In vitro measurements of the competitive interactions between two saprobic basidiomycetes on Typha latifolia

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Abstract: Psathyrella typhae (Kalchbr.) Pearson & Dennis forms small basidiomata (mushrooms) and Sclerotium hydrophilum Saccardo in Rothert numerous minute sclerotia at the base of senescent shoots of Typha latifolia L. To assess how the two might compete in nature, isolates of these fungi were paired on autoclaved leaf segments of T. latifolia and incubated at 15 and 25 °C. The relative abundance of each species in the segments was determined by macerating the leaf tissues and then transferring fragments of macerate to microplates containing two types of media: one conclusively demonstrated the presence of P. typhae while the other demonstrated the presence of S. hydrophilum. Relative numbers of microplate wells showing positive reactions for each species on each medium indicated the proportion of the segment occupied following single and paired inoculations. These data demonstrated that competition was asymmetric, with P. typhae the stronger competitor at both temperatures, and uninhibited by the presence of S. hydrophilum. In contrast, S. hydrophilum was competitively excluded by P. typhae.

Key words: fungal competition, competitive exclusion.

Introduction

Typha latifolia L. (common cattail), a large emergent macrophyte, produces a high volume of detritus in many wetland ecosystems (Brinson et al. 1981). This material is primarily decomposed while in a standing-dead state (Bärlocher and Biddiscombe 1996) and fungi are the predominant saprobes (Hackney et al. 2000). Sclerotium hydrophilum Saccardo in Rothert (incertae sedis) is well known as a weak parasite of aquatic macrophytes in temperate and tropical regions (Hausner and Reid 1999; Punter et al. 1984) and produces minute black sclerotia (400–600 μm in diameter) on the basal sheathing leaves of senescent and decomposing shoots of T. latifolia (Schulz and Thormann 2005). Psathyrella typhae (Kalchbr.) Pearson & Dennis (Agaricales, Coprinaceae) forms small, brown basidiomata (mushrooms) (pileus 0.5–1.7 cm across; stipe 2.5–3.5 cm long) also on decaying, standing or floating, Typha leaves (Redhead 1979, 1981). Considering that the production of relatively large basidiomata ostensibly requires more temporal and spatial stability than the conversion of vegetative hyphae to minute sclerotia, we suspected that P. typhae would be the stronger competitor in their shared environment in stands of T. latifolia. In culture-based assays, P. typhae and S. hydrophilum both degraded gelatin, cellulose, and starch, and gave positive reactions on media that detect the presence of polyphenol oxidases (Schulz and Thormann 2005).
When grown for 12 weeks on sterile *T. latifolia* leaves in moist chambers, *P. typhae* and *S. hydrophilum* caused mass losses of about 50% and 25%, respectively (Schulz and Thor mann 2005). These observations suggest that even though both species break down a similar range of macromolecules, *P. typhae* degrades leaf tissue faster and would be a stronger competitor for substrate than *S. hydrophilum* (Pugh 1980).

When grown together on agar media, hyphae of each species intermingled and there were no obvious effects of competition. Neither was it possible to make a direct microscopic assessment of their interactions because the vegetative hyphae of both species are hyaline and similar in diameter. *Psathyrella typhae* differs from *S. hydrophilum* in having clamp connections, but these were not always present and were impractical to use as a distinguishing characteristic. In any case, because competitive fungal interactions detected on agar media likely have limited relevance to what occurs in nature (Shearer 1995), we pursued alternative methods of identifying the mycelial phases of these two basidiomycetes so that we could measure their relative rates of growth and interactions in the leaves of *T. latifolia*.

A search for simple differential media that could distinguish between the vegetative phases of each species yielded two that provided clear binary characters (e.g., growth vs. no growth) and that would allow the two species to be tracked during colonization of an opaque substrate, that is, leaves. We then used these media to obtain indirect measurements of the relative amounts of *T. latifolia* leaf tissues occupied by *S. hydrophilum* and *P. typhae* after being grown together on autoclaved leaf segments. Because temperature could significantly affect fungal interactions (Magan and Lacey 1984; Widden 1984; Widden and Hsu 1987; Marín et al. 1998), measurements were obtained using material incubated at 15 and 25 °C.

**Materials and methods**

Pure cultures of both fungal species are deposited at the University of Alberta Microfungus Collection and Herbarium in Edmonton (UAMH). *Sclerotium hydrophilum* (UAMH 10282) was isolated from surface-sterilized senescent *T. latifolia* leaves collected in Elk Island National Park, Alberta, Canada (EINP). *Psathyrella typhae* (UAMH 10280) was obtained from stipe explants from basidiocarps growing on *T. latifolia* near EINP.

