species. For each species pair, six mating combinations (i.e., a_1 and a_2 sporidia) were randomly chosen from among the samples available per *Microbotryum* species. The six inoculum combinations were then applied under four different treatments (S-pair, S-mix, T-high, T-low, see below). A total of 144 individual crosses were performed (six pairs of haploid genotypes for each of six species pairs × four treatments). These 144 crosses are detailed in Table S1. Between 15 and 25 plants were inoculated for each cross.

The four treatments were designed to contrast the rates of selfing with intraspecific outcrossing or interspecific hybridization under conditions of either (1) forced hybridization or intraspecific outcrossing, (2) hybridization or selfing possible via sporidia (autogamy), or (3) hybridization or selfing possible via sporidia and intrapromycelial mating (automixis) (Fig. 1). The treatment S-pair (for Sporidial pair) comprised inoculation with a_1 sporidia from one haploid genotype and a_2 sporidia from a second haploid genotype, such that hybridization or intraspecific outcrossing could be forced. The treatment S-mix consisted of inoculation with equal quantities of four distinct sporidial types, the a_1 and the a_2 from each of two fungal individuals (except when a second mating type was unavailable for the species to be crossed with MvSl; see Table S1). Under the S-mix treatment, competition between outcrossed/hybrid and nonhybrid (selfed) progeny could occur because selfing was possible; however, selfing via intrapromycelial mating was absent. In the T-high (for Teliospore, high concentration) and T-low treatments, the inoculum consisted of a suspension of teliospores from two *Microbotryum* diploids, which allowed intrapromycelial mating. Because teliospores are the transmissible stages of *Microbotryum* and intrapromycelial mating is the more common form of mating in nature, these teliospore treatments are most reflective of processes in natural populations (Hood and Antonovics 2000, 2004; Schäfer et al. 2010). With teliospores, outcrossing or hybridization was not forced, and competition between outcrossed/hybrid and nonhybrid progeny could occur. The T-high and T-low treatments differed in that the T-low inoculum was diluted 100-fold relative to the T-high inoculum to assess the role of teliospore density in the balance of selfing and outcrossing/hybridization. For each treatment, three intraspecific crosses, consisting of selfing MvSl genotypes from three populations, were conducted. These crosses were designed to obtain a baseline infection rate by selfed progeny for estimations of the reduction in fitness of hybrid progeny. To simplify the description of the results, the term "hybrid" will henceforth be used as an umbrella term for both interspecific hybrid and intraspecific outcrossed progeny.

Under the hypothesis that selfing, and in particular the developmental propensity for intrapromycelial mating, plays a role in reproductive isolation, the rate of hybrid infection was predicted to be highest in the absence of competition (S-pair) and moderate under competition with nonhybrids in the absence of intrapromycelial selfing (S-mix). Intrapromycelial mating was predicted to reduce rates of hybrid infection to their lowest level, even below that projected by intrinsic hybrid fitness and selfing rates. The rate of hybrid infection was therefore expected to be lower in the presence of selfing by intrapromycelial mating (T-high), and lower still under reduced teliospore concentrations (T-low), due to less frequent contact between teliospores and a decreased density of hybrids relative to nonhybrids, yielding a stronger sibling competition arena.

DATA COLLECTION AND GENOTYPING

After 2–4 days of incubation, seedlings were transplanted to soil in the greenhouse. Upon flowering, plants were visually assessed for symptoms of anther-smut disease. All flowering plants were removed from the flowerbeds as soon as the first flower appeared to avoid secondary contamination. The number of plants that flowered for each cross is reported in Table S1. The identity of healthy and diseased plants was noted, and 1–2 flowers of diseased plants were retained for genetic analysis. Anthers were desiccated on silica gel (Silica gel blue 2–5 mm Prolabo) and stored at 4°C.

DNA was extracted, using the Chelex (Bio-Rad) method (Bucheli et al. 2001), from —one to two anthers from a single flower derived from each diseased sample. Artificial inoculation at the single meristem stage of seedlings largely prevents coexistence of multiple infections, resulting in systemic infection by the single pathogen genotype that persists in subsequently derived meristems (Hood 2003; Gold et al. 2009). Anthers from a single flower were therefore considered accurate for genetic typing of an infection under our inoculation protocol. Even in the unlikely event of multiple infections, they would segregate in different stems (Hood 2003; Gold et al. 2009; López-Villavicencio et al. 2011), and our genotyped strains would represent an unbiased sample of the infecting strains.

Microsatellite genotyping was conducted as described in Giraud (2004). The microsatellite markers SVG8 and SL16 (Giraud et al. 2008c) were used to identify interspecific and intraspecific hybrids, respectively. The majority of strains used in this study were homozygous at the SVG8 microsatellite marker, and different species carried discriminating alleles. Therefore, heterozygotes at this marker indicated infection by a hybrid pathogen. The marker SL16 was additionally used to distinguish two *M. lychnidis-dioicae* (MvSl) strains (728.6, 729.2), which were not distinguishable using the marker SVG8.

All raw data are deposited in the Dryad repository: doi:10.5061/dryad.rg148qj4.

DATA ANALYSIS

To assess variation in the overall infection rate, logistic regressions were performed with JMP 3 (SAS Institute Inc., Cary, NC). For