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Anther smut disease caused by *Microbotryum* on berry campion *Silene baccifera*: endemic pathogen or host shift?

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This research investigated whether anther smut disease caused by *Microbotryum violaceum* agg. on *Silene baccifera* in Europe is caused by a host-specific lineage, or if it represents a host shift of the pathogen from a related species. Characterization of ITS sequences of anther smut from *S. baccifera* confirmed their strong similarity to the pathogen endemic on *Silene latifolia*. Cross-inoculation studies showed that *S. baccifera* was susceptible to anther smut isolates from *S. latifolia*, *S. dioica* and *S. vulgaris*; conversely isolates from *S. baccifera* could cause disease on *S. latifolia*. In an experimental field study, spore transmission from diseased *S. latifolia* to healthy *S. baccifera* was rare relative to intraspecific transmission within *S. latifolia*. The distribution of anther smut in natural populations based on herbarium specimens indicated that disease occurrence on *S. baccifera* was very sporadic. These findings strongly suggest that anther smut disease on *S. baccifera* in Europe is usually a temporary host shift from the self-sustaining populations of *Microbotryum* on other species.

Keywords: cross-species transmission, *Cucubalus baccifer*, host specificity, *Silene dioica*, *Silene latifolia*, *Silene vulgaris*

Introduction

Infectious diseases and the pathogens that cause them are often shared between host species that are closely related, with distantly related species being at much less risk of host shifts (Pedersen & Davies, 2009; Antonovics *et al.*, 2012; Parker *et al.*, 2015). However, there are often exceptions; for example, interkingdom host shifts of fungal pathogens among plants and insects have been documented (Spatafora *et al.*, 2007; Sung *et al.*, 2007). Factors such as geographical overlap and ecological similarity also contribute to determining whether a host shift will occur and whether it will be successful (Davies & Pedersen, 2008).

Fungi in the genus *Microbotryum* (Basidiomycota, Microbotryales) cause anther smut disease on a large number of species in the Caryophyllaceae (carnation or pink family), especially on the genus *Silene*. Anther smut disease is commonly found on *Silene latifolia*, *S. dioica* and *S. vulgaris* throughout Europe (Vercken *et al.*, 2010; Abbate & Antonovics, 2014) but observations of its presence on *S. baccifera* are more sporadic (Table S1).

Phylogenetic studies have shown that anther smut disease on different hosts in the Caryophyllaceae and related families is caused by highly divergent lineages in the genus *Microbotryum* (Kemler *et al.*, 2009, 2013), many of which appear to be highly host-specific (Le Gac *et al.*, 2007; de Vienne *et al.*, 2009). Several, but by no means all, of these have been given species names (Lutz *et al.*, 2008; Denchev *et al.*, 2009), and the taxonomy remains in a state of flux; therefore, in this report, the term 'lineages' is used in a general sense, and species names are assigned only in specific cases.

In *Silene*, as with other members of the Caryophyllaceae, the host specificity of *Microbotryum* is not absolute and some lineages are found on multiple host species (Lutz *et al.*, 2008; Refrégier *et al.*, 2008). Transient host shifts have also been observed (Antonovics *et al.*, 2002; Gladieux *et al.*, 2011), and it is of interest to ask what factors limit the establishment of such pathogens on new hosts. For example, although inoculation studies have shown that annuals are susceptible to anther smut (Gibson *et al.*, 2013), the disease is not self-sustaining in annuals because the pathogen can only persist across seasons in the vegetative parts of the plant (Thrall *et al.*, 1993; Hood *et al.*, 2010). More generally, the degree of host specificity in the smut fungi has been a long-standing debate (Zillig, 1921), but is being increasingly resolved through phylogenetic (de Vienne *et al.*, 2009) and experimental approaches (Antonovics *et al.*, 2002; Gilbert & Webb, 2007).

This study focused on anther smut disease found on *Silene baccifera* (=*Cucubalus baccifer*; berry campion or catchfly). *Silene baccifera* is found in all warm temperate regions of Eurasia (Clapham *et al.*, 1952). In Europe, it occurs frequently in riparian areas, along riverbanks and low-lying moist areas, often exclusively so in parts of its range (Slavik, 1980). It is a perennial that has a 'scrambling' habit and can reach several metres high when associated with shrubs, especially in hedgerows. In more

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open situations it can form dense, low mats several metres across.

