47	3	water bath	?	-	Lane (1984)
Compost - Nematode								
Globodera pallida	cysts	onion waste	40	168	flasks	hatching juveniles	root diffusate	Noble (2005)
Globodera rostochiensis	cysts	onion waste	40	168	flasks	hatching juveniles	root diffusate	Noble (2005)
Globodera rostochiensis	cysts	household, green waste	60 max	696	compost	hatching juveniles	root diffusate	Bøen et al. (2006)
Globodera rostochiensis	cysts	household, green waste	50 min	192	compost	reproduction	potato bioassay	Bøen et al. (2006)
Other systems - Nematode								
Aphelenchoides subtenuis	*Narcissus* bulbs	water	47	0.1	water bath	?	-	Woodville (1964)
Ditylenchus dipsaci	*Narcissus* bulbs	water	47	0.4	water bath	?	-	Woodville (1964)
Ditylenchus dipsaci	suspension	water	50	0.25	water bath	microscopy	water	Qiu et al. (1993)
Ditylenchus dipsaci	*Narcissus* bulbs	water	48	1.5	water bath	microscopy	water	Qiu et al. (1993)
Ditylenchus dipsaci	garlic bulbils	water	49	1	water bath	microscopy	-	Jaehn (1995)
Meloidogyne incognita	grape rootstock	water	50	0.33	water bath	bioassay & microscopy	sodium hypochlorite	Gokte and Mathur (1995)
Meloidogyne javanica	grape rootstock	water	50	0.33	water bath	bioassay & microscopy	+ acid fuchsin	Gokte and Mathur (1995)

[a] Compost temperatures were constant unless stated as maximum or minimum values.

Table 9. Availability of detection methods for quarantine fungi, oomycetes and plasmodiophoromycetes.

Pathogen	Survival spores/ structures	Inoculum required for infection	Detection methodology	Limit of detection	Viability	Reference
Guignardia citricarpa	asexual pycnidiospores on fruit surface	no data	culturing on potato dextrose agar, oatmeal agar, cherry decoction agar	no data	√	EPPO (2003) Agostini et al. (2006)
	conidia/ascospores on infected leaves		PCR	20 pg DNA		Bonants et al. (2003) Peres et al. (2007)
			Real Time PCR	10 fg DNA		Van Gent-Pelzer et al (2007)
Synchytrium endobioticum	winter sporangia in tubers/soil	10 sporangia g^{-1} soil	bioassay	1–5 sporangia g^{-1}	√	Hampson and Wood (1997) EPPO (1999b) EPPO (2004b)
			spore viability microscopic assay	80–95% spore recovery	√	Pratt (1976) Laidlaw (1985)
			PCR	10 sporangia		Van den Boogert et al. (2005)
Tilletia indica	teliospores	in theory, 1 teliospore	soil sieving followed by sucrose centrifugation and plating on agar to assess germination	recovery rate 74.3% or 10 teliospores/g soil	√	Murray et al. 2005 Bonde et al. (1997) Babadoost and Mathre (1998) Inman et al. (2008)
Phytophthora ramorum	chlamydospores and possibly oospores	in theory, 1 zoospore	culturing.			Lane et al. (2007)
			serological screen followed by PCR for species identification	1pg DNA		Kox et al. (2002) Schena et al. (2006) Wagner and Werres (2003) Guglielmo et al. (2007)
			Real Time PCR	15-50 fg DNA 10 pg DNA		Hayden et al. (2004) Hughes et al. (2005) Belbahri et al. (2007) Bilodeau et al. (2007)
			pear baiting method	no data	√	Swain et al. (2006)
			rhododendron leaf baiting method	no data	√	Fichtner et al. (2007)
			culturing on selective PARP agar	no data	√	Swain et al. (2006)
			mRNA detection		√	Chimento and Garbelotto (2007)
			field detection using PCR	no data		Tomlinson et al. (2005)
			lateral flow kit	87.6% efficient compared with laboratory tests		Lane et al. (2007)
Phytophthora kernoviae	sporangia oospores		similar to *P. ramorum*	no data		Schena et al. (2006)
Polymyxa spp	cystosori in roots or soil	100-150 cystosori	detection of spores from soil using Real Time PCR	1.39×10^3 cystosori g^{-1} soil		Ward et al. (2004)
			bait tests followed by glutathione-S-transferase ELISA or microscopy	no data	√	Kingsnorth et al. (2003)
			MPN method bait test method	50–100 cystosori	√	Tuitert (1990)

Table 10. Availability of detection methods for quarantine bacteria, viruses and viroids

Organism	Survival spores/ structures	Inoculum required for infection	Detection methodology	Limit of detection	Viability	Reference
Clavibacter michiganensis subsp. *sepedonicus*	not a spore former, cells in infected plant material or possibly potato seed	<10cfu for aubergine artificial infection	eggplant bioassay	10^3 cells ml^{-1}	√	Lelliot and Sellar (1976) EPPO (2003)
			MTNA medium	no data	√	EPPO (2006) Jansing and Rudolph (1998)
			NCP88 medium	potato tubers – 3.0 $x10^5$- 1.2 × 10^9 $cfug^{-1}$ stems – 1.1$x10^9$-9.3$x10^9$ cfu g^{-1}	√	de la Cruz et al. (1992)
			immunofluorescence antibody staining (IFAS)	5 x 10^3 cells ml^{-1} of extract		De Boer and Naughton, (1986)
			ELISA	similar to IFAS –10^6cfu g^{-1} fresh weight		Gudmestad et al. (1991)
			multiplex and Real Time PCR methods	5 × 10^3 cells per ml of extract		Pastrik et al. (2000), Schaad et al. (1999)
			Taqman BIO PCR	2–3 cells ml^{-1}		Pastrik et al. (2000), Schaad et al. (1999)
Pepino mosaic virus	virus in plant material or tomato seed	?	indicator plants to confirm viability followed by:		√	
			serological methods using lateral flow kits	1: 2049 dilution of infected leaf		Danks and Barker (2000)
			TAS ELISA using monoclonal	1:256 dilution of infected leaf		Salome and Roggero (2002)
			Real Time PCR	0.1-0.5 ug ul^{-1} RNA		Mansilla et al. (2003)
			Real Time PCR	1:100,000 dilution of infected leaf in uninfected leaf		Martinez-Culebras et al. (2002)
Potato spindle tuber viroid (*PSTVd*)	viroid in plant material, possibly in seed	potato – 0.12ng tomato – 0.24ng	bioassay using indicator plants	5.6 × 10^2 propagules ml^{-1}	√	Grasmich and Slack (1985), Spence (2004), Singh et al. (1990)
			Real time PCR for seed transmission	0.062 mg infected tissue		Jeffries and James (2005)
			Real Time TaqMan PCR assay	×1000 more sensitive than chemiluminescent system		Boonham et al. (2004) Roenhorst et al. (2005)