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HERBARIUM STUDIES ON THE DISTRIBUTION OF ANTHER-SMUT FUNGUS (*MICROBOTRYUM VIOLACEUM*) AND *SILENE* SPECIES (CARYOPHYLLACEAE) IN THE EASTERN UNITED STATES[1]

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We used herbarium specimens of *Silene virginica, S. caroliniana, S. rotundifolia,* and *S. latifolia* to survey the incidence of anther-smut disease (caused by *Microbotryum violaceum* sensu lato) in the eastern USA. We found no evidence of a collector bias against diseased specimens. Diseased specimens were frequently found in collections of *S. virginica* and *S. caroliniana,* but not in those of *S. rotundifolia* or *S. latifolia*. Disease incidence in *S. virginica* and *S. caroliniana* increased significantly over the past century and was higher in marginal populations. The absence of disease in specimens of *S. rotundifolia* is consistent with field observations, but its presence in natural populations of *S. latifolia* (especially in Virginia) suggests that the disease is recently introduced. Changes in the host distributions were also evident. The relative abundance of *S. caroliniana* declined over time (especially further north), while the relative abundance of *S. virginica* increased. *Silene latifolia* was absent or rare south of Pennsylvania before ca. 1920, indicating that *S. latifolia* and its anther smut are likely to be recent introductions in Virginia. Methods are also presented that quantify the completeness of coverage provided by herbarium specimens.

Key words: Caryophyllaceae; fungal pathogen; invasive species; *Microbotryum violaceum;* plant distribution; *Silene antirrhina*; *Silene noctiflora*; *Silene ovata*; *Silene stellata*; *Silene vulgaris*.

Although gathering data on disease distribution is routine in agriculture and medicine, disease distributions are far less well understood in natural populations, largely because the institutional infrastructure for gathering such data does not exist or is put in place only when natural populations are seen as likely disease reservoirs (rabies: Krebs et al., 2000; West Nile virus: Eidson et al., 2000; Hanta virus: Abbott et al., 1999). With regard to plant populations specifically, there is almost no source of information on disease distributions on different plant species, other than in general catalogs (Farr et al., 1989; Büchen-Osmond et al., 2000) that are not comprehensive enough to obtain quantitative information on the disease distribution or long-term disease trends. It has been recently argued that understanding the spatial scales of plant–pathogen interactions is essential to explaining their dynamics (Burdon and Thrall, 1999, 2001). For natural plant–pathogen systems in particular, such regional studies are far fewer. Rust flax on *Linum* (Jarosz and Burdon, 1991) and anther smut and other diseases on island archipelagos (Carlsson et al., 1990; Carlsson and Elmqvist, 1992; Ericson et al., 1999) are a few examples.

A major source of information on plant species distribution comes from herbarium collections. While these collections have been primarily intended to support taxonomic and floristic studies, they have also been a useful source of information on life-history traits (Primack, 1978, 1980), stomatal densities (Parkhurst, 1978), phenology (Borchert, 1996), history of invasive weeds (Forcella et al., 1986; Forcella and Harvey, 1988), and antibiotic effects of plant extracts (Eloff, 1999). They are also increasingly being used to document the occurrence of pathogens (McCain and Hennen, 1986; Plowman et al., 1990; Vergeer and Denhartog, 1991; Clay, 1993; Rabeler, 1993; Fraile et al., 1997; Barreto et al., 1998; Mouchacca and Horak, 1998; Pimentel et al., 1998; Ristaino, 1998; Koponen et al., 2000; Ristaino et al., 2001) and herbivores (Graham, 1995).

In this study, we used herbarium collections to investigate the distribution of anther-smut disease, caused by *Microbotryum violaceum*, on several eastern U.S. species of *Silene*. Our goals were both to identify localities where the disease was likely to be found and to obtain data on the distribution and long-term dynamics of the disease and its hosts. Because these herbarium specimens span time periods back into the 1800s, we were also able to investigate whether there have been any changes in the distribution or abundance of the disease and its host species over time. We also asked if the occurrence of disease is related to host density or to the presence of disease in neighboring regions.

