

# Infectivity of asexual propagules of *Phytophthora infestans* in sterile peat exposed to various temperature regimes

## Infectivité de propagules asexuelles du *Phytophthora infestans* dans de la mousse de tourbe exposée à divers régimes de température

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Résumé de l'article

La survie de propagules asexuelles (spores et mycélium) du *Phytophthora infestans*, dans de la mousse de tourbe stérilisée à l'autoclave et exposée à diverses températures, a été évaluée par leur capacité à infecter des disques flottants de feuilles de pomme de terre. Le pouvoir infectieux des propagules dans la mousse de tourbe a été mesuré après 0, 21, 35, 63 et 79 jours d'exposition à des températures allant de -33 C à 20 C. Les propagules exposées à des températures sous le point de congélation n'étaient plus infectieuses après 21 jours. L'exposition des propagules à des températures de 15 ou de 20 C a entraîné un déclin rapide du pouvoir infectieux; aucune propagule n'était infectieuse lors du dernier échantillonnage. À l'opposé, les propagules exposées à une température de 5 C durant 79 jours sont demeurées infectieuses.

**Infectivity of asexual propagules of *Phytophthora infestans* in sterile peat exposed to various temperature regimes**

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Survival of asexual (sporangia and mycelium) propagules of *Phytophthora infestans* in autoclaved peat moss exposed to various temperatures was assessed by ability to infect floating potato leaf discs. Infectivity of propagules in peat was measured after 0, 21, 35, 63, and 79 days of exposure to temperatures ranging from -33°C to 20°C. Propagules exposed to temperatures below freezing were no longer infective after 21 days. Exposure of propagules to temperatures of 15 or 20°C resulted in a rapid decline in infectivity over time; by the final sampling date, none were infective. In contrast, propagules exposed to a temperature of 5°C for 79 days remained infective.

**[Infectivité de propagules asexuelles du *Phytophthora infestans* dans de la mousse de tourbe exposée à divers régimes de température]**

La survie de propagules asexuelles (sporangies et mycélium) du *Phytophthora infestans*, dans de la mousse de tourbe stérilisée à l'autoclave et exposée à diverses températures, a été évaluée par leur capacité à infecter des disques flottants de feuilles de pomme de terre. Le pouvoir infectieux des propagules dans la mousse de tourbe a été mesuré après 0, 21, 35, 63 et 79 jours d'exposition à des températures allant de -33°C à 20°C. Les propagules exposées à des températures sous le point de congélation n'étaient plus infectieuses après 21 jours. L'exposition des propagules à des températures de 15 ou de 20°C a entraîné un déclin rapide du pouvoir infectieux; aucune propagule n'était infectieuse lors du dernier échantillonnage. À l'opposé, les propagules exposées à une température de 5°C durant 79 jours sont demeurées infectieuses.

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Infectivity of asexual inoculum of *Phytophthora infestans* (Mont.) de Bary (primarily sporangia; Zan 1962) is an important factor in the epidemiology of late blight. Sporangia washed from foliar lesions by rain onto the soil surface are primarily responsible (often by indirect germination producing zoospores) for tuber infections (Lapwood 1977; Sato 1980). Inoculum on tuber surfaces also can cause infection in storage (Dowley and O'Sullivan 1991). Tuber infection causes loss in marketable product. Furthermore, infected tubers are the means by which the pathogen overwinters (Andrison 1995). Diseased plants resulting from infected seed pieces can produce disease foci the following cropping season (Hirst and Stedman 1960). Sporangia are formed on blighted tubers buried in soil (Sato 1980) and, when splashed onto leaves near the soil, foliar infection occurs (Boyd 1980; Hirst and Stedman 1960). Also, the distribution of infected seed potatoes can introduce new genotypes of *P. infestans* into a potato-producing region (Goodwin *et al.* 1995).

Sporangia harvested from agar cultures and incorporated into soil remained infective for 15 to 77 d at 15°C depending on soil type (Andrison 1994; Zan 1962). Survival (as measured by infection of tuber slices) of sporangia in artificially-contaminated soil samples maintained in glass jars tended to increase with a decrease in temperature to above freezing (Zan 1962). Lacey (1965) found that surface soil layers in potato fields retained higher concentrations of sporangia than deeper soil layers and could remain infective for at least 32 d after haulm destruction.

