

# Composting of Rhododendron and Bilberry Wastes To Contain Spread of Exotic Plant Pathogens *Phytophthora kernoviae* and *Phytophthora ramorum*

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Plant material infected with the exotic pathogens *Phytophthora kernoviae* and *Phytophthora ramorum*, particularly of the invasive and highly susceptible *Rhododendron ponticum*, can pose a risk to indigenous host flora in Britain. Areas of infected bilberry (*Vaccinium myrtillus*) can also threaten surrounding non-infected heathland. Composting was examined as a more environmentally acceptable method of disposal of infected plant material than burning. Three types of low cost composting systems were developed and tested on shredded rhododendron and chopped bilberry wastes: permanent and mobile insulated bays, and insulated cargo containers, located at six different sites. In addition to temperature-time profiles of the composting wastes, the discoloration of the waxy leaves of *R. ponticum* and Portugal laurel (*Prunus lusitanica*) was developed as a potential indicator of the sanitising effect of the composting process. The relationship between the mean compost temperature and the percentage of green area of leaves positioned in the compost enabled the sanitising effect of a composting process to be immediately assessed. Mean compost temperatures and exposure times achieved in shredded rhododendron or chopped bilberry wastes in the majority of the compost in the insulated composting systems were above those needed to reduce *P. ramorum* and *P. kernoviae* to below detectable limits, and to eliminate any green colour in the indicator leaves. The exception was in the corners of the systems that contained >4 m<sup>3</sup> waste, and in the outer surfaces at one site where the volume of waste was only 2.9 m<sup>3</sup>. Temperature-time profiles of the composts and positioned indicator leaves demonstrated that the main pathogen survival risk was in the corners of the insulated composting systems; pathogen survival risk could be minimised by positioning the corner material into the centre of the composting system during sequential refilling.

## Introduction

*Rhododendron ponticum* is an introduced and invasive evergreen plant in Britain and can spread rapidly by seeds and vegetative means. Bilberry (*Vaccinium myrtillus*) is a British native deciduous species of heaths and moorlands. Both woody species are highly susceptible hosts to the introduced exotic pathogens *Phytophthora kernoviae* and *P. ramorum*, but attacked plants, particularly of less susceptible species, can survive for several years (Sansford & Woodhall 2007; Sansford 2008; Fichtner *et al.* 2009; Beales *et al.* 2009; Anon 2010). During this time, large amounts of inoculum of these pathogens can be produced which then threatens non-infected areas of bilberry and other indigenous susceptible host flora. Attempts to eradicate *R. ponticum* using herbicide applications have not been very successful (Anonymous 2010) and clearance usually involves cutting the plants to ground level followed by burning of the waste. However, the high

moisture content of the waste means that fuel needs to be added to enable burning and significant amounts of smoke are produced, which is environmentally unacceptable.

Composting has been shown to be an effective method for reducing inoculum of *P. ramorum* from infected plant wastes to below a detectable level (Swain *et al.* 2006; Noble *et al.* 2011a). Both *P. kernoviae* and *P. ramorum* have been shown to be susceptible to compost temperatures and exposure times which are achieved in well managed composting systems (Noble *et al.* 2009).

Temperature and exposure time are usually the most important and easily verified factors in eradicating pathogens during composting, but other factors such as moisture and gaseous conditions in the compost may also have an influence (Noble and Roberts 2004; Noble *et al.* 2009). The viability of indicator organisms in the compost has therefore been used to provide additional information on sanitisation (Noble *et*

al. 2011a,b). The discoloration of waxy leaves such as those of *R. ponticum* during composting could be a potential indicator of the sanitising effect of the composting process; this would have the advantages of the indicator being present throughout the composting mass rather than only at specific monitoring points, as well as the results being immediately apparent.

The aims of this work were to (1) assess the discoloration of rhododendron and other waxy leaves in response to compost temperature and exposure time to provide supplementary information on the efficacy of compost sanitisation (2) develop low cost composting systems that could be used for sanitising infected rhododendron and bilberry waste and (3) assess the pathogen survival risk from temperature profiles and the use of indicator leaves following sequential turning in the composting processes.