MATERIALS AND METHODS

Study species—*Microbotryum violaceum* sensu lato causes anther-smut disease in over 100 species in the Caryophyllaceae and related families (Thrall et al., 1993). The disease is characterized by the production of dark-colored fungal spores instead of pollen in the anthers of an infected plant. Diseased flowers also have reduced ovaries and are therefore completely sterile. The disease is largely pollinator transmitted. Although formerly classified as *Ustilago violacea*, anther smut is now considered to be in the order Microbotryales (Urediniomycetes), which is evolutionarily quite distant from the grass smuts (*Ustilago* spp.) in the Ustilaginomycetes (Begerow et al., 1997). *Microbotryum violaceum* is itself almost certainly a species complex. Distinct

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host races have been established experimentally (Goldschmidt, 1928; Liro, 1938; Antonovics et al., 1996), and extensive genetic divergence has been shown between the anther smuts on native North American species and anther smut on the introduced *S. latifolia* (Perlin et al., 1996). In Europe, *Microbotryum* isolates from different species that have morphological differences have often been given species recognition (Vanky, 1998). Because the systematics of the anther smut fungi is in a state of flux, we use the name *M. violaceum* for all isolates regardless of the host, but with no implication that they represent a single species or host-race of the fungus.

We investigated the distribution of anther smut on four species of *Silene* that have been the focus of more detailed genetic and ecological investigations in our laboratory. Three of these species are native to North America (*S. virginica* L.—fire-pink; *S. caroliniana* Walter—Carolina pink; and *S. rotundifolia* Nutt.—roundleaf catchfly) and one is introduced (*S. latifolia* Poiret—white campion [= *S. alba* (Miller) Krause, = *Melandrium album* (Miller) Garcke, = *Lychnis alba* Miller]).

Herbarium collections—Specimens were borrowed from the following herbaria, chosen to cover the expected range of these species in the eastern USA (alphabetically by herbarium code; Holmgren et al., 1990): Alabama Museum of Natural History (ALU); Cornell University, Bailey Hortorium (BH); Ohio University (BHO); University of Cincinnati (CINC); Clemson University (CLEMS); Carnegie Museum of Natural History, Pittsburgh (CM); University of Georgia (GA); Gray Herbarium, Harvard University (GH); University of Illinois (ILL); Illinois Natural History Survey (ILLS); Northern Kentucky University (KNK); University of Kentucky (KY); University of Michigan (MICH); Missouri Botanical Garden (MO); North Carolina State University (NCSC); New England Botanical Club (NEBC); New York Botanical Garden (NY); New York State Museum, Albany (NYS); Pennsylvania State University (PAC); Academy of Natural Sciences, Philadelphia (PH); University of Tennessee (TENN); University of Missouri (UMO); Smithsonian Institution, Washington (US); United States National Herbarium (USNH); Vanderbilt University (VDB); Virginia Polytechnic Institute and State University (VPI); Valdosta State University, Georgia (VSC); West Virginia University (WVA).

Collection coverage—To assess the degree to which the collections provided an adequate description of the species distributions, we created a model based on random resampling. Data on specimens of a species were chosen at random without replacement, and a record kept of whether choosing an additional specimen resulted in the recording of a new county. This random drawing was iterated 100 times to assess the average number of new counties found per additional specimen drawn. The following three-parameter model was then fitted to the data: $y = k + cx + a\exp(-bx)$, where y = the number of new counties per additional specimen, x = the number of specimens examined, and the other parameters are estimated from the data. This model provided the best fit of several two- and three-parameter models that were investigated. The number of specimens that would have been required to obtain complete coverage was obtained from the intercept of this curve on the x-axis (i.e., when no new counties would be found). The predicted total number of counties occupied with complete coverage was obtained by integrating the function to find the area under the curve from 0 to the x-intercept.

Disease assessment—Nearly all specimens were flowering and could be scored visually for the presence of anther-smut disease. The presence of spores in the anthers was confirmed with a binocular microscope. Overall we examined 1022 specimens of *S. virginica*, 888 of *S. caroliniana*, 96 specimens of *S. rotundifolia*, and 1104 of *S. latifolia*. Plant species identification was confirmed on all specimens and the sheets annotated appropriately. Although several subspecies have been recognized within *S. caroliniana* (Wilbur, 1976) we made no attempt at subspecies identification. We noted the date of collection and the county and state where the collection was made. We pooled duplicate sheets (representing specimens from one collecting event at one time) that had been distributed to several herbaria, as well as multiple collections made from the same county on the same date (because this might have represented deliberate intensive local search for a particular species, thus biasing the overall sampling). We considered any collection diseased if that herbarium sheet or its duplicates contained at least one diseased plant. Because multiple individuals were only rarely sampled from any one locality, it was not possible to estimate disease prevalence within individual collecting sites.

Data analysis—The data were analyzed using ArcView GIS 3.3 (Environmental Systems Research Institute, 2002) and Mathematica 4.1 (Wolfram Research, 2000). Because precise locality data were frequently not noted on the labels, we assigned the coordinates of the county capital (or county seat) as the location of a specimen collected in that county. Statistical analysis of derived data was carried out using SAS version 8.02 (SAS Institute, 2001) and SigmaPlot 5.00 (SPSS, 1999).

