CSL RESEARCH FUNDED BY DEFRA PLANT HEALTH DIVISION FROM THE *PHYTOPHTHORA RAMORUM* AND *P. KERNOVIAE* PROVISION

FINAL SUMMARY REPORT

Project Title: Determining the susceptibility of key / dominant UK heathland species to *Phytophthora kernoviae*

Summary:

The susceptibility of eight commonly occurring heathland species to the recently described pathogen *P. kernoviae* (Brasier *et al.*, 2006) was tested. Larger leaved species such as *Vaccinium vitis-idaea*, *V. myrtillus* and *Arctostaphylos uva-ursi* were highly susceptible, the pathogen killing both leaves and stems. Smaller-leaved species such as heathers and heaths, e.g. *Erica cinerea*, *E. tetralix* and *Calluna vulgaris* were less susceptible, showed limited infection when the plant was wounded prior to inoculation. The sporulation potential of these smaller-leaved varieties was, however, significantly greater than the more susceptible plant species.

Background:

Phytophthora kernoviae is potentially a serious pathogen of UK woodland environments. The disease was first discovered in historic woodland gardens in the heart of Cornwall in late 2003, where both large rhododendron bushes and beech trees were infected with this new species of phytophthora.
To date, the organism has been found affecting eight genera of plants, including rhododendron, pieris, magnolia, beech and oak trees both in woodland environments, established gardens and one commercial premises.
Recently, *P. kernoviae* has been detected on rhododendron in woodlands adjacent to heathland. These findings have raised concerns of the potential of the organism to affect heathland plant species.

Aim:

To test the susceptibility of selected British heathland plant species to *P. kernoviae*.

Objectives, Methods and Results:

All objectives have been completed.

Objective 1:

1 (a) Ascertain the range of heathland plant species in areas where *P. kernoviae* has predominantly been detected (south west England)

1 (b) In consultation with PHSI, determine range of heathland plant species in land adjacent to recent *P. kernoviae* findings on rhododendron.

1 (c) Based on the findings from 1(a) and 1(b), select key / dominant plant species for *P. kernoviae* susceptibility testing studies (maximum 20).

To date *P. kernoviae* has predominantly been found in established gardens or wild sites in the South West of England, with further findings in South Wales and a single location in a garden centre in Cumbria that has since been eradicated.
A literature survey was carried out to ascertain the most prevalent heathland flora in the UK and Southwest of England. This list can be found in appendix A.

Following consultation with inspectors and further literature study, it was determined that the heathland flora in areas adjacent and in the general vicinity of *P. kernoviae* infected bushes was diverse. The most prevalent plants included, *Calluna vulgaris, Erica cinerea, E. tetralix, E. vagans, Vaccinium myrtillus, V. vitis-idaea.* Other less prevalent / rare species include *V. macrocarpon,* and *Empertrum nigrum*

Objective 2:

2 Obtain plant species selected from 1 (c).

Some difficulties were encountered when attempting to obtain all plant species identified from 1. It was not possible to obtain plants from the wild. Commercially cultivated (pot grown) plants were therefore purchased. Available plants were as follows:- *Calluna vulgaris* (cv. Winter chocolate), *Erica cinerea* (cv. Glen Cairn) , *E. tetralix, , Vaccinium myrtillus, V. vitis-idaea*, *V. macrocarpon* and *Empertrum nigrum. Arctostaphylos uva-ursi,* (another heathland species of more Northern UK habitat) was also available and included within this study.

Objective 3:

3 (a) Test suitability of *P. ramorum* host susceptibility method using *P. kernoviae*.
3 (b) Write standard protocol for testing of susceptibility of plants to *P. kernoviae* infection.
3 (c) Using the standardised protocol, test susceptibility of plants obtained in 2, using CSL-type isolate and a Cornwall isolate of *P. kernoviae*.

A zoospore-dip method has previously been developed for testing susceptibility of heathland plants to *P. ramorum* (Defra project PH0193s). (http://www.defra.gov.uk/science/project_data/DocumentLibrary/PH0193S/PH0193S_2686_FRP.pdf)

The suitability of this method was evaluated using an isolate of *P. kernoviae* (CSL culture ref: 2246) and *P. ramorum* (BBA 9/95 (Werres *et al.*, 2001)). Unwounded and wounded (leaf tip removed with surface sterilised scissors), detached leaves of the susceptible plant, *Rhododendron catawbiense* were dipped into a zoospore suspension (ca. 1 x 10^5 zoospores ml^{-1}) of *P. kernoviae* and *P. ramorum* for 10 seconds. Sterile distilled water (SDW) (without inoculum) was used for control tests. Each test was replicated five times. Individual leaves were incubated on moist paper towels in moist chambers at 19°C with an 18-hour, white-light day-length cycle and assessed for disease after 7 days. The presence or absence and extent of necrosis on each leaf was recorded (table 1).