## Materials and Methods

### Discolouration of Waxy Indicator Leaves In Bench-Scale Flask Composts

Composting was conducted under controlled temperature and aeration in bench-scale equipment consisting of flasks immersed in thermostatically controlled water baths. Each 2-L flask contained 1200 g of green waste compost (moisture content 41% w/w) (Noble *et al.* 2011a). Mature, 9-12 month old leaves were obtained from rhododendron (*R. ponticum*) and Portugal laurel (*Prunus lusitanica*) bushes and inserted in the flasks of compost. The following compost temperature  $\times$  exposure time treatments were examined on the leaves: 18, 35, 40, 50, 55, 60 and 65°C  $\times$  1, 3, 5, 7 and 10 days. Two replicate flasks of each compost temperature treatment were prepared, each flask containing three rhododendron and three laurel leaves for each of the five specified exposure times. Day 0 leaves were used as controls. After exposure to compost, the percentage of area of the retrieved leaves that

remained intact and green was measured, i.e. leaf areas that matched RHS colour cards Green Group 131-143 and Yellow-Green Group 147 were considered green and colour cards Greyed-Orange Group 165-177, Greyed-Brown Group 199 and Brown Group 200 were considered brown (Anonymous 1967).

### Insulated Composting Systems

Composts were prepared in insulated bays at five sites: (A) Warwick HRI, Wellesbourne, (B) National Trust Trengwainton Gardens, Penzance, Cornwall, (C) National Trust Biddulph Grange Gardens, Biddulph, Staffordshire, (D) Coombe Abbey Country Park, Coventry, and (E) Tregonning Hill, Helston, Cornwall, and in insulated cargo containers at site (A) and at site (F) Cannock Chase, Staffordshire. The insulated bays were constructed of insulated wooden walls on three sides mounted either on a concrete base (sites A, B and C) or a double layer of thick polythene (sites D and E). Between one and six composts were prepared at each site. After filling with plant wastes, the open ends of the bays were closed by horizontal wooden boards fitted into vertical slots on the ends of the side walls. A waterproof, insulating cover was put on the surface of the composting wastes. The dimensions of the insulated bays and cargo containers, the types of waste (predominantly rhododendron or bilberry) and the waste fill weights and heights used at each site are shown in Table 1.

Rhododendron branches were cut at ground level and the stems and leaves were mechanically shredded to produce pieces of 25-105 mm in length (Table 1). Bilberry waste pieces of about 80-120 mm in length were harvested with a tractor mounted flail mower on accessible areas of site F; on inaccessible areas and at site E with a strimmer fitted with a brush cutter was used (Table 1). Due to the inaccessibility of large mechanisation at site E, the quantity of bilberry waste was smaller than used at the other sites. Water was added to the wastes at 100 L t<sup>-1</sup> during filling of the composting sys-

TABLE 1.  
Composting system types and internal dimensions, and waste types, filling weights and shredding machines used at each of six sites.

Site	Composting System	Dimensions L $\times$ W $\times$ H (m)	Waste Type	Waste		Mechanised Shredder <sup>a</sup> , Strimmer <sup>b</sup> Or Tractor Mounted Flail <sup>c</sup>
				Weight (t)	Height (m)	
A	Insulated bay	1.8 $\times$ 1.8 $\times$ 2.0	rhododendron	1.5	1.3	Green Mech CS100 <sup>a</sup>
A	Cargo container	2.9 $\times$ 2.2 $\times$ 2.4	rhododendron	3.0	1.7	Seko 500/130 GT <sup>a</sup>
B	Insulated bay	2.9 $\times$ 2.2 $\times$ 2.0	rhododendron	2.9	1.5	Jensen Wessex A528 <sup>a</sup>
C	Insulated bay	3.4 $\times$ 1.7 $\times$ 1.6	rhododendron	2.1	1.2	Timberwolf 150 <sup>a</sup>
D	Insulated bay	1.8 $\times$ 1.8 $\times$ 2.1	rhododendron	1.4	1.5	Green Mech CS100 <sup>a</sup>
E	Insulated bay	1.8 $\times$ 1.8 $\times$ 2.1	bilberry	0.7	0.9	Stihl HL 100 <sup>b</sup>
F	Cargo container	3.7 $\times$ 2.4 $\times$ 2.6	bilberry	3.9	1.7	Major MT10 155 <sup>c</sup> and Stihl HL 100 <sup>b</sup>