Changes in Silene abundance and geography over time—To examine changes in the frequency of collections over time, visual maps of the geographic range were created by categorizing the data into quartiles over time, with each quartile containing an equal number of specimens. This enabled comparisons of distributions over time without the confounding effects of sampling intensity.

We quantified the change in geographic range for the collections of each *Silene* species by calculating the north–south and east–west distance from a fixed point for each specimen. Then we took the average of these distances for each decade to obtain the change in the average distribution of the collections for each 10-yr period. The relative abundance of the collections of each plant species over time was measured by counting the number of specimens for each species in a decade, then expressing that number as a percentage of the total number of specimens for that species.

Differential changes in broad geographic patterns among the species collections were examined statistically by dividing the study region into five regions. We then used PROC FREQ in SAS to test if temporal changes in the distribution of the collections of each species were significantly different from each other. The following regions were defined: Northeast (Maine, New Hampshire, Vermont, Massachusetts, Rhode Island, Connecticut), Mid-Atlantic (New York, Pennsylvania, Delaware, New Jersey, Maryland, and District of Columbia), South (Virginia, North Carolina, South Carolina, Tennessee, Georgia, Florida, Alabama, and Mississippi), Midwest (West Virginia, Ohio, Kentucky, Indiana, Michigan, Illinois, and Wisconsin) and West (all states west of the Mississippi River). These regions were chosen because together they included the distribution of all four species, and because they were, to some extent, separable phytogeographically and historically.

Factors influencing prevalence of disease—For the two species in which diseased specimens were present (*S. caroliniana* and *S. virginica*), we examined whether the likelihood of disease in a specimen was correlated with either collection density (as indicated by the number of specimens of that species collected in a county or region) or with the prevalence of disease in the neighboring region (as indicated by the fraction of diseased specimens). We did not correct for county area, because the variation in specimen number was far greater than the variation in county size; there was no significant correlation of specimen number with county size in either species.

We calculated the likelihood of disease in a specimen as the fraction of diseased specimens in a given county. We then calculated the number of specimens collected in that ''focal'' county, and the number collected in surrounding counties within 30, 30–40, 40–50, and 50–60 miles (1 mile = 1.6093 km). The number collected within 30 miles excludes other specimens within the focal county. Distances from the focal county to neighboring counties were determined by calculating the distances from the seat of the focal county to the seats of the neighboring counties. To assess if there was a statistically significant relationship between the fraction of diseased specimens in a focal county and the number of specimens collected in that same county or in adjacent counties, we used logistic regression (LOGISTIC procedure in SAS). Each specimen within a county was given a score of 0 (healthy) or 1 (diseased) to use as the dependent variable in the logistic regression. We used the same method to establish whether the likelihood of disease in a specimen was related to the fraction of diseased specimens in neighboring counties.

We first carried out these analyses using all counties as focal counties. Because the vast majority of the counties had no diseased specimens, we then

TABLE 1. Estimated coverage of the total species range for *Silene caroliniana*, *S. latifolia*, *S. virginica*, and *S. rotundifolia*, using the methods described in the text and in Fig. 1. The estimated number of necessary sheets represents the estimated average number of herbarium sheets that would have to be drawn in order to identify the entire range of occupied counties for a species.

Species	Occupied counties			Collections		
	No. observed	Estimated total	Percent coverage	No. sampled	Estimated no. necessary sheets	Percent more specimens needed
S. caroliniana	230	246.0	93.50	888	1306.1	47.10
S. latifolia	421	508.9	82.70	1104	2036.9	84.40
S. virginica	402	480.3	83.70	1022	1842.9	80.20
S. rotundifolia	45	91.9	49.00	96	531.0	453.10

restricted the analyses to include as focal counties only those having disease specimens at some time during the study. To eliminate the "propositus effect," we estimated the frequency of disease in a focal county as $(d - 1)/(n - 1)$, where d = the number of diseased plants and n = the total number of plants. This frequency takes into account the fact that there has to be at least one diseased individual in a population for it to be recognized as diseased; failing to make this correction would result in an automatic expectation that counties with a few samples would have a higher disease frequency.

RESULTS

Collection intensity—Although extrapolating curves beyond the actual data is always to be interpreted cautiously, our analysis (Table 1; Fig. 1) suggested that we had identified over 80% of the counties that were occupied by the three most abundant species (*S. caroliniana*, *S. virginica*, and *S. latifolia*), and ca. 50% of the counties occupied by *S. rotundifolia*. The model also showed that considerably more sampling effort would be needed to obtain complete coverage for these species. Thus, in *S. caroliniana* we would have to examine half as many herbarium specimens again to find all the counties, while in *S. virginica* and *S. latifolia* about 80% more samples would be needed. In *S. rotundifolia* almost five times as many specimens would be needed to identify all the counties.