Previous phylogenetic studies have suggested that the pathogen causing anther smut on *S. baccifera* is closely related, if not identical, to that on the widespread species *S. latifolia* (Lutz *et al.*, 2008). *Silene baccifera* was once placed in a separate genus (*Cucubalus*), largely based on its unusual fruit morphology. The fruit appears to be a berry, as its common name suggests, especially on herbarium specimens, but it is in fact a shiny, black, thin-walled capsule mimicking a berry (Rohweder & Urmi, 1978). More recent phylogenetic studies have shown that *S. baccifera*, far from being in a separate genus, is nested within the *Silene* clade (Greuter, 1995; Oxelman & Lidén, 1995). Nevertheless, the phylogeny for the clade containing *S. baccifera*, *S. latifolia* and *S. vulgaris* is not well resolved (B. Oxelman, University of Gothenburg, Sweden, personal communication), so the precise relationship of these host taxa to each other is not known.

To establish the phylogenetic status of *Microbotryum* on *S. baccifera*, studies were carried out on a broader range of samples than previously. The susceptibility of *S. baccifera* to *Microbotryum* isolated from other species of *Silene* that overlap with *S. baccifera* in their distribution was investigated, and spore transmission between diseased *S. latifolia* and healthy *S. baccifera* in experimental field arrays was studied. In addition, herbarium and field studies were carried out to examine the distribution of anther smut disease on *S. baccifera* in nature. The results strongly support the hypothesis that anther smut disease on *S. baccifera*, at least in most areas of Europe, is not self-sustaining but is the result of transient host shifts of the pathogen from other co-occurring species.

Materials and methods

Phylogenetic status

To extend the phylogenetic analysis of Lutz *et al.* (2008), which was based on two samples of *Microbotryum* from *S. baccifera* collected within 40 km of each other in Germany, two further samples were obtained, one from the Czech Republic and one from Germany. The Czech Republic sample came from a herbarium specimen in the Natural History Museum, London while the German sample came from the Bayreuth Botanical Garden, Germany (Table 1). The two host populations studied by Lutz *et al.* (2008) were revisited, but the disease could not be found. For the Bayreuth isolate, DNA was extracted using two diseased anthers suspended in 150 μL of 5% chelex, which were incubated at 56 °C for 4 h and 95 °C for 30 min. For the Czech Republic isolate in the herbarium, DNA was extracted using a CTAB-chloroform extraction followed by precipitation with isopropanol. DNA specific to the ITS1 sequence of *Microbotryum* was amplified by PCR using primers ITS-710-F (5′-CTGTTTAACCAGGGCGTGAC-3′) and ITS-710-R (5′-TGATCTCGAAGGTTAGGATGC-3′). A negative control of ddH_2O was included as well as a positive control of DNA extracted from *Microbotryum* spores found on *S. latifolia*. PCR products were visualized using gel electrophoresis with ethidium bromide on a 1% agarose gel. The PCR products were then cleaned using Exo-SAP-IT reagent and sequenced at Yale sequencing facility. Sequences were of high quality and *c.* 300 bp long from the beginning of the ITS sequence.

Table 1 Sources of material used in the inoculation experiments.

	Host	Population
Microbotryum inoculum	*Silene vulgaris*	Italy, San Damiano (lat 44.504, long 7.252), August 2011. Population with 12 diseased and 87 healthy plants
	Silene latifolia	Germany, Brandenburg, Blossin near Friedersdorf (lat 52.262, long 13.805), September 2013. Heavily diseased population
	Silene dioica	Germany, Oberstdorf, hiking trail to Kemptner Hütte, (lat 47.325, long 10.305) 24 August 2013. Heavily diseased population
	Silene baccifera	Germany, Bayreuth, Botanical Gardens 14 October 2011. A single diseased plant
Seeds	*S. latifolia*	Italy, Piemonte, Chiusa di Pesio, trail to Castello Mirabello (lat 44.325, long 7.684), July 2013. Healthy population, but diseased individuals within 100 m
	S. baccifera	Italy, Piemonte, Beinette (lat 4.3434, long 7.673), August 2011. Seeds collected from plants in the greenhouse, grown originally from seeds sampled from a healthy population with 8 individuals