tems. The temperature of the composting wastes was then allowed to rise for about 7 days. Three rhododendron and three laurel leaves as described above were enclosed in 500 × 300 mm nylon mesh sacks containing 2 kg of the same waste that was in the large-scale systems and a temperature probe connected to a data logger (Grant Instruments, Cambridge). The sacks were inserted into different locations of the large-scale systems (centre, corners, sides), from the surface to depths of up to 0.7 m, to obtain a range in exposure temperatures. The air temperature during the composting tests was also recorded. After 10 days, the sacks were removed and the leaves retrieved and assessed for discoloration as described above. The composting wastes were emptied and refilled in the composting systems on two occasions at 11-14 day intervals. At sites A, B, D and F, additional water was added to compost at 50 L t<sup>-1</sup> during each turn. Fresh rhododendron and laurel indicator leaves were inserted in the sacks of compost, which were replaced with the temperature probe in the same relative positions in the composting systems. After each test was completed, the compost was spread as a 50 mm layer mulch over soil and observed for 6 months for the regrowth of rhododendron or bilberry.

Samples of the initial and final materials were analysed for dry matter and ash contents and pH and electrical conductivity before and after composting using methods described in Noble *et al.* (2011b). Gas conditions in the composts were determined using gas detector tubes as described in Noble *et al.* (2011b).

#### Statistical Analysis

An initial examination of the data from the flask experiment revealed large numbers of extreme values (0 or 100%) for the percentages of leaf areas that were green. It was decided to use non-parametric statistics and Steel's many-one-rank test (Steel 1959) for comparing treatments with two controls: the values from the day 0 leaves, and from the most extreme combination of temperature and time examined (65°C for 10 days).

The relationship between mean compost temperature and the percentage green leaf area after large-scale composting was investigated using a non-linear regression. Since an initial examination of the data revealed that this relationship followed an S-shaped curve, oriented to the left, i.e. decreasing with increasing temperature, it was decided to fit a left-oriented sigmoid curve. This analysis investigated whether the relationship between compost temperature and the percentage green leaf area differed between the two species, i.e. whether the slope and inflection point (the temperature at which an estimated 50% of leaf area remained green) differed. Analyses were conducted using Genstat 12.1.

## Results

### Discolouration of Waxy Leaves In Bench-Scale Flask Composts

After exposure to any of the compost temperatures for up to 10 days, the leaves of both species remained sufficiently intact to be retrieved and assessed for colour (less than 60% disintegrated). Only leaves exposed to 40-50°C for >7 days were more than 40% disintegrated. Compared with the percentage of green leaf area (G) of the day 0 leaves, i.e. 100% green, using Steel's non-parametric test, G was significantly smaller after the 35°C for 10 days treatment for both species, and for rhododendron also after the 18°C for 10 days treatment (Figure 1a and 1b). G was also significantly