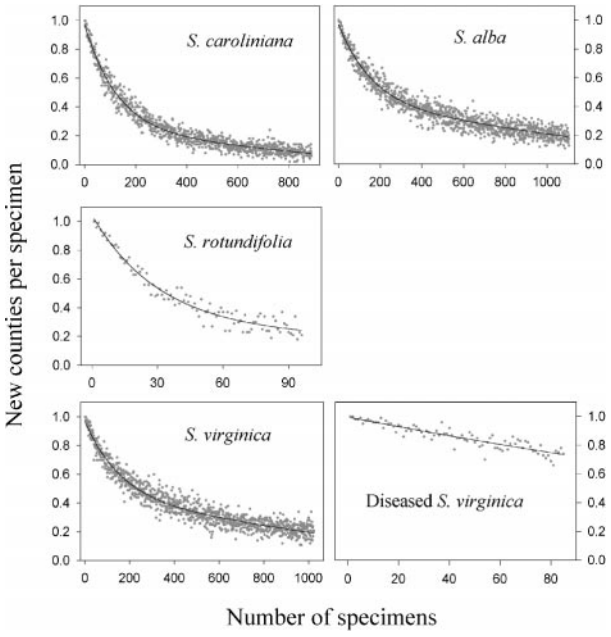


Fig. 1. The likelihood of discovering a specimen in a new county for each additional herbarium sheet chosen, for the four *Silene* species studied, and for diseased *S. virginica*. Each data point reflects the average of 100 random selections, given the actual recorded number of specimens and occupied counties for each species. The fitted curves are of the form $y = k + cx + a\exp(-bx)$.

Disease distribution—*Silene virginica*—In *S. virginica*, anther-smut disease was found in 85 of 1022 collections. Diseased collections were present throughout the species range (Fig. 2a). Analysis of disease frequency in 20-yr intervals indicated that the frequency of herbarium specimens that are diseased has increased from ca. 3.2% before 1900 to ca. 14.9% for specimens collected after 1980 (Fig. 3).

The fraction of diseased specimens in a county was not significantly related to the density of specimens in that county, either when all counties were included in the analysis (regression coefficient = −0.0008; $P = 0.40$) or when the analysis included only those counties with disease (regression coefficient = −0.0009; $P = 0.21$). In all cases, year was used as a covariate and had a highly significant effect.

The fraction of diseased specimens in a county was also not significantly related to the number of specimens within 0–30, 30–40, 40–50, and 50–60 miles when all counties were included as focal counties in the analyses (P values for effect of neighbor distance classes 30–60 miles ranged from 0.32 to 0.86; 1 mile = 1.6093 km). However, when only those focal counties with diseased specimens were used in the analysis, the logistic regression of disease vs. healthy on year and number of neighbors in the different distance classes was highly significant ($P < 0.0001$); both year and distance class contributed significantly to the model. Selective elimination of the furthest distance classes showed no significant contributions of the numbers at 50–60 miles, while the effects of the other three distances were significantly negative. Their relative effects depended on how many variables were included in the model (see Table 2). All year by distance interactions were not significant, regardless of the number of distance classes entered into the model. Therefore, there is evidence that counties that are more isolated (i.e., have fewer close neighbors) have a higher prevalence of disease.

There was no significant relationship between the fraction of diseased specimens in a given county and the fraction of diseased specimens in neighboring counties, although the trend was for more disease within the immediate neighboring area (i.e., 0–30 miles) (logistic regression for all counties = 0.84, $P = 0.08$; logistic regression when only diseased counties are included = 0.52, $P = 0.37$; year included in the model).

Because we had a relatively large number of diseased specimens of *S. virginica*, we also examined whether the collections provided an adequate description of the disease distributions in a manner analogous to our analysis for the host distributions (see Materials and Methods: Collection coverage). The analysis showed that we were far from attaining complete coverage for the disease (Fig. 1). Almost every new

