Literature review on detection and eradication of plant pathogens in sludge, soils and treated biowasteDeskstudy on bulk density

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Contents

1. GENERAL INTRODUCTION

The European STREP "HORIZONTAL-HYG" project to develop "Horizontal Standards on Hygienic Microbiological parameters for implementation of EU Directives on sludge, soil and treated biowaste" started on 1st December 2004. This project is carried out under the umbrella of the main project HORIZONTAL "Development of horizontal standards for soil, sludge and biowaste".

The strategic objectives of this HORIZONTAL-HYG project focus on the development of reliable and harmonised European standards for sampling and hygienic microbiological parameters in the field of sludge, soil and treated biowastes and similar matrices. These methods are of fundamental importance to properly evaluate the environmental problem they may pose and to facilitate regulation of these parameters related to different uses and disposal governed by EU Directives. The Working document on revision of the Sewage Sludge Directive (86/278/EEC; draft April 2000) and the Working Document on Bio-waste (draft February 2001) called for standards on sampling, and analysis of hygienic and biological parameters, inorganic parameters and organic pollutants.

This project is concentrated only on the development of horizontal standards (if possible) for **microbiological parameters**, including **sampling and sample handling** taking into account the limited stability of microbiological parameters. Defining test organisms and test methods for the validation of safe treatment processes (biotechnological, chemical and physical treatment) forms part of the project.
Besides sampling and sample handling (WP 1) and process control and process validation (WP3), the central work package (WP 2) deals with methods by which microbiological parameters describing the microbiological quality of the final product or applicable for the re-isolation of test organisms applied in validation procedures shall be determined in a reliable way :
For *Salmonella* spp. and *Escherichia coli* (SubWP2/1) drafted CEN standards are available and therefore a co-normative work will be performed consisting in the validation of those methods (performance data). This work will consist in three main steps : (i) a training in a central laboratory of 16 EU laboratories for methods to be validated, (ii) an intralaboratory suitability study of methods to be validated (fit for purpose on the nine different matrices that are to be targeted) and finally (iii) an interlaboratory round robin test with selected laboratories to validate the methods.
For Enterococci and *Clostridium perfringens* (SubWP2/2), **viable helminth ova** (SubWP2/3) and **bacteriophages** (SubWP2/4), all relevant from the point of view of human and animal health as well as plant protection and environmental safety, only a pre-normative work will be performed (no validation study). This will consist in two main steps : (i) a critical review including an European workhop with experts first leading to a decision if and for which substrates standards shall be drafted and (ii) an intralaboratory suitability study of identified draft standards (fit for purpose on the nine different matrices that are to be targeted).
For plant pathogens (SubWP2/5), a 12 months desk study will be performed.

Literature review on detection and eradication of plant pathogens in sludge, soils and treated biowaste

1.1. Introduction

There is an increasing amount of sewage sludge and composted biowaste being recycled to soils used for plant production. As a result, there is increasing concern that plant diseases may originate from primary infection by plant pathogens present in waste material recycled to field crops, gardens, forests or greenhouse crops. Another concern is the introduction of exotic plant pathogens with waste material from outside the EU or the spread of a plant pathogen into areas in EU, which are protected zones for the pathogen in question. Furthermore, increased use of recycled composted greenwaste instead of non-renewable peat in soil conditioners and growth media may lead to increased risks of seedling diseases and root rot caused by species of *Pythium* and *Rhizoctonia* in greenhouse and other horticultural operations. The risks of spreading plant pathogens through recycling of biowaste have yet to be quantified. Any assessment of biowaste management, treatment or handling capacity in relation to such risks will also need to take into account cost-efficiency and environmental acceptability.

There are, however, few examples in the literature where sewage sludge or composts have been found to be major sources of plant pathogens for agricultural or horticultural crops. In contrast, the addition of well conditioned organic matter to soils is usually reported to be beneficial in suppressing those plant pathogens which are already soil-borne. The potato cyst nematodes *Globodera rostochiensis* and *G. palida* are amongst the few organisms which have been proven to survive in sewage sludge, due to the formation of resistant cysts. Other organisms with hardy resting structures may also survive composting and other common biowaste treatments. These include fungal pathogens such as *Plasmodiophora brassicae* (the cause of club root disease of *Brassica* spp.), *Fusarium oxysporum* f.sp. *lycopersici* (tomato wilt), *Macrophomina phaseolina* (dry root rot), *Synchytrium endobioticum* (potato wart disease), *Olpidium brassicae* (fungal vector of *Tobacco necrosis virus* and *Lettuce big vein virus*), *Polymyxa betae* (fungal vector of *Beet necrotic yellow vein virus*) and *Spongospora subterranea* (powder scab in potato and vector of *Potato mop top virus*). *Phytophthora ramorum,* causing sudden oak death, is an example of a plant pathogen which appears to have been newly introduced into parts of the EU and which has recently been found on *Rhododendron* spp. and other hosts in the UK. As yet, it is unknown whether this organism could spread through recycling of green waste which may contain infected plant material.

A number of plant viruses, namely, cucumber mosaic virus, tomato mosaic virus and tomato bushy stunt virus have been detected (Tomlinson and Faithful, 1984) in tomato plants growing on dried sewage sludge from tomato seeds which had survived sewage treatment.

Whereas bacterial plant pathogens are generally not thought to survive composting or sewage treatment, their spread in biowastes has occurred when such treatments have either failed or have been inadequate. For example, *Ralstonia solanacearum* (the quarantine bacterium causing potato brown rot disease) has been shown to colonise solanaceaous weeds (e.g. *Solanum dulcamara*) in river systems when introduced via industrial or domestic waste disposal. Transmission to potato crops has been observed in several EU n contaminated river water has been used for irrigation.

In contrast to viewing sludge and biowaste as a waste problem or a low grade nutrient supply product on arable land, there is evidence that quality compost can enhance soil biological activity and suppress soilborne diseases and even foliar pathogens by inducing disease resistance. Fuchs *et*

al. (2004) suggests that instead of solving a biowaste management problem only, biowaste should be used for production of quality compost in order to improve soil fertility and plant growth. Maturation and conditions of storage (oxygen diffusion) are important factors affecting compost quality. The three most important factors are the moisture of the materials, the air composition and temperature. At the end of maturation pH, salt content (i.e. electrical conductivity), ammonium nitrite and nitrate content have to be analysed. Plant tests and disease suppressive properties against "Rhizoctonia" and "Pythium" are also recommended. With this information, it is possible to choose the most appropriate compost for each end-use (Fuchs *et al*. 2004).

1.2. Scope

This report reviews

- qualitative assessment of the risk of spreading plant pathogens with sludge and biowaste of vegetable origin
- national regulations, guidelines and standards for sludge and biowaste on plant health safety
- selected plant pathogens and their sensitivity to sludge and biowaste treatment
- methods for detection of plant pathogens in composted and otherwise-treated biowastes

This review does not consider the possible risk for plant diseases from spreading of plant pathogen infested soil on arable land from e.g. building or construction sites. Phytotoxicity from sludges and biowastes is considered elsewhere in project HORIZONTAL**.**

2. Definitions

In this report the term "plant" is used with two different meanings:

(i) Photosynthetic, eukaryotic, multicellular organisms of the kingdom *Plantae* characteristically producing embryos, containing chloroplasts, having cellulose cell walls, lacking the power of locomotion, and are the subject of plant pathogen attack and reproduction

(ii) The equipment, including machinery, tools, instruments, and fixtures and the buildings/location containing them, necessary for treatment of sludge and biowaste

2.1. Plant pathogens

Description of plant pathogens

A plant pathogen is an organism or a virus that can inhabit and survive on plants and can compromise the health of the plant causing disease symptoms. Plant pathogens may be fungi, bacteria, viruses or nematodes, covering different levels of host specificity, some with a broad host range, others host species specific.

Plant pathogen inoculum is the biological structure (e.g. spore, conidium, sclerotium, mycelium, cell, egg, cyst, particle) able to cause primary infection of a plant.

Some structures can survive a long time in soil, some survive in living plant parts or in plant debris, and may be easily eradicated.

There may be a difference in the level of inoculum needed to cause infection at various conditions (pH, temperature, humidity, etc.).

Table 1. Plant pathogens and pests of plant health concern covered in this report.

Plant pathogen or pest	Hosts	Disease or nematode

Bacteria:		
Clavibacter michiganensis subsp. *michiganensis*	tomato	bacterial canker
Clavibacter michiganensis* subsp. *sepedonicus	tomato	ring rot
Clavibacter xyli subsp. *xyli*	sugar cane	stunting disease
Erwinia amylovora	*Rosacea*	fire blight
Erwinia chrysanthemi	various	soft rot, blight
Ralstonia solanacearum	*Solanacea*	bacterial wilt
Pseudomonas savastanoi pv. *phaseolicola*	*Phaseolus* beans	halo blight
Xanthomonas campestris pv. *campestris*	*Brassicae*	black rot
Fungi:		
Armillaria luteobubalina	various woody	honey fungus
Botrytis cinerea	various	grey mold
Botrytis allii (syn. aclada)	onion	neck rot
Fusarium oxysporum f.sp. -	various	wilt
Macrophomina phaseolina	various	dry root rot
Microdochium nivale	grasses	snow mold
Olpidium brassicae	various	vector of LBVV & TNV
Phytophthora kernoviae	various woody	
Sclerotinia sclerotiorum	various woody	sudden oak death
Phytophthora spp.	various	root rot, blight
Plasmodiophora brassicae	*Brassicaceae*	clubroot
Polymyxa betae	*Chenopodiacea*	vector of BNYV
Polymyxa graminis	cereals	vector of various viruses
Pythium sp.	various	damping off, root rot
Rhizoctonia solani	various	damping off, potato black scurf
Sclerotinia sclerotiorum	various	watery soft rot
Sclerotinia cepivorum	*Allium*	white rot
Sclerotium rolfsii	various	southern blight
Spongospora subterranean	potato	powdery scab
Synchytrium endobioticum	potato	potato wart disease
Thielaviopsis basicola	various	black root rot
Tilletia indica	wheat	karnal bunt

Note: Quarantine plant pathogens and pests are indicated in bold

Plant pathogens can be divided according to the level of severity and economic loss that they may cause in plant production. It is also relevant to group the plant pathogens into ubiquitous or quarantine plant pathogens. Table 1 includes the plant pathogens covered in this report **and the quarantine plant pathogens are indicated in bold.**

Quarantine plant pathogens:
The risk of spreading plant pathogens with plant material used for planting as well as import/export of plants from EU is regulated according to EU Council Directive 2000/29/EC on protective measures against the introduction into the Community of organisms harmful to plants or plant products and against their spread within the Community. This directive includes living plants or plant parts used for planting, cut flowers, branches with foliage and cut trees retaining foliage. Also included are plant products understood as products of plant origin, unprocessed or having undergone simple preparation, in so far as these are not plants. Wood only in so far as it retains all or part of its natural round surface, with or without bark, or as it is in the form of chips, particles, sawdust, wood waste or scrap, and also in the form of dunnage, spacers, pallets or packing material which are actually in use in the transport of objects of all kinds, provided that it presents a plant health risk are included in the directive.
The quarantine plant pathogens included in the directive are divided into three groups:

-harmful organisms not known to occur in any part of the Community and relevant for the entire Community
-harmful organisms known to occur in the Community but not endemic or established throughout the Community and relevant for the entire Community
-other harmful organisms against the protected zone for which they are relevant.

Sources of plant pathogens:
All material of plant origin in sludge, soil and biowaste may harbour the potential risk of being contaminated with plant pathogens. The composition of plant pathogens is dependent on the type and species of plant material included in the waste.

Waste originating from plants or plant products originating in third countries from outside of the EU may contain plant pathogens, which are not established within the European flora. However import from such sources is already regulated according to the EU plant health directive (2000/29/EC).

2.2. Types of sludge and biowaste

Sludge
Sludge (= a "mixture of water and solids separated from various types of water as a result of natural or artificial processes") (European Standardisation Committee, CEN)

Sludge: Sewage sludge (sludge from urban waste water)
Septic tank sludge

Industrial sludge

Definition from the Sludge directive 86/278/EEC:
(a)'sludge' means:
(i)residual sludge from sewage plants treating domestic or urban waste waters and from other sewage plants treating waste waters of a composition similar to domestic and urban waste waters;
(ii)residual sludge from septic tanks and other similar installations for the treatment of sewage;
(iii)residual sludge from sewage plants other than those referred to in (i) and (ii);
(b)'treated sludge' means:
sludge which has undergone biological, chemical or heat treatment, long-term storage or any other appropriate process so as significantly to reduce its fermentability and the health hazards resulting from its use.

