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Effects of sanitation processes on survival of *Synchytrium endobioticum* and *Globodera rostochiensis*

Silke Steinmöller · Martina Bandte · Carmen Büttner · Petra Müller

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Abstract The two quarantine pests *Synchytrium endobioticum*, the causal agent of potato wart disease and *Globodera rostochiensis*, the yellow potato cyst nematode are currently present in Germany. Winter sporangia of *Synchytrium endobioticum* and cysts of *Globodera rostochiensis* can be spread with waste from potato processing industries, if infected tubers are processed. The German Biowaste Ordinance prescribes sanitation of organic waste before it can be used on arable land as fertilizer or filling material. Sanitation parameters prescribed by the German Biowaste Ordinance include composting for 7 days at 65°C or 14 days at 55°C or pasteurisation for 60 min at 70°C. The effect of composting and pasteurisation processes on winter sporangia of *Synchytrium endobioticum* and cysts of *Globodera rostochiensis* was tested with varying time-temperature relations. Cysts of *Globodera rostochiensis* were killed by composting for 7 days at 50–55°C and by pasteurisation for 30 min at 70°C. In contrast, viable winter sporangia of *Synchytrium endobioticum* could be extracted from sample material after composting for 70 days at 30–45°C, composting for 21 days at 50–55°C and after composting for 12 days at 60–65°C. Likewise viable winter sporangia could be extracted after pasteurisation for 90 min at 70°C and heating in a water bath at 80°C and in a dry oven at 90°C for 8 h. The parameters prescribed in the German Biowaste Ordinance are sufficient to kill cysts of *Globodera rostochiensis* but not sufficient to kill winter sporangia of *Synchytrium endobioticum* in organic waste.

Keywords Potato wart · Sanitation · Potato cyst nematode · Potato residues · Bio waste · Composting · Heating · Pasteurisation

Abbreviation
EPPO European and Mediterranean Plant Protection Organisation

Introduction

The chytridiomycete *Synchytrium endobioticum* (Schilbersky) Percival, the causal agent of potato wart disease, was one of the most important potato pathogens in Europe (Langerfeld 1984) but has recently become less important as a result of strict phytosanitary control and resistant potato cultivars. However, in addition to the formerly widely spread pathotype 1

S. Steinmöller (✉) · P. Müller
Julius Kühn-Institut, Federal Research Centre for Cultivated Plants, Institute for National and International Plant Health, Stahnsdorfer Damm 81,
14532 Kleinmachnow, Germany
e-mail: silke.steinmoeller@jki.bund.de

M. Bandte · C. Büttner
Faculty of Agriculture and Horticulture, Department of Crop and Animal Sciences, Phytomedicine, Humboldt-Universität zu Berlin, Lentzeallee 55-57,
14195 Berlin, Germany

more pathotypes have been detected which occur in different climatic areas and for which resistance in current potato cultivars is very limited (EPPO/CABI 1997a). The European and Mediterranean Plant Protection Organisation (EPPO) states *S. endobioticum* as being present in the European Union. The pathogen is listed in Annex 1, Section II of Council Directive 2000/29/EC as a pathogen which is known to occur in the European Union but for which the introduction into and spread within all Member States is banned. *S. endobioticum* can develop persistent winter sporangia which can survive in soil for many years without host plants (Rintelen et al. 1983). McDonnell and Kavanagh (1980) described that winter sporangia of *S. endobioticum* survived for 31 years under field conditions in Ireland. The EPPO standard on soil tests and de-scheduling of previously infested plots (OEPP/EPPO 1999) allows complete de-scheduling of previously infested fields after a minimum of 20 years.

Another important quarantine pest of potato listed in the same section of Council Directive is *Globodera rostochiensis* (Wollenweber) Behrens, the golden potato cyst nematode. The pest occurs worldwide and is present in the European Union. *G. rostochiensis* has different pathotypes (EPPO/CABI 1997b) with varying resistances in potato cultivars. The cysts of *G. rostochiensis* can also survive some years in soil without host plants. Turner (1996) extracted cysts from fields 25 to 40 years after potato production, even though with a low number of eggs and juveniles. The EPPO recommends application of a period of 7 years without potato growing to reduce the nematode population in the soil to a significant degree (EPPO/CABI 1997b).