Cross inoculation

To assess the susceptibility of *S. baccifera* to isolates of *Microbotryum* from other host species, anther smut from *S. latifolia* and *S. dioica* were sampled from Germany, where diseased *S. baccifera* had been found previously. In the absence of diseased *S. vulgaris* collections from Germany, *Microbotryum* from *S. vulgaris* in the Piemonte province, Italy was used, where it occurred in close proximity to *S. baccifera*. Seeds of *S. latifolia* and *S. baccifera* from Italy were used to determine whether *S. baccifera* was resistant to *Microbotryum* from *S. latifolia*; in the detailed study area in Italy (Fig. S1), *Microbotryum* had never been found on *S. baccifera*. *Silene baccifera* seedlings were inoculated with teliospores collected from diseased flowers of *S. latifolia*, *S. dioica* and *S. vulgaris*, and, conversely, seedlings of *S. latifolia* and *S. baccifera* (positive control) were inoculated with *Microbotryum* spores collected from the *S. baccifera* plant in the Bayreuth Botanic Garden. Throughout, sterile water was used as a negative control. Seed and smut collection locations are given in Table 1 and sample sizes in Table 2.

All seeds were sterilized prior to germination by steeping them in a bleach-ethanol solution (40% Clorox bleach with 8.25% NaOCl, 20% ethanol) for 6 min, and rinsing them five times in sterile water. To promote germination of *S. baccifera*, seeds were nicked with a razor blade and placed in Petri dishes on 0.75% agar supplemented with 10% strength Murashige and Skoog basal medium. They were then subjected to a cold treatment (4 °C) for 7 days and placed in the growth chamber at 16 °C for 23 days in alternating light and dark cycles, with a 23:00–07:00 night. Most seeds germinated after *c.* 7 days and seedlings were inoculated 16 days after germination. The

Table 2 Infection rates following inoculation of *Silene baccifera* and *Silene latifolia* with *Microbotryum* from different *Silene* host species.

Inoculated species	Inoculum source	No. of plants			Diseased (%)
		Healthy	Diseased	Vegetative	
S. baccifera	*S. latifolia*	5	2	10	29
	S. dioica	6	4	4	40
	S. vulgaris	4	1	13	20
	S. baccifera (positive control)	3	1	16	25
	Water only (negative control)	3	0	1	0
S. latifolia	*S. baccifera*	5	4	6	44
	S. latifolia (positive control)	6	5	4	45

inoculum, consisting of 800 viable teliospores per μL in 2 μL water plus Triton-X surfactant, was applied to the apical meristem between the two cotyledons. The inoculated seedlings were kept in the growth chamber for two more days, and then transplanted into 2.5 cm diameter 15 cm deep pots (Cone-tainers; Stuewe & Sons) containing a peat:perlite mixture (ProMix BX), randomized and maintained under standard greenhouse conditions. After 2 months, when the plants had outgrown the Cone-tainers, they were transplanted into 15 cm diameter pots, until flowering.

To test whether *Microbotryum* spores originating from *S. baccifera* could cause disease in *S. latifolia*, similar protocols were followed for germinating and inoculating *S. latifolia*, except that seeds were not nicked or cold treated.

Spore deposition

To investigate spore transmission from diseased *S. latifolia* to healthy *S. baccifera*, experimental arrays were set up in a garden area at the offices of the Parco Naturale del Marguareis near Chiusa di Pesio, Piemonte, Italy. Diseased and healthy inflorescences of locally collected *S. latifolia* and healthy inflorescences of *S. baccifera* were placed in water containers. Each container had *c*. 20–40 open flowers and 20–40 unopened flower buds, on three or four stems. Both the *S. latifolia* and the *S. baccifera* inflorescences came from close-by sites in Chiusa di Pesio, and inflorescences were put in water and placed in the experimental area immediately after collection.

A container with diseased *S. latifolia* inflorescences was placed in the centre of the experimental array and it was surrounded by containers with alternating healthy *S. baccifera* and *S. latifolia* (six of each) in circles at 1 and 5 m from the centre. All open flowers on healthy plants were removed at the start of the experiment. After 2 days, all newly opened flowers on healthy plants were collected for spore counting. This procedure was repeated for another 2 days. Spores were counted on these healthy flowers as follows: after sampling and being placed in sterile envelopes to air dry, each flower was suspended in 1 mL Pohl's solution (25% methanol, 1.2% aqueous Aerosol OT) for 1 day to soften the tissues, vortexed and counted using a haemocytometer. All flowers per array location were counted separately, and then averaged per location to give a rate of spore deposition per flower per location.