To determine characteristics that could assist in distinguishing between the morphologically similar mycelial phases of *S. hydrophilum* and *P. typhae*, isolates of each species were grown on 14 different media. Two provided characters that clearly distinguished between *S. hydrophilum* and *P. typhae*. Both fungi grew on casamino acids media (CAS; Hutchison 1990), with *P. typhae* causing a permanent purple-to-yellow color shift (acidification); there was no color shift with *S. hydrophilum*. On corn meal agar (CMA, Difco, Detroit, Michigan) adjusted to pH 4 with HCl (CMA4), *S. hydrophilum* grew well and produced numerous characteristic minute black sclerotia; the growth of *P. typhae* was negligible.

A 5 cm × 1 cm *Typha latifolia* leaf segment, taken along the margin of an inner basal sheathing leaf of a living plant collected from the wild, was autoclaved twice and then placed directly on a layer of 150 mL perlite moistened with 50 mL distilled water in a 100 mm × 80 mm glass storage dish. To assess the interaction of *S. hydrophilum* and *P. typhae* in leaf segments incubated at different temperatures and over time, the fungi were co-inoculated on the leaf segments and incubated at 15 and 25 °C for 9, 21, and 36 d. Inoculum consisted of a 3 mm diameter plug from the growing edge of a colony growing on CMA and was placed at one end of a leaf segment. A plug of the other species was placed at the opposite end. Treatments without competition were inoculated at one end with either *P. typhae* or *S. hydrophilum*. Following inoculation, each dish (each containing an inoculated segment and hereafter referred to as a sample) was sealed with Parafilm® and incubated. Samples were incubated simultaneously but staggered over 4 d intervals to allow time for processing at the end of each incubation period. Six replicate samples of each treatment were harvested at 9, 21, and 36 d.

Leaf segments from each sample were macerated individually and a representative set of particles from each was transferred to 24-well tissue culture plates (see below). Macerate was prepared by first shipping entire leaf segments into 1 mm wide transverse slices, using sterile scissors, to reduce the length of fibers that otherwise would wind around the blade of the macerator. Slices were placed in 10 mL sterile distilled water (sd-H2O) in a 25 mL scintillation vial and ground at level “4” using a handheld macerator (X-120,
rose Scientific, Mississauga, Ontario), fitted with a T10 10 mm dispersing tool, for up to 60 s (time varied depending on the level of decomposition of the segment) to form a slurry of minute fragments. The slurry was diluted with approximately 35 mL sd-H2O in a 12.5 mm diameter plastic Petri plate. Fragments, each with at least one vascular bundle, were rinsed with water and transferred, one per well, to all the wells of four 24-well tissue culture plates (VWR, West Chester, Pennsylvania). These included two plates with 1 mL CAS per well, and two with 1 mL CMA4 per well. In treatments without competition, fragments from leaves that had been inoculated with S. hydrophilum were transferred to all the wells of two tissue culture plates containing CMA4, and fragments from leaves inoculated with P. typhae were transferred to two tissue culture plates containing CAS. Tissue culture plates were covered with Parafilm® to prevent hyphae from growing between adjacent wells and incubated at ambient light and temperature (approximately 20 °C) in the lab.

At 10 and 20 d (for CAS and CMA4, respectively), wells with positive reactions were counted. Positive reactions were a purple-to-yellow color shift in wells containing CAS, indicating the presence of P. typhae, and mycelium with sclerotia in wells containing CMA4, indicating the presence of S. hydrophilum. Random checks of the microscopic morphology of hyphae growing from leaf particles confirmed that the simple septate hyphae of S. hydrophilum were found only in wells of CMA4 showing positive reactions, and clamped hyphae characteristic of P. typhae were found only in wells of CAS showing positive reactions. The percentage of wells with positive reactions on each type of medium and for each set of samples was assumed to indicate the proportions of the leaf segment that had been occupied by S. hydrophilum and P. typhae, with and without competition, at the two different temperatures. Wells that were contaminated (with bacteria or other fungi) or accidentally missed (i.e., had no leaf fragments) were excluded from analyses (2.9% of wells).

Data were analyzed with SYSTAT 8.0 (SYSTAT 1998) using a series of Kruskal–Wallis tests. Nonparametric analyses were used because data transformations failed to normalize the data. In separate analyses, all of which had percent colonization as the response variable, we tested for differences between the two species, as a function of competition and temperature, and among the 9, 21, and 36 d incubation periods.

### Results

In treatments without competition, positive reactions for S. hydrophilum had developed in 80% of the CMA4 wells and positive reactions for P. typhae had developed in 100% of the CAS wells by the end of the study in both temperature treatments (Fig. 1). When the fungi competed, 100% of the CAS wells were positive for P. typhae and no wells of CMA4 were positive for S. hydrophilum at the end of the 36-day incubation period at both temperatures (Fig. 1).