In addition to their persistence, both pathogens may spread easily with soil, potato tubers or residues from potato processing industries used as fertilizer or for watering (Efremenko and Yakovleva 1981; Spaull and McCormack 1989; Langerfeld 1984). In 2003, *S. endobioticum* was detected in a potato field in Rhineland. The occurrence was restricted to an area of approximately 100 m² where a former dip had been filled in with soil from potato processing (Steinmöller et al. 2004). Potato processing is an important industrial sector in Germany. Almost six million tons of potatoes are processed every year to produce various products like french fries or crisps, resulting in approximately 3 million tons of waste whereof the major part is suitable for reuse as fertilizer or for similar purposes. In Germany, the Biowaste Ordinance (Anonymous 1998) regulates that this waste has to be sanitised before application to agricultural or horticultural fields or in landscaping to avoid the introduction of human, veterinary or plant pathogens. The recommended parameters include composting for 2 weeks at a temperature of 55°C or for 1 week at a temperature of 65°C, or pasteurization of the waste for 1 h at 70°C. Composting and pasteurisation are two common treatments to sanitise various substrates. Cysts of *G. rostochiensis* have been subject of several studies on the effect of composting. Bøen et al. (2006) described that composting at 40–60°C for 8 days reduced the number of hatched juveniles by more than 99.9% and none of the hatched juveniles was able to fulfil its life cycle on potatoes. But there is hardly any information available on the effect of composting on winter sporangia of *S. endobioticum*. Composting experiments on *S. endobioticum* and *Clavibacter michiganensis* ssp. *sepedonicus* showed that both pathogens can withstand composting (Steinmöller et al. 2007). Previous studies reported on the effect of heat treatment on winter sporangia (Glynne 1926; Weiss and Brierley 1928). They concluded that wet winter sporangia were no longer infectious after being treated at 70°C for 60 min, whereas dry winter sporangia could survive at 60°C for a few days and at 100°C for 11–12 h.

The aim of the experiments presented here was to study the effect of sanitation processes on winter sporangia of *S. endobioticum* and cysts of *G. rostochiensis* and to test whether the recommended parameters for duration of the processes and necessary temperatures prescribed in the German Biowaste Ordinance are sufficient to inactivate these persistent pests.

Material and methods

Sample material

For *G. rostochiensis*, viable cysts of pathotyp Ro1 were provided by the Plant Protection Service of Lower Saxony. Potatoes of the susceptible cultivar ‘Hela’ were planted in 9×9 cm pots filled with sterile potting soil to propagate young cysts with viable content. The soil was inoculated with approximately 6000 eggs and larvae per pot. After completing the life cycle and maturation of the nematodes, cysts were extracted from soil by a flotation process. Extracted cysts were calibrated and only cysts with a size between 500 and 900 μm were used for the experiments. The average multiplication

rate of the used population was determined by bioassays as 10.1 and an average content of eggs and larvae of more than 200 per cyst was observed. A minimum of five infective eggs or juveniles per ml soil is considered necessary for resistance testing of potato varieties (OEPP/EPPO 2006).

For *S. endobioticum* infested potato compost or quartz sand mixed with viable winter sporangia of pathotype 1 (D1) (Baayen et al. 2006) was used (provided by Julius Kühn-Institut, Institute for Plant Protection in Field Crops and Grassland, Kleinmachnow). The potato compost consisted of rotten potato residues infested with *S. endobioticum* and sand, composted for 2 years and then stored at the dark at a temperature of 15°C. For the quartz sand, potato warts were cut and mixed with the quartz sand, kept slightly wet and mixed regularly for 6 months. After this time, the mixture was stored in a climate chamber at 15°C for 2 years before being used for the experiments.

Extraction of winter sporangia of *S. endobioticum*

The average content of winter sporangia in compost and quartz sand was determined by a method according to Van Leeuwen et al. (2005) with sieving of 100 g material, using a set of sieves with 210, 125, 75 and 25 μm sieves. The fraction held in the 25-μm sieve was washed with tap water into a beaker; 2 g Kaolin was added and then centrifuged at 1050 g for 5 min. The pellet was resuspended with 10–20 ml $CaCl_2$ and centrifuged again at 1050 g for 5 min. The supernatant was poured using the 25-μm sieve. Residues in the sieve were washed into a beaker with 2 ml tap water. Eight aliquots of 3.2 μl from each sample were examined under the microscope in a Fuchs-Rosenthal chamber. Winter sporangia are golden brown and thick-walled and are 25–75 μm in diameter. Winter sporangia in sample material were counted and the average numbers of winter sporangia per gram sample material were determined by extrapolation. Untreated quartz sand contained between 2300–8900 winter sporangia/g quartz sand, untreated compost contained between 500–2000 winter sporangia/g compost.