Note: the term biosolid equals sewage sludge in this report.

Biowaste
Biodegradable waste (=any waste capable of undergoing anaerobic or aerobic decomposition)(EU, 2001):

Sources of biodegradable waste (*Lepeuple et al. 2004)****:***
Agriculture and forestry – crop residues, fruit/vegetable waste, pruning waste, spent growth media, forests maintenance waste, bark and wood chips, sawmills/wood industry, manure, slurry, etc.
Municipalities - yard waste, green waste, household waste, food waste, septic tank sludge, sewage sludge
Industries – food and feed processing waste

Biowaste is in the broadest sense any biodegradable waste. However, biowaste mentioned in this report may be partly or all of plant (vegetable) origin for relevance in the plant pathogen perspective.

2.3. Soil

Soil for agricultural purposes is defined as the plant root growth zone, normally the upper 0-25 cm of cultivated soil.
Transport and spread of soil from beet (*Beta vulgaris* L.) within the EU and import and spread of soil from outside EU are restricted according to the EU directive 2000/29/EC.
Surface soil from construction or building sites may be infected with plant pathogens, and may thus cause a potential risk for spread of disease. However, most of this soil is not spread on agricultural land and will not be covered further in this study.

Soilborne plant pathogens:
Soilborne plant pathogens can significantly reduce the yield and quality of crops. Soilborne pathogens can be defined as pathogens that cause plant diseases via inoculum that comes to the plant by way of the soil (Koike *et al.*, 2003). The most familiar diseases are probably rots that affect below ground tissues and vascular wilts initiated through root infections. Soilborne pathogens can be divided into soil inhabitants which are able to survive in soil for a relatively long time and soil transients which are only able to survive in soil for a relatively short time. Survival of the pathogens in soil depends on the survival structure (resting spores, sclerotia, oospores, cysts) or some

pathogens are even able to survive as saprophytes living on dead plant debris. The most important soilborne plant pathogens belong to very different classes: Plasmodiophoramycetes, Oomycetes, Zygomycetes, Ascomycetes, Basidiomycetes and Deuteromycetes. Fewer plant diseases are caused by soilborne bacterial pathogens. For example species of *Pseudomonas, Erwinia, Ralstonia* and *Xanthomonas* usually persist in the soil for only a short time. Various nematodes and a few soilborne viruses affect crops (Koike *et al*., 2003).

3. Risk of spread of plant pathogens via organic waste streams

Plant based organic wastes in the different countries have been quantified as part of the recent EU project RECOVEG (www.hri.ac.uk/recoveg/recoveg.htm) and are shown in Table 2. The predominant waste streams available for composting and subsequent use in vegetable crop production differed between Northern European countries (UK, Ireland, France) and Southern European countries (Greece, Israel). In Northern countries, the predominant wastes were spent mushroom compost (SMC), green wastes (tree prunings, grass clippings etc.) and waste vegetables. Waste potatoes were also a significant quantity, but these could largely be disposed off as animal feed. In Southern countries, wastes from the olive, wine-making and fruit/vegetable industries predominated. Spent mushroom composts are also available but in smaller quantities than in Northern Europe.

Table 2: Annual production of plant-based wastes in different countries (www.hri.ac.uk/recoveg/recoveg.htm).

Waste Material	Country	1000 Tonnes	Ref.
Spent Mushroom Compost	UK	157	7
	Ireland	273	8
	Greece	10	9
	France	250	16
	Israel	15	15
	Spain	320	9
	Netherlands	700	16
Green wastes that are composted	UK	640	6
	Ireland	8	10
	France	1900	13
Waste Vegetables	UK	173	1-4
	Ireland	2	11
Waste Citrus Fruit	Spain	124	9
Waste Potatoes	UK	186	1, 5
	Ireland	23	10
Greenhouse Crop Residues	Ireland	5	12
	Israel	29	15
	Spain	1317	9
Grape Marc Waste	Greece	140	14
	Spain	296	9
Olive Leaves	Greece	95	14
Olive Press Cakes	Greece	950	14
	Spain	1100	9
Olive Mill Waste Waters	Greece	1150	15
	Spain	1651	9
Other Organic Wastes that are Composted	UK	192	6
	Israel	417	15

1. Basic horticultural statistics for the United Kingdom. Calendar and crop years 1991/2 - 2001/2. Department for Environment, Food and Rural Affairs. DEFRA Publications, London, 73pp.
2. Monthly report on the fruit and vegetable crops in England and Wales. March 2001. Department for Environment, Food and Rural Affairs, York, 199pp.
3. Coventry, E., Noble, R., Whipps, JM (2001) Composting of onion and other vegetable wastes, with particular reference to control of *Allium* white rot. Horticulture LINK Project CSA 4862.
4. Organic Recycling Ltd, Spalding, Lincs.
5. Potato Statistics in Great Britain 2000-2001. Potato Marketing Board, Oxford. 24pp.
6. The State of Composting in the UK (1999). The Composting Association, Wellingborough, Northants, 54pp.
7. Peatering Out - towards a sustainable UK growing media industry. Royal Society for the Protection of Birds. Sandy, Beds. 26pp.
8. Richardson SJ, Holmes SJ (1999) Supply of spent mushroom compost: Monitoring and assessment of peat and alternative products for growing media and soil improvers in the UK. ADAS, Wolverhampton, 8pp.
9. Spanish Ministry of Agriculture, Madrid.
10. Dublin Corporation, Ireland.
11. Bord Glas Census, Ireland.
12. Teagasc, Dublin, Ireland.
13. AND International, ADEME – Quels debouches pour les compost, Decembre 2002.
14. National Agricultural Research Foundation, Greece.
15. Volcani Center, Israel.

Two main pathways by which plant pathogens may be transmitted to their host plants via biowaste streams are considered:

1. Through incorporation of biosolids, concentrated during wastewater/sewage treatment, into arable land.

2. Through generation of composts from greenwaste which may then be used as soil improvers or used in plant growth media.

There is little published information on the survival of individual plant pathogens during wastewater treatment, sludge treatment/maturation or after incorporation of sewage sludge into soil. Of the few reports on the fate of plant pathogens during sewage treatment, most concern is on the survival of potato cyst nematodes (*Globodera* spp.) and potential transmission to potato crops (Heinicke, 1989; Spaul and McCormack, 1989). The authors therefore recommended that sewage sludge should not be applied to land intended for the growth of seed potatoes, bulbs or other nursery stock affected by these nematodes. Storage of sludge over a 6 month period reduced but did not eliminate viable nematode cysts (Heinicke, 1989). Whereas anaerobic digestion (mesophilic or thermophilic) or pasteurisation of the sludge did effectively eliminate viable cysts, viability was not acceptably reduced by treatment with lime at pH 11.5 (20°C, 24 hours), aerobic stabilisation in an oxidation ditch (7 weeks) or activated-sludge treatment (5 days) (Spaul and McCormack, 1989).

A number of plant viruses, namely, cucumber mosaic virus, tomato mosaic virus and tomato bushy stunt virus have been detected (Tomlinson and Faithful, 1984) in tomato plants growing on dried sewage sludge from tomato seeds which had survived sewage treatment. Tomato bushy stunt virus and other similar plant viruses have also been detected in river water, thought to originate from the liquid effluent from sewage treatment works. Numerous other plant viruses that are resistant to biological degradation have similarly been isolated from river waters (Koenig, 1986). Many of these viruses undoubtedly survive sewage treatment and, hence, could be transmitted to horticultural crops, if untreated sludge were used on land.

Godfree and Farrell (2005) have considered the fate of plant pathogens in wastewaters, the range and numbers of which vary with the level of endemic disease in the community, discharges from commercial activities, and seasonal factors. Wastewater treatment reduces number of pathogens by concentrating them with the solids in the sludge. Although some treatment processes are designed specifically to inactivate pathogens, many are not, and the actual mechanisms of microbial inactivation are not fully understood for all processes. Regulations to control pathogen risk in the United States and Europe arising from land application of biosolids are based on the concept of multiple barriers to the prevention of transmission. The barriers are (i) treatment to reduce pathogen content and vector attraction, (ii) restrictions on crops grown on land to which biosolids have been applied, and (iii) minimum intervals following application and grazing or harvesting. It was concluded that effective pathogen risk management requires control to the complete chain of sludge treatment, biosolids handling and application, and post-application activities. This may be achieved by adherence to quality management systems based on hazard analysis critical control point (HACCP) principles.

A number of treatment options can render sewage sludge safe for use as an agricultural fertiliser. Composting and pelleting currently offer the most acceptable means to produce biosolids with no

detectable pathogens (Brown, 2005). Drying and pelleting are now being applied at much smaller plants (treatment facilities) because of the introduction of indirect dryers, which have fewer air pollution problems than the direct dryers still used at some larger plants (Oleszkiewicz and Mavinic, 2002). Stabilisation of biosolids in newer plants is more often combined with disinfection by composting at thermophillic temperatures, in anaerobic and particularly in aerobic regimes. For the smallest plants, dewatering is now available in drying bags or vacuum drying beds, and larger plants benefit from an array of new devices offering sludge cakes as dry as 22 to 40% total solids.

Ryckeboer *et al.* (2002a) showed that a number of plant pathogens and nematodes (including *Plasmodiophora brassicae*, *Heterodera schachtii*, *Meloidogyne incognita* and *Ralstonia solanacearum*) with the exception of tobacco mosaic virus (TMV) could be eliminated during anaerobic digestion of source separated household wastes. High temperature composting (at 58-68 ºC for 12-19 days) following anaerobic digestion reduced but did not eliminate infectivity by TMV. Termorshuizen *et al.* (2003) further studied the efficiency of plant pathogen elimination from vegetable, fruit and garden waste during 6 weeks of mesophilic anaerobic digestion at maximum temperature of 40 ºC and a ratio of 50:50% digested and undigested material. They concluded that many pathogens could be inactivated readily (including *Fusarium oxysporum* f.sp. *asparagi* and *Ralstonia solanacearum*), but that anaerobically digested compost may involve some significant phytohygienic problems. Viable *Plasmodiophora brassicae* was found to survive at low level and viable sclerotia of *Sclerotium cepivorum* were also recovered from the digestion vessel.

Termorshuizen *et al.* (2005) assessed the phytosanitary risks associated with compost utilization. Although, the risks are often related to pathogen inactivation at lethal temperatures, several additional factors were considered, all relating to tracing-and-tracking principles. The following parameters were considered to affect risk: (1) the proportion of host plant biomass relative to the total quantity of biowaste, (2) the proportion of host plant material infected with a pathogen, (3) the density of the infected host material, (4) the proportion of propagules of a pathogen that survived the process, and (5) the threshold density of the pathogen in soil above which transmission to host plants may be expected. , Little knowledge exists in the literature on the density of pathogens in host materials or on threshold values.

The RECOVEG project has examined the risks of plant pathogens surviving different large-scale composting systems using different composting feedstocks. It has been concluded that most plant pathogens, including the heat tolerant *Brassica* club root fungus (*Plasmodiophora brassicae*), can be eliminated from plant waste provided that suitable conditions (heating for at least 7 days at 50 - 60°C, and at a high moisture content of 50% or higher) can be maintained during the process. The exception amongst the pathogens studied was the mechanically transmissible *Tobacco mosaic virus* which was confirmed to be resistant to composting and could not be eliminated from flasks of compost held at 60 ºC for 6 weeks. Temperatures in excess of 60 ºC have been shown to be achieved in different commercial composting systems, with a wide range of organic feedstocks (Noble and Roberts, 2004).

Tee *et al.* (1999) evaluated the risks of spreading weeds and plant pathogens through the recycling of green waste (municipal waste plant material) over a 2-year period in waste streams at 6 sites across the greater Melbourne region, Australia. They included a series of trials to assess the survival of specific plant pathogens including *Plasmodiophora brassicae*, *Sclerotium rolfsii* (*Corticium rolfsii*), *Tobacco mosaic virus*, *Armillaria luteobubalina* and *Sclerotinia sclerotiorum*. Conditions

within compost heaps were found to be highly variable, resulting in variation in the ability of the process to kill plant pathogens. They concluded that an efficient turning regime was necessary to expose all of the green waste feedstock to temperatures of 55 ºC for at least 3 days.