Field and herbarium studies

The presence of anther smut disease is rarely noticed by collectors and so herbarium collections can be used to study disease distribution (Antonovics *et al.*, 2003; Hood & Antonovics, 2003; Hood *et al.*, 2010). Samples from several herbaria were examined or images and information from them were accessed to assess the occurrence of diseased and healthy plants on *S. baccifera* (Table 3).

The status of *S. baccifera* was also investigated in two areas where diseased plants had been found previously. Plants from a site in Norfolk, UK were investigated in 2008 and plant records of this site were obtained from the Botanical Society of Britain and Ireland database and from the Cambridge University Herbarium. In addition, two populations in Saxony, Germany, where diseased plants had been found previously (Lutz *et al.*, 2008), were investigated in 2014.

Results

Phylogenetic status

The ITS sequences of *Microbotryum* isolates from *S. baccifera* were closely related to those of isolates from *S. latifolia* (identified as *Microbotryum lychnidis-dioicae*; Fig. 1) and very similar to those isolated by Lutz *et al.* (2008). There was also close similarity with the ITS sequences of the *Microbotryum* isolate normally found on *S. dioica* (*M. silenes-dioicae*), which is closely related to that on *S. latifolia* (Vercken *et al.*, 2010).

Table 3 Occurrence of diseased *Silene baccifera* in herbarium collections.

Herbarium	Number of sheets	Number diseased
Natural History Museum, London (BM)	66	1
Royal Botanic Gardens, Edinburgh (E)	185	0
Botanical Museum, Berlin (B)	116	1
Natural History Museum, Paris (P)[a]	537	0
Various herbaria[b]	45	0
Total[c]	949	2

[a]https://science.mnhn.fr/institution/mnhn/collection/p/item/list?specificEpithet=baccifer&genus=Cucubalus.

[b]Italy: Collegio Rosmini di Domodossola (DOMO); Natural Science Museum Torino (MRSN); University of Torino Herbarium (TO); Liceo Salesiano Valsalice, Torino (TOGR); Giardini Hanbury, Ventimiglia (HMGBH); Civico Museo Alba (ALB), Erbario MSN Doria Genova (GDOR); Università di Pavia (PAV), Orto Botanico de Bergamo (BER). France: Jardin Botanique de la Ville de Nice (JBVN).

[c]Does not include one diseased specimen from Cambridge University Herbarium (CGE), which was searched with prior knowledge that a diseased specimen was there.