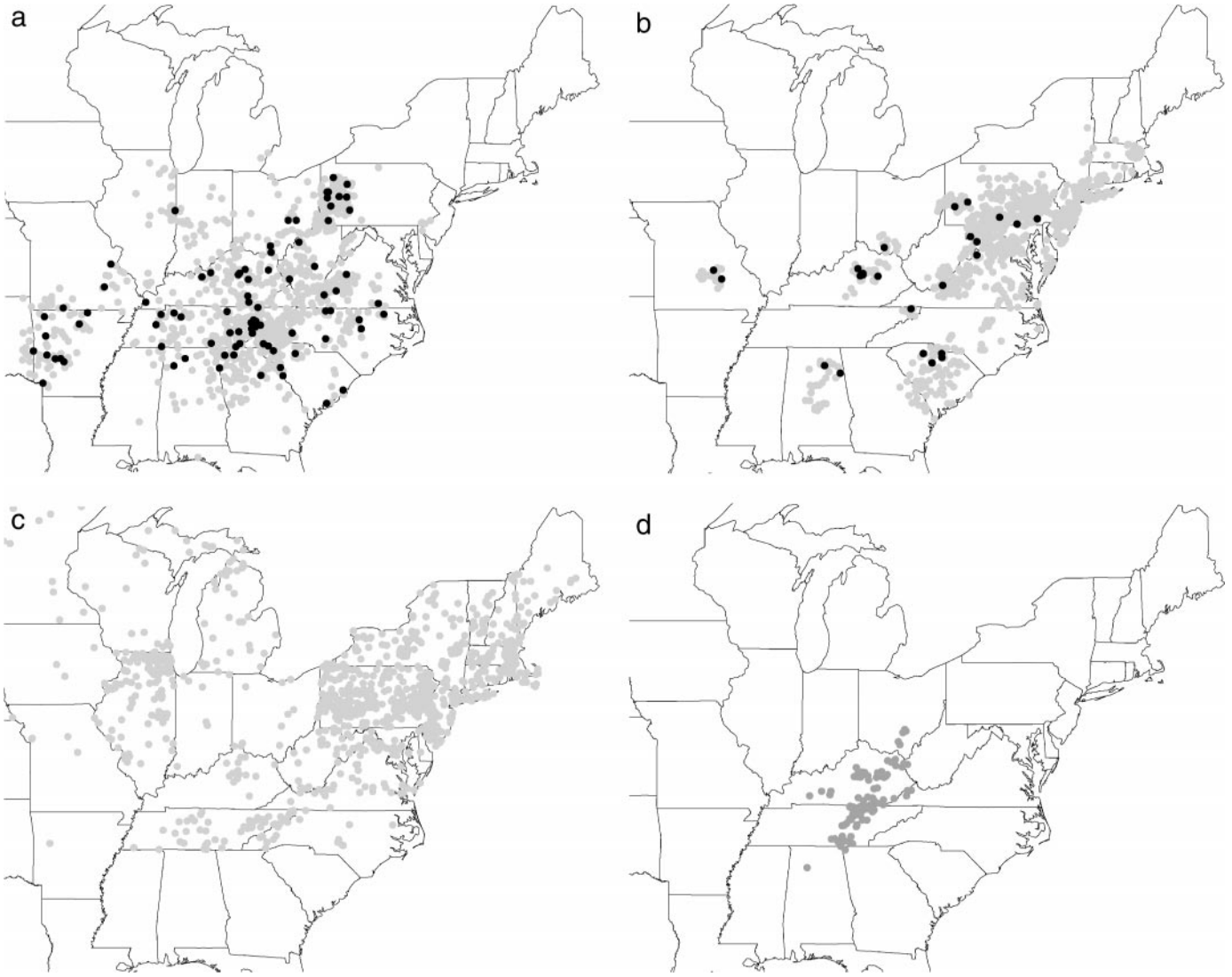


Fig. 2. The distribution of collections of four *Silene* species in the eastern United States. Diseased specimens are shown as black dots, and healthy specimens are shown as gray dots. Each dot is placed in a randomly generated location within the county of the collection's origin in order to minimize overlapping points. (a) *Silene virginica*. (b) *S. caroliniana*. (c) *S. latifolia*. (d) *S. rotundifolia*.

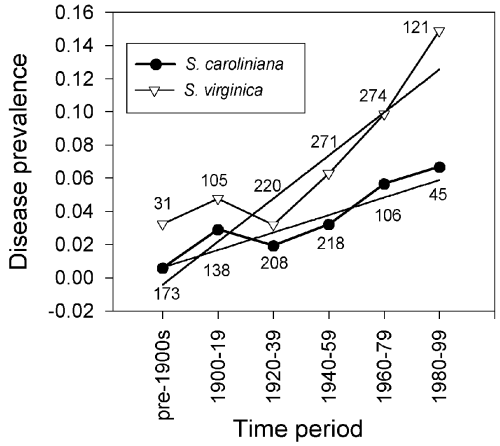


Fig. 3. The prevalence of disease in *Silene virginica* and *S. caroliniana* over 20-yr periods. Straight lines are regressions of prevalence vs. time weighted by number of specimens at each time (as indicated).

TABLE 2. Effect of year and number of specimens of *Silene virginica* sampled in adjacent counties on the likelihood of disease on a specimen in a focal county (including only those counties where diseased specimens had been found). The model estimates are based on multivariate logistic regression. One mile = 1.6093 km.