Pathogens introduction

As both pathogens are quarantine pests which require specific safety precautions, carriers were used to introduce the pathogens into the composting and pasteurisation processes. The carriers for *G. rostochiensis* were provided by the Plant Protection Service of Lower Saxony and consisted of closed nylon gauze bags (3×4 cm) with a pore size of 100 μm or glass tubes (2×1 cm), closed at both ends with the same nylon gauze. Twenty-five viable cysts were placed into the bags or put into the glass tubes.

For *S. endobioticum*, a carrier system was developed consisting of plastic jars (120 ml) with bottoms and tops closed with poly-ethylene gauze with a pore size of 17 μm. This pore size was small enough to prevent diffusion of the winter sporangia, whereas smaller microorganisms that are part of the composting process could diffuse. The warming of the carrier content compared to the surrounding substrate was tested in preparation of the experiments. Temperature probes were placed in and around carriers during a composting process to confirm, that the substrate in the carriers warmed up to the same temperatures as the surrounding substrate with a maximum delay of only 5–10 min. For the experiments, 100 g quartz sand mixed with viable winter sporangia was filled into each carrier.

Sanitation experiments

Composting experiments were conducted in 60-l composters under conditions comparable to a commercial composting facility (see Fig. 1). For each experiment 27 samples were used for *G. rostochiensis* and nine samples were used for *S. endobioticum* and placed in three layers in the substrate (near the bottom of the composter, in the middle and at the top). In the first two experiments (1 and 2), the composted substrate consisted of a mixture of fresh gardening compost, pulp and potato residues in a ratio of 1:2:1. The composting process was operated for a period of 70 days because temperatures did not exceed 45°C. In the succeeding experiments temperatures were increased by changing the composting substrate. Instead of pulp, only potato residues and old potatoes were mixed with fresh garden compost in a ratio of 2:1. Composting duration was 12 days at temperatures between 60–65°C (experiment 3) and 21 days at temperatures between 50–60°C (experiment 4). In experiment 5 only cysts of *G. rostochiensis* were examined. The composting duration was 7 days at temperatures between 50–58°C. Control samples of *S. endobioticum* were kept dry at room temperature for the duration of the composting experiments.

Fig. 1 Composters (60 l) used for experiments in a quarantine station

Control samples containing 25 viable cysts of *G. rostochiensis* were kept dry at 6°C or in put in tap water for the duration of the composting experiments.

For pasteurisation, carriers with quartz sand mixed with winter sporangia and carriers with cysts were put into beakers filled with pulp. The beakers were placed in a water bath heated to 70°C for 60 min (experiment 6), 90 min (experiment 7) and 120 min (experiment 8). The warming-up period of the pulp was determined with temperature probes. It took 30 min for the pulp to reach the 70°C, therefore the real pasteurisation time correlated to 30 min, 60 min and 90 min. The experiment was replicated three times.

In an additional experiment, winter sporangia of *S. endobioticum* were heated under dry conditions in an oven and under wet conditions in a water bath for 8 h. For heating of samples in a water bath, winter sporangia were extracted from contaminated quartz sand according to the method described above, washed in a beaker with 50 ml tap water and used as a spore suspension. Heating of samples in a dry oven was carried out with infested potato compost. Four samples each were heated for 8 h in a water bath at 80°C (experiment 9), or in a dry oven at 90°C (experiment 10).

Test of pathogen viability

Samples with cysts of *G. rostochiensis* were sent to the Plant Protection Service of Lower Saxony for testing of viability. Treated cysts and cysts of untreated controls were each mixed with 200 g sterilised potting soil and filled in transparent plastic pots. Potato plants of cultivar 'Hela' were planted in the soil and cultivated in a glasshouse. Young larvae penetrate the roots of the potato plants and develop inside the roots to adults. Male nematodes are able to leave the roots again but the females persist in the roots. Only their back bursts through the roots tissue so that they are copulated by the male nematodes. The female nematodes die during the development of the eggs and remain as cysts in the soil. In the bioassays, only the females on the roots that developed from hatched juveniles out of the treated cysts or the control cysts were counted after 10 weeks. Juveniles that hatched from treated cysts but were not able to complete their life cycle were neglected and defined as not viable.

