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Phytophthora kernoviae oospore maturity, germination, and infection

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ABSTRACT

Limited information is known on the basic biology of the recently described *Phytophthora kernoviae* that produces homothallic oospores. In this study, different *P. kernoviae* isolates were used to investigate oospore maturity, germination, and infection. All isolates produced oospores in V8 broth at 20 °C in the dark by 6 d. Oospores also formed at 10 and 15 °C, but did not form at 25 and 28 °C. Continuous light inhibited oospore production of some isolates but had no negative effect on others. Maturation time of the oospores, as noted by germination and staining with tetrazolium bromide, was not much different among the isolates between 2 and 14 weeks. Oospore germination was optimal at 18 and 20 °C, and did not occur at 5, 25, and 30 °C. Oospore germination under continuous light was higher than in the dark, but individual isolates showed variable results. *Rhododendron* leaf disks inoculated with oospores and maintained in the dark at 20 °C were necrotic after 1 week, while those kept under continuous light did not develop necrosis. The percentage of leaf disks infected with *P. kernoviae* was lower in the leaves exposed to continuous light (40 %) compared to those kept in the dark (100 %).

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Introduction

Phytophthora kernoviae was first isolated in the wild from beech (*Fagus sylvatica*), rhododendron (*Rhododendron ponticum*), English oak (*Quercus robur*), and tulip tree (*Liriodendron tulipifera*) in several woodlands and public gardens in southwest England (Brasier *et al.* 2005). A *Phytophthora* species isolated in New Zealand in the 1950s and 2002 from soil and custard apple (*Annona cherimola*) was later identified as *P. kernoviae* based on DNA sequence data (Ramsfield *et al.* 2007). Recently, *P. kernoviae* has been found in Scotland (Anonymous 2008) and on bilberry (*Vaccinium myrtillus*) in both woodlands and open moorland in Cornwall, England (Beales *et al.* 2009). To date, *P. kernoviae* has been isolated in nature from 31 different plant and tree species, representing 10 different plant families, with additional species infected under laboratory inoculations

(DEFRA 2008). Although to date the known host range for *P. kernoviae* is less than *Phytophthora ramorum*, *P. kernoviae* was demonstrated to be more aggressive on beech stems and foliage of *L. tulipifera* and *Aesculus hippocastanum* than *P. ramorum* (Brasier *et al.* 2006). Therefore, there is reason to believe that *P. kernoviae* may be more of a specific threat to some species in a forest than *P. ramorum*.

Phytophthora kernoviae produces caducous sporangia that are readily dislodged from infected tissues during rain, spreading to nearby hosts. Although no studies have been reported on the dispersal patterns of *P. kernoviae* sporangia, it is unlikely that it is different from that of other caducous *Phytophthora* spp. Short-range dispersal up to 70 cm through rain splash has been demonstrated with *Phytophthora palmivora* (Gregory *et al.* 1984) and up to 3000 m through aerial dispersal of *Phytophthora infestans* (Zwankhuizen *et al.* 1998). Because it is apparent

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that sporangia do not survive long distance transport due to desiccation (Ristaino & Gumpertz 2000), it is unlikely that *P. kernoviae* will arrive in the United States (USA) through this mechanism. Therefore, spread will be most likely through infected live plant material or infested soil. Since *P. kernoviae* is not known to produce chlamydospores, the propagule most likely involved in soil infestation will be oospores.

Oospores are often considered the main survival propagule in soil of many *Phytophthora* spp. (Sneh & McIntosh 1974; Stack & Millar 1985). Germination of *Phytophthora* spp. oospores is influenced by oospore age, nutrition, temperature, light, chemical treatments, and treatments with enzymes (Erwin & Ribeiro 1996). If conditions become favorable, oospores germinate and develop mycelia or produce sporangia. Currently, very little is known about the basic biology of *P. kernoviae* oospores. It is known that *P. kernoviae* is homothallic and produces abundant oospores in culture (Brasier et al. 2005). In general, oospores of homothallic species usually are formed in diseased plant tissue (Erwin & Ribeiro 1996) and can be an inoculum source when, for example, leaves dehisce and degrade in the soil (Harris 1979). Preliminary tests from our laboratory revealed that *Rhododendron* leaves contained *P. kernoviae* oospores after inoculation with sporangia (personal observation). A field study in unmanaged woodlands in the United Kingdom (UK) reported *P. kernoviae* oospores in naturally infected *R. ponticum* leaves and roots (Fichtner et al. 2008).