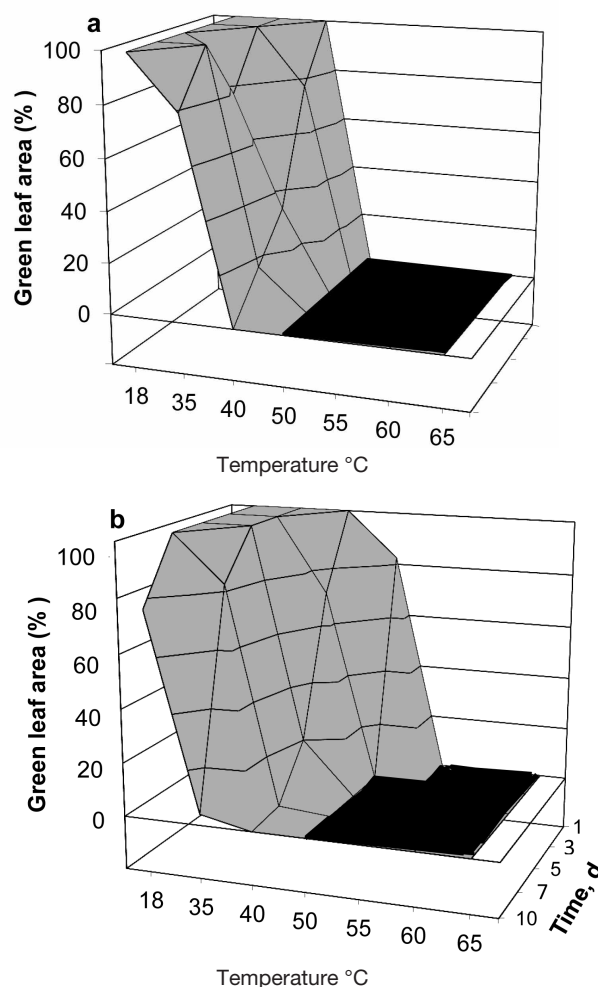


FIGURE 1. Percentage of green leaf area of (a) laurel and (b) rhododendron leaves after various combinations of compost temperature and exposure time in a flask system. The black zone indicates treatment combinations that were not significantly different from the 65°C for 10 days treatment (0% green) using Steel's many-one-rank test. Each value is the mean of two replicate flasks and three replicate leaves. At the start of the test, all leaves were 100% green.

less than 100% after 3 or more days at 40°C or 1 or more days at 50°C for both species. Compared with G after the 65°C for 10 days treatment, i.e. 0% green, all the leaves of both species had significantly larger G after 10 days or less at 18°C, 7 days (10 days for rhododendron) or less at 35°C, 3 days or less at 40°C or 1 day at 50°C (Figure 1a and b).

*Compost Temperature Profiles  
In Insulated Composting Systems*

After filling with shredded rhododendron waste, the mean temperatures in the composting systems

ranged from 53-62°C at the centre to 37-41°C in the corners (Table 2). Mean temperatures in bilberry waste (sites E and F) were lower than in equivalent systems and positions in rhododendron waste. At site E this was at least partly due to the smaller quantity of waste used than in the insulated bays at the other sites (Table 1). After subsequent turns, mean temperatures in the same relative positions generally became progressively lower, although at sites A (cargo container), D and E (insulated bays), mean compost temperatures remained similar before and after each of the two turns. Maximum compost temperatures (Table 3) were about 7°C and 12°C higher than

TABLE 2.  
Mean (± S.D.) compost and air temperatures (C°) in different positions of the insulated bays and cargo containers at sites A to F, during each turning stage of n rhododendron or bilberry waste composts.

Position	After Turn	Insulated Bays					Cargo Containers	
		A n = 2 Rhododendron	B n = 4 Rhododendron	C n = 6 Rhododendron	D n = 1 Rhododendron	E n = 1 Bilberry	A n = 1 Rhododendron	F n = 4 bilberry
Centre	0	54 ± 7.2	57 ± 4.8	53 ± 6.9	61	38	62	51 ± 2.4
	1	48 ± 7.3	58 ± 6.2	43 ± 5.9	63	45	65	47 ± 1.1
	2	38 ± 4.0	53 ± 2.7	37 ± 6.0	61	43	64	46 ± 3.2
Surface	0	49 ± 6.5	52 ± 8.1	46 ± 6.4	58	35	62	46 ± 2.9
	1	42 ± 2.2	52 ± 4.3	39 ± 6.0	63	41	61	42 ± 2.4
	2	41 ± 5.4	50 ± 2.3	32 ± 3.6	57	38	61	42 ± 4.5
Side	0	49 ± 6.3	49 ± 6.8	45 ± 8.5	50	31	57	38 ± 7.5
	1	42 ± 9.9	52 ± 2.5	39 ± 4.8	47	37	56	37 ± 4.9
	2	41 ± 5.1	48 ± 2.5	30 ± 4.0	51	34	36	37 ± 4.5
Corner	0	37 ± 1.6	39 ± 8.0	38 ± 6.5	37	23	41	26 ± 3.0
	1	26 ± 0.1	34 ± 6.9	33 ± 4.2	36	28	40	26 ± 4.5
	2	22 ± 0.1	34 ± 3.1	28 ± 2.5	33	25	37	25 ± 4.1
Air	0	10 ± 3.3	10 ± 5.2	13 ± 4.4	10	10	14	14 ± 1.0
	1	9 ± 3.0	10 ± 5.1	15 ± 6.6	11	11	17	13 ± 0.7
	2	9 ± 2.9	10 ± 5.2	12 ± 4.6	17	7	14	12 ± 1.0