From each sample of *S. endobioticum*, 100 g were used for extraction of winter sporangia according to the method of Van Leeuwen et al. (2005) described above. According to the EPPO standard PM/3/59 (2) on soil tests and de-scheduling of previously infested soils (OEPP/EPPO 1999), extracted winter sporangia can be differentiated between viable and dead with direct examination. Viable winter sporangia have a homogeneous, greyish, granular content or a slightly rounded-off cytoplasm when germinating. Winter sporangia that are permanently plasmolyzed, without apparent contents or disintegrated are considered dead. Figure 2 shows the difference between dead and viable winter sporangia under the microscope. Another 100 g of sample material was mixed with garden mould and used for a bioassay according to the tube test of Potocek et al. (1991) to determine the infectiousness of the winter sporangia. Mixtures of sample material and garden mould were placed in ten conical plastic tubes for each sample. Potato tubers of the susceptible cultivars 'Deodora' and 'Tomensa' were attached beneath the tubes with rubber bands and then cultivated at 15°C and with 15 h light per day. Sprouts grew through the tubes for 3 months with appropriate watering. After 3 months potato sprouts were examined visually for proliferations. Five tubes with garden mould were used as negative controls and ten tubes with untreated quartz sand from control samples were used as positive control for each experiment.

Statistical analysis

No statistical analysis was conducted for *G. rostochiensis*, as all experiments gave a clear black/white picture (yes/no reaction).

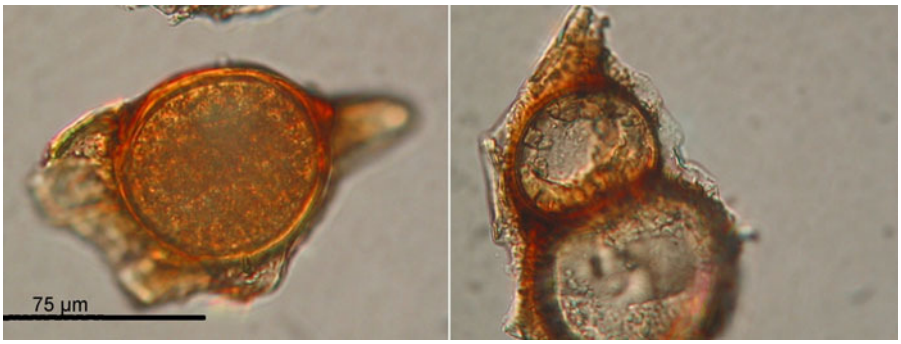

Fig. 2 Viable resting spore with visible content (left side) and empty dead resting spore (right side) of *S. endobioticum*

The winter sporangia of *S. endobioticum* that were extracted after the composting processes were counted and basic statistical data as range, median and quartiles were determined. A t-test for normally distribution (Shapiro-Wilk-Test) and a signed-rank test (Mann–Whitney-Rank-Sum-Test) were run to determine significant differences in the data from the control and from the composting experiments to determine whether the experiments had any generally influence on the number of winter sporangia.

Results

The composting experiments showed a considerable variation in temperature progression related to the applied composting substrate and environmental temperatures. During composting for 70 days (experiment 1), temperatures exceeded 40°C for 3 weeks, and then dropped below 40°C. In experiment 2, temperatures did not exceed 40°C during the whole composting duration. For the next experiments conditions were adapted to the parameters defined in the German Biowaste Ordinance. Changing the mixture of the composting substrate attained higher temperatures for a defined time. In experiment 3, temperatures were between 60–65°C for 12 days; in experiment 4, temperatures were between 50–60°C for 21 days. In experiment 5, only cysts of *G. rostochiensis* were introduced for 7 days to test shorter composting periods with temperatures between 50–58°C. Figure 3 displays the temperature development in the three compost layers in the five experiments.

No females of *G. rostochiensis* could be detected in the bioassays after composting and pasteurisation including composting for 7 days at temperatures between 50–58°C and pasteurisation for just 30 min at 70°C. Cysts from control samples stored at 6°C developed females on the roots of the bioassay plants with an average multiplication rate of 5. Table 1 gives an overview on treatment parameters and the results from the bioassays for *G. rostochiensis*.

Results for *S. endobioticum* were completely different. Viable winter sporangia could be extracted after all composting/pasteurisation processes, regardless of temperature progression or duration including maximum parameters for sanitation processes of composting for 12 days at temperatures between 60–65°C and pasteurisation for 90 min at 70°C. Viable winter sporangia could be extracted after heating of spore suspensions (wet spores) for 8 h in a water bath at 80°C and heating of infested compost (dry spores) in a dry oven for 8 h at 90°C. Table 2 provides the data on treatment parameters and the results for survival of winter sporangia of *S. endobioticum* from direct examination and bioassays.

Only very few plants from the bioassay (tube tests) showed proliferations on the newly grown sprouts after composting. Proliferations could only be observed on four tubers out of 90 after experiment 2 and on one tuber out of 90 after experiment 4. Similar results were obtained from the positive controls, as proliferations were observed only on four tubers out of 10 in the control of experiment 1 and on three tubers out of 10 in the control of experiment 4 (see Table 2). Figure 4 shows potato tubers from the bioassay after composting at 30–45°C for 70 days.