One of the missions of the Foreign Disease Weed Research Unit in Fort Detrick, Maryland, USA, is to gain information on plant diseases that are perceived to be a threat to agriculture in the USA. In line with this mission, the objective of this study reported here was to investigate different factors such as oospore age, light, and exposure to different suspension solutions on *P. kernoviae* oospore germination. In addition, the effect of light on symptom development of a selected host plant inoculated with oospores was examined. The information from this study will be important in understanding some basic biology of *P. kernoviae*.

Materials and methods

Isolate maintenance

The *Phytophthora kernoviae* isolates used in this study were received from P.W. Tooley (Fort Detrick, MD, USA) and are

described in Table 1. All isolates were maintained on clarified 20% V8™ (Campbell Soup Co., Camden, NJ, USA) agar at 20 °C in the dark. To ensure the pathogenicity of the isolates, cultures were periodically (approximately every 6 m) re-isolated from infected, surface-sterilized *Rhododendron* leaf disks on a *Phytophthora* selective medium (PARPH + V8; Ferguson & Jeffers 1999). For all studies, cultures grown under continuous lighting were done so in a Sanyo MLR-350H Versatile Environmental Test Chamber (Sanyo Electric Co., LTD, Osaka, Japan) with Sanyo FL40SS-W/37 40 W fluorescent bulbs at 3180 lx, unless stated otherwise.

Oospore production and maturity

Oospores were formed and released from the mycelia by following a modified method described by Singh et al. (2004). Five 6-mm-diameter plugs of clarified 20% V8 agar containing mycelia from the edge of an actively growing colony of *Phytophthora kernoviae* isolates PK-1, PK-2, PK-3, and PK-4 (isolate PK-5 was not available during this test) were transferred to a Petri dish (100 × 15 mm) containing approximately 20 ml of sterile, clarified 20% V8 broth. The plates were sealed with Parafilm and stored in the dark at 10, 15, and 20 °C or under continuous fluorescent lighting at 20 °C. After 2 d when mycelial growth from the agar plugs was observed but before oospore formation, some cultures grown in the dark at 20 °C were removed and transferred to incubators with no artificial lighting at temperatures of 25 and 28 °C. It was necessary to start the cultures at 20 °C because very little mycelial growth occurs in V8 broth above 25 °C and it was difficult to distinguish oospores previously formed in the agar plug from those that might be forming on any new mycelial growth. Brasier et al. (2005) reported that the maximum growth of *P. kernoviae* is at 26 °C and so oospore development would not be expected above this temperature; however, tests were conducted at 28 °C to verify an upper limit. The plates were observed daily for production of oospores.

The maturation time of oospores, which can be defined as the time needed to complete cytological and physiological processes necessary for germination, was determined by separating oospores from the mycelium in liquid cultures incubated at 20 °C in the dark at different times and germinating them in water or staining with tetrazolium bromide (MTT). Starting 1 d after oospores were first observed in culture, samples of mycelium containing oospores were removed at

Table 1 – Description of *Phytophthora kernoviae* isolates used in the present study.

Isolate	Original designation ^a	Origin	Original isolation	Year ^b	Source ^c
PK-1	ICMP 15082	Trounson Kauri Park, New Zealand	Soil	2003	TR; MC
PK-2	ICMP 14761	Northland, New Zealand	<i>Annona cherimola</i>	2002	TR; MC
PK-3	CSL 2300	Cornwall, UK	<i>Rhododendron ponticum</i>	2004	KH
PK-4	CSL 2378	Cornwall, UK	<i>Rhododendron ponticum</i>	2004	KH
PK-5	CSL 2286	Cornwall, UK	<i>Rhododendron ponticum</i>	Unknown	KH

a Isolate name designated by source.

b Year isolated.

c Name of originator: MC, Mike Coffey, University of California, Riverside, CA, USA; KH, Kelvin Hughes, The Food and Environment Research Agency (Fera), York, UK; and TR, Tod Ramsfield, Scion, Rotorua, New Zealand.

different times and transferred to a 50-ml beaker containing approximately 10 ml of sterile deionized water. The mycelial suspension was sonicated with a Tekmar Sonic Disruptor model TM-50T (Teledyne Tekmar, Mason, OH, USA) at 40 % output of a 25 W power at 20 KHz frequency for two 1-min bursts followed by one 1-min burst at 40 % output of 50 W power with a 1-min rest in between bursts. The resulting suspension containing detached oospores and mycelial fragments was poured through four layers of sterile cheesecloth into a 50-ml conical tube and frozen at -20°C for 24 h to kill any mycelial fragments prior to use. The oospore suspension was thawed quickly in a 40°C water bath and used immediately.