Sansford (2003) and Noble and Roberts (2004) have recently reviewed the diverse literature on the fate of plant pathogens during composting of plant material. Detailed information on the fate of individual plant pathogens is further discussed below. In general, the authors concluded that:

1. Composting is not a sterilisation process and some plant pathogens can survive commercial composting processes.

2. A peak temperature of 64-70 degrees C and composting duration of 21 days, is sufficient to reduce numbers of most plant pathogens to below detection limits of currently available tests. Shorter periods and/or lower temperatures may be satisfactory for eradication of many plant pathogens.

3. Plant pathogens at most risk of surviving composting and other biowaste treatment processes include heat tolerant mechanically transmissible viruses such as *Tobacco mosaic virus* and *Cucumber green mottle mosaic virus*.

4. Heat tolerant obligate biotrophic plant pathogenic fungi with hardy resting spores may also survive biowaste treatments since the spores are protected from microbial competition and antagonism or chemical effects, remain viable over large temperature fluctuation and thus persist for long periods (often for decades). These include *Plasmodiophora brassicae* (clubroot of *Brassica* spp.), *Fusarium oxysporum* f.sp. *lycopersici* (tomato wilt), *Macrophomina phaseolina* (dry root rot), *Synchytrium endobioticum* (potato wart disease), *Olpidium brassicae* (fungal vector of *Tobacco necrosis virus* and *Lettuce big vein virus*), *Polymyxa betae* (fungal vector of *Beet necrotic yellow vein virus*) and *Spongospora subterranea* (powder scab in potato and vector of *Potato mop top virus*).

5. In addition to the potential efficiency of pathogen inactivation by the process, risk assessments should take into account knowledge of the proportion and severity of infected plant material in biowaste, monitoring of traceability of waste sources and treated waste, and quality management (including HACCP principals) applied in handling and treating waste and final products.

4. Regulations, standards and guidelines for sludge and biowaste

4.1. Regulation of plants and plant products

The risk of spreading plant pathogens with plant material used for planting as well as import/export of plants and plant parts into EU member-states is regulated according to EU Council directive 2000/29/EC on protective measures against the introduction into the Community of organisms harmful to plants or plant products and against their spread within the Community.

All living plants and plant material with intention of planting/seeding that may cause a risk of spreading plant pathogens/pest are included. Plant products (eg. wooden chips, fruits, potatoes, plant parts) used for e.g. consumption, soil or growth medium supplements that may cause a risk of

spreading plant pathogens/pests are also included.
This directive also includes plant products understood as products of plant origin, not treated or undergone simple treatment.
A plant health certificate or plant pass is needed for import or transportation into or within EU of plant material that may pose a potential risk for spread of plant pathogens and pests (quarantine pathogens and pests). Such plant material may end up in household waste, green waste or in the sewage/sludge system as fruit and vegetable peelings/waste. The plant material origination from such certified plant products may not cause any risk of spread of quarantine pests/pathogens. Lists of plant pests and pathogens regulated by EU Council directive 2000/29/EC are listed in Annex 1 and 2.

4.2. Regulation of waste of plant origin

Spread of sludge and biowaste on soil is normally restricted mainly for the purpose of preventing toxic pollution of soil and health hazards to livestock and human consumers.
Article 7 of the Council directive 86/278/EEC of 12 June 1986 on the protection of the environment, and in particular of the soil, when sludge is used in agriculture says:
-Member States shall prohibit the use of sludge or the supply of sludge for use on:

(a)grassland or forage crops if the grassland is to be grazed or the forage crops to be harvested before a certain period has elapsed. This period, which shall be set by the Member States taking particular account of their geographical and climatic situation, shall under no circumstances be less than three weeks;
(b)soil in which fruit and vegetable crops are growing, with the exception of fruit trees;
(c)ground intended for the cultivation of fruit and vegetable crops which are normally in direct contact with the soil and normally eaten raw, for a period of 10 months preceding the harvest of the crops and during the harvest itself.

Each memberstate may have tightened these restrictions on the use of sludge to further secure the health of livestock and human consumers with the side effect of preventing potential infection of plants with plant pathogens in part of the growth season and for certain crops.
In addition, spread of waste products of plant origin on arable land may be regulated for fields used for certain crops to prevent infection and spread of plant diseases.

4.3. Standards for compost sanitization

Various standards for production of compost from sludge and biowaste have been elaborated by different associations as a tool for the safe production and declaration of products.

USA: The Composting Council of the United States (Leege & Thompson, 1997)
DE: Ordinance on the Utilisation of Biowastes on Land used for Agricultural, Silvicultural and Horticultural Purposes (Ordinance on Biowastes - BioAbfV) September 1998.
UK: The Publicly Available Specification for composted materials from British Standardisation Institute (BSI PAS 100:2005)
AU: Kompostverordnung, Bundesministerium für Land und Forstwirtschaft, Umwelt und Wasserwirtschaft (2000)
EU: Eco-label "Commission decision of 28 August 2001 establishing ecological criteria for the award of the Community eco-label to soil improvers and growing media (2001/688/EC).

Criteria from decision 2001/688/EC relevant for protection against pathogens: 5.b. Products shall not adversely affect plant emergence or subsequent growht. This may be fullfilled by performing a plant response test/phytotox text, but no reference is indicated to such a test.

4.4. German legislation

In German legislation "Ordinance on the Utilisation of Biowastes on Land used for Agricultural, Silvicultural and Horticultural Purposes" (Ordinance on Biowastes – BioAbfV, 1998) standard tests for *Plasmodiophora brassicae* and *Tobacco mosaic virus* (TMV) are required for validation of production plants and sanitation processing plants.
Standard methods for detection are described for these two plant pathogens, and tests are carried out on the basis of the methods described by Bruns *et al*. (1994), based on the method developed by Knoll *et al.* (1980) (see Annex 3 for details).

The reduction of defined indicator plant pathogens by means of direct process validation is a parameter for evaluation of the sanitary quality (Lorenz and Buchenaur 2004). Reduction of TMV and *P. brassicae* was investigated during anaerobic digestion. TMV was very heat tolerant and complete inactivation was not achieved during mesophilic digestion (max. retention time 70 days) or thermophilic digestion (max. retension time 28 days) in full-scale facilities using carrier bags with inoculum.
P. brassicae was not detectable after 24 hour thermophilic anaerobic digestion or half hydraulic retention time in mesophilic anaerobic conditions. Tomato seed germination was eliminated with these same conditions (Lorenz and Buchenaur 2004).

Standard methods have to be optimised for TMV, as a ten fold higher lesion number was observed using Celite instead of Bentonit-Carborundum powder. However, the results from a standard bioassay detection method from 5 independent laboratories indicated that the present bioassay detection methods for TMV and *P. brassicae* are only qualitative, not quantitative (Lorenz and Buchenaur 2004).

4.5. Switzerland

So far only heavy metal and organic compound levels in compost and digestate are explicitly regulated for farmland use in Switzerland (Ordinance on Substance). The hygiene parameters are currently under review although 3 weeks at 55 degrees is mandatory for compost and digestate (Fuchs *et al*., 2004).
Fuchs *et al*. (2004) put focus on high quality input material for the production of high quality compost with plant pathogen suppressive effects.

4.6. France

For agricultural use a product should be either homologated or follow waste legislation or the standards for biowaste or sludge (ADEME, 2001; Déportes, ADEME, pers. comm.).
For homologation the products have to be authorised (as for chemicals), consistent composition, agronomic benefit and safe to use. This procedure is rare and concerns only products, which are not covered by the waste legislation and where no standards exist.
In the waste legislation phytopathogenic sanitation is not required.
In the standards covering nearly all biowaste compost except sludge, it is recommended to analyse for *Pythium* species but not required.

No phytopathogenic sanitation is required in the sludge standard.
Isabelle Déportes, ADEME, (pers. comm.) states that *Pythium* is most likely to be the choice for a standard test organism in sludge and biowaste products for agriculture.

4.7. Denmark

According to Danish regulations (Bekendtgørelse no. 623, 2003) untreated sewage sludge, except of vegetable origin, is not allowed for agricultural purposes. Stabilised and composted sewage sludge is allowed only with crops for processing and one year before growth of crops for direct consumption or silage.
Non-treated or stabilised organic food waste is not allowed for agricultural purposes, controlled composting (55ºC for 14 days) or controlled sanitisation (70ºC for 1 hour or thermophillic anaerobic digestion, effectiveness controlled just after sanitisation process with Enterococcus < CFU per gramme wet weight as well as in end-product delivered to end-user with E.coli < 100 CFU per gramme product wet weight and no Salmonella present per 25 gramme product wet weight) of food waste are required.

Danish GAP is a certification standard for potato, fruit and vegetable production to be implemented in the growth season 2006 (http://www.lr.dk/planteavl/informationsserier/info-planter/danishgap-fruit-veg.htm). The following restrictions are given to prevent plant pathogen infection:
-Sludge of vegetable origin is not allowed on fields one year prior to fruit and vegetables growth and three years prior to potato growth.
-Solid organic waste and soil debris from potato processing industry is not allowed on fields 3 years prior to potato production, and not allowed for seed potato production.
-Process water from potato processing industry is allowed on potatoes for industrial processing only.

4.8. Nordic investigation

Nordic Council of Ministers supported development of a Nordic System for Evaluating the Sanitary Quality of Compost described in Christensen *et al.* (2000a, 2000b, 2001, 2002a, 2002b).
In this Nordic investigation at four large scale compost facilities, eradiction of different pathogens and indicator organisms, including the plant pathogens *Tobacco mosaik virus* (TMV) and *Plasmodiophora brassicae*, were studied (Christensen *et al.* 2001). TMV is known to be quite tolerant to elevated temperatures and may be useful for validating sanitation in waste handling facilities.

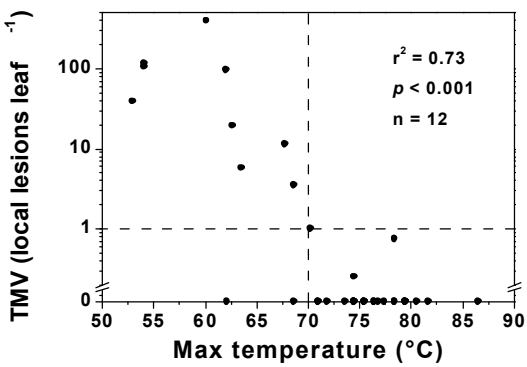

Figure 1.
Survival of *Tobacco Mosaik Virus* (TMV) correlated with the maximum temperature obtained during 2-4 week sanitation in four large scale compost facilities (Christensen et al. 2001)

Christensen *et al*, (2001) concluded that the eradication of TMV takes place when a max

temperature of 70 °C is achieved in the entire mass for minimum 1 hour (temperatures were logged every hour, no differences in eradication whether 70 °C was achieved for one hour or during a few days). Therefore control and documentation of temperature was sufficient and biotests not needed. Eradication of TMV during long term composting was not investigated.

4.9. United kingdom experiences of plant pathogens in biowaste

The disposal of sewage sludge on agricultural land is currently goverened by the Sludge (Use in Agriculture) Regulations 1989 (SI 1293 as amended by SI 880 in 1990). These regulations were introduced under Section 2 (2) of the European Communities Act 1972 to transpose Directive 86/278/EEC of 12 June 1986 on the protection of the environment, and in particular of the soil, when sludge is used in agriculture. An associated non-statutory code of practice on the agricultural use of sewage sludge was also introduced to facilitate compliance with the regulations. Proposals to modify the regulations and code of practice have been made to introduce a statutory ban on the use of untreated sludge on agricultural land used to grow food crops. All sludge applied to land is treated either conventionally (to reduce *Escherichia coli* by 99% with a maximum allowed poulation of 10^5 cells per g of dry solid) or by an enhanced treatment (which reduced *E. coli* by 99.9999% with a maximum allowed population of 10^3 cells per g of dry solid). No specific testing for plant pathogens is required but the required treatments are expected to reduce many phytopathogenic spp.

A recent report conducted on behalf of Defra (Department for Environment, Food and Rural Affairs), Plant Health Division summarises current knowledge on sanitisation of biowaste to control pathogens and pests of plant health concern. This report lists the current legislation, directives and regulations of the EU and UK that may govern in the choice of suitable biowaste disposal method for plant health, environmental protection and animal welfare together with quality standards and codes of practice.