The range in the numbers of extracted winter sporangia was very broad, and went from a minimum of 150 winter sporangia per gram sample material up to a 10,000 winter sporangia per gram sample material. Tests for normally distribution of the data failed for the numbers of winter sporangia from all experiments and the control. Therefore the non-parametric Mann–Whitney

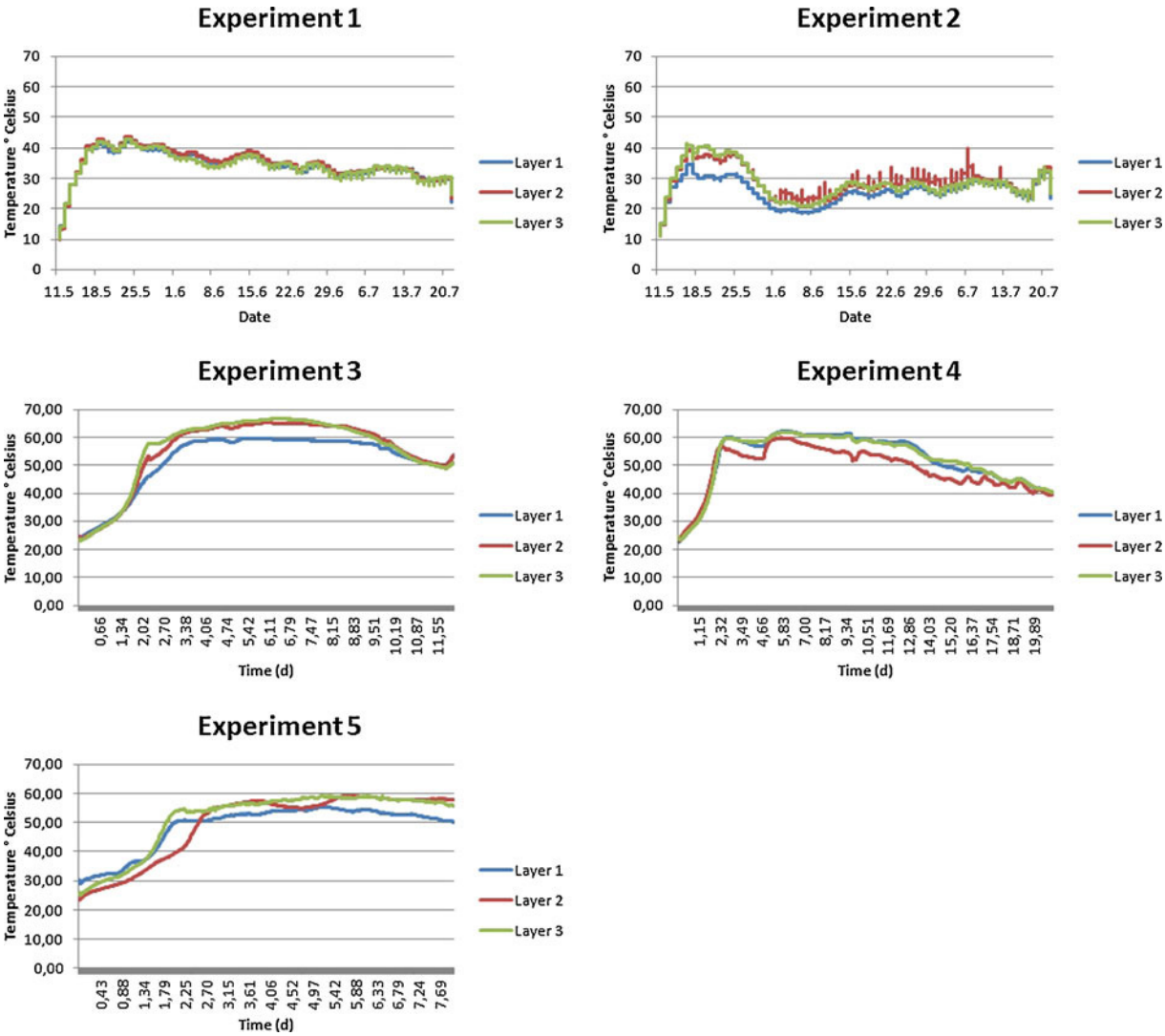

Fig. 3 Temperature progression in the five composting experiments; different colours indicate the three layers used for pathogen introduction

Rank Sum Test was used on the median values on a pairwise comparison to determine whether any of the experiments resulted in a significant lower number of winter sporangia after composting compared to the untreated control samples. Significant differences between the median values of the experiments and the control appeared for all experiments except for experiment 3. These results indicate that the number of winter sporangia in the composted samples of experiments 1, 2 and 3 was considerably lower than without composting, even so high numbers of winter sporangia were counted in some composted samples. For experiment 3 no reduction in the number of winter sporangia appeared compared to the control. The statistical data for the control and all experiments are shown in Fig. 5.