Wells with positive reactions for either fungus were significantly fewer when inoculated with fragments from leaf segments that had been incubated at 15 °C, compared with those incubated at 25 °C, at 9 d (Table 1). Significant differences in the colonization of the material incubated at 15 °C were also apparent in treatments with P. typhae without the competitor. The number of wells with positive reactions increased significantly as the incubation period increased. There were significant differences between species and in all treatments, except at 9 d. There was also a significant difference between the treatments with and without competition: S. hydrophilum showed significantly lower levels of colonization in competition with P. typhae while the colonization of P. typhae was unaffected by the presence of S. hydrophilum.

At 9 d at 15 °C, S. hydrophilum appeared in more wells than P. typhae, in this experiment (Fig. 1) and in all preliminary trials (data not shown). This was also observed at 5 d at 25 °C in preliminary trials (data not shown).

### Discussion

Competitive success for a fungus is directly related to its ability to occupy substrate units (e.g., Keddy 1989; Malley et al. 1994, 1996). Thus measurements of the relative growth of two fungi occupying the same material over the same period of time should indicate if competition occurs and, if so, which of them is able to occupy the greater portion of the shared resource and exhibit competitive dominance over the other species. Traditionally, obtaining such measurements of the vegetative increase of fungi in natural materials has been problematic and has been limited to studies of fungi that either sporulate heavily (Khan 1987; Adhikari and Tiwari 1991) or differ substantially in cultural morphology (Widden 1984; Widden and Hsu 1987). The technique used here allowed vegetatively similar, and nonsporulating fungi to be distinguished based on their response to differential media.

Our hypothesis that P. typhae would be the stronger competitor at either 15 or 25 °C was clearly supported. Sclerotium hydrophilum was inhibited to the point of competitive exclusion by P. typhae at both temperatures but
grew well when alone, indicating that *P. typhae* had a strong competitive effect. In marked contrast, *S. hydrophilum* had no apparent effect on *P. typhae*, despite its apparent ability to grow through the substrate more rapidly. These contrasting results for the two species clearly indicate asymmetric competition, rather than competitive equivalence, and are consistent with our hypothesis that the weakly parasitic *S. hydrophilum* would be competitively inferior to the more vigorous saprobe, *P. typhae*. Widden (1984), Widden and Hsu (1987), and Marin et al. (1998) also found that some fungal species were dominant regardless of temperature. Data were interpreted with the assumption that the number of counts for each species on each indicator medium would be proportional to the amount of leaf occupied. Several sources of bias in these results should be considered. First, in selecting macerated leaf segment fragments containing vascular bundles, it is possible that a fungus preferentially occupying the bundles would be favored. However, the even distribution of vascular bundles across *T. latifolia* leaves, the usual presence of other tissues attached to the bundles, and the consistency of the results strongly suggest that fragment selection was not a source of bias. Second, there is a possibility that reactions in wells could be misread if both fungi were present, although random comparisons of physiological results and microscopic hyphal morphologies provided partial assurance of the validity of positive and negative scores. Third, the positive reactions for *Sclerotium hydrophilum* were obtained on an inhibitive medium (i.e., low pH) and thus could lead to a negative score if only a very few viable hyphae remained in a leaf fragment. *Sclerotium hydrophilum* grew in approximately 80% of the wells inoculated with fragments of leaves in which this fungus was the sole colonist while *P. typhae* grew in 100% of wells when it was the sole colonist (Fig. 1). This suggests that either approximately 20% of the *S. hydrophilum* fragments were false negatives, or that the colonization of leaf segments by *S. hydrophilum* was less extensive than by *P. typhae*. In either case, the percentage (80%) was high enough to indicate unequivocally heavy colonization of the substrate.

Relating our specific findings to natural *T. latifolia*-dominated communities is difficult due to the artificial methods used, as well as the paucity of observations on the life cycles of these organisms, which is the case for most saprobic fungi, in their natural habitat (Trappe and Luoma 1992). First, with respect to methodology, although Tsuneda et al. (2001) found no discernable changes in *Sphagnum* leaf cell morphology after autoclaving, chemical alterations (e.g., loss of volatiles, leaching of nutrients, etc.) of the *T. latifolia* leaf segments used in this study could limit the extent to which our conclusions can be applied to interactions of these two fungi on unprocessed and nonsterile leaf tissue. Almost certainly the activities of other members of the microbial community, as well as microfauna, would be expected to influence the outcome of these interactions. Finally, because only a single isolate of each fungus was used in this experiment, the effects of strain variation on competitive interactions have not been assessed. Despite these concerns, we offer that our method gives a more realistic approximation of the fungal interactions occurring in nature than techniques that use agar media in Petri plates.

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**References**