The oospores were chemically tested for viability with MTT as described by Sutherland & Cohen (1983). For each isolate at each sample time, a 250- μl aliquot of the suspension was removed and transferred to an Eppendorf tube containing 250 μl of a 1 g/100 ml MTT (Sigma Chemical Co., St. Louis, MO, USA) solution in water. The capped tubes were covered with aluminum foil and placed in a 35°C incubator. After 2 d, two 25 μl aliquots were placed on a glass slide and 100 oospores were examined from each aliquot. An oospore was counted as positive for viability if it stained a rose or purple color. Non-stained or black stained oospores were deemed non-viable. The experiment was conducted three times.

The percentage of oospores that germinated at each sample time was determined using the following procedure. A 50 μl aliquot of the oospore suspension was added to a well in a 24-well Cellstar[®] (Greiner Bio-One International AG, Kremsmünster, Austria) microtiter plate containing 1 ml of sterile deionized water. Two replications were prepared for each sample. The plates were randomly placed in an incubator at 20°C in the dark. After 3 d, the germination percentage of each replication was determined by observing 100 random oospores through a Nikon SMZ 1500 dissecting microscope at $20\times$ (Nikon, Inc., Melville, NY, USA). An oospore was considered germinated if an existing germ tube was at least half the diameter of the oospore or if a sporangium was present. The average of the replications for each isolate at each sample time was calculated and used as the data point for analysis. The experiment was conducted three times.

To determine if any differences existed in oospore maturity among the different isolates and over time, statistical analyses were conducted on the data. The percentage of stained oospores and percentage of germination data failed the normality test based on the Shapiro–Wilk test ($P \geq 0.05$) and were first transformed with arcsine square root before further analyses (Gomez & Gomez 1984). The transformed data were subjected to repeated measures analysis of variance (ANOVA) using MIXED procedure in SAS for Windows (Version 9.1, SAS Institute, Inc., Cary, NC, USA). The covariance structure for the repeated factor is modeled using a spatial exponential structure since the intervals between temperature and time are unequal. The mean separations were performed using a Bonferroni least significant difference (LSD) multiple comparison technique at $\alpha = 0.05$, which allows for comparisons at different times. The data presented in the figure were back-transformed for clarity.

Effect of temperature, light, and solution on oospore germination

For the following experiments, 6- to 8-week-old oospore cultures of isolates PK-1, PK-2, PK-4, and PK-5 were sonicated to release oospores from the mycelia and treated as described above. Isolate PK-3 was not included in these studies because of low germination observed in the previous study.

The effect of temperature on oospore germination was conducted by exposing oospore suspensions to different temperatures. A 50 μl aliquot of each of the oospore suspensions was added to 3 ml of sterile deionized water in 60×15 mm Petri dishes. One dish of each isolate suspension was placed at a temperature of 5, 10, 15, 18, 20, 22, 25, and 30°C on a temperature gradient plate (an aluminum plate with a hot water bath at one end and a cooling bath at the other end) under fluorescent lighting at 3000 lx with GE F15T8-RS-CW 15 W tubes (General Electric, NY, USA). After 3 d, 100 oospores in a random field as viewed by means of a dissecting microscope were observed and the number germinated counted. An oospore was considered germinated if an existing germ tube was half the diameter of the oospore or if a sporangium was present. The experiment was conducted three times.

The effect of light on oospore germination was determined by exposing the oospore suspension to continuous light or kept in the dark. In two separate 24-well microtiter plates, a 50 μl aliquot of oospore suspension was added to a well containing 500 μl of deionized water. Two replications were prepared in each plate for each sample. One plate was placed directly on a shelf in an incubator at 20°C exposed to fluorescent lighting. The other plate was wrapped in aluminum foil to block out all light and placed in the same 20°C incubator. After 3 d, 100 oospores in a random field as viewed by means of a dissecting microscope were observed and the number germinated counted. The average of the two replications for each isolate was used as the data point for analysis. The experiment was conducted three times.