Discussion

Composting is a common treatment and widely used for sanitation of organic material. Information on eradication of robust plant pathogens and nematodes is given by Mikkelsen et al. (2006) and Noble et al. (2004). For some pathogens varying results can be found in the literature concerning the time-temperature conditions needed for complete inactivation, due to the complexity

Table 1 Parameters of sanitation treatments and resulting number of females per potato in the bioassay for *G. rostochiensis*

Number of Experiment	Treatment	Number of samples	Females on bioassay plants	
			Nr. of females/plant	Nr. of all test plants
1	Composting 70 d 20–35°C	27	0	27
	Control 6°C	2	130/100	2
	Control in water	2	250/120	2
2	Composting 70 d 30–45°C	27	0	27
	Control 6°C	2	130/110	2
	Control in water	2	150/120	2
3	Composting 12 d 60–65°C	27	0	27
	Control 6°C	2	250/170	2
	Control in water	2	100/400	2
4	Composting 21 d 50–55°C	27	0	27
	Control 6°C	2	180/100	2
	Control in water	2	20/70	2
5	Composting 7 d 50–55°C	27	0	26*
	Control 6°C	2	80/50	2
	Control in water	2	90/110	2
6, 7, 8	Pasteurisation 30, 60, 90 min at 70°C	9	0	9
	Control 6°C	2	230	250

*Loss of test plant

of the composting process and the variations resulting from different sample material or composting substrate. Some of the variability in the results may be due to difficulties to recover the pathogens from the tested substrates. For the validation of eradication measures it is therefore relevant to ensure that each pathogen that had been entered into the sanitation process can at the end be extracted from the composting substrate to test its viability. The introduction of all winter sporangia and cysts via carriers in the experiments described here, not only ensured that quarantine regulations were maintained, but it also allowed for the reliable extraction of the pathogens after composting. Nevertheless, winter sporangia and cysts were completely exposed to all temperature progressions and other chemical processes which are part of composting, because the gauze of the carriers had pore sizes small enough to keep the pathogens inside the carriers but large enough to allow bacteria, small microorganisms and fluids to pass.

The inactivation of cysts of *G. rostochiensis* in the experiments described in this paper supports other research results already conducted on this subject. Bøen et al. (2006) observed a reduction of cysts by 99.9% after 8 days of composting at 50°C. In the experiments described in this paper no completion of life cycle of the eggs or larvae in the cysts could be observed in the bioassays after any composting experiment. This included composting for 7 days at 50–55°C and pasteurisation for 30 min at 70°C. Eggs and larvae from cysts from control samples stored during the experiments beneath 6°C completed their life cycle and hatched females could be counted on the roots of the bioassay plants. As the parameters prescribed in the German Biowaste Ordinance for sanitation of organic waste include composting at maximum temperatures of 55°C for 14 days or 65°C for 7 days it can be concluded that these parameters are adequate to secure inactivation of cysts of *G. rostochiensis*. In some European countries,

Table 2 Parameters of sanitation treatments and results from direct examination of winter sporangia and the bioassay for survival of *Synchytrium endobioticum*

Number of Experiment	Treatment	Sample material	Number of samples	Viable winter sporangia extracted from samples	Positive bioassay plants	
					Nr. positive plants	Nr. of all test plants
1	Composting 70 d 20–35°C	Contaminated quartz sand	9	Yes	0	90
	Control at room temperature	""	2	Yes	4	10
2	Composting 70 d 30–45°C	Contaminated quartz sand	9	Yes	4	90
	Control at room temperature	""	2	Yes	0	10
3	Composting 12 d 60–65°C	Contaminated quartz sand	9	Yes	0	90
	Control at room temperature	""	2	Yes	0	10
4	Composting 21 d 50–55°C	Contaminated quartz sand	9	Yes	1	90
	Control at room temperature	""	2	Yes	3	10
6, 7, 8	Pasteurisation 30, 60, 90 min at 70°C	Contaminated quartz sand	3	Yes	Not tested	
9	Water bath 8 h 80°C	Spores suspension	4	Yes	Not tested	
10	Dry oven 8 h at 90°C	Contaminated compost	4	Yes	Not tested	

minimum parameters for sanitation of biowaste include composting where maximum temperatures of 55°C have to be attained for three or four days (Hogg et al. 2002). It was not tested here, whether composting for such a short time at this temperature is also sufficient to inactivate cysts of *G. rostochiensis*.