TABLE 3.  
Maximum (± S.D.) compost and air temperatures (C°) in different positions of the insulated bays and cargo containers at sites A to F, during each turning stage of n rhododendron or bilberry waste composts.

Position	After Turn	Insulated Bays					Cargo Containers	
		A n = 2 Rhododendron	B n = 4 Rhododendron	C n = 6 Rhododendron	D n = 1 Rhododendron	E n = 1 Bilberry	A n = 1 Rhododendron	F n = 4 bilberry
Centre	0	66 ± 4.1	71 ± 5.2	68 ± 7.8	73	46	72	59 ± 1.3
	1	61 ± 7.1	68 ± 3.7	58 ± 7.7	65	47	74	56 ± 4.8
	2	50 ± 7.8	56 ± 2.4	52 ± 8.3	68	50	72	4 ± 3.5
Surface	0	59 ± 3.7	65 ± 5.0	66 ± 9.7	68	41	71	54 ± 3.1
	1	50 ± 3.1	57 ± 4.9	53 ± 7.2	64	43	70	51 ± 4.5
	2	46 ± 2.9	56 ± 4.9	48 ± 6.3	67	46	68	49 ± 4.6
Side	0	56 ± 3.5	69 ± 9.9	67 ± 9.9	62	38	68	46 ± 8.0
	1	49 ± 3.1	56 ± 2.3	53 ± 4.8	55	39	63	41 ± 6.5
	2	44 ± 2.8	53 ± 2.1	48 ± 4.3	60	42	54	42 ± 8.2
Corner	0	46 ± 1.4	58 ± 9.9	52 ± 8.2	59	31	59	33 ± 4.8
	1	45 ± 2.8	52 ± 6.2	49 ± 8.3	49	33	54	34 ± 4.4
	2	39 ± 2.5	42 ± 2.9	41 ± 6.5	55	36	49	31 ± 7.5
Air	0	21 ± 5.9	25 ± 6.3	25 ± 4.1	15	18	27	19 ± 3.2
	1	17 ± 5.7	23 ± 4.7	28 ± 5.6	16	16	24	17 ± 2.6
	2	19 ± 5.1	25 ± 5.4	24 ± 6.4	25	13	21	16 ± 2.5

TABLE 4.

Moisture and organic matter contents, pH and electrical conductivities ( $\pm$  S.D.) rhododendron and bilberry wastes before, at the start, and after composting in insulated bays and cargo containers at sites A to F.