These potentially include:

- Plant Health (Great Britain) Order 1993 (as amended) and related EC plant health legislation including:
  - Council Directive 2000/29/EC on protective measures against the introduction into the Community of organisms harmful to plants or plant products and against their spread within the Community,
  - Council Directive 69/464/EEC on the control of potato wart disease,
  - Council Directive 69/465/EEC on the control of potato cyst nematode,
  - Council Directive 93/85/EEC on the con trol of potato ring rot,
  - Council Directive 98/57/EC on the control of Ralstonia solanacearum (Smith)Yabuuchi *et al.*
- Environmental Protection Act 1990 and associated Regulations.
- EU Landfill Directive 1999/31/EC,
- Waste Framework Directive 75/442/EEC as amended by 91/156/EEC,
- Incineration of Waste Directive 2000/76/EC,
- Possible EU directive on the biological treatment of biowaste,
- Council Directive 96/25/EC and amendments on the circulation and use of animal feed materials,
- Defra (Department for Environment, Food and Rural Affairs) code of practice for the management of agricultural and horticultural waste,

· BSI PAS 100 specification for composted materials,
· Proposed EPPO phytosanitary procedure on the management of plant health risks associated with the use of biowaste of plant origin.

In addition to controlled incineration, controlled landfill and controlled heating, additional scope for the use of more environmentally acceptable and cost-efficient disposal methods may be considered, provided that there is evidence that:

(a) Specificiations of the proposed treatment are sufficient to eliminate the pathogen in question.
(b) The waste is treated such that there is no identifiable risk of escape of the organism to the environment.

Additional potential waste treatment methods may include:
On-farm burial; On-farm burning; Industrial processing; Feeding to animals; Aerobic composting; Anaerobic digestion

The Waste and Resources Action Programme (WRAP) has supported investigations in UK on the effect of the composting process on particular plant, animal and human pathogens known to be of concern for the end-uses (Noble *et al.* 2004). They concluded that propagules of *Fusarium oxysporum* f.spp. *lycopersici* and *radicis-lycopersici*, *Pythium ultimum*, *Thielaviopsis basicola*, *Rhizoctonia solani*, *Verticillium dahliae* and *Xanthomonas campestris* pv. *campestris* in affected plant material were eradicated in laboratory tests by a compost temperature of 52°C or less, held for 7 days. Propagules of *Phytophthora nicotianae* required a temperature of up to 58°C for 7 days for eradication. These conditions were sufficient to eradicate all except the most temperature tolerant plant pathogens tested (*Microdochium nivale, Plasmodiophora brassicae and Tobacco Mosaic Virus*). Propagules of *M. nivale* required a temperature of up 64°C for 7 days for eradication from composting material.
In a large-scale aerated tunnel with green waste (waste plant material) *Fusarium oxysporum* f.sp. *lycopersici* in affected tomato plant material and *Verticillium dahliae* in infected oat grains were eradicated from compost that exceeded 50°C for 4 days and peaked at 70°C.

4.10. EPPO (OEPP) European and Mediterranean Plant Protection Organization draft guideline

A draft EPPO Phytosanitary Procedure proposes standardised procedures for the management of plant health risks associated with the use of biowaste of plant origin.

General requirements for the treatment process

General requirements aiming at eliminating most of the plant pests are defined below. Specific requirements related to biowaste known or suspected to contain quarantine pests or heat-tolerant pests are presented in the next section.
The processes at composting facilities should be managed in such a way to guarantee a thermophilic temperature range and a high level of biological activity over a period of several weeks. This can be achieved when conditions are favourable with regard to humidity and nutrients, as well as an optimum structure and optimum air conduction. In general water content should be at least 40%. In the course of the composting process, the entire quantity of materials being treated should be exposed either to

• a temperature of at least 55°C for a continuous period of two weeks,
• or, alternatively, to a temperature of at least 65°C (or, in the case of enclosed

composting facilities, at least 60°C) over a continuous period of one week.
A minimum number of turnings may be required to ensure that the whole mass will be exposed to this temperature.
These time-temperature combinations will eliminate most plant pests. It should be noted that there are reports in the scientific literature based on varying methodologies showing that some heat tolerant organisms (fungi with hardy resting spores e.g. *Plasmodiophora brassicae* and heat resistant viruses, e.g. *Tobacco mosaic virus* and viroids e.g. *Potato spindle tuber pospiviroid*) have survived these time temperature combinations and further studies are needed to determine the necessary time-temperature combinations to eliminate these and other similarly hardy pests. For further verification of the sanitation effect of a composting process it may be useful to apply a direct process validation.

There are few references on the effectiveness of anaerobic digestion treatment against plant pests. Consequently the waste matrix for anaerobic digestion should be subjected to:
- either a minimum thermal pre-treatment of input materials or post-treatment of residues (70° C for 1 hour, preferably wet heat),
- or, an aerobic secondary decomposition of the residues (composting).

The pre or post-treatment time-temperature combinations mentioned above will eradicate most plant pests. It should be noted that Tobacco mosaic virus survives this pre or post treatment and other heat-tolerant organisms (e.g. fungi with hardy resting spores and other heat-resistant viruses and viroids) may survive.
For the thermal treatment process to be effective the particle size of the biowaste should preferably not be larger than 12 mm. The biowaste should be homogenised. During the thermal treatment a moisture content of the biowaste is necessary that guarantees a sufficient heat transfer between and inside the particles. The temperature should act upon the whole material over a contiguous period of time.
In order to prevent potential contamination, treated material should be stored in such a way that any contact (e.g. direct contact or through run-off water, wind, machinery, tools, storage containers) with untreated materials is avoided.

Special requirements for biowaste containing quarantine pests. Biowaste of plant origin known or suspected to contain any quarantine pest or heat-tolerant pest should not enter into the biowaste treatment process, unless it can be pre- or post- heat-treated to 74° C for 4 hours, preferably wet heat. However, exceptions could be made, at the discretion of the National Plant Protection Organization (NPPO), when the organism is well known to be inactivated by the biowaste treatment process. In all cases of waste known or suspected to contain quarantine pests, the treatment should be authorized and supervised by the NPPO (including confinement conditions to prevent escape of the quarantine pest, testing of the resulting treated biowaste which should be found free from the quarantine pest and specification of a 'non-risk' outlet for the final use of the treated biowaste).

Standard tests for *Plasmodiophora brassicae* and *Tobacco mosaic virus* (TMV) is proposed by EPPO for validation of production plants and for testing of the end product.

5. Fate of some plant pathogens during biowaste treatment

5.1. Viruses and viroids

***Tobacco mosaic virus* (TMV) and other plant viruses**

Some viruses are known to tolerate long periods of exposure to high temperatures. *Tobacco mosaic virus* (TMV) requires temperatures in excess of 68 ºC for longer than 20 days (Noble and Roberts, 2004) or 70 ºC in 1 hour or more (Christensen *et al*, 2001) for complete eradication from infected plant material. In affected leaf material, a compost temperature of 80°C for 7 days was required for eradication whereas the pathogen was shown to survive a compost temperature of 60°C for 35 days. For this reason it has been selected as a useful indicator of successful sanitization. *Tobacco necrosis virus* (TNV) was inactivated by exposure to 55 ºC for 72-96 hours or 70 ºC for 24-48 hours. Other viruses known to be temperature tolerant include *Cucumber Green Mottle Mosaic Virus, Pepper Mild Mottle Virus, Tobacco Mosaic Virus* (TMV) and *Tobacco Rattle Virus* (TRV). TRV survived exposure over 6 days to 68 ºC during composting of infected plant material (Bollen, 1985) although this process effectively eliminated the nematode vector of the virus. *Tomato Mosaic Virus* (ToMV) was very temperature tolerant when tested in an incubator (Avgelis and Manios,1989), but eradicated from a compost heap at 47°C for 10 days. *Cucumber Mosaic Virus*, *Melon Necrotic Spot Virus* and *Tobacco Necrosis Virus* could be eradicated by a composting temperature of 55°C held for 14 days, whereas *Tomato Spotted Wilt Virus* required a temperature of 60°C for 3 days (Noble and Roberts, 2004). Studies at Central Science Lab, UK, (Mumford, unpublished) demonstrated *Pepino mosaic virus* (PepMV) was successfully eradicated from shredded infected tomato plants during windrow composting in which all parts of the heap attained a minimum of 60 ºC for at least 5 days. As a further precaution, the finished compost was not used for horticultural purposes.

***Potato spindle tuber viroid* (PSTVd)**

Viroids are particularly persistent and heat tolerant. The *Potato spindle tuber viroid* (PSTVd) can remain infective in hydrated plant material for several months and in dry material for over a year, and is expected to survive composting processes. The only suitable disposal methods for high-risk biowaste infected with PSTVd are incineration or containment in an approved landfill site.

5.2. Bacteria

Clavibacter michiganensis* subsp. *sepedonicus

A minimum temperature of 82 ºC for at least 5 min has been shown to completely inactivate the potato ring rot bacterium (Secor *et al*., 1987). No information is available on the behaviour of this organism during composting or anaerobic digestion but Turner *et al* . (1983) concluded that the related pathogen *Clavibacter michiganensis* subsp. *michiganensis* (Cmm) was effectively reduced during anaerobic digestion at 35 ºC. Cmm, the causal agent of bacterial canker of tomato, was also shown to be eradicated from naturally infected tomato seeds that were soaked in water at 52 ºC for 20 min or at 56 ºC for 30 min (Shoemaker and Echandi, 1976; Fatmi *et al*., 1991). Treatment at 51 ºC for 1 h was also shown to control the related pathogen *Clavibacter xyli* subsp. *xyli* in infected sugarcane seed (Ramallo and de Ramallo, 2001).

Ralstonia solanacearum

In Korea, *R. solanacearum* was eliminated from recycling nutrient solution by heating to 70 °C for only 3 minutes (Lee *et al.* 1998). In the laboratory the organism was eliminated in liquid culture or

infected potato pieces at temperatures as low as 55 ºC applied for at least 10 minutes (Elphinstone, unpublished). All biovars of *R. solanacearum* were eliminated from liquid cultures by exposure to 43 °C (Date *et al*., 1993). However, the bacterium survived in diseased plant residues and in soil at 43 °C for 2 days and at 40 °C for 5 days. Hot air treatment at 75% relative humidity of infected ginger rhizomes eliminated *R. solanacearum* when their internal temperatures were allowed to reach either 49 °C for 45 minutes or 50 °C for 30 minutes (Tsang and Shintaku, 1998).

Ryckeboer *et al.* (2002a) 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. Elphinstone (unpublished) also showed reduction of *R. solanacearum* below detectable levels within 48 hours during anaerobic digestion of sewage sludge at 37 °C, further indicating that factors other than temperature alone are important in elimination of this pathogen.

5.3. Fungi

Botrytis cinerea

Survival during composting of *B. cinerea*, the grey mould fungus, varied in different experiments with eradication conditions ranging widely between 35 ºC for 4 days (Lopez-Real and Foster, 1985) and 50 (+/- 10) ºC over 70 days (Hoitink *et al*., 1976). During windrow composting of waste from a food factory, *B. cinerea* survived for less than 1 week at 70 ºC (Ylimaki *et al*., 1983). Wijnen *et al.* (1983) showed that the related *B. allii* was eradicated during composting of infected onions over 21 days at 60 (+/- 13) ºC. Under controlled laboratory conditions *B. cinerea* was killed at 40 ºC over 21 days and 50 ºC over 7 days (Hoitink *et al*., 1976).

Phytophthora ramorum* and *P. kernoviae

Phytophthora spp. have the potential to produce two types of hardy resting spores, oospores and chlamydospores. Nobel *et al.* (2004) have shown in laboratory studies that the number of surviving propagules of *Phytophthora nicotianae* cultures containing oospores and mycelium declined as temperature increased from 18 – 52 ºC with complete eradication occurring in onion waste and pure cultures at 58 ºC over 7 days and in green waste at 52 ºC over 7 days. The results obtained for *Phytophthora nicotianae* were similar to those obtained previously for other *Phytophthora* species. Bollen *et al.* (1989) showed that peak compost temperatures of 55 and 60 °C and composting for 21 days eradicated *P. infestans* and *P. cryptogea* respectively. Hoitink *et al.* (1976) eradicated *P. cinnamomi* in compost at only 40 °C over 7 days.

New research on the eradication of *Phytophthora ramorum* (the cause of sudden oak death) during composting of infected plant material at 55°C over 14 days (Garbelotto, 2003) is providing hope that composting will provide a reliable means of sanitizing plant material (bay laurel leaves and woody chips and stems) infected with this quarantine pathogen. One week of heat treatment '*sanitised'* the woody substrates (chips and stems) but not the bay laurel leaves, probably because chlamydospores rarely form in wood but frequently form in leaves. Because the chlamydospores are embedded in the leaf parenchyma they may be more resistant to high temperatures than when tested on agar in the absence of leaf tissue. Significant variation detected among different plant substrates, post treatment environmental conditions, and composting parameters (specifically

temperature) mean that further investigation will be required to assess the risks. It is possible that time of year may play an important role in the final outcome of the sanitation process.