Rainfall washes sporangia onto the soil and provides moisture for infection of tubers (Lapwood 1977; Sato 1980). However, Sato (1979) reported that soil temperature during and immediately after rain had a greater effect on the frequency of tuber rot than amount of rain. Soil temperatures of 18°C or less were necessary for tuber infection, probably because cool water favoured indirect germination of sporangia and prolonged swimming of zoospores (Sato 1979).

The recent influx of A2 mating types of *P. infestans* into Europe (Drenth *et al.* 1993), the United States (Deahl *et al.* 1991; Goodwin *et al.* 1995) and Canada (Chycoski and Punja 1996; Deahl *et al.* 1991; Peters *et al.* 1999a, 1999b) predicated a need to determine the survival capacity and infectivity of asexual propagules of new populations of this pathogen. Isolates of the traditional US-1 (A1) and new US-8 (A2) genotypes were selected to represent recent (1994) populations occurring in Canada. The infectivity of asexual (sporangia and mycelium) propagules of *P. infestans* in peat moss was assessed following exposure to a range of temperatures.

Isolates of *P. infestans* used in this study were collected from the Canadian provinces of Manitoba, Ontario, Quebec, and New Brunswick in 1994 and represented the commonly occurring US-1 (A1) and US-8 (A2) genotypes (nomenclature and isolate characteristics as in Goodwin *et al.* 1995). A total of 50 isolates (25 A1 and 25 A2) were grown in petri dishes (60 mm x 15 mm, Fisher Scientific Co., Ottawa, ON) containing a clarified rye extract medium (Peters *et al.* 1998) in the dark at 15°C for two wk.

Inoculum of *P. infestans* was prepared by blending cultures in sterile, distilled water (200 petri dishes L<sup>-1</sup> of water) for 2 min using a kitchen blender (Model C50107, Proctor-Silex Canada, Inc., Picton, ON). Concentration of total propagules (sporangia and mycelial fragments in approximately equal ratios) in the resulting agar slurry was measured with a haemocytometer (Bright-Line Improved, Neubauer, 1/10 mm deep, Spencer, Buffalo, NY). Concentration was adjusted to 1 x 10<sup>4</sup> propagules mL<sup>-1</sup> by dilution with sterile distilled water. Samples of 10 g of autoclaved peat (Pro-Gro Canada, Mix #4, Annapolis Valley Peat Moss Co. Ltd., Berwick, NS) were placed in plastic petri dishes (100 mm x 15 mm, Fisher Scientific Co., Ottawa, ON) and mixed with 10 mL of the inoculum suspension (dispensed using a Fisherbrand disposable 25 mL pipet). The inoculum mixture (slurry) consisted of a diverse population of sporangia and mycelium ob-

tained by mixing 50 distinct single isolates (25 A1 and 25 A2) of *P. infestans*. Controls consisted of blended, clarified rye agar (no propagules) or distilled water. Petri dishes were sealed with two layers of sealing film (Nescofilm, Nippon Shoji Kaisha Ltd., Osaka, Japan) and infested peat samples were then randomly placed in clear, plastic boxes (1.5 L, Rubbermaid Canada, Inc., Mississauga, ON). These boxes were exposed to one of seven possible temperature regimes in freezers set at -5, -10, -20, or -33°C or growth cabinets set at 5, 15, or 20°C.