The effect of incubation solution on oospore germination was determined simultaneous to the light study using identical oospore suspensions. Sterile solutions of deionized water, 0.1 mM 2-[N-morpho-lino]ethanesulfonic acid (MES; Sigma Chemical Co.) buffer, pH 6.2 (herein referred to as MES buffer), and 10 g l^{-1} soil extracts collected from two different sites (SE1: Duffield–Ryder silt loam collected in Fort Detrick, MD; SE2: Mt. Zion gravelly silt loam collected in Myersville, MD; Englander et al. 2006) were added to wells in a 24-well microtiter plate (0.5 ml per well per solution). A 50 μl aliquot of oospore suspension was added to each of the solutions and placed in a 20°C incubator exposed to constant fluorescent lighting. After 3 d, 100 oospores in a random field as viewed by means of a dissecting microscope were observed and the number germinated counted. The experiment was conducted three times.

The effects of light and solution on oospore germination were analyzed statistically using SAS for Windows with two-factor ANOVA to test for differences between isolates and light conditions or suspension solution. The data were first transformed using arcsine square root to stabilize variance (Gomez & Gomez 1984). Means were separated using Duncan's

multiple range test at the $P = 0.05$ confidence level. The effect of temperature was analyzed by repeated measures analysis of variance using the MIXED procedure in SAS. Bonferroni LSD at $\alpha = 0.05$ was used to perform the multiple comparisons of the temperatures and the isolates. The data presented in the tables and figure were back-transformed for clarity.

Oospore infection of plant material

Infection of *Rhododendron* leaf disks inoculated with oospores was determined by the following procedure. Mature oospores, taken from cultures 6- to 8-weeks-old, were separated from mycelia and treated as described above for isolates PK-1, PK-2, PK-4, and PK-5. Ten 11-mm-diameter disks of mature *Rhododendron* 'Cunningham's White' leaves were placed on a mesh screen on a moist paper towel in a Petri plate for each isolate. An aliquot of oospore suspension containing 1000 oospores was pipetted directly into a droplet of sterile, deionized water sitting on the abaxial-side of a leaf disk so that the final volume on each leaf disk was 50 μl . Since no previous studies have been conducted to determine the minimum number of oospores needed to get consistent infection, this quantity of oospores was chosen to ensure that good infection would occur. The plates were sealed and randomly placed in a 20 °C incubator in the dark or under continuous fluorescent light. After 1 week, the leaf disks were photographed and the percentage necrosis for each leaf disk calculated using ASSESS software (American Phytopathological Society Press, St. Paul, MN, USA). The average necrosis of the 10 disks for each treatment was calculated and used as the data for the analysis. The leaf disks were surface sterilized for 30 s in 70% ethanol, rinsed three times for 10 min each in sterile water, and plated on PARPH + V8 agar medium. After 5 d, the selective medium was observed for mycelial growth originating from the disks, which was confirmed to be *Phytophthora kernoviae* by visual observation of propagules (Brasier et al. 2005). The experiment was conducted three times.

The percentage of the leaf disk that was necrotic was analyzed statistically using SAS for Windows with two-factor ANOVA to test for differences between isolates and light conditions. The data were first transformed using arcsine square root to stabilize variance (Gomez & Gomez 1984). Means were separated using Duncan's multiple range test at the $P = 0.05$ confidence level.

Results

Oospore production and maturity

Within 6 d, oospores formed abundantly in liquid cultures at 20 °C for all isolates maintained in the dark and for isolates PK-1 and PK-2 kept under continuous light. However, oospores were rare in cultures of PK-4 and non-existent in PK-3 when maintained under continuous light. For all isolates, oospores formed at 10 and 15 °C in the dark by 10 d. No oospores formed in any of the isolates tested at 25 and 28 °C in the dark by 6 weeks, at which time the experiment was terminated.