The effect of temperature alone under laboratory conditions on chlamydospores and sporangia of *P. ramorum* in the absence of plant material was investigated at the Central Science Laboratory in the UK (Defra Project PH0194). Viability of chlamydospores decreased with time at 40 ºC and no spores could be germinated after 24hrs. Sporangia were less robust and none were able to germinate after 1 hour at 40 ºC. It is however, questionable whether chlamydospores of *P. ramorum* are dormant or dead when they appear to be non-viable. It is not possible to get 100% of chlamydospores to germinate in the laboratory and it is therefore difficult to determine whether chlamydospores have been fully killed during composting or whether some of the remaining population may be dormant rather than dead.

The newly identified species *Phytophthora kernoviae* produces sporangia and the more hardy oospores in nature but has not yet been found to produce chlamydospores and may therefore be more easily eradicated than *P. ramorum* during a well managed composting process.

Plasmodiophora brassicae

Data obtained for the fate in treated biowaste of *Plasmodiophora brassicae*, the causal agent of clubroot of Brassicas, are very variable. Laboratory tests in water have shown that temperatures in excess of 65°C over a 10 minute period are required for eradication of *P. brassicae* (Lopez-Real and Foster 1985). Ylimäki *et al.* (1983) found that a high level of inoculum of *P. brassicae*, the clubroot fungus, survived composting in a windrow system after three weeks exposure at 60-65 ºC, whereas one week at 70 ºC with optimum moisture and pH was sufficient to result in eradication. Bruns *et al.* (1993) and Christensen *et al.* (2001) also found eradication at temperatures of 60 - 70°C for 7 - 21 days, but shorter time might have been sufficient. However, Lopez-Real and Foster (1985) and Bollen *et al.* (1989) showed that P. brassicae was fairly heat resistant but was effectively destroyed in a simulated compost by a 1-21 day exposure at 54-55 ºC or 24-48 hours at 70 ºC. Both Bollen *et al.* (1989) and Ryckeboer *et al.* (2002b) found that *P. brassicae* could survive for several weeks at lower temperatures during the maturation phase of composting. Bollen (1985) states that fungi such as *P. brassicae* which are obligate parasites with hardy resting spores are likely to require higher temperatures within a composting situation than other types of fungi such as those which form sclerotia since they remain unaffected by microbial antagonism. The effect of compost moisture content and feedstock composition on the temperature tolerance of P. brassicae has been studied at INRA Dijon, France as part of an EU funded project 'RECOVEG' (www.hri.ac.uk/recoveg/recoveg.htm). *P. brassicae* was eradicated in spent mushroom compost and green waste heated for 7 days at 50 and 60°C at a high moisture content (50% or higher). Eradication of this pathogen was found to depend on the moisture content of the composts. Bollen and Volker (1996) tentatively suggest that *P. brassicae* could be eliminated after exposure in compost to 60 ºC for one day. However, the earlier evidence suggests that this organism could potentially survive composting processes and recent studies (Termorshuizen *et al.*, 2003) confirmed that low levels of *P. brassicae* survived mesophilic (maximum temperature 40 ºC) anaerobic digestion during 6 weeks with a 50/50% (v/v) ratio of recycled digestate and fresh vegetable, fruit and garden waste.

Polymyxa betae* and *Polymyxa graminis

Dickens *et al.* (1991) investigated the effect of heat treatment on effluent from sugar beet processing on the survival of *Polymyxa betae*, the vector of beet necrotic yellow vein virus (BNYVV) which causes sugar beet rhizomania. Viable resting spores (cystori) containing infective virus could be recovered from soil/water suspensions following 30 minutes exposure to temperatures up to 75 ºC. Populations were reduced but not eliminated at 55 ºC but not at 50 ºC.

Nishinome *et al.* (1996), however, found that lethal temperatures were achieved during composting of dewatered waste soil (80% soil) from sugar beet processing, using an aerated static pile process. The lethal temperature for rhizomania infectivity was determined as 40 ºC for 14 days or 60 ºC for 1 day, and temperatures within the aerated pile reached 60 ºC over the one month duration.

***Pythium* spp.**

P. irregulare was shown to be killed in 7 days at 40 ºC under laboratory conditions and at 50 (+/- 10) ºC over 77 days during composting of hardwood tree bark containing infected *Rhododendron* crowns and roots (Hoitink *et al.*, 1976). There are no published data on the fate of *Pythium ultimum*, an important causal agent of root rot, during composting or other biowaste treatments (Nobel and Roberts, 2004).

Rhizoctonia solani

Christensen *et al.* (2001) found that *Rhizoctonia solani* required a compost temperature of 60°C, held for 10 days, for eradication. During windrow composting of waste from a food factory, *R. solani* survived for up to 1 week at 70 ºC (Ylimäki *et al.*, 1983). However, several other workers using infected plant material or mycelium as inoculum, did not find *R. solani* to be particularly temperature tolerant during composting.

Sclerotinia sclerotiorum and Sclerotinia cepivorum

S. cepivorum, cause of white rot of onion, was killed under laboratory conditions over 3 days at 48 ºC (Coventry *et al.* 2002). In contrast, temperatures of 57 (+/- 12) ºC were required over 21 days to eradicate the pathogen during composting of infected onions. Furthermore, viable sclerotia of *S. cepivorum* were found following mesophilic (maximum temperature 40 ºC) anaerobic digestion during 6 weeks with a 50/50% (v/v) ratio of digested and fresh vegetable, fruit and garden waste (Termorshuizen *et al.*, 2003). The related *S. sclerotiorum* could be eliminated during composting at 55-57 ºC over 5-12 days (Bollen *et al.* 1989; Dittmer *et al* . 1990).

Synchytrium endobioticum

Resting bodies (sori) of the potato wart disease fungus are able to survive for over 30 years in soil and are resistant to temperature extremes and microbial antagonism and competition. Survival of *S. endobioticum* in water at 60°C for 2 hours has been reported (Nobel and Roberts 2004), but there is little information on its behaviour during composting. One report from Russia showed that the pathogen was not recovered from waste from processing of infected potatoes which had been composted for 2 to 3 months together with animal manure and saturated with ammonia (Efremko and Yakoleva, 1981). Zoosporangia were able to maintain viability for 2-3 months in untreated processing waste (sludge) at temperatures below 21 ºC but were effectively killed by heat treatment.

The only reports of effective control of this organism involve fumigation of infested soils, e.g. using 98% methyl bromide at 50-200 g per m^2 for 72 h (Noehr Rasmussen and Mygind, 1977). Potocek

(1991) reported successful fumigation of soil using granulated urea at 500 g per m^2, followed by calcium cyanamide (500 g per m^2) and dazomet (as Basamid, 60 g per m^2). Other effective treatments included AITK (as Allyspol 75 EC, 50 ml per m^2), DNOC (as Nitrosan 25, 100 g per m^2), metam Na (as Nematin, 100 ml per m^2) and MITK + dichlorpropene (as Di-Trapex, 50 ml per m^2).

5.4. Nematodes

Nobel and Roberts (2004) found that all seven nematode species affecting plants studied during composting were eliminated within 1 day at temperatures of at least 52 ºC. These were:

- *Globodera pallida*
- *Globodera rostochiensis*
- *Heterodera schachtii*
- *Meloidogyne hapla*
- *Meloidogyne incognita*
- *Meloidogyne javanica*
- *Pratylenchus penetrans*

However, some cyst-forming spp. such as the beet cyst nematode (*Heterodera schachtii*) has the ability to survive in compost at lower temperatures (31°C) for long periods (Ryckeboer *et al.* 2002b). All nematode pests are expected to be eliminated during well managed composting processes under quality standards (e.g. BSI PAS100).

Globodera pallida and Globodera rostochiensis
The lethal temperature for potato cyst nematodes (PCN) in sugar beet compost has been determined as 40 ºC for 10 days or 50 ºC for 5 days (Nishinome *et al.*, 1996). PCN was also killed in potato processing sludge below the lethal temperature at only 34 ºC, probably due to toxicity of the sludge (Bollen, 1985). However viable PCN cysts have been recovered from sewage sludge after anaerobic digestion although populations were reduced (Turner *et al.*, 1983). Similar studies by Catroux *et al.* (1983) showed that almost 100% of the cysts of *G. rostochiensis* and *G. pallida* were killed during anaerobic sewage digestion; composting with temperatures rising to more than or equal to 40 ºC also killed most of the cysts whereas aerobic digestion destroyed only a small proportion of the cysts. Live cysts could survive up to 3 months of storage in the outer layer of sludge. In limed sewage sludge at pH 10 or more, the cysts were killed in 14 days.

6. Detection methods

Reports on the fate of plant pathogens in biowaste are only as accurate as the methods used to detect them and to determine their viability. Nobel and Roberts (2004) recently reviewed the methods used to detect plant pathogens in composts. The majority of methods have been adapted from those designed to detect fungal and bacterial pathogens in soil and are mostly based on bioassays (particularly for fungi) and dilution plating (particularly for bacteria). Critical comparisons of detection methods have rarely been performed and little or no information has been provided on the limits of detection of target organisms in biowastes and composts. There is therefore a need for

independent validation of detection and quantification methods so that the reliability of data on pathogen survival can be assessed. No detection assay can give an absolute guarantee that composts or otherwise treated biowastes are free from a particular pathogen. It is desirable to ensure that test methods can reliably detect the concentrations of pathogens which present a risk to end-users but, for most plant pathogens, these risks have yet to be quantified.

Noble and Roberts (2003, 2004) collated the available data on survival and eradication of plant pathogens together with estimated detection limits, leading to recommendations for the phytosanitary requirements of composting. Eradication was defined as a reduction in the levels of a pathogen to below the limit of detection of the specific detection method used.

Definitions:
Direct process evaluation: Inoculation of the raw material with defined indicator organisms/pathogens
Spot test analysis: Analysis for indicator organisms/ pathogens in samples taken from the raw material and compost at different stages during the process

6.1. Bioassays and bait tests

Bioassays are the most commonly used methods for the detection of fungal pathogens in soils and composts (Nobel and Roberts, 2004) and are particularly useful for detecting non-culturable obligate parasites (e.g. *Plasmodiophora brassicae*, *Olpidium brassicae* and *Polymyxa betae*). Detection usually involves growing sensitive indicator plants in the test material either directly or mixed into inert material such as sterilized sand, soil or compost. Presence of the target organism is indicated by the development of typical symptoms such as damping-off (e.g. caused by *Pythium* spp. or *Rhizoctonia*), wilting/vascular discoloration (e.g. *Fusarium*), root galls (e.g. *Plasmodiophora*) or rots (e.g. *Phytophthora*, *Pythium* or *Pyraenochaeta*).

Bioassays for virus detection involve direct standard inoculations of growing plants with suspensions containing soil, compost or plant waste. Panels of different indicator plants are often inoculated together and viruses are detected according to the development of characteristic symptoms on specific hosts, usually within 7-14 days. Symptom severity can be used as a measure of virus concentration in the sample extract.

Bioassays can indicate both viability and pathogenicity of the target organism and can effectively test for multiple pathogens. However, they can take several weeks to perform and results can vary with environmental conditions, so year-round reliability cannot always be guaranteed. Furthermore, infection and/or symptom development may not occur under low inoculum levels or due to microbial interactions, as has been demonstrated with *Rhizoctonia* (Christensen *et al.* 2001). Nevertheless they are inexpensive and easily performed and can be readily adapted for *in situ* testing of composts and otherwise-treated biowastes prior to end-use. Indications of detection limits obtained with bioassays are rarely found in the literature. Staniaszek *et al.* (2001) were able to detect as few as 10^3 *Plasmodiophora brassicae* spores per g of peat and 10^6 spores per g of soil, whereas, Tuitert and Bollen (1993) demonstrated a 1.7% recovery rate for *Polymyxa betae*. Although detection limits are likely to vary according to the test material, environmental conditions, host plants and bioassay methods used, the results obtained provide a useful indication of the

phytosanitary risks posed by the material to end-users.