Table 1. Evaluation of zoospore-dipping method for *P. kernoviae* using rhododendron leaves.

Isolate tested	Control		*P. ramorum*		*P. kernoviae*	
	Wounded	Unwounded	Wounded	Unwounded	Wounded	Unwounded
Number leaves showing symptoms (total 5)	0	0	5	5	5	5
Average lesion length (mm)	0	0	28	25	27	24

All wounded and unwounded leaves tested using zoospore suspensions were necrotic after seven days incubation. No necrotic symptoms were observed on control leaves. The extent of lesions was comparable for both *P. ramorum* and *P. kernoviae*, indicating that the zoospore dipping method is suitable for future susceptibility studies using *P. kernoviae* inoculum. A standard protocol was written and used for all subsequent heathland susceptibility tests.

Five *P. kernoviae* isolates were selected and used for all susceptibility studies within this project (Table 2).

Table 2. *P. kernoviae* isolates used for susceptibility studies.

Isolate number	Host	Origin
2246	Rhododendron	St. Austell, Cornwall
2493	Rhododendron	Penzance, Cornwall
2486	Rhododendron leaf litter	Penzance, Cornwall
2441	Rhododendron	Swansea
2497	Drymis winteri	Cornwall

Using the standard protocol each of the host plants was tested with the *P. kernoviae* isolates (Table 2). For each heathland species, three replicates were wounded and

three unwounded. *Rhododendron catawbiense* detached leaves were used as positive control for each isolate and SDW as a negative control.

Leaves / sprigs were assessed for the extent of necrosis observed after incubation relative to negative control samples. Results are shown in Table 3.

Table 3. Number of test plants showing symptoms (/3) after inoculation with various isolates of *P. kernoviae*.

	A		**B**		**C**		**D**		**E**		**F**		**G**		**H**		**I**	
Isolate	W	U	W	U	W	U	W	U	W	U	W	U	W	U	W	U	W	U
2246	3	3	3	0	1	0	0	0	1	0	0	0	3	3	3	3	3	3
2493	3	3	2	0	3	0	3	0	0	0	0	0	3	3	3	3	3	3
2486	3	3	3	0	1	0	2	0	2	0	0	0	3	3	3	3	3	3
2441	3	3	3	0	3	0	3	0	3	0	0	0	3	3	3	3	3	3
2497	3	3	3	1	3	3	3	0	3	0	0	0	3	3	3	3	3	3

Key:
A: *A. uva-ursi*
B: *C. vulgaris*
C: *Em. nigrum*
D: *Er. cinerea*
E: *Er. tetralix*
F: *V. macrocarpon*
G: *V. myrtillus*
H: *V. vitis-idaea*
I: *R. catawbiense*

W= wounded
U=unwounded

Each isolate responded similarly on each host plant. Both wounded and unwounded leaves of rhododendron control (I) were infected with each isolate, and no evidence of infection was observed on any negative control test plant. Unusually for isolate 2497, infection was observed on unwounded test plants of *C. vulgaris* and *Em. nigrum*. All other isolates did not cause any lesions on unwounded sprigs of these plants. As this isolate was comparable with other isolates for all other host plants tested, it is believed that there may have been a natural wound on these plants (e.g. from abscised leaf or flower) which has allowed the inoculum to enter the plant and cause infection. *P. kernoviae* was re-isolated from each of the infected test sprigs.

The susceptibility of each heathland plant was further classified into one of four groups according to the amount of necrosis observed as follows:

Resistant : No evidence of necrosis on either wounded or unwounded plants for all *P. kernoviae* isolates tested.

Tolerant : Necrosis only observed on wounded test plants (any isolate).

Slightly Susceptible : Necrosis observed on all wounded plants (all isolates) and one isolate on unwounded test plants.

Highly Susceptible : Necrosis observed on all wounded plants (all isolates) and more that one isolate of unwounded.

These data are shown in Table 5.

Table 5. Susceptibility of heathland plants to *P. kernoviae.*

Heathland species	**Susceptibility**
Arctostaphylos uva-ursi	Highly susceptible
Calluna vulgaris	Slightly susceptible
Empertrum nigrum	Slightly susceptible
Erica cinerea	Tolerant
Erica tetralix	Tolerant
Vaccinium macrocarpon	Resistant
Vaccinium myrtillus	Highly susceptible
Vaccinium vitis-idaea	Highly susceptible

Discussion

The susceptibility of heathland species tested ranged from resistant (*V. macrocarpon*) to highly susceptible (*V. myrtillus, V. vitis-idaea*). The dominant heathland species, *C. vulgaris* and *Erica* species, were shown to be not particularly susceptible to *P. kernoviae* infection. Some similarities and differences were observed when compared with previous work using *P. ramorum* inoculum (Defra project PH0193s) (see Table 6).