Statistically, the age of the culture affected oospore viability ($P < 0.001$), as determined by positive staining with MTT,

and germination ($P < 0.001$). This effect is especially evident 1 d after oospores first appeared in culture (1 week from inoculation) for all isolates where a very low percentage of oospores stained positive and none germinated (Fig 1). Although there was not a general isolate effect on oospore viability ($P = 0.27$) or germination ($P = 0.16$), there were some differences in the statistical groupings at the different culture ages among the isolates. For example, the percentage of oospores staining positive at week 1 was significantly lower than weeks 2 through 10 for isolate PK-1, but only at weeks 2 through 6 for isolate PK-2. Isolate PK-4 was significantly higher at weeks 2 through 8 than weeks 1, 12, or 14. These grouping results were similar for germination where the percentages were higher at weeks 2 through 14 than week 1 for isolates PK-1 and PK-2, while the maximum germination of isolate PK-4 at week 8 was significantly higher than at 1, 12, or 14 weeks. Oospore germination was evident by the production of germ tubes that directly developed into hyphae or developed a sporangium on its tip (Fig 2) that released zoospores. The exact number of oospores that produced sporangia in contrast to only mycelium was not quantified in this study. Oospores of isolate PK-3 appeared to be aborted, which was reflected in very low viability staining and germination rate (maximum of less than 10%). In addition, other unusual

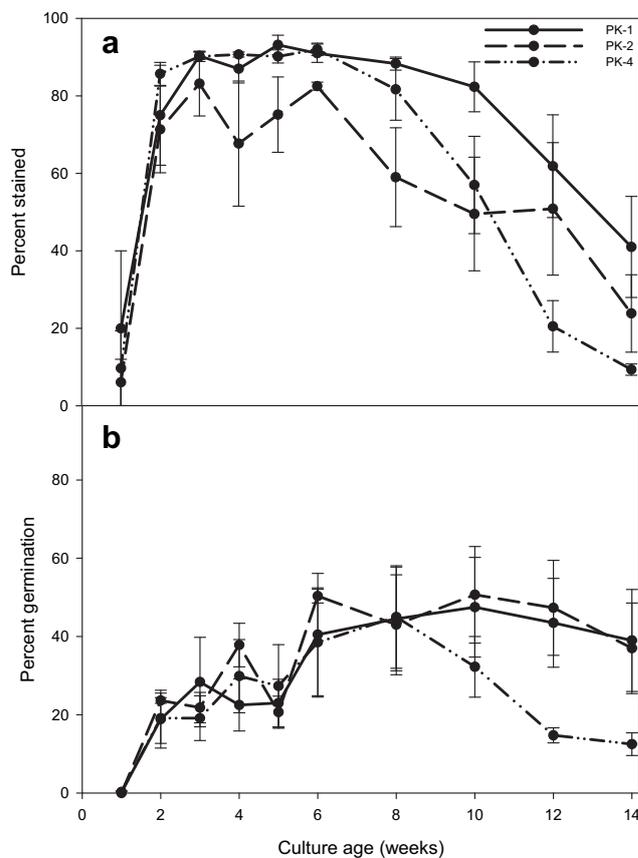


Fig 1 – Percentage of *Phytophthora kernoviae* oospores stained red or purple with tetrazolium bromide to determine viability (a) and germinated in water after 3 d at 20 °C under continuous fluorescent light (b). Bars represent the standard error of the means.

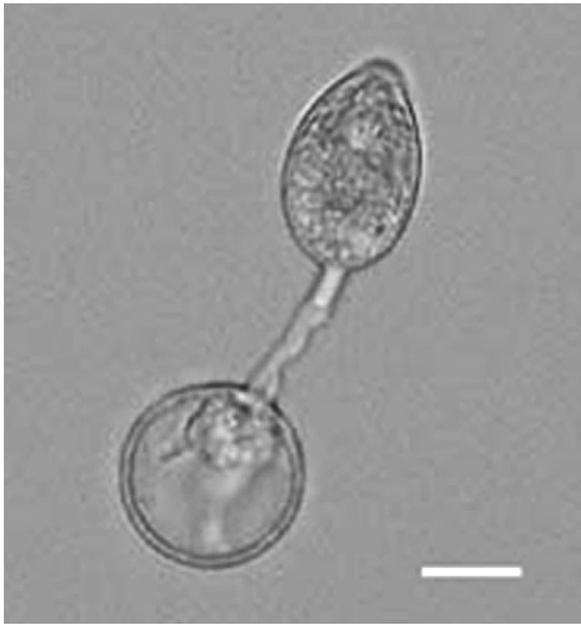


Fig 2 – Germinated *Phytophthora kernoviae* oospore with sporangium. Bar = 10 μ m.

characteristics of this isolate were observed in a previous study (Widmer 2008). For these reasons, isolate PK-3 was determined to be abnormal and so was removed from all analyses.