Bait tests use susceptible plants or plant material to attract target pathogens or increase their numbers to enable them to be detected by secondary methods such as selective isolation or serological or DNA-based assays. Bait tests therefore have the advantage of detecting viable and infective pathogen populations, even in the absence of typical disease symptoms. Baiting and pathogen isolation is commonly used for oomycete fungi such as *Pythium* and *Phytophthora* spp. where sterilised seeds are used as the bait for a water extract of the soil/compost and after incubation (e.g. overnight) the seeds are plated on a suitable agar medium (Nobel and Roberts, 2004). More recently baiting has been combined with either a serological test (Christensen *et al.*, 2001; Thornton *et al.* 2004; Yuen *et al.* 1998) or PCR (Lees *et al.* 2002; Nechwatal *et al.* 2001) in which the bait plant material is tested directly for the presence of the pathogen. In the case of serological methods this may overcome the problem of relatively high detection thresholds, and in the case of PCR avoids inhibitors of the assays, which are usually present in high concentrations in composts, soils and decomposing plant material.

6.2. Pathogen isolation

For detection of most bacteria and many culturable fungi in soils and composts, direct plating of serially diluted sample extracts on selective media is the method which has been most widely used (Nobel and Roberts, 2004). Direct plating usually offers a cost-effective and reliable approach. Furthermore, viability of the pathogen is determined during isolation whereas molecular and serological methods also detect dead cells and therefore may not be appropriate to indicate disease risks from composts or otherwise-treated biowastes.

Theoretical detection limits of isolation tests are usually around 10 cells per ml of extract and the sensitivity of detection using isolation methods therefore usually exceeds that expected by newer molecular and serological methods. In a comparison of methods for detection of *Ralstonia solanacearum* in soils (Pradhanang *et al.*, 2000) dilution plating on selective media had the lowest detection limit, which was only matched by PCR if combined with a pre-enrichment step.

6.3. Serological methods

The serological tests which have been most commonly used to detect plant pathogens in soils and composts (Nobel and Roberts, 2004) are based on ELISA (enzyme linked immunosorbent assay). Further development of this technology is now providing on-site test kits based on dipsticks, or increasingly on lateral flow device (LFD) technology (Danks and Barker, 2000; Pettitt *et al.*, 2002).

The accuracy of all serological methods depends on the specificity of antibodies used. In most cases monoclonal antibodies are more specific than polyclonal antibodies although for screening purposes it may be beneficial to use the less specific test with follow-up laboratory confirmation of positive screening tests. The sensitivity of serological tests is usually limited, meaning that only moderate to high pathogen populations are detected. In such cases it is necessary to ensure that the detection limit is sufficient to indicate the presence of pathogen populations which present a significant phytosanitary risk to the end-user. Recovery and detection limits can vary with soil/compost type and extraction method (Otten *et al.*, 1997). The detection limits of serological methods (typically around 10^4 to 10^5 cells per ml of extract) may be improved by combining with enrichment or baiting (Thornton *et al.* 1994; Pradhanang *et al.* 2000; Thornton *et al.* 2004), or trapping (Pettitt *et al.* 2002),

or plating as in the immunofluorescent colony staining (IFC) method of (van Vuurde *et al*., 1995).

An ELISA method for detection of *Pythium* spp. that cause cavity spot of carrots (White *et al.* 1996) has been used successfully to provide a predictive indication of the suitability of particular fields for carrot production. However, serological methods are most useful for secondary confirmation following plating or baiting tests. A useful example is the LFD assay for *Rhizoctonia* detection in matured composts (Thornton *et al*., 2004), which is being used in quality control of composting processes by testing bait plants growing in samples of matured composts.

6.4. Nucleic acid-based detection methods

Schoen *et al.* (2005) recently reviewed novel molecular and biochemical techniques for monitoring plant pathogens in plant material, soil, compost and water and other environments in the agrofood production chain. Recent research has largely focused on detection of plant pathogens using assays based on DNA polymerase chain reaction (PCR) technology in which specific target DNA sequences are amplified using specific primers. Numerous PCR assays are now available, as are RNA amplification tests (such as RT-PCR, NASBA and AmpliDet RNA) which can be used to descriminate live populations of target pathogen. There are, as yet, few examples of practical applications of PCR to monitor plant pathogens in soils, composts or biowastes. In Australia PCR-based detection is being offered to growers as a commercial indexing service for the soil-borne pathogens *Gaumanomyces graminis* and *Rhizoctonia solani* (Keller *et al.* 2003) and a club root indexing has been proposed (Porter *et al.* 2003).

The more recently developed real-time PCR (RT-PCR) technology allows detection of fluorescently labeled amplified DNA targets. RT-PCR methods are already available which can detect plant pathogens in soils and infected plant material (Weller *et al*., 2000; Cullen *et al*., 2000). The assay of Weller *et al.* (2000) has also been used to monitor populations of *Ralstonia solanacearum* in sewage sludges and effluents. This technology is more easily automated (allowing high throughput sample testing) and also provides quantitative estimation of target populations. Mobile RT-PCR equipment also now offers the opportunity for on-site testing, although equipment and reagent costs are currently very high.

Whilst the nucleic acid-based methods offer potential benefits in terms of speed, specificity and sensitivity of detection, there are few examples in the literature where they have been successfully applied for testing biowastes for target organisms. This is probably due to the combination of the high cost of this technology, the small sample sizes, which can be tested and the tendency for DNA and RNA amplification to be inhibited by common components of decomposing plant material, soils, composts and digested biowastes. For example, Pradhanang *et al.* (2000), when testing soils for *Ralstonia solanacearum*, showed that PCR results were only comparable to selective isolation after an initial enrichment step in the same selective medium. To overcome problems of PCR inhibition, Expert *et al.* (2000) found immuno-magnetic separation and DNA magnetic capture procedures potentially useful for purification and concentration of *R. solanacearum* target DNA from environmental substrates such as water, soils, sewage sludge and potato processing wastes. At present, however, PCR methods are mainly useful and cost effective for secondary laboratory confirmation of primary screening tests conducted using isolation, bioassay or baiting.

7. Conclusions

7.1. Legislations and regulations

Germany is the only country in Europe, so far, having a biowaste legislation, where biowaste treatment plants have to be validated for plant pathogen eradication of the thermotolerant *Tobacco mosaic virus* and *Plasmodiophora brassicae* with guide value in the biotest of ≤ 8 lesions per plant or infection index ≤ 0.5, respectively (annex 3).

Most regulations for use of biowaste of plant origin in agriculture are restrictions for application on fields with specific crops (e.g. seed potatoes) for prevention of infection and spread of plant disease.

Standards for treated biowaste products are available but most standards do not specifically mention any plant pathogen precausions other than the biowaste products must not adversely affect plant emergence or subsequent growth.
The draft guideline of The European and Mediterranean Plant Protection Organization (EPPO) for a standardised phytosanitary procedure for the management of plant health risks associated with the use of biowaste of plant origin focus mainly on aerobic composting at either - at least 55 ºC for two weeks or, - at least 65 ºC for one week or, - wet heat treatment at 70 ºC for 1 hour. Quarantine plant pathogen (known or suspected) infected biowaste should not enter into biowaste treatment unless it is heat treated at 74 ºC for 4 hours under the authorization and supervision of the national plant protection organization.

7.2. Eradication of plant pathogens

Temperature is the most important factor for eradication of plant pathogens although treatment time is also important. The water content together with nutrient composition and pH are important factors for obtaining the highest temperature during composting.
Bacterial plant pathogens, nematode pests and less thermo-tolerant fungi and vira seem to relatively easy to eradicate in biowaste treatment plants.
Eradication of bacterial plant pathogens is in general obtained at 50 ºC for 4-7 days in aerobic composting or thermophilic anaerobic digestion at 50 ºC for one day. Mesophilic anaerobic digestion at 37 ºC for 2 days eradicated the quarantine pathogen *Ralstonia solanacearum*. Even for quarantine bacterial plant pathogens sludge and biowaste treatment is useful for eradication. However, it will be very important that the treated biowaste is not contaminated from untreated biowaste.
Mesophilic or thermophilic anaerobic digestion, aerobic composting or pasteurisation of biowaste for eradication of nematode pests seems to be effective in well managed treatment plants, whereas storage, liming or stabilisation was not sufficiently effective for eradication.
Fungi with hardy resting structures and some viruses needs higher temperature treatments and/or longer treatment time. *Fusarium oxysporum* f.spp. *lycopersici* and *radicis-lycopersici*, *Pythium ultimum*, *Thielaviopsis basicola*, *Rhizoctonia solani*, and *Verticillium dahliae* in affected plant material were eradicated by a compost temperature of 52°C or less, held for 7 days. *Phytophthora nicotianae* required a temperature of up to 58°C for 7 days for eradication. *Microdocium nivale* required a temperature of up 64°C for 7 days for eradication from composting material. These conditions were sufficient except for *Plasmodiophora brassicae and Tobacco Mosaic Virus* where a peak temperature of at least 70 °C was required for 1 hour during a 21 day composting treatment.

Several viruses are heat tolerant and *Cucumber Green Mottle Mosaic Virus, Pepper Mild Mottle Virus, Tobacco Mosaic Virus* (TMV) and *Tobacco Rattle Virus* (TRV) need 70 °C or more for several days for eradication.

There are no investigations on plant pathogen eradication rates. It is not known whether the level of infection structures (spores, cysts, particle, etc.) in biowaste is important to the subsequent requirement of temperature-time treatment for eradication of plant pathogens. This may, however, be of importance only with heavily infested biowaste.

7.3. Detection methods

Many different methods for detection of plant pathogens, such as bioassays, direct plating, dilution plating, serology, and direct microscopic examination have been developed and described for epidemiological studies during time. Some methods have been developed for detection of plant pathogens in various substrates as growth media, soils and water fluids. However, not many methods for detection of plant pathogens in sludges and biowastes are standardised and validated.
Standard methods for detection of *Plasmodiophora brassicae* and *Tobacco Mosaic Virus* in composted sludge and biowaste are used regularly in Germany according to their legislation (see annex 3).
Bioassays can indicate both viability and pathogenicity of the target organism and can effectively test for multiple pathogens. However, they can take several weeks to perform and results can vary with environmental conditions, so year-round reliability cannot always be guaranteed.
Baiting with susceptible plants to attract or increase the concentration of a plant pathogen is often followed by selective isolation, serological or DNA-based assays. Viability of the pathogen is determined with baiting as serological or PCR based methods may also detect non-viable cells. Baiting is also useful to increase the pathogen concentration, as the detection treshold with serological tests is often high. Baiting also overcomes the inhibitors of PCR usually present in decomposing plant material, composts or soils.

8. Recommendations

There are different strategies that may be used singly or in combination to obtain a reliable degree of sanitary safety of sludge and biowaste used in agriculture:

- Knowledge of plant pathogen status of the biowaste of plant origin (e.g. quarantine plant pathogens)
- Validation of sludge and biowaste treatment and registration of process parameters
- Test for indicator organisms or plant pathogens in the end product
- Restrictions for utilisation of the final product

Untreated sludge and biowaste of plant origin of unknown plant pathogen infection status is recommended not to be spread on agricultural land.

8.1. Further Research Work recommended

Lethal temperature/time requirements for elimination of high-risk organisms

Whereas the limited published information has been presented on particular lethal temperatures for

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Literature review on detection and eradication of plant pathogens in sludge, soils and treated biowaste

different organisms, there are no examples in which the full range of temperatures and required exposure times are known for any particular organism. A systematic study is therefore required to identify the range of lethal temperatures and exposure times for each organism of concern, particularly the heat tolerant plant pathogens. Such a study should take into account the full range of growth stages and spore types of each organism, which may differ in tolerance to heat. Furthermore, the behaviour of each organism inside the host plant should be determined to account for variation in heat transfer through different plant parts or host species.

Specifications for composting and digestion processes

Lethal temperatures and exposure times are important factors in the elimination of high-risk organisms during composting and anaerobic digestion but other factors are also involved. These can be particularly important in the assessment of the suitability of processes for biowaste containing organisms for which critical temperature-time conditions may be difficult to achieve in full. Careful selection and preparation of particular feedstocks can influence the efficiency of the processes as can the type of composting or digestion process used. Further research is needed to accurately determine specifications required for elimination of key high-risk organisms such as the biotrophic fungi with hardy resting spores and heat tolerant viruses. Survival of such organisms during commercial composting and digestion processes should be assessed in response to the use of different feedstocks, particle dimensions, moisture contents, turning frequencies, chemical analyses (especially ammonia and pH) and microbiological interactions.

Detection of key organisms in biowastes before and after processing

Current detection methodology relies mostly on the use of bioassays for assessment of biowastes. Recent evaluation at Central Science Lab, UK, of the recommended EPPO method for process validation with indicator organisms found this to be laborious, time consuming and expensive. A new method for rapid analysis of seedlings planted into processed green waste allows early detection of *P. brassicae* using a lateral-flow device (LFD) serological test kits. The development of similar procedures for detection of key pathogens of quarantine importance would facilitate post-processing quality analysis of treated wastes to confirm eradication of key target pathogens.