Property	Stage	Insulated Bays					Cargo Containers	
		A n = 2 Rhododendron	B n = 4 Rhododendron	C n = 6 Rhododendron	D n = 1 Rhododendron	E n = 1 Bilberry	A n = 1 Rhododendron	F n = 4 bilberry
Moisture (% w/w)	before	49 $\pm$ 1.6	48 $\pm$ 1.5	46 $\pm$ 1.2	49	64	44	45 $\pm$ 1.0
	start	59 $\pm$ 5.9	63 $\pm$ 4.7	59 $\pm$ 6.2	53	64	53	64 $\pm$ 2.8
	end	63 $\pm$ 3.5	56 $\pm$ 6.3	60 $\pm$ 5.5	55	62	54	61 $\pm$ 3.3
Ash (% DM)	before	3.0 $\pm$ 0.27	2.6 $\pm$ 0.84	2.8 $\pm$ 0.89	2.2	2.6	3.0	3.8 $\pm$ 1.46
	after	4.6 $\pm$ 0.27	3.7 $\pm$ 1.27	3.9 $\pm$ 0.97	3.7	4.2	5.8	8.2 $\pm$ 2.55
pH	before	5.9 $\pm$ 0.21	6.1 $\pm$ 0.14	5.8 $\pm$ 0.06	5.6	4.7	5.7	5.1 $\pm$ 0.17
	after	6.0 $\pm$ 0.23	6.5 $\pm$ 0.09	6.0 $\pm$ 0.11	5.9	5.3	5.9	5.9 $\pm$ 0.32

the mean temperatures for bilberry and rhododendron wastes respectively, although the difference between maximum and mean temperatures was generally greater in the corners than in the centres of the composting systems.

The initial moisture content of the waste was about 47% w/w, which increased to about 57% w/w after wetting (Table 4). During the composting process, the moisture content of the wastes remained stable whereas the ash content increased. The bilberry waste was more acidic than the rhododendron waste; the pH of both types of waste increased during composting. Mean O<sub>2</sub> and CO<sub>2</sub> concentrations in the composting plant wastes were 11.9 $\pm$ 4.1 and 6.6 $\pm$ 2.3 % v/v respectively. Mean ammonia concentration in the composting rhododendron waste was 0.2 $\pm$ 0.07 mg m<sup>-3</sup>; no ammonia was detected in the composting bilberry wastes.

*Discolouration of Waxy indicator Leaves In Large-Scale Systems*

Rhododendron and laurel leaves retrieved from the sampling sacks after the 10-day composting periods were intact. Leaves retrieved from sampling sacks positioned in the centre or surface of the composts were completely brown and only leaves retrieved from sampling sacks positioned in the sides or corners of the composting systems had a proportion of green area. The strong negative effect of mean compost temperature (*T*) on the percentage of green leaf area (*G*) in the large-scale systems (*P* < 0.001) was similar to that observed in the flask composts, with a sharp decline in *G* between *T* values of 35 and 40°C over a 10-day period. The fitted relationship between *T* and *G* was:

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

where *M* is the inflection point and *B* is the slope of the curve at the inflection point. It was assumed that for sufficiently high temperatures, the percentage of green leaf area will decrease towards zero, whilst for sufficiently low temperatures, it will be 100%. Although it should be possible to estimate these values instead of fixing them at 0 and 100%, this was not felt appropriate, given the range of values observed in this experiment. There was no significant difference between the two species in the values of *B* or *M* in Equation (1) of the fitted curves; a single curve was therefore fitted to both species (Figure 2). The estimated value of *T* at *M*, at which *G* = 50% of leaf area, was 35.4 (S.E.  $\pm$ 0.15)°C (*P* < 0.001), and the estimated value of *B* was -1.035 (S.E.  $\pm$ 0.15) (*P* < 0.001).

There was no regrowth of rhododendron or bilberry from any of the composted wastes after three composting cycles in the large-scale systems, and then spreading as a mulch.

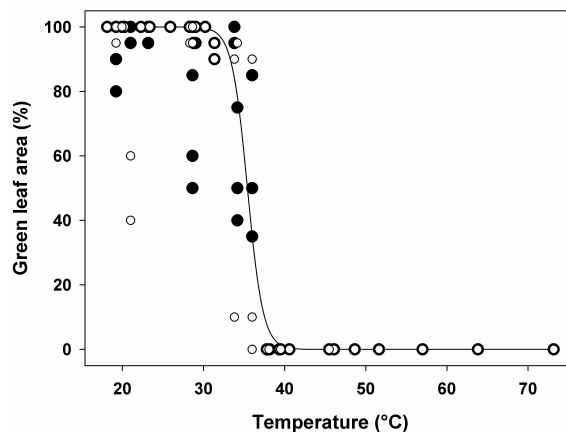


FIGURE 2. Fitted regression between mean compost temperature and the percentage of leaf area remaining green after exposure to composting plant wastes in large-scale systems for 10 days (○ laurel leaves; • rhododendron leaves).