Effect of temperature, light, and solution on oospore germination

The optimum temperature range for maximum oospore germination was 18–20 °C for all isolates (Fig 3). Temperature had a significant effect on oospore germination ($P < 0.001$), but there was not a significant difference among the isolates

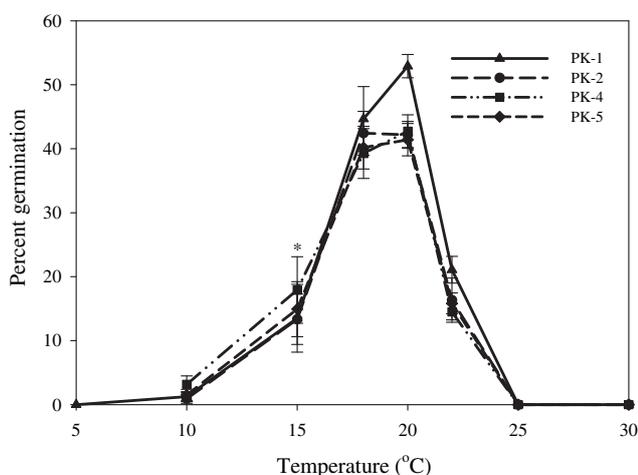


Fig 3 – Effect of temperature on germination of different isolates of *Phytophthora kernoviae* oospores. Bars represent the standard error of the means. An asterisk (*) indicates significant difference between PK-1 and PK-4.

tested at these temperatures ($P = 0.93$). Under the conditions in this experiment, oospores germinated by 2 d but data were collected at 3 d to assure the maximum number. Beyond this time, it was difficult to discern which oospores germinated due to excessive growth of mycelia. No oospore germination was observed after 3 d at 5, 25, and 30 °C. Continuous light either significantly increased or had no significant effect on oospore germination, depending upon the isolate tested (Table 2). No interaction between isolate and lighting was detected ($P = 0.17$). Exposure of oospores to soil extracts negatively affected the germination of PK-1, but not PK-2, PK-4 or PK-5 (Table 2).

Oospore infection of plant material

The lighting condition significantly affected ($P < 0.001$) necrosis of the inoculated *Rhododendron* leaf disks. Inoculated leaf disks exposed to continuous light did not develop necrosis after 1 week when inoculated with any of the *Phytophthora kernoviae* isolates tested and was not different from the non-inoculated controls ($P = 0.14$; Fig 4). In contrast, all of the leaf disks inoculated with the tested isolates and maintained in the dark had significantly higher necrosis than the non-inoculated controls ($P < 0.001$). Differences in necrosis of the inoculated *Rhododendron* leaf disks occurred depending on the isolate used ($P < 0.001$) when maintained in the dark. All inoculated leaf disks that were maintained in the dark were infected as evidenced by the mycelial growth from the leaf disks when plated on the PARPH + V8 medium. However, of those leaf disks maintained under continuous light, there were on average only four out of 10 disks infected, regardless of the isolate. The software ASSESS calculated a minimal amount of necrosis on the non-inoculated controls, primarily around the edges of the leaf disk. However, no mycelium was observed from these disks when plated on the PARPH + V8 selective medium, confirming that the necrosis was probably due to injury.

Discussion

Phytophthora kernoviae oospores first appeared in liquid cultures after 6 d in the dark at 20 °C. Harnish (1965) reported oospores appeared within 3–4 d for the 10 species he tested. Light inhibited oospore formation for the two isolates originating from the UK (PK-3 and PK-4), but not for those from New Zealand (PK-1 and PK-2). However, due to the limited number of isolates used in this study a conclusion on the effect of geographic origin cannot be made. Continuous light also inhibited oospore formation of the homothallic *Phytophthora heveae* (Brasier 1969) and a majority of other *Phytophthora* spp. previously tested (Banihashemi 2004; Harnish 1965; Yu et al. 1981). Yu et al. (1981) attributed this to inhibition to reduction of hormone production by light in heterothallic species. It is unknown what role, if any, this might have on homothallic species such as *P. kernoviae*.

