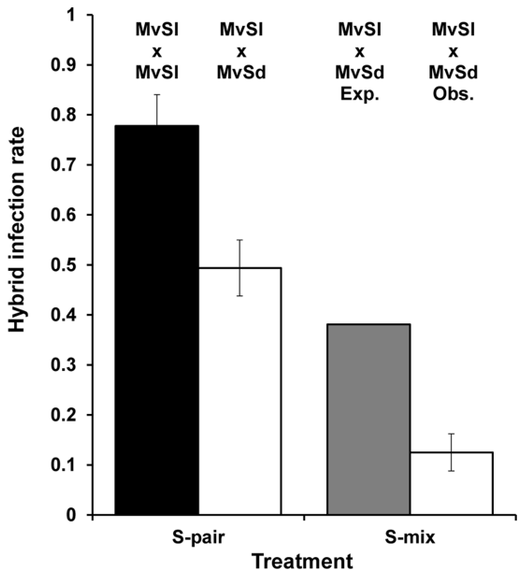

Figure 4. Reduction of hybrid infection rate resulting from selfing and the sibling competition arena. For the forced mating treatment (S-pair), infection rates are shown for MvSl × MvSl (outcrossed) and MvSl × MvSd (hybrid) crosses. For the hybridization choice treatment (S-mix), the MvSl × MvSd hybrid infection rate is shown relative to the expected infection rate based on the results in the forced mating treatment (S-pair). Assuming no reduction in hybrid success due to the sibling competition arena, the expected hybrid infection rate of MvSl × MvSd in sporidial mixtures (S-mix) is estimated as the rate of intraspecific outcrossing (MvSl × MvSl) in S-mix corrected by the reduction in MvSl × MvSd hybrid fitness observed under forced hybridization (S-pair). The observed rate of hybrid infection is significantly less than that predicted based upon hybrid fitness and selfing rate alone ($\chi^2 = 14.070$, df = 1, $P = 0.0002$), indicating a role for competition between selfed and hybrid progeny (i.e., sibling competition arena). Error bars show the standard error of the proportion for observed rates.

rate attributable to intrapromycelial mating ($\chi^2 = 3.190$, df = 1, $P = 0.0741$).

As noted above, the hybrid infection rates in the S-mix and T-high treatment for the remaining four crosses were too low for an informative statistical test comparing the observed and expected hybrid infection rates. However, the reduction of hybrid infection rates to zero when competition is introduced is consistent with our hypothesis.

Discussion

Selfing in combination with the competition arena is shown here to be an important barrier to gene flow between closely related, sympatric species of the phytopathogenic fungi *Microbotryum*. Intrapromycelial mating, a developmentally promoted form of automixis, in combination with early, intense competition between selfed and hybrid progeny, yields nearly complete reproductive isolation between species of *Microbotryum*. Results of artificial inoculations confirm our prediction that fewer hybrids successfully establish infection when selfing and intrapromycelial mating are possible, in comparison to the forced hybridization treatment. Furthermore, our results confirm the prediction that fewer hybrids successfully establish infection under competition with nonhybrid siblings than expected based upon selfing rates and intrinsic fitness reductions. Moreover, we demonstrate that low teliospore density further reduces rates of hybrid infection, potentially by reducing the frequency with which hybrids are generated. Reproductive isolation in this fungal system thus appears to be strongly influenced by selfing and the associated competition between nonhybrid and hybrid genotypes, which we refer to as the sibling competition arena.

Treatments differing in the potential for selfing and intrapromycelial mating yielded significantly different rates of hybrid infection. Intrapromycelial selfing is the most common form of mating in nature and is promoted by the development of physically linked gametes upon germination of the teliospore, the transmissible stage of *Microbotryum* in natural populations (Hood and Antonovics 2000, 2004; Schäfer et al. 2010). The potential for intrapromycelial selfing alone reduced hybrid infection rates by 76–78% across both the MvSl × MvSl and MvSl × MvSd species pairs, most likely indicating a constant average rate of intrapromycelial mating within the MvSl pathogen species. In natural populations, rates of selfing vary but have been estimated to be as high as 88–94% (Gladieux et al. 2011), which is higher than the rate observed in our experimental crosses. In nature, teliospore concentrations and/or the frequency of occurrence of different mating partners on a given plant may be lower or more variable than they are under artificial inoculation, influencing estimations of selfing rates. Moreover, Oudemans et al. (1998) and Thomas et al. (2003) reported that haplolethal alleles, which limit outcrossing for the sporidia of one mating type, may be as frequent as 100% in some field populations. This was not the case for the three MvSl strains used in this study.

It is possible that mechanisms promoting high frequencies of selfing represent adaptations to limit gene flow, but identifying such adaptations has proven notoriously problematic (Ramsey et al. 2003; Martin and Willis 2007; Matsubayashi and Katakura 2009). Refrégier et al. (2010) found no evidence for a mating preference for conspecifics in sympatric versus allopatric populations of MvSl and MvSd. They attributed this to the presence of a powerful prezygotic isolating mechanism (i.e., selfing) that limits selection for additional reproductive barriers, such as assortative mating by mate choice. That study, however, also found no evidence for adaptive reinforcement in the form of higher rates of selfing in sympatric populations. Likely, alternative hypotheses for the observed frequencies of selfing are facilitation of mating