Effect	Slope estimate	Chi square	*P*
Four-variable model			
Year	0.018	9.01	0.0027
0–30 miles	−0.01	0.77	0.38
30–40 miles	−0.022	3.68	0.055
40–50 miles	−0.038	6.69	0.0097
Three-variable model			
Year	0.016	8.06	0.0045
0–30 miles	−0.026	6.42	0.011
30–40 miles	−0.031	9.08	0.0026
Two-variable model			
Year	0.019	13.36	0.0003
0–30 miles	−0.031	8.93	0.0028

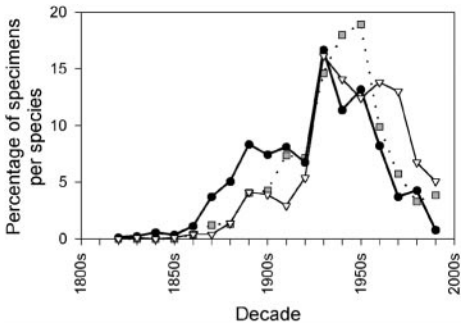


Fig. 4. The relative abundance of collections of *Silene caroliniana, S. latifolia*, and *S. virginica* over 10-yr intervals. Each data point represents the percentage of all the specimens for the particular species that was collected within the decade. See Fig. 8 for key to symbols.

collection was from a new county (there were a total of 85 collections from 73 counties). Because of the need to extrapolate well beyond the data, estimates of the percentage of counties in which the disease might be expected to occur differed greatly between replicate runs (each with 100 randomizations) but were generally between 40% and 100% of the total counties currently occupied by the host.

Silene caroliniana—In *S. caroliniana*, anther-smut disease was found in 25 of 888 collections (Fig. 2b). Analysis of disease frequency in 20-yr intervals indicated that the frequency of herbarium specimens that are diseased has increased significantly from ca. 0.6% before 1900 to ca. 6.7% for specimens collected after 1980 (Fig. 3). *Silene caroliniana* has a highly disjunct distribution, but the disease was found in specimens from all these disjunct regions. Three subspecies of *S. caroliniana* have been recognized (Wilbur, 1976). *Silene caroliniana* subsp. *pennsylvanica* occurs in all the eastern states from North Carolina to New England. *Silene caroliniana* subsp. *caroliniana* includes the plants in South Carolina, Georgia, and southern North Carolina. *Silene caroliniana* subsp. *wherryi* includes the populations located in Kentucky/Ohio, Tennessee, and Alabama/Georgia. These subspecies have been distinguished on the basis of detailed morphological characters (Wilbur, 1976). Using geographic location to define the subspecies, we found that anther-smut disease was present in all of the subspecies. However, disease frequency among the three subspecies was significantly different (disease frequency for *S. c. pennsylvanica* = 1.44%, $n = 693$; *S. c. caroliniana* = 4.49%, $n = 89$; *S. c. wherryi* = 10.58%, $n = 104$; $P < 0.0001$). Pairwise tests showed the overall significance was largely due to *S. c. wherryi* and *S. c. caroliniana* specimens having higher disease levels than *S. c. pennsylvanica*. The difference between *S. c. caroliniana* and *S. c. wherryi* was not significant, $P = 0.17$.

We also investigated disease relationships in the same way as in *S. virginica*, but almost none of the distance-related effects were significant, perhaps because the sample size of diseased specimens was much smaller than in *S. virginica*. With all counties included, the fraction of diseased specimens in a focal county correlated positively with the percentage fraction of disease in neighboring counties, although the only significant values were for neighbors within 30–40 miles (logistic regression = 0.089, $P = 0.074$) and 40–50 miles (logistic regression = 0.042, $P = 0.028$). When the analysis included only those focal counties with disease, the trend was similar but no values were significant (P values for effect of neighbor distance classes = 0.36–0.80).

TABLE 3. Comparison of geographical changes according to species of *Silene*. The data represent the number of collections for each species in each region for four equally divided temporal quartiles (see Fig. 5 for discussion of temporal quartiles).

Species	Quartiles 1	2	3	4	Total of region	Percent of total
New England, All	161	29	13	19	222	7.37
S. latifolia	103	26	12	18		
S. caroliniana	58	3	1	1		
S. virginica	0	0	0	0		
Mid-Atlantic, All	386	260	322	122	1090	36.16
S. latifolia	100	147	203	72		
S. caroliniana	237	85	84	25		
S. virginica	49	28	35	25		
Midwest, All	71	149	157	208	585	19.41
S. latifolia	24	49	79	81		
S. caroliniana	9	40	30	30		
S. virginica	38	60	48	97		
South, All	103	275	224	354	956	31.72
S. latifolia	3	37	34	50		
S. caroliniana	33	85	71	79		
S. virginica	67	153	119	225		
West, All	32	47	33	49	161	5.34
S. latifolia	21	22	10	13		
S. caroliniana	3	8	1	5		
S. virginica	8	17	22	31		
Total of quartiles	753	760	749	752	3014	100
Percent of total	24.98	25.22	24.85	24.95		