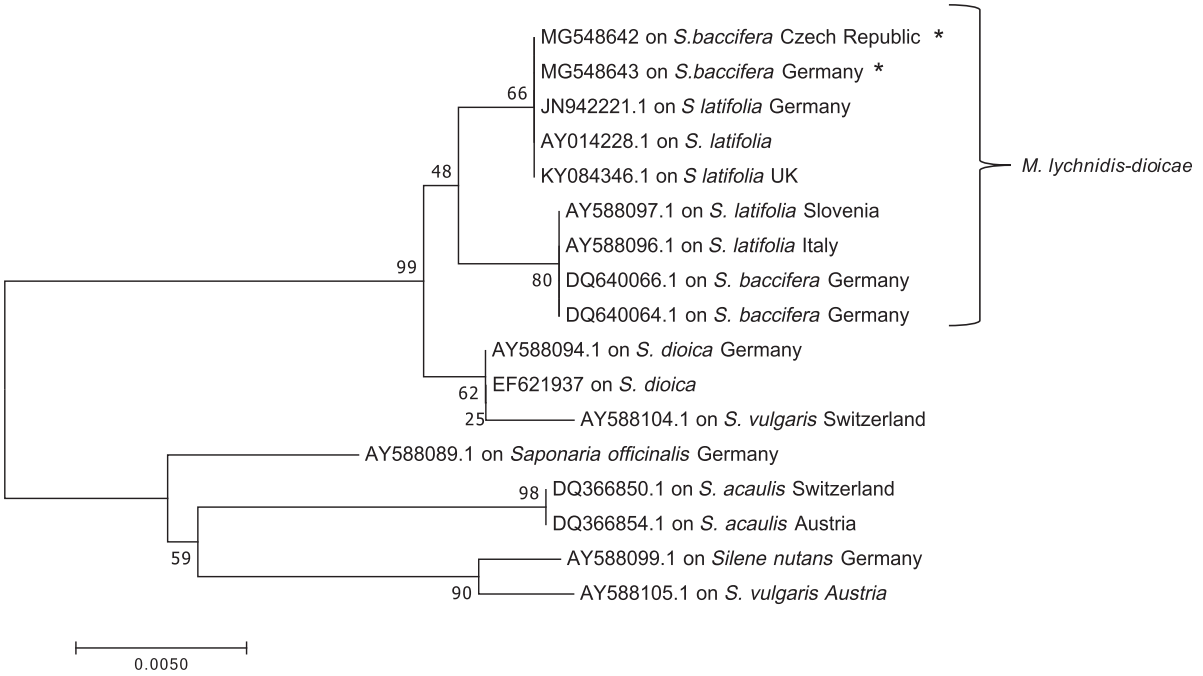

Figure 1 Phylogeny showing relationships of *Microbotryum* isolates from *Silene baccifera* based on the ITS region. Those newly studied here are indicated by asterisks, while the other two *S. baccifera* sequences are from Lutz *et al.* (2008). All belong to the clade identified as *Microbotryum lychnidis-dioicae*. The tree was inferred using the neighbour-joining method in MEGA 7. Numbers next to the branches show the percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (500 replicates). The tree is drawn to scale, with branch lengths in units of base substitutions per site (computed using the maximum composite likelihood method). Indicated at the branch tips are the GenBank accession numbers of the *Microbotyrum* sequences, the host species on which they occurred, and their provenance (when known).

Cross inoculation

Silene baccifera was susceptible to infection by *Microbotyrum* collected from *S. latifolia*, *S. dioica* and *S. vulgaris* (Table 2), as well as the pathogen originating from *S. baccifera* used as a positive control. Sample sizes were small and so no attempt was made to assess whether the differing relative rates of infection were statistically significant, but it was clear that *S. baccifera* was susceptible to anther smut disease from a variety of sources. Conversely, *Microbotryum* infecting *S. baccifera* in the Bayreuth Botanical Gardens was also able to infect *S. latifolia* (Table 2).

Spore deposition

The results of the spore deposition experiment showed that diseased *S. latifolia* occurring close to *S. baccifera* resulted in some cross-species spore transmission. However, spore deposition was much greater onto the conspecific *S. latifolia* than onto *S. baccifera* (Table 4).

Herbarium and field studies

Out of a total of 949 herbarium sheets of *S. baccifera* examined, plants with diseased flowers were found on only two of them (Table 3). Comparing this with the data on other perennial species in the genus *Silene* (see supplementary material in Hood *et al.*, 2010) and restricting the analysis to species with at least 100 herbarium samples, *S. baccifera* ranked 38th out of 53 species in terms of the percentage of specimens that were diseased (including 13 in which no anther smut disease was found).

Silene baccifera was not found in the 2008 survey of the area in Norfolk where it had been previously recorded as diseased, suggesting that it may have become locally extinct, or at least is currently very rare. There has been no record of the plant in this area since then (Botanical Society of British Isles database). This view was supported by correspondence with the recorder responsible for that vice-county, and conversations with local people. However, during the survey in Norfolk, anther smut disease was found in two populations of *S. dioica* within 0.5 km of the Merton estate, an area where *S. baccifera* had been previously recorded as diseased. The occurrence of collections of *S. baccifera* in this area of Norfolk is shown in Table 5, based on records from the Botanical Society of the British Isles and the Kew herbarium. It appears that *S. baccifera* plants were introduced by the early 1900s, but the disease was first recorded in 1944.

The following populations where disease had previously been recorded were visited in Saxony, Germany:

Area 1: Bad Düben (51°35′06.9″N, 12°34′50.4″E). This was a small population of six plants, all healthy.

Table 4 Spore deposition on flowers of *Silene baccifera* and *Silene latifolia* from a central disease source of *Microbotryum* on *Silene latifolia*.

	1 m away				3 m away		
Species	Day	Flowers	Plants with spores (%)	Spores per flower	Flowers	Plants with spores (%)	Spores per flower
S. latifolia	1–2	32	67	1.1	66	17	1.0
	3–4	36	33	5.9	39	33	0.5
	5–6	34	50	2.0	44	50	0.75
S. baccifera	1–2	47	0	–	126	0	–
	3–4	15	0	–	58	17	0.5
	5–6	16	0	–	26	0	–

Table shows the number of flowers on which spore deposition was counted, the percentage of plants in each group on which spores were found, and the mean number of spores per flower when spores were present, as seen in a 1 × 1 mm haemocytometer field.