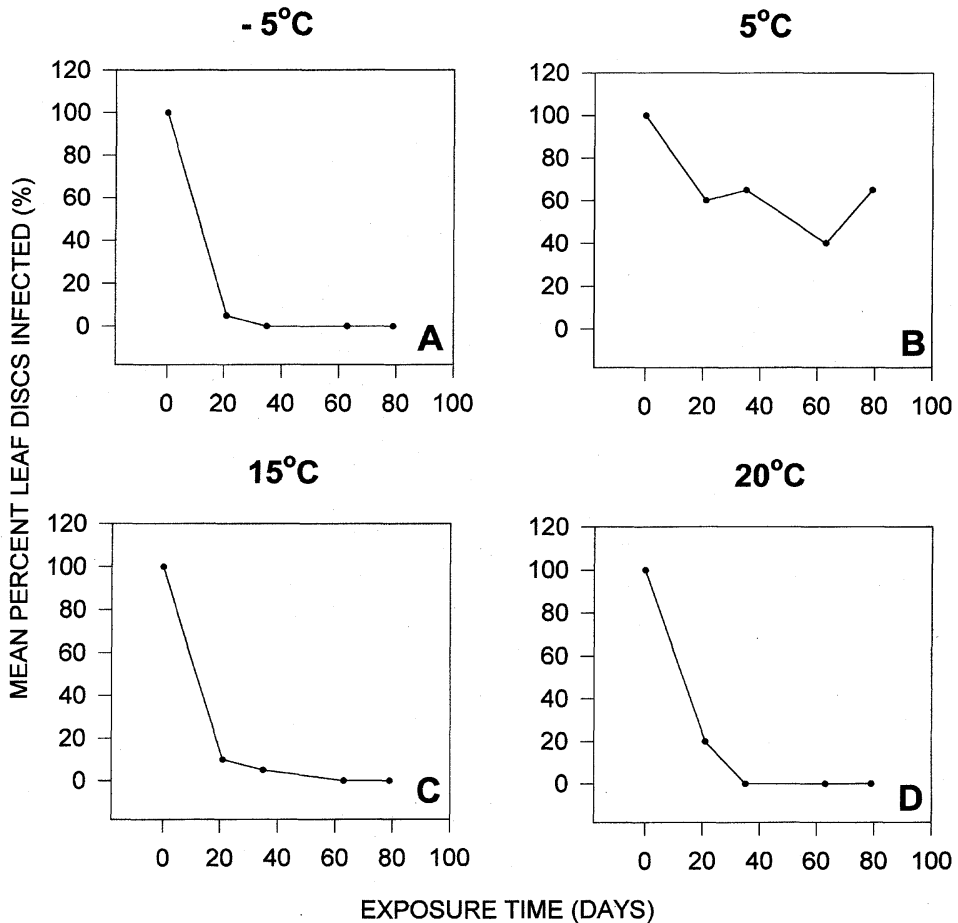
Infectivity of propagules was assessed using a floating leaf disc assay modified from Drenth *et al.* (1995). At each of five sampling times (0, 21, 35, 63, and 79 d of temperature exposure), two petri dishes (replications) per treatment were removed from each incubation chamber. The peat from each dish was scraped into a clear, plastic container (21.5 cm x 7 cm x 6 cm, 4 compartments, Tri State Molded Plastics, Inc., Dixon, KT) using a sterilized spatula. Each sample was flooded with 100 mL of sterile, distilled water and placed in a growth cabinet at 15°C (16 h daylength) for 2 d to encourage germination of sporangia.

After 2 d, 10 leaf discs were cut with a #6 cork borer (12 mm) and floated (abaxial surface uppermost) on the liquid surface of each flooded peat sample. Potato (*Solanum tuberosum* L. cv. Green Mountain) leaflets harvested from just beneath the apex of plants growing in a growth cabinet at 20°C (16 h daylength) were the source of leaf discs. Leaf discs were incubated on flooded peat for 7 d at 15°C (16 h daylength) and then scored for the presence/absence (+/-) of sporulation of *P. infestans* on the abaxial surface of the leaf discs. Infection of leaf discs at time 0 (prior to exposure to treatment temperatures) provided a baseline for comparison of results at subsequent sampling times. The completely randomized experimental design consisted of three treatments (infested peat and two control treatments), seven temperature regimes, two replications and five sampling times. Data were visualized graphically using

SigmaPlot (Version 1.02 for Windows, Jandel Scientific, San Rafael, CA) software.

No leaf discs became infected when exposed to the two control treatments (blended agar or distilled water alone). At time 0, soils infested with asexual inoculum had 100% of leaf discs with sporulating lesions of late blight. Thereafter, infectivity of propagules declined over time (Fig. 1). The decline in propagule infectivity was most rapid in peat samples kept at temperatures below freezing; only a mean of 5% leaf discs were infected after 21 d exposure of the infested peat sample to freezing temperatures. Decline in propagule infectivity was less rapid at 15 or 20°C; a mean of 10 and 20% leaf discs were infected after 21 d exposure of the infested peat sample to 15 and 20°C, respectively. However, 79 d after exposure to these two temperatures, no evidence of propagule infectivity was found (Fig. 1). By contrast, asexual inoculum exposed to a temperature of 5°C remained infective for the duration of the study (Fig. 1).