## Discussion

### *Waxy Leaves as Compost Sanitisation Indicators*

The consistent response of waxy leaf discoloration to compost temperature and exposure time indicates that this method can provide useful information on the sanitising effect of a composting process. This response is similar to the effects of temperature and exposure time on inocula of *P. kernoviae* and *P. ramorum*, as well as other plant pathogenic *Phytophthora* species during composting (Noble *et al.* 2011a,b). The presence of green rhododendron or laurel leaf areas after the composting of infected plant wastes would provide a useful risk assessment of the compost, and indicate need for further phytosanitary treatment. The method has advantages over the use of other indicator organisms of compost sanitisation such as fungi, bacteria, and seeds which can only be positioned in specific locations in the waste, and which require time for viability to be assessed post-composting (Christensen *et al.* 2002; Noble *et al.* 2011b).

### *Composting of Rhododendron and Bilberry Wastes In Insulated Systems*

Mean temperatures and exposure times achieved in shredded rhododendron or chopped bilberry wastes in the majority of insulated bays and cargo containers were above those which were previously found to reduce inocula of *P. kernoviae* and *P. ramorum* to below their detectable limits (Noble *et al.* 2011a). The exception to this was in the upper and lower corners of the systems, as well as on the surface and in the sides of the insulated bay at site E where the quantity of waste was smaller than at the other sites. This was confirmed by the green leaf areas on the indicator leaves positioned in these zones. However, by positioning the corner material into the centre of the composting system on refilling, the risk of pathogen survival can be reduced.

The volume of compost in a vessel has been shown to be critical in producing sufficient heat to destroy pathogens; Alexander (2007) found that temperatures in a 790 L vessel containing plant wastes remained only a few degrees above ambient. The large surface to volume ratio in small masses of compost mean that heat losses are large compared with the microbial heat produced by the compost (Miller 1991). The volume of waste in most of the systems used here ranged from 4.2-9.6 m<sup>3</sup> in the bays at sites A, B, C and D to 10.8-15.1 m<sup>3</sup> in the cargo containers at sites A and F. The exception was at site E where the waste volume was only 2.9 m<sup>3</sup>. This indicates that a volume of 4.2 m<sup>3</sup> is adequate in terms of generating

sufficient heat in the majority of the rhododendron or bilberry waste to reduce inocula of *P. kernoviae* and *P. ramorum* to below their detectable limits, if the system is insulated against heat loss. However, the 'edge effect' and potential for pathogen survival will be greater than in systems with a larger capacity.

For many waste feedstocks that have a high oxygen demand during composting, some form of forced aeration is usually necessary to maintain aerobic conditions (MacGregor *et al.* 1981; de Bertoldi *et al.* 1988). Oxygen levels recorded in the centre of the composting systems showed that forced aeration to maintain aerobic conditions in the rhododendron and bilberry wastes was unnecessary, where the composts were emptied and refilled at 11-14 day intervals. This significantly reduces the cost of the installation and enables composting in locations that are remote from a power supply.

## Conclusions

1. The relationship between the mean compost temperature and the percentage of green area of rhododendron or laurel leaves positioned in the compost enabled the sanitising effect of a composting process to be immediately assessed.

2. Mean compost temperatures and exposure times achieved in shredded rhododendron or chopped bilberry wastes in the majority of two insulated composting systems (bays and cargo containers) were above those needed to reduce inocula of *P. ramorum* and *P. kernoviae* to below detectable limits, and to eliminate any green colour in the indicator leaves. The exceptions were in the corners of the systems, and in the outer surfaces at one site where the volume of waste was only 2.9 m<sup>3</sup>.

3. Temperature-time profiles of the composts and positioned indicator leaves demonstrated that the main pathogen survival risk was in the corners of the insulated composting systems; pathogen survival risk could be minimised by positioning the corner material into the centre of the composting system during sequential refilling.

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