Suitability of indicator organisms for process validation

The proposed EPPO phytosanitary procedure recommends a validation procedure in which *Plasmodiophora brassicae* and *Tobacco mosaic virus* (TMV) in infected plant material are placed into batches of biowaste during treatment and used as indicators of effective sanitisation. Studies comparing the survival of these indicators during commercial processes with the heat tolerant organisms of plant health concern (including *Phytophthora*, *Polymyxa*, *Synchytrium* and *Tilletia* species) will enable determination of their suitability in validation of the processes for treatment of biowaste with high plant health risk.

Improvement of detection methods

No detection method can give an absolute guarantee that treated biowastes are free from a particular pathogen. It is desirable to ensure that a method can reliably detect the concentration of a pathogen, which presents a risk to the end-user. For most plant pathogens this risk has yet to be quantified.

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ANNEX 1

From COUNCIL DIRECTIVE 2000/29/EC (2000) on protective measures against the introduction into the Community of organisms harmful to plants or plant products and against their spread within the Community. Official Journal of the European Communities L169, p 112.

PART A.
HARMFUL ORGANISMS WHOSE INTRODUCTION INTO, AND SPREAD WITHIN, ALL MEMBER STATES SHALL BE BANNED

Section I
HARMFUL ORGANISMS NOT KNOWN TO OCCUR IN ANY PART OF THE COMMUNITY AND RELEVANT FOR THE ENTIRE COMMUNITY

(a) Insects, mites and nematodes, at all stages of their development 1. Acleris spp. (non-European) 2. Amauromyza maculosa (Malloch) 3. Anomala orientalis Waterhouse 4. Anoplophora chinensis (Thomson) 5. Anoplophora malasiaca (Forster) 6. Arrhenodes minutus Drury 7. Bemisia tabaci Genn. (non-European populations) vector of viruses such as: (a) Bean golden mosaic virus (b) Cowpea mild mottle virus (c) Lettuce infectious yellows virus (d) Pepper mild tigré virus (e) Squash leaf curl virus (f) Euphorbia mosaic virus (g) Florida tomato virus 8. Cicadellidae (non-European) known to be vector of Pierce's disease (caused by Xylella fastidiosa), such as: (a) Carneocephala fulgida Nottingham (b) Draeculacephala minerva Ball (c) Graphocephala atropunctata (Signoret) 9. Choristoneura spp. (non-European) 10. Conotrachelus nenuphar (Herbst) 10.1. Diabrotica barberi Smith and Lawrence 10.2. Diabrotica undecimpunctata howardi Barber 10.3. Diabrotica undecimpunctata undecimpunctata Mannerheim 10.4. Diabrotica virgifera Le Conte 11. Heliothis zea (Boddie) 11.1. Hirschmanniella spp., other than Hirschmanniella gracilis (de Man) Luc and Goodey 12. Liriomyza sativae Blanchard 13. Longidorus diadecturus Eveleigh and Allen 14. Monochamus spp. (non-European) 15. Myndus crudus Van Duzee 16. Nacobbus aberrans (Thorne) Thorne and Allen 17. Premnotrypes spp. (non-European) 18. Pseudopityophthorus minutissimus (Zimmermann) 19. Pseudopityophthorus pruinosus (Eichhoff) 20. Scaphoideus luteolus (Van Duzee) 21. Spodoptera eridania (Cramer) 22. Spodoptera frugiperda (Smith) 23. Spodoptera litura (Fabricus) 24. Thrips palmi Karny 25. Tephritidae (non-European) such as: (a) Anastrepha fraterculus (Wiedemann) (b) Anastrepha ludens (Loew)	

S e c t i o n I I

HARMFUL ORGANISMS KNOWN TO OCCUR IN THE COMMUNITY AND RELEVANT FOR THE ENTIRE COMMUNITY

(a) Insects, mites and nematodes, at all stages of their development 1. Globodera pallida (Stone) Behrens 2. Globodera rostochiensis (Wollenweber) Behrens 3. Heliothis armigera (H¸bner) 4. Liriomyza bryoniae (Kaltenbach) 5. Liriomyza trifolii (Burgess) 6. Liriomyza huidobrensis (Blanchard) 6.1. Meloidogyne chitwoodi Golden *et al.* (all populations) 6.2. Meloidogyne fallax Karssen 7. Opogona sacchari (Bojer) 8. Popilia japonica Newman 8.1. Rhizoecus hibisci Kawai and Takagi 9. Spodoptera littoralis (Boisduval) **(b) Bacteria** 1. Clavibacter michiganensis (Smith) Davis *et al.* ssp. sepedonicus (Spieckermann and Kotthoff) Davis *et al.* 2. Pseudomonas solanacearum (Smith) Smith **(c) Fungi** 1. Melampsora medusae Th¸men 2. Synchytrium endobioticum (Schilbersky) Percival **(d) Viruses and virus-like organisms** 1. Apple proliferation mycoplasm 2. Apricot chlorotic leafroll mycoplasm 3. Pear decline mycoplasm	

PART B

HARMFUL ORGANISMS WHOSE INTRODUCTION INTO, AND WHOSE SPREAD WITHIN, CERTAIN PROTECTED ZONES SHALL BE BANNED

(a) Insects, mites and nematodes, at all stages of their development	**Protected zone(s)**
1. Bemisia tabaci Genn. (European populations)	DK, IRL, P (Entre Douro e Minho, Tr·s-os-Montes, Beira Litoral, Beira Interior, Ribatejo e Oeste, Alentejo, Madeira and Azores), UK, S, FI
2. Globodera pallida (Stone) Behrens	FI
3. Leptinotarsa decemlineata Say	E (Menorca and Ibiza), IRL, P (Azores and Madeira), UK, S (Malmˆhus, Kristianstads, Blekinge, Kalmar, Gotlands L‰n, Halland), FI (the districts of Åland, Turku, Uusimaa, Kymi, H‰me, Pirkanmaa, Satakunta)
(b) Viruses and virus-like organisms	
1. Beet necrotic yellow vein	virus DK, F (Brittany), FI, IRL, P (Azores), S, UK
2. Tomato spotted wilt virus	DK, S, FI

ANNEX 2

From COUNCIL DIRECTIVE 2000/29/EC (2000) on protective measures against the introduction into the Community of organisms harmful to plants or plant products and against their spread within the Community. Official Journal of the European Communities L169, p 112.

PART A

HARMFUL ORGANISMS WHOSE INTRODUCTION INTO, AND SPREAD WITHIN, ALL MEMBER STATES SHALL BE BANNED IF THEY ARE PRESENT ON CERTAIN PLANTS OR PLANT PRODUCTS

Section I

HARMFUL ORGANISMS NOT KNOWN TO OCCUR IN THE COMMUNITY AND RELEVANT FOR THE ENTIRE COMMUNITY

(a) Insects, mites and nematodes, at all stages of their development	Subject of contamination
1. Aculops fuchsiae Keifer	Plants of Fuchsia L., intended for planting, other than seeds
2. Aleurocantus spp.	Plants of Citrus L., Fortunella Swingle, Poncirus Raf., and their hybrids, other than fruit and seeds
3. Anthonomus bisignifer (Schenkling)	Plants of Fragaria L., intended for planting, other than seeds
4. Anthonomus signatus (Say)	Plants of Fragaria L., intended for planting, other than seeds
5. Aonidella citrina Coquillet	Plants of Citrus L., Fortunella Swingle, Poncirus Raf., and their hybrids, other than fruit and seeds
6. Aphelenchoïdes besseyi Christie (*is not present on Oryza spp. in the Community)	Seeds of Oryza spp.
7. Aschistonyx eppoi Inouye	Plants of Juniperus L., other than fruit and seeds, originating in non-European countries
8. Bursaphelenchus xylophilus (Steiner and Buhere) Nickle et al.	Plants of Abies Mill., Cedrus Trew, Larix Mill., Picea A. Dietr., Pinus L., Pseudotsuga Carr. and Tsuga Carr., other than fruit and seeds, and wood of conifers (Coniferales), originating in non-European countries
9. Carposina niponensis Walsingham	Plants of Cydonia Mill., Malus Mill., Prunus L. and Pyrus L., other than seeds, originating in non-European countries
10. Diaphorina citri Kuway	Plants of Citrus L., Fortunella Swingle, Poncirus Raf., and their hybrids, and Murraya Kˆnig, other than fruit and seeds
11. Enarmonia packardi (Zeller)	Plants of Cydonia Mill., Malus Mill., Prunus L. and Pyrus L., other than seeds, originating in non-European countries
12. Enarmonia prunivora Walsh	Plants of Crataegus L., Malus Mill., Photinia Ldl., Prunus L. and Rosa L., intended for planting, other than seeds, and fruit of Malus Mill. and Prunus L., originating in non-European countries
13. Eotetranychus lewisi McGregor	Plants of Citrus L., Fortunella Swingle, Poncirus Raf., and their hybrids, other than fruit and seeds
14. Eotetranychus orientalis Klein	Plants of Citrus L., Fortunella Swingle, Poncirus Raf. and their hybrids, other than fruit and seeds
15. Grapholita inopinata Heinrich	Plants of Cydonia Mill., Malus Mill., Prunus L. and Pyrus L., other than seeds, originating in non-European countries
16. Hishomonus phycitis	Plants of Citrus L., Fortunella Swingle, Poncirus Raf., and their hybrids, other than fruit and seeds
17. Leucaspis japonica Ckll.	Plants of Citrus L., Fortunella Swingle, Poncirus Raf., and their hybrids, other than fruit and seeds
18. Listronotus bonariensis (Kuschel)	Seeds of Cruciferae, Gramineae and Trifolium spp., originating in Argentina, Australia, Bolivia, Chile, New Zealand and Uruguay
19. Margarodes, non-European species, such as:	Plants of Vitis L., other than fruit and seeds
(a) Margarodes vitis (Phillipi)	
(b) Margarodes vredendalensis de Klerk	
(c) Margarodes prieskaensis Jakubski	Plants of Pyrus L., other than seeds, originating

S e c t i o n I I

HARMFUL ORGANISMS KNOWN TO OCCUR IN THE COMMUNITY AND RELEVANT FOR THE ENTIRE COMMUNITY

(a) Insects, mites and nematodes, at all stages of their development	**Subject of contamination**
1. Aphelenchoides besseyi Christie	Plants of Fragaria L., intended for planting, other than seeds
2. Daktulosphaira vitifoliae (Fitch)	Plants of Vitis L., other than fruit and seeds
3. Ditylenchus destructor Thorne	Flower bulbs and corms of Crocus L., miniature cultivars and their hybrids of the genus Gladiolus Tourn. ex L., such as Gladiolus callianthus Marais, Gladiolus colvillei Sweet, Gladiolus nanus hort., Gladiolus ramosus hort., Gladiolus tubergenii hort., Hyacinthus L., Iris L., Trigridia Juss, Tulipa L., intended for planting, and potato tubers (Solanum tuberosum L.), intended for planting
4. Ditylenchus dipsaci (K¸hn) Filipjev	Seeds and bulbs of Allium ascalonicum L., Allium cepa L. and Allium schoenoprasum L., intended for planting and plants of Allium porrum L., intended for planting, bulbs and corms of Camassia Lindl., Chionodoxa Boiss., Crocus flavus Weston ëGolden Yellowí, Galanthus L., Galtonia candicans (Baker) Decne, Hyacinthus L., Ismene Herbert, Muscari Miller, Narcissus L., Ornithogalum L., Puschkinia Adams, Scilla L., Tulipa L., intended for planting, and seeds of Medicago sativa L.
5. Circulifer haematoceps	Plants of Citrus L., Fortunella Swingle, Poncirus Raf., and their hybrids, other than fruit and seeds
6. Circulifer tenellus	Plants of Citrus L., Fortunella Swingle, Poncirus Raf., and their hybrids, other than fruit and seeds
7. Radopholus similis (Cobb) Thorne	Plants of Araceae, Marantaceae, Musaceae, Persea spp., Strelitziaceae, rooted or with growing medium attached or associated
(b) Bacteria	
1. Clavibacter michiganensis spp. insidiosus (McCulloch) Davis et al.	Seeds of Medicago sativa L.
2. Clavibacter michiganensis spp. michiganensis (Smith) Davis et al.	Plants of Lycopersicon lycopersicum (L.) Karsten ex Farw., intended for planting
3. Erwinia amylovora (Burr.) Winsl. et al.	Plants of Chaenomeles Lindl., Cotoneaster Ehrh., Crataegus L., Cydonia Mill., Eriobotrya Lindl., Malus Mill., Mespilus L., Pyracantha Roem., Pyrus L., Sorbus L. other than Sorbus intermedia (Ehrh.) Pers. and Stranvaesia Lindl., intended for planting, other than seeds
4. Erwinia chrysanthemi pv. dianthicola (Hellmers) Dickey	Plants of Dianthus L., intended for planting, other than seeds
5. Pseudomonas caryophylli (Burkholder) Starr and Burkholder	Plants of Dianthus L., intended for planting, other than seeds
6. Pseudomonas syringae pv. persicae (Prunier et al.) Young et al.	Plants of Prunus persica (L.) Batsch and Prunus persica var. nectarina (Ait.) Maxim, intended for planting, other than seeds
7. Xanthomonas campestris pv. phaseoli (Smith) Dye	Seeds of Phaseolus L.