Several plants of *S. latifolia* and one plant of *S. dioica* were in the vicinity (within 100 m), but these were also healthy.

Area 2: Dessau, near Riesigk (51°49′49.00″N, 12°28′07.86″–12°29′03.14″E). This was a population of 37 mostly large plants extending along 200 m of the northern banks of an east–west drainage channel between two fields. No diseased plants were found. There was also a population of 28 plants of *S. latifolia* near the road crossing the drainage channel in a north–south direction, but all the plants were healthy. Although no systematic search was done, a roadside population of *S. latifolia* with anther smut disease was encountered 20 km north of Area 2.

Silene baccifera was also common in the southern Piemonte area of Italy and, although often in very close proximity to diseased *S. latifolia*, anther smut disease on *S. baccifera* was not observed, either in formal mapping of the populations (Fig. S1) or over a 5-year period (2012–2016) of informal but consistent search.

Table 5 Historical incidence of anther smut disease on *Silene baccifera* in Norfolk, UK.

Year	No. of records	No. of sheets examined	No. of sheets with disease
Pre-1910	0	0	0
1910–19	9	7	0
1920–29	4	2	0
1930–39	5	2	0
1940–49	3	2	1[a]
1950–59	9	1	0
1960–69	9	2	1[b]
1970–79	2	0	0
1980–89	7	0	1[c]
1990–99	11	0	0
2000–09	4	0	0
2010–present	0	0	0

Duplicate records have been excluded. None of these records mentioned disease.

[a]Kew Fungarium, accession 186853/4.

[b]Cambridge Herbarium, accession 24097.

[c]Kew Fungarium, accession 186855.

Discussion

This study supports the hypothesis that anther smut disease caused by *Microbotryum* on *S. baccifera* is commonly the result of a temporary host shift of *Microbotryum* from other related species of *Silene*. First, the ITS sequences of the two samples included in the phylogenetic studies agreed with the results of Lutz *et al.* (2008) who also found that isolates of *Microbotryum* from *S. baccifera* in Germany belong to the *M. lychnidis-dioicae* lineage commonly found on *S. latifolia*. Secondly, even though the sample sizes were small, the inoculation studies confirmed that *S. baccifera* could be infected with anther smut found on *S. latifolia*, *S. dioica* and *S. vulgaris* and, conversely, that *Microbotryum* spores from a diseased *S. baccifera* plant could infect *S. latifolia* (even though this test was only possible with one live sample of *Microbotryum* from *S. baccifera*). Thirdly, the disease has been found on *S. baccifera* only sporadically even though the plant is common, and well documented in central and southern Europe. Moreover, a search of two localities in Germany where the disease had been recorded within the past 10 years failed to find the disease, suggesting the disease is not self-sustaining. Nor was the disease ever found on *S. baccifera* in the southern Piemonte region of Italy which was a focal area for the present study of the distribution of anther smut and where the disease has previously been found on over 20 other species (Bruns *et al.*, 2018).

Host shifts have been documented in anther smut on other species of *Silene*. For example, anther smut on *S. vulgaris* in the USA represented a host shift of *M. lychnidis-dioicae* from *S. latifolia* (Antonovics *et al.*, 2002; Cafuir *et al.*, 2007). The host shift was observed in one field where *S. vulgaris* was growing intermixed with a heavily diseased population of *S. latifolia*. This host shift was probably also not self-sustaining as experimental studies showed that transmission rates within *S. vulgaris* were very low (Antonovics *et al.*, 2002). *Microbotryum lychnidis-dioicae* from *S. latifolia* can also be found on *S. vulgaris* in several parts of Europe, especially in lowland areas, but its occurrence appears to be transient (M. E. Hood, Amherst College, MA 01002, USA, personal communication). Instead, in Europe,

heavily diseased populations of *S. vulgaris* are generally the result of infection by three endemic lineages, two of which also infect the closely related species or subspecies *S. uniflora* (=*S. maritima*) and *S. prostrata* (Chung *et al.*, 2012; Abbate & Antonovics, 2014). It appears that the lineages endemic to *S. vulgaris* have never reached the USA where *S. vulgaris* is abundant and where, other than the recorded host shift mentioned above, the disease has never been recorded.