Asexual survival of *P. infestans* in soil is primarily as sporangia (Zan 1962). Although mycelium can grow in sterilized soil, its growth is inhibited by microorganisms in nonsterilized soil (Andrison 1995; Lacey 1965). In this study, asexual propagules (probably sporangia) showed excellent survival in peat at 5°C. This is probably due to reduced metabolism of sporangia at lower temperatures. Propagules of US-1 and US-8 genotypes were not directly compared, but were treated as a composite to reflect the range of response of recent (1994) populations of *P. infestans*. The duration of sporangia infectivity in Canadian genotypes of *P. infestans* at 5°C (at least 79 d) is important, since similar soil temperatures would occur in autumn (prior to and during harvest) and in spring (during and after crop planting) in Canada. Surface (5 cm depth) soil temperatures are typically about 5 to 10°C in May and October in Prince Edward Island, Canada (on-site atmospheric monitoring, Agriculture and Agri-Food Canada, Harrington Research Farm, Harrington,



**Figure 1: Effect of temperature and exposure time on infectivity of asexual propagules of *Phytophthora infestans* in sterile peat.** Peat was infested with sporangia and mycelium of *P. infestans*. Infested peat was incubated at: A) -5°C, B) 5°C, C) 15°C, or D) 20°C for 0, 21, 35, 63, or 79 days and assessed for infectivity of propagules using a floating leaf disc assay. Results for -10, -20, and -33°C incubations are not shown, as they were identical to those for -5°C.

P.E.I.; B. Sanderson, personal communication). Sporangia produced on foliar lesions would therefore remain viable in soil, given adequate moisture conditions, for long enough to infect tubers in autumn. In addition, sporangia deposited on tuber surfaces during harvest could survive under normal storage temperatures (commonly 5°C), providing inoculum for tuber infection. Finally, sporangia produced on infected seed would be available for a considerable time to cause initial disease foci in the spring. These findings emphasize the importance of planting pathogen-free seed and managing infective sporangia present in fall soils.

Freezing would appear to be effective in reducing inoculum levels in soils, as would extended periods of warmer weather if they increased soil temperatures to 15°C or higher. Inoculum exposed to freeze/thaw cycling behaved similarly to inoculum exposed to constant freezing temperatures (after initial exposure to temperatures below 0°C, infective inoculum could not be detected; Peters *et al.*, unpublished data). In our study, warmer peat temperatures (>15°C) may have increased the metabolic activity in pathogen propagules resulting in quick germination and rapid loss of infectivity. Zoospore release would also be hindered by higher temperatures. In Canada, conditions not conducive to the persistence of asexual inoculum occur at various times during the year. For example, surface (5 cm depth) soil temperatures in Prince Edward Island are commonly less than 0°C from January to March and greater than 15°C in July and August (on-site atmospheric monitoring, Agriculture and Agri-Food Canada, Harrington Research Farm, Harrington, P.E.I.; B. Sanderson, personal communication).

Based on our results, we believe that the poor correlation often observed between rainfall amount (Lacey 1965) or the severity of foliar blight (Lapwood 1977) and the incidence of tuber infection may be partly explained by the effect of soil temperature on inoculum survival. Temperatures not conducive to the maintenance of viable sporangia in soils would limit the time available

for the successful infection of tubers. In addition, as Sato (1979) noted (presumably by observation of the US-1 genotype), field soil temperatures of 18°C or less favour indirect germination of sporangia and prolong swimming of zoospores, thereby increasing the potential for tuber infection to occur. Water temperatures greater than 20°C reduce zoospore release and motility (Sato 1979).

The results of this study refer to survival (in terms of infectivity) of propagules of *P. infestans* in autoclaved peat. Microorganisms which directly lyse sporangia or inhibit sporangial germination are present in field soils (Andrison 1994; Lacey 1965). In this context, some soils can be termed disease suppressive (Andrison 1994). Antagonistic microorganisms could therefore decrease soil infectivity more rapidly in nature than in the sterile environment used in this study. Research on the biological control of soil-borne phases of *P. infestans* would be beneficial.

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