Germination was higher when oospores were exposed to continuous light compared to those in the dark, although not significantly for all isolates. Some *Phytophthora* spp. require light for oospore germination. Banihashemi (2004) noted

Table 2 – Effects of different solutions and conditions on the percent germination (\pm S.E.) of *Phytophthora kernoviae* oospores.

Solution ^b	Isolate ^a							
	PK-1		PK-2		PK-4		PK-5	
	Light ^c	Dark ^d	Light	Dark	Light	Dark	Light	Dark
Water	59.8 \pm 2.7 a	37.4 \pm 6.9*	73.8 \pm 3.0 a	69.2 \pm 5.5 ^{NS}	61.0 \pm 2.6 a	54.4 \pm 2.6 ^{NS}	67.8 \pm 1.9 a	46.8 \pm 6.5*
MES buffer	58.4 \pm 1.7 a	–	79.4 \pm 1.2 a	–	66.0 \pm 2.3 a	–	70.2 \pm 2.3 a	–
Soil extract 1	44.9 \pm 5.6 b	–	65.6 \pm 4.5 a	–	44.4 \pm 10.4 a	–	52.8 \pm 10.7 a	–
Soil extract 2	39.5 \pm 9.5 b	–	67.0 \pm 13.0 a	–	50.5 \pm 22.5 a	–	69.0 \pm 5.0 a	–

a Isolate of *Phytophthora kernoviae* tested (original designation of isolates: PK-1 – ICMP 15082; PK-2 – ICMP 14761; PK-4 – CSL 2378; PK-5 – CSL 2286).

b Solution used to suspend *P. kernoviae* oospores: Water – sterile distilled water; MES buffer – sterile 0.1 mM 2-[N-morpho-lino]ethanesulfonic acid buffer, pH 6.2; Soil extract 1–10 g l⁻¹ sterile soil extract prepared with Duffield–Ryder silt loam in deionized water; Soil extract 2–10 g l⁻¹ sterile soil extract prepared with Mt. Zion gravelly silt loam in deionized water.

c Values followed by the same letter are not significantly different at $P = 0.05$ level according to Duncan's multiple range test.

d Not significantly (NS) or significantly different (*) at $P \leq 0.05$ for the isolate between the light and dark treatments according to Duncan's multiple range test. Dashes (–) represent experiment was not conducted under those conditions.

that *Phytophthora cactorum* needs a minimum exposure to light for photoactivation of mature oospores. Chang & Shu (1988) observed that the homothallic species, *P. cactorum* and *P. heveae* did not germinate in the dark after 3 d. However, Jimenez & Lockwood (1982) and Sneh et al. (1981) observed that *Phytophthora megasperma* f.sp. *glycinea* did not need light to germinate. Germination of *P. kernoviae* oospores in the dark was higher than those observed for *P. cactorum* and *P. heveae* (Chang & Shu 1988), but similar to *P. megasperma* f.sp. *glycinea* (Jimenez & Lockwood 1982).

Oospores often need a maturation phase before germination can occur. In this study, a relatively short maturation time of 1 week after formation (culture age of 2 weeks) was needed for some *P. kernoviae* oospores to germinate. Other *Phytophthora* spp. have been reported to require longer maturation periods. Chang & Shu (1988) reported germination of *P. cactorum* and *P. heveae* oospores was higher in 30-d-old

cultures compared to 10-d-old cultures. Schmitthenner (2000) also noted that *Phytophthora sojae* (synonym = *P. megasperma* f.sp. *glycinea*) oospores germinated about 30 d after formation and Förster et al. (1983) observed higher germination in *P. megasperma* f.sp. *medicaginis* as the culture age increased. However, in all of these previous studies statistical analyses were not conducted on the data so it not known if the differences were significant.