Table 6. Comparison of heathland species susceptibility to *P. kernoviae* and *P. ramorum*

Heathland species	***P. kernoviae* susceptibility**	***P. ramorum* susceptibility**
Arctostaphylos uva-ursi	Highly susceptible	Tolerant
Calluna vulgaris	Slightly susceptible	Highly susceptible
Empertrum nigrum	Slightly susceptible	Resistant
Erica cinerea	Tolerant	Tolerant
Erica tetralix	Tolerant	Tolerant
Vaccinium macrocarpon	Resistant	ND
Vaccinium myrtillus	Highly susceptible	Highly susceptible
Vaccinium vitis-idaea	Highly susceptible	Slightly susceptible

ND = not done

Using the arbitrary susceptibility scale determined in this project, similarities were observed between the two pathogens for *Erica* spp. and for *V. myrtillus.* For *V. vitis-idaea* , *C. vulgaris* and *A. uva-ursi*, susceptibilty varied with the pathogen: *V. vitis-ideae* and *A. uva-ursi* appeared significantly more susceptible to *P. kernoviae* than to *P. ramorum*; *C. vulgaris* appeared less susceptible to *P. kernoviae* than to *P. ramorum. Em. nigrum*, was resistant to *P. ramorum* but slightly susceptible when challenged with *P. kernoviae*.

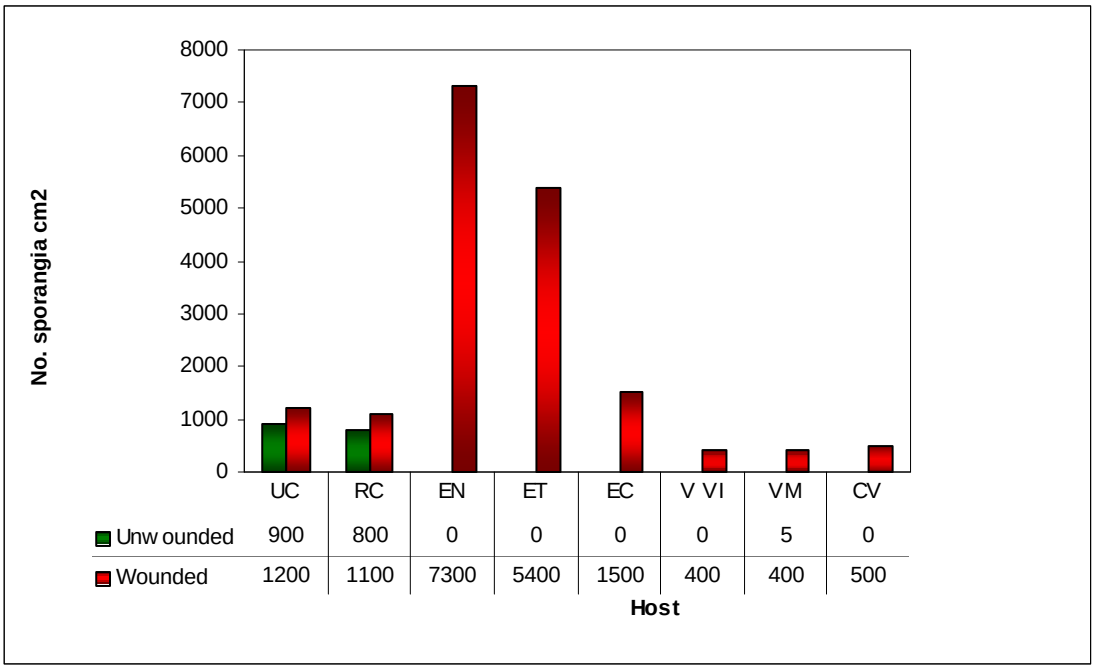
Although this study shows that certain heathland species are susceptible to *P. kernoviae* infection, the tests were carried out using detached plant material and conditions were optimised for *P. kernoviae* (i.e. warm, high humid).
Heathland environments vary from exposed dry areas to shady woodland habitats, so infection in the field may or may not favour *P. kernoviae* infection and establishment on susceptible genera. Further work would investigate the susceptibility of these species growing in natural conditions.