S. latifolia and S. rotundifolia—No disease was found in the collections of these species (Fig. 2c, d).

Host distribution—Collections of the three most abundant species were most frequent between 1930 and 1960 (Fig. 4). *Silene caroliniana* was the most commonly collected species before 1910, while *S. virginica* was most commonly collected after 1960. The relative changes in collection frequencies were highly significant ($P < 0.0001$ for year × species interaction, logistic regression). To examine whether these changes were different in different regions, we divided the overall number of collections into four time periods, each with an approximately equal number of collections (i.e., quartiles), and examined whether the relative abundance of the species differed among regions over the time quartiles (Table 3). The results had a highly significant interaction for region × quartile × species ($P < 0.0001$, Proc Catmod in SAS), showing that the relative abundance of the species also changed differently in the different regions.

Visual inspection suggested that there had been substantial change in the geographical range of the collections of the three more abundant species. In *S. latifolia*, collections were largely restricted to the Northeast and Mid-Atlantic regions before 1930 (Fig. 5a, b). After that, the collections become more abundant both in the South and Midwest regions.

In *S. virginica*, the overall collection distribution remained unchanged, but there was an increase in the frequency of collections in the South and West regions (Fig. 6a, b).

In *S. caroliniana*, the early collections were mainly from

the Mid-Atlantic region, while later collections extended its distribution to Ohio, Kentucky, and Missouri (Fig. 7a, b). There was a substantial increase in the number of collections in the South and a decrease in the number of collections in the Mid-Atlantic region. Statistical analysis of average distance of the collections from a set point confirmed the trend of increasing numbers of collections towards the south and west for all three species (Fig. 8).

For *S. rotundifolia*, there was no evidence of change in abundance or distribution with time (Fig. 2d).

DISCUSSION

Disease distribution—Our study shows that it is possible to identify herbarium specimens of *Silene* that are diseased with anther smut and that such specimens provide information on temporal and spatial patterns of both host and pathogen distribution. Thus, our data show that the incidence of anther-smut disease in both *S. virginica* and *S. caroliniana* has increased over the previous century. It is very unlikely that these results are caused by any increase in tendency to collect diseased specimens. Indeed, we could find no evidence that diseased specimens were recognized by collectors, let alone that the propensity to collect such specimens had increased with time. We only found one herbarium sheet (of *S. virginica*) that was annotated to indicate the diseased specimens were in any way unusual, and then it was not recognized as diseased—the label simply noted that the plants had ''purple stamens.'' In *S. virginica*, the disease is relatively inconspicuous because the flowers have deep red petals and dark-colored anther sacs. Indeed, the disease was first noted on *S. virginica* in 1988 in a natural roadside population (Antonovics et al., 1996), even though it was collected more than 100 years ago! In *S. caroliniana*, diseased plants are much more easily recognizable by their dark anthers, and this may have introduced some bias. However, a systematist who had extensively examined both field and herbarium material of this species (Wilbur, 1976) had never noticed the disease (R. Wilbur, Duke University, personal communication), suggesting that collection bias by other investigators might have been minimal.