PART B

HARMFUL ORGANISMS WHOSE INTRODUCTION INTO, AND WHOSE SPREAD WITHIN, CERTAIN PROTECTED ZONES SHALL BE BANNED IF THEY ARE PRESENT ON CERTAIN PLANTS OR PLANT PRODUCTS

(a) Insect mites and nematodes, at all stages of their development	**Subject of contamination**	**Protected zone(s)**
1. Anthonomus grandis (Boh.)	Seeds and fruits (bolls) of Gossypium spp. and unginned cotton	EL, E (Andalucia, Catalonia, Extremadura, Murcia, Valencia)
2. Cephalcia lariciphila (Klug)	Plants of Larix Mill., intended for planting, other than seeds	IRL, UK (Northern Ireland, Isle of Man and Jersey)
3. Dendroctonus micans Kugelan	Plants of Abies Mill., Larix Mill., Picea A. Dietr., Pinus L. and Pseudotsuga Carr., over 3 m in height, other than fruit and seeds, wood of conifers (Coniferales) with bark, isolated bark of conifers	EL, IRL, UK (*)
4. Gilphinia hercyniae (Hartig)	Plants of Picea A. Dietr., intended for planting, other than seeds	EL, IRL, UK (Northern Ireland, Isle of Man and Jersey)
5. Gonipterus scutellatus Gyll.	Plants of Eucalyptus l'Herit., other than fruit and seeds	EL, P
6.(a) Ips amitinus Eichhof	Plants of Abies Mill., Larix Mill., Picea A. Dietr. and Pinus L., over 3 m in height, other than fruit and seeds, wood of conifers (Coniferales) with bark, isolated bark of conifers	EL, F (Corsica), IRL, UK
(b) Ips cembrae Heer	Plants of Abies Mill., Larix Mill., Picea A. Dietr. and Pinus L. and Pseudotsuga Carr., over 3 m in height, other than fruit and seeds, wood of conifers (Coniferales) with bark, isolated bark of conifers	EL, IRL, UK (Northern Ireland, Isle of Man)
(c) Ips duplicatus Sahlberg	Plants of Abies Mill., Larix Mill., Picea A. Dietr. and Pinus L., over 3 m in height, other than fruit and seeds, wood of conifers (Coniferales) with bark, isolated bark of conifers	EL, IRL, UK
(d) Ips sexdentatus Bˆrner	Plants of Abies Mill., Larix Mill., Picea A. Dietr. and Pinus L., over 3 m in height, other than fruit and seeds, wood of conifers (Coniferales) with bark, isolated bark of conifers	IRL, UK (Northern Ireland, Isle of Man)
(e) Ips typographus Heer	Plants of Abies Mill., Larix Mill., Picea A. Dietr., Pinus L. and Pseudotsuga Carr., over 3 m in height, other than fruit and seeds, wood of conifers (Coniferales) with bark, isolated bark of conifers	IRL, UK
7. Matsucoccus feytaudi Duc.	Isolated bark and wood of	F (Corsica)

(*) (Scotland, Northern Ireland, Jersey, England: the following counties: Bedfordshire, Berkshire, Buckinghamshire, Cambridgeshire, Cleveland, Cornwall, Cumbria, Devon, Dorset, Durham, East Sussex, Essex, Greater London, Hampshire, Hertfordshire, Humberside, Kent, Lincolnshire, Norfolk, Northamptonshire, Northumberland, Nottinghamshire, Oxfordshire, Somerset, South Yorkshire, Suffolk, Surrey, Tyne and Wear, West Sussex, West Yorkshire, the Isle of Wight, the Isle of Man, the Isles of Scilly, and the following parts of counties: Avon: that part of the county which lies to the south of the southern boundary of the M4 motorway; Cheshire: that part of the county which lies to the east of the western boundary of the Peak District National Park; Derbyshire: that part of the county which lies to the east of the western boundary of the Peak District National Park, together with that part of the county which lies to the north of the northern boundary of the A52 (T) road to Derby and that part of the county which lies to the north of the northern boundary of the A6 (T) road; Gloucestershire: that part of the county which lies to the east of the eastern boundary of the Fosse Way Roman road; Greater Manchester: that part of the county which lies to the east of the western boundary of the Peak District National Park; Leicestershire: that part of the county which lies to the east of the eastern boundary of the Fosse Way Roman road together with that part of the county which lies to the east of the eastern boundary of the B4114 road together with that part of the county which lies to the east of the eastern boundary of the M1 motorway; North Yorkshire: the whole county except that part of the county which comprises the district of Craven; Staffordshire: that part of the county which lies to the east of the eastern boundary of the A52 (T) road together with that part of the county which lies to the east of the western boundary of the Peak District National Park; Warwickshire: that part of the county which lies to the east of the eastern boundary of the Fosse Way Roman road; Wiltshire: that part of the county which lies to the south of the southern boundary of the M4 motorway to the intersection of the M4 motorway and the Fosse Way Roman road, and that part of the county which lies to the east of the eastern boundary of the Fosse Way Roman road).

ANNEX 3

Standard tests for detection of Tobacco Mosaic Virus and Plasmodiophora brassicae Ordinance on the Utilisation of Biowastes on Land used for Agricultural, Silvicultural and Horticultural Purposes (Ordinance on Biowastes - BioAbfV) Of 21 September 1998

2.3.2 Direct process validation and product analysis with regard to plant health requirements
The tests needed to ascertain compliance with plant health requirements in the case of anaerobic treatment must be carried out using similar testing methods to those specified for composting.
2.3.2.1 Test organisms and guide values
Out of the variety of plant pathogens and seeds that may be present in feedstocks for biological waste treatment facilities, the following indicator organisms are used in direct process validation:
-*tobacco mosaic virus* (TMV)
guide value in the biotest: ≤ 8 lesions per plant;
-*Plasmodiophora brassicae* (clubroot)
guide value in the biotest: infection index ≤ 0.5

If, in the case of samples which have been passed through either the entire process or the process stage responsible for thermal inactivation, the guide values for tobacco mosaic virus or tomato seeds are exceeded by more than 30 per cent, direct process validation is considered to have failed. In the case of the parameter Plasmodiophora brassicae, the guide value must not be exceeded at all.

2.3.2.2 The tobacco mosaic testing method
Testing is carried out on the basis of the method according to BRUNS *et al.* (1994), based on the method developed by KNOLL *et al.* (1980).

2.3.2.2.1 Buried samples
Every sample used in the process of biological treatment contains a quantity of 10 g of TMVinfected tobacco leaves (Nicotiana tabacum Samsun) as well as 100 g of the compost raw materials to be tested. The two components are mixed and packaged in 15 x 15 cm sacks made of non-decomposable gauze (mesh size 1 x 1 mm), and it must be ensured that no test organisms can transfer into the surrounding compost. In the case of fermentation facilities, 10 g of ground TMV-infected tobacco leaves are used in carriers with semi-permeable membranes mixed with substrate which are introduced into the process in stable, non-decomposable sample containers.
Propagation of the virus takes place in tobacco plants (Nicotiana tabacum var. Samsun), where it spreads systemically. The tobacco plants are grown under normal greenhouse conditions up to the five-leaf stage. For inoculation, two or three of the lower leaves are thinly powdered with carborundum, cellite or bentonite, and the TMV-infected suspension (pressed-out juice from TMV-infected tobacco plants in 0.05 mol/l of phosphate buffer; pH value: 7) is carefully applied to the powdered leaves with a brush, glass spatula or with a gauze wad. Two to three weeks after inoculation, leaves infected with the virus and showing mosaic-style discolourations

can be used for the tests. The control samples are kept in the freezer at a temperature of approx. -18 °C.

2.3.2.2.2 Proof of infectivity of TMV

Immediately after removal of the sample carrier from the composting or fermentation facility, its content is removed, and any coarse components that are not decomposed (wood, stones, etc.) are screened. The sample is ground in a mixer, with 30 ml of phosphate buffer (0.05 mol/l; pH value: 7) being added. The sample homogenate should have a pulpy rather than liquid consistency. The pulp is returned to the gauze bag, and any surplus liquid is pressed out (extracted). The same is done with the control samples. To prove infection, the extracts from the samples and from the control samples are applied to the test plant Nicotiana glutinosa.

The detection method used here is the "half-leaf method" (WALKEY, 1991). The plants should be in the 6-8 leaf stage. The leaf tips and the lower leaves are removed, so that four fully grown leaves are left on the plant. The second and third leaves are inoculated with the extracts containing the virus, one half of each leaf being rubbed with the control extract, the other with the sample extract. The tobacco variety referred to above reacts to TMV by showing so-called local lesions; these are small round stains, the centres of which consist of dead, necrotic tissue. The local lesions are counted ten days after inoculation. Evaluation consists in adding up the number of lesions on the two leaf halves of each plant which are infected with the sample solution.

2.3.2.3 The Plasmodiophora brassicae testing method

The testing is carried out on the basis of the method according to BRUNS *et al.* (1994), based on the method developed by KNOLL *et al.* (1980).

2.3.2.3.1 Buried samples

Every sample used in the process contains a quantity of 30 g of gall material with Plasmodiophora brassicae from affected cabbage plants, as well as 430 g of infectious soil and 200 g of the compost raw materials to be tested. In the case of fermentation facilities, 10 g of the ground gall material is mixed with substrate and introduced into the process using carriers with semi-permeable membranes. This corresponds to a ratio of 5 per cent gall material, 65 per cent soil and 30 per cent compost. The gall material is deep-frozen at -25 oC until the test start. The three components of the sample are thoroughly mixed and packaged in nondecomposable bags (maximum mesh size 1 x 1 mm). Here it is important to ensure that no test organisms can transfer into the surrounding compost. Instead of the compost raw material, the control samples consist of a mixture of gall material, infectious soil and sterile sand, with the ratio of components as described above. The control samples are stored in damp, sterilised sand at room temperature during the test period.

2.3.2.3.2 Proof of infectivity by means of biotests

After being recovered from the process, all pathogen samples are freed from coarse wood and thoroughly ground, and, in each case, 325 ml of sample are mixed with 275 ml of a sand/peat mixture (30 % : 70 % = V:V; sand steamed for five hours at 80 °C). The resulting total substance quantity is approx. 600 ml per sample, the pH value being > 6 ($CaCl_2$). To determine the pH value, which has a strong influence on the infectivity of Plasmodiophora brassicae, a blank sample is used which does not contain any gall material but has been stored in the tested

compost or fermentation residue during the entire test period. This blank sample is first used to produce the mixture, then to determine the pH value, which is corrected (by increasing or reducing the proportion of peat) if the value measured exceeds or is below 6 ($CaCl_2$).
For each sample, a container with test plants is established in the biotest. The plant species used is Brassica juncea of the Vittasso variety. Four plants are pricked in each container and pathogen sample. The test plants in the control sample are fertilised with culture substrate to which 250 mg N, 100 mg P_2O_5, 300 mg K_2O and 100 mg Mg are added per litre. Due to the normally very high P and K concentrations in the composts, there is usually no need to add these nutrients in the containers holding the samples. The biotest is carried out as a randomised split-plot design in light thermostats at 8000 Lux and at a temperature of 16 to 18 °C in the first week, and 22 °C from the second week onwards. The growing period for the biotest is five weeks.
After completion of the biotest, the number of affected plants is counted, and root galling is graded on a valuation scale from 0 to 3 according to BUCZACKI *et al.* (1975). Category 0: no swelling on lateral and/or tap roots; category 3: severe swelling on lateral and/or tap roots.
The scores are aggregated in the infection index according to the following formula:

$$\text{Infection index} = \frac{\Sigma(\text{Number of plants infected} \times \text{infection category})}{\text{Total number of plants}}$$

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