The history of disease incidence on *S. baccifera* introduced into the UK is somewhat similar to *S. vulgaris* in the USA. It seems unlikely that *S. baccifera* would have been diseased when it was introduced into the UK as the disease is not seed transmitted and the introductions were most probably as seeds. The frequent co-occurrence of diseased *S. dioica* in the vicinity suggests that anther smut on *S. baccifera* in Norfolk probably resulted from a host shift of the pathogen from *S. dioica*. It is possible that the disease played a role in the eventual extinction of *S. baccifera* in this area, but there were insufficient herbarium records to establish anything but an anecdotal record that it was present. A number of studies have shown that anther smut disease can have a strongly negative effect on population growth of its host populations (Thrall & Jarosz, 1994; Bernasconi *et al.*, 2009; Bruns *et al.*, 2017) and such disease-driven extinction is theoretically feasible (Best *et al.*, 2011).

This study emphasizes the difficulty of inferring host specificity from one-time observational studies, especially where quite divergent pathogen lineages cause similar symptoms. Clearly, much more intensive sampling would be necessary to be confident that *S. baccifera* has no endemic 'host-specific' *Microbotryum* lineage or that, even if host shifts from *S. latifolia* or *S. dioica* do occur, they are never self-sustaining. In England and northern Germany, where field populations of *S. baccifera* were studied, this species is near to the northern edge of its range and the disease might be endemic elsewhere in Europe. Disease ranges can be quite limited; for example *S. nutans* and *Atocion rupestre* (=*Silene rupestris*) have host-specific lineages in France and Switzerland (Hepper, 1956; Piatek *et al.*, 2012), but in the southern Piemonte region of Italy, where populations have been sampled intensively, the disease has never been found on these species (authors' unpublished data).

The experiments on spore transmission from *S. latifolia* to *S. baccifera* showed fairly strong pollinator specificity for *S. latifolia*. How *S. baccifera* plants are pollinated is not known, but it is probably by night-flying moths, whose larval stages also eat the seeds (Kravchenko *et al.*, 2007; Infusino *et al.*, 2017); no pollinators have ever been seen visiting *S. baccifera* flowers in the field sites in Italy, even after several hours of observation both day and night (authors' unpublished data). Nevertheless, fruit set is copious in the field, suggesting either sporadic pollinator visitation or selfing. Given that individual plants in the field are often large with several hundred flowers, inter-individual movement may be rare and only over short distances, thus potentially limiting disease transmission. *Silene latifolia* is pollinated by a wide range of pollinators, including night-flying moths (Altizer *et al.*, 1998) and the latter are a probable route of cross-species transmission. Further studies to characterize the mating system and pollination mechanism of *S. baccifera* would be informative, especially to determine if it has any specialist pollinators or if pollinator movement among individuals is indeed rare. It is possible that in areas where *S. baccifera* is not endemic, it is more likely to be pollinated by generalist pollinators. For example, because *S. baccifera* was only recently introduced to England, it may have been visited mostly by pollinators of other related *Silene* species.

Habitat preferences may also contribute to the relative paucity of cross-species disease transmission between *S. baccifera* and other species. *Silene baccifera* is generally found in riparian areas (Slavik, 1980) while *S. latifolia* is typically found at edges of agricultural fields and in disturbed areas (Goulson & Jerrim, 1997). However, in the southern Piemonte region of Italy where the authors' observations have been systematic, *Microbotryum* has never been seen on *S. baccifera*, even where it is often found close to diseased *S. latifolia*.

In conclusion, and by analogy with studies of animal and human diseases, *Microbotryum* on *S. baccifera* is probably the result of a cross-species transmission event or a 'spillover' of the pathogen from a closely related host species (Quammen, 2012; Wood *et al.*, 2012). This study emphasizes the difficulty of understanding the factors determining the frequency and fate of such spillovers and emphasizes the dangers of assuming host-species specificity based on limited pathogen sampling and characterization.

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site.

Figure S1. Distribution map of populations of *Silene baccifera* (all healthy) and *S. latifolia* (diseased and healthy) found in a 25 × 25 km region near Chiusa di Pesio, Piemonte, Italy. Populations were surveyed in summers of 2011–2014.

Table S1. Known records of anther smut disease on *Silene baccifera*.