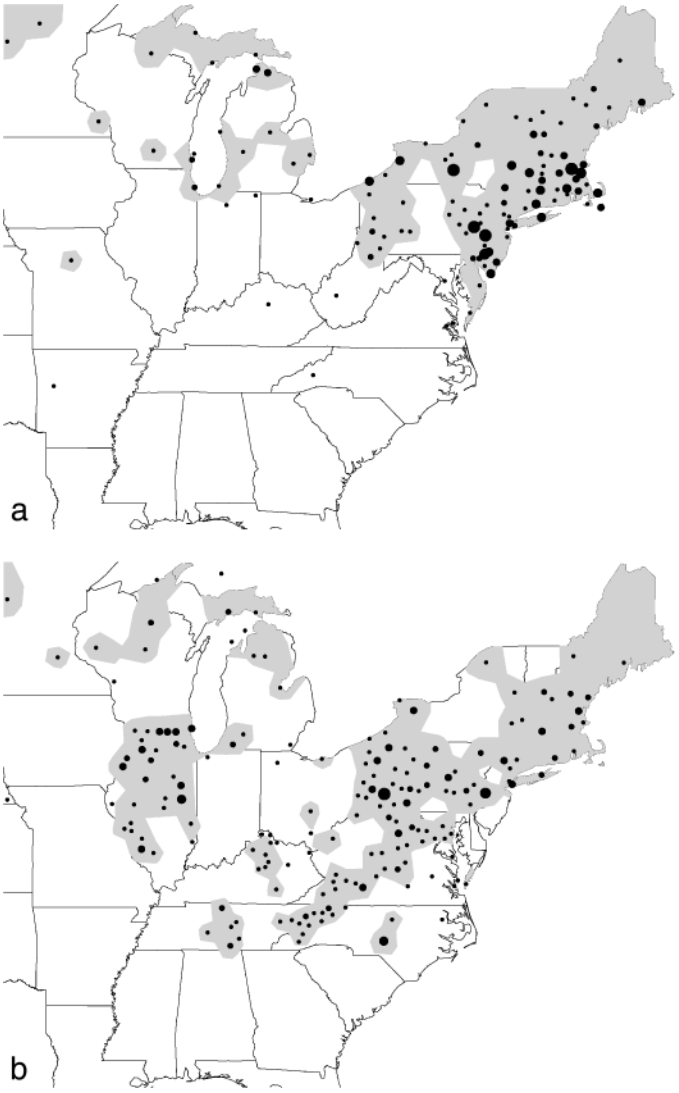
Explaining the reason for the increase in anther smut on these species is more difficult. In both *S. virginica* and *S. caroliniana*, diseased specimens were more likely to have other diseased specimens in neighboring regions, suggesting an ''epidemiological signal'' in the data; however, this relationship was only significant in *S. caroliniana*. There was no evidence that counties where more plant specimens had been collected had more disease. This suggests that counties with more specimens (and therefore possibly more populations) do not result in greater disease incidence. Rather the trend was in the opposite direction in *S. virginica* in that there was a highly significant *negative* relationship between disease incidence and the overall number of specimens collected in the immediately surrounding counties. In *S. caroliniana*, the trend was in the same direction, but it did not approach significance. One explanation for this pattern is that disease might be more common in recently established and/or smaller populations.

Our results show that all three subspecies of *S. caroliniana* are susceptible to the disease, and the pathogen occurs in highly disjunct regions of the host. This supports the idea that this host–pathogen association is a very old one. It is interesting that *S. c.* subsp. *pennsylvanica* has a lower disease incidence than the other two subspecies, but the reason for this is unclear. Transfer of the disease between *S. caroliniana* and *S. virginica* cannot be excluded. We have found diseased populations of each species within several hundred meters of each other, and although *S. caroliniana* generally flowers earlier in the season, there is substantial overlap in their flowering times. The two species are also known to hybridize (Mitchell and Uttal, 1969). We have found two obviously hybrid populations in the field, but neither was diseased with anther smut. We therefore do not know if such hybrids might form a bridge through which host shifts might occur. The issue of whether *Microbotryum* represents one or two host races on these two native North American species needs to be resolved experimentally and phylogenetically.

No diseased plants were seen in the collections of *S. rotundifolia*. Far fewer collections of this species were available, but no disease has been seen in numerous populations that were visited in the field as part of a study investigating genetic differentiation in this species (Leonie Moyle, University of California, Davis, personal communication). *Silene rotundifolia* is a habitat specialist found almost exclusively on sandstone bluffs in West Virginia and Tennessee. Although it is often close to populations of *S. virginica* and also has bright red flowers that are hummingbird pollinated, it flowers in August, much later than *S. virginica*. There have been no studies of the susceptibility of *S. rotundifolia* to anther-smut disease.

Because our own research has been focused largely on anther smut on *S. latifolia*, we examined numerous herbarium specimens of this introduced species in an attempt to understand its disease history. We were therefore surprised that we found no diseased specimens of *S. latifolia* in over 1000 herbarium sheets that we examined. This may be due to collection bias (the disease is particularly conspicuous on *S. latifolia* because of the white flowers and because female plants produce diseased anthers and have aborted ovaries). Alternatively, the disease on this species may be a relatively recent introduction from Europe. We know from field studies that diseased *S. latifolia* are commonly found in the mountains and western regions of Virginia (Antonovics et al., 1996), and recent studies have recorded it occasionally in Pennsylvania (A. M. Jarosz and E. Lyons, Michigan State University, personal communication) and Michigan (J. Antonovics, University of Virginia, personal observation). The disease has not been found in New England, but does occur on Nantucket Island (T. Meagher, University of St. Andrews, UK, personal communication). One possibility that might explain the present-day distribution of disease is that *M. violaceum* has ''followed'' the relatively recent spread of *S. latifolia* into the southeastern USA and that it has been largely lost in areas where *S. latifolia* has been established for a long time. Therefore, in Virginia, where most of our studies have been carried out, both the host and the disease are