The same results were obtained by pasteurisation of cysts of *G. rostochiensis* with an actual exposure time of 30, 60 and 90 min at 70°C. None of the nematodes could complete its life cycle on potatoes after the treatment, leading to the conclusion that the parameters for pasteurisation in the Biowaste Ordinance (1 h at 70°C) are sufficient to inactivate cysts of *G. rostochiensis.* Moreover a pasteurisation time of 30 min at 70°C would already be sufficient. Van Loenen et al. (2003) could inactivate cysts of *G. rostochiensis* with aerated steam and temperatures at 60°C and an exposure time of 3 min and 8 min resting period in the steamed soil. These results back up the assumption, that cysts of *G. rostochiensis* can be inactivated already by exposure to a temperature of 70°C for a very short time.

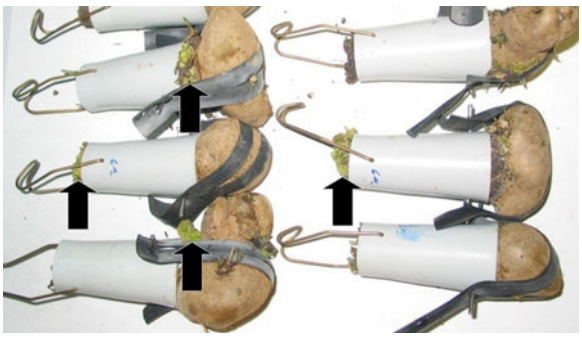

Fig. 4 Potato tubers from bioassay (tube test) to verify infectiousness of winter sporangia from *S. endobioticum* after composting for 70 days at 30°–45°C; arrows indicate proliferations induced by infection with *S. endobioticum*

In contrast, viable winter sporangia of *S. endobioticum* could be extracted after all composting experiments. Determination of viability of winter sporangia was done by direct examination of the spores according to the EPPO standard PM/3/59 (2) on soil tests and descheduling of previously infested soils (OEPP/EPPO 1999). The bioassays (tube tests) on infectiousness did lead to reasonable results, because only very few potato tubers showed proliferations after 3 months. This applied as well to the positive controls from untreated quartz sand; therefore no reliable conclusion concerning the effect of the composting experiments on the

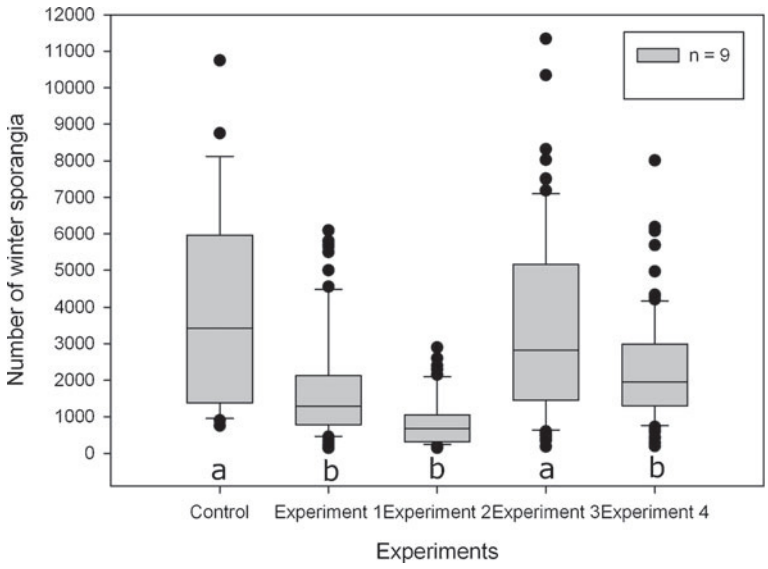

Fig. 5 Range, Quartiles, medians and outliers for the numbers of winter sporangia of *S. endobioticum* in the untreated control and after four different composting experiments; different letters under the box plots indicate significant differences in the medians of the numbers of winter sporangia in the composted samples and in the control determined with the Mann–Whitney Rank Sum test ($p<0.05$)

infectiousness of the winter sporangia can be drawn. The reasons for the poor results of the tube tests are not completely obvious. One cause might be the irregular germination of winter sporangia. Even though many studies on the germination of winter sporangia exist (Langerfeld 1984), no method for reliable stimulation of the germination has been found so far (Lange and Olson 1981). The EPPO standard PM/3/59 (2) on soil tests and de-scheduling of previously infested soils (OEPP/EPPO 1999) states that the observation of viable winter sporangia by direct examination is sufficient to prohibit de-scheduling of a former infested field. However, the differentiation between viable and dead winter sporangia might be difficult if spores have incomplete or heterogeneous contents. In case of doubt winter sporangia which seem to be plasmolyzed or look empty should be considered viable (OEPP/EPPO 2004). In the experiments described here, only complete winter sporangia without visible cracks in the walls and with visible content were counted as viable.