Additional work:

Sporulation potential of heathland species infected with *P. kernoviae.*

A further investigation examined the sporulation potential of heathland plants infected with *P. kernoviae*. The method (adapted from method described in a previous defra project (Defra project PH0193s, section 1.4) involved zoospore dipping using *P. kernoviae* isolate number 2486 (5×10^5 zoospores ml^{-1}) and assessing the number of sporangia formed on the leaves after incubation for 7 days in moist chambers at 19°C with an 18 hour white light day-length cycle). Image analysis software (Axio-Vision) enabled calculation of number of sporangia per unit leaf area infected. *R. catawbiense* and *Umbellullaria californica* (Californian bay laurel) were tested alongside the heathland plants as a comparison. All heathland species apart from *A. uva-ursi* (leaves unavailable at time of testing) and *V. macrocarpon* (resistant to *P. kernoviae*) were tested.

Figure 1. shows differences in sporulation of *P.* kernoviae observed on wounded and unwounded heathland species.



Key:
UC – *U. californica*
RC – *R. catawabiense*
EN – *Em. nigrum*
ET – *Er. tetralix*
EC – *Er. cinerea*
VVI – *V. vitis- idaea*
VM – *V. myrtillus*
CV – *C. vulgaris*

Figure 1. Sporulation potential of heathland species infected with *P. kernoviae*.

The non-heathland reference species, *U. californica* and *R. catawbiense*, produced similar numbers of sporangia per cm^2, with wounded leaves producing slightly more than unwounded leaves. Very little sporulation was observed on unwounded leaves of all heathland species tested. Smaller leaved heathland plants (particularly *Em. nigrum*, *Er. tetralix* and to a lesser extent *Er. cinerea*), that are only susceptible to the pathogen when wounded, produced considerably more sporangia than any other host (including non-heathland) host plants tested.
These results show that all heathland species infected with *P. kernoviae* produce varying amounts of inoculum that could potentially initiate new infections and perpetuate an epidemic. If, however, infection established on the less susceptible species (the greatest sporulators) (apart from *C. vulgaris*), the potential inoculum source from these infections could be considerable.

Appendix A.

Heathland Plants present in the UK (Preston (ed.) 2005)

1. *Erica cinerea* * (Bell Heather)
2. *Erica ciliaris* * (Dorset Heath)
3. *Erica vagans* * (Cornish Heath)
4. *Erica tetralix* * (Cross-leaved Heath)
5. *Erica x watsonii** (sterile hybrid between *E.cliaris* and *E. tetralix*)
6. *Erica x williamsii** (A naturally occurring sterile hybrid between *E. vagans* and *E. tetralix*)
7. *Calluna vulgaris** (Heather)
8. *Ulex europaeus* * (Gorse)
9. *Ulex Gallii* * (Western Gorse)
10. *Ulex europaeus x U. gallii**
11. *Vaccinium macrocarpon** (Cranberry)
12. *Vaccinium myrtillus* * (Bilberry)
13. *Vaccinium vitis-idaea* *(Cowberry)
14. *Empetrum nigrum** (Crowberry)
15. *Arctostaphylos uva-ursi* (Bearberry)
16. *Pteridium aquilinium** (Bracken)
17. *Prunus spinosa* * (Blackthorn)
18. *Equisetum arvense, E. fluviatile, E. palustre* * (Horsetail)
19. *Dryopteris dilatata* * (Broad Buckler fern)
20. *Osmunda regalis* * (Royal Fern)
21. *Ribes* sp*
22. *Molinea caerulea* *
23. Grasses, E.g. *Agrostis curtisii* *
24. *Shoenus nigricans* * (black bog rush)
25. *Narthecium ossifragum* * (Bog asphodel)
26. *Sorbus aucuparia* * (Rowan)

* Heathland plants found in Cornwall.

Appendix B

Representative symptoms observed on detached test plant sprigs (isolate 2497).

Unwounded Wounded

Arcotostaphylos sp. Unwounded and wounded: Extensive necrosis extending down shoot and into lower leaves

Calluna vulgaris unwounded : Light brown leaf necrosis. Lesions did not extend beyond the depth the sprig was immersed into inoculum.

Em. *nigrum*: light brown watersoaked leaf lesions that darken to deeper brown. No evidence of stem infection observed.

Er. cinerea wounded : Necrosis of occasional leaf in contact with inoculum. No lesions observed on stem.

Er. tetralix wounded: Light brown necrotic lesions observed on leaves in contact with inoculum.

V. macrocarpon wounded : No infection observed.

V. myrtillus unwounded

V. myrtillus wounded

Dark necrotic lesions on inoculated leaves extending throughout leaf tissue into stem and beyond into lower leaves.

V. vitis-idaea unwounded :

Deep brown necrosis observed on leaves in contact with inoculum. Lesions extend into stem tissue and lower leaves.

References:

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