When the two measurements for oospore viability (staining positive with MTT and germination) are compared, a significantly higher ($\alpha = 0.05$) percentage of oospores stained positive for viability than actually germinated among each of the isolates between 2 and 5 weeks. After 6 weeks, no significant differences were observed ($\alpha = 0.05$). This may indicate that between 2 and 5 weeks, oospores of *P. kernoviae*, although viable, have not completed all of the cytological or biochemical processes needed for germination. Jiang et al. (1989) observed that delays in oospore germination of three homothallic species, *P. megasperma* f.sp. *medicaginis*, *P. megasperma* f.sp. *glycinea*, and *P. cactorum*, were related to a cytological event involving the fusion of nuclei. It was noted in that study that the fusion of *P. megasperma* f.sp. *glycinea* oospore nuclei occurred much later than in *P. megasperma* f.sp. *medicaginis* and *P. cactorum* and was correlated to a lower percentage of germination.

Some studies have suggested that root exudates, enzymes, and other chemicals can enhance germination (El-Hamalawi & Erwin 1986). *Phytophthora megasperma* f.sp. *medicaginis* oospores germination was significantly higher in alfalfa root extract, root exudate or soil extract when compared to distilled water (Förster et al. 1983). Other studies also showed that oospore germination of different *Phytophthora* spp. increased after incubation in soil extracts, root exudates, and root extracts (Hord & Ristaino 1991; Strömberg et al. 2001). However, the soil extracts used in this study did not increase *P. kernoviae* oospore germination.

An interesting result in this study was the complete inhibition of necrotic symptoms on inoculated *Rhododendron* leaf material when exposed to continuous light. In solutions such as water and soil extracts, it was observed that oospores readily germinated and formed sporangia under continuous

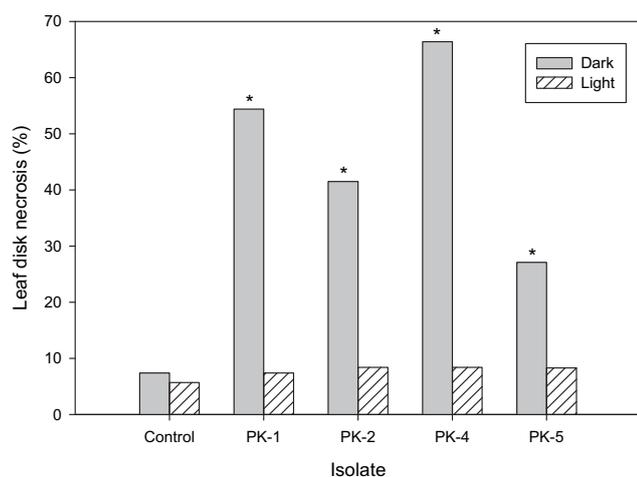


Fig 4 – Percentage of *Rhododendron* ‘Cunningham’s White’ leaf disk area that was necrotic after 7 d from inoculation with different *Phytophthora kernoviae* oospores and kept in the dark or under continuous light at 20 °C. An asterisk (*) indicates significantly different from control at $P = 0.05$ level according to Duncan’s multiple range test.

light and released active zoospores. This demonstrated that these processes were not affected by light and, therefore, infective propagules should have been present. Since a lower percentage of the disks was infected when inoculated with *P. kernoviae* oospores and exposed to continuous light, it appears that light may affect the initial infection process, thereby reducing or inhibiting the visual necrotic symptoms. There have been very few studies examining the effect of light on infection involving *Phytophthora* spp. Victoria & Thurston (1974) demonstrated that a higher light intensity negatively affected the size of lesions caused by *Phytophthora infestans* on potato leaflets. However, in their study where even the lowest light intensity was much higher than the light intensity used in this study (11 840 lx compared to 3180 lx), infection and lesions did occur, while in this study no visible lesions appeared under continuous light. In the present study, photos were taken 1 week after inoculation. It is not known if necrosis would have developed over a longer period of time in those disks that were shown to be infected. Future work will need to be conducted to examine this in more depth and its relationship to reports of sporulation and infection of asymptomatic tissue (Bush 2002; Bienapfl et al. 2005; Denman et al. 2008).

It is very important to increase our knowledge in all aspects of the basic biology of *Phytophthora* spp. In this manner, oospores are a very important propagule regarding long-term survival and their role in initiating and sustaining epidemics. They are also important in the exchange of genetic material through sexual out-crossing, even among homothallic species. The results presented here provide a foundation of knowledge and should facilitate future studies involving *P. kernoviae* oospores.

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