Although many pests and pathogens might be inactivated during those sanitation processes some robust pathogens are difficult to eradicate by composting (Christensen et al. 2002; Downer et al. 2008; Hermann et al. 1994; Hoitink and Fahy 1986; Noble and Roberts 2004). Bollen et al. (1989) state heat to be the most crucial factor for pathogen inactivation during composting even though other factors can have an effect on the pathogens as well. In the experiments presented here, viable winter sporangia could be extracted from samples after composting for 12 days at 60–65°C, as well as after composting for 14 days at 50–55°C and for 70 days at 30–45°C.

In addition, viable winter sporangia were extracted after pasteurisation at 70°C for 90 min as well as after heating spore suspensions in a water bath at 80°C and spores in organic material in a dry oven at 90°C for 8 h. These results are in contradiction to the former studies of Glynne (1926), who reported that wet winter sporangia were inactivated after heating with 70°C for 1 h. Weiss and Brierley (1928) reported that winter sporangia in dried wart material resisted dry heat at 100°C for 12 h and heating to 60°C for four to seven days with apparently undiminished viability, still inducing infections on test plants. The authors concluded that winter sporangia might survive exposures even at higher temperatures.

The German Biowaste Ordinance prescribes pasteurisation for 1 h at 70°C for the sanitation of organic waste. The EPPO (OEPP/EPPO 2008) recommends treatment with wet heat at 74°C for 4 h, 80°C for 2 h or 90°C for 1 h for the treatment of waste contaminated by quarantine or heat-tolerant pests. The results presented in this paper show that winter sporangia of *S. endobioticum* are able to survive the treatments prescribed in the German Biowaste Ordinance and those recommended by EPPO.

The persistence of winter sporangia is not surprising, since other members of the *Chytridiomycota* are also known to survive extreme conditions. Gleason et al. (2004) showed that sporangia of *Rhizophlyctis rosea* could survive air-drying as well as heating up to 90°C for 48 h. Of other tested species of *Chytridiomycota* five

survived high temperatures about 80° or 90°C for 48 h. The authors considered that the cytoplasm of the fungi is resistant to drying and to heat. Hampson et al. (1994) presume that the robust structure is responsible for the persistence of the winter sporangia of *S. endobioticum*. The spores are enclosed by a solid wall consisting of an exospore (Curtis 1921), a chitin containing mesospore (Bal et al. 1981) and an endospore (Hampson et al. 1994). Hampson et al. (1996) examined winter sporangia of *S. endobioticum* for evidence of melanin or melanin-like compounds and concluded that the pigmentation in these spores is caused by an allomelanin. In some fungi melanin or melanin like pigments protect fungi from irradiation, enzymatic lysis or even high temperatures (Butler and Day 1998).

The statistical analysis of the data shows a clear reduction in the median values of the winter sporangia from composting experiment 1, 2 and 4 but not for composting experiment 3. That means that a reduction in the number of winter sporangia was observed after composting at 30–45°C and at 40–50°C for 70 days as well as after composting at 50–60°C for 21 days. Composting at 60–68°C for 12 days did not result in a lower number of winter sporangia. These results contradict the results from Bollen et al. (1989) that heat is the most important factor concerning the eradication of winter sporangia. The clearest reduction in the median values can be observed after experiments 1 and 2, where temperatures did not exceed 50°C and that lasted for 70 days. It is possible that the longer composting duration was crucial for these results. Winter sporangia disintegrate and release the zoosporangia into the substrate when germinating (Lange and Olson 1981). The zoosporangia are only able to survive for a very short time (Curtis 1921) and cannot be detected after composting. However, the fact that experiment 3 with the highest temperatures during the composting process did not result in a significant reduction in the median values of the number of winter sporangia is somehow unexpected. A possible explanation might be that these unfavourable conditions prevented germination of the winter sporangia.

Overall the experiments lead to the conclusion that the treatments specified in the German Biowaste Ordinance for sanitising organic waste before their application to arable land are sufficient to kill cysts of *G. rostochiensis*. Winter sporangia of *S. endobioticum* however could neither be killed by composting nor pasteurisation according to the German Biwaste Ordinance nor through heat treatment. Further research on alternative methods to destroy winter sporangia of *S. endobioticum* is required to ensure a secure sanitation of waste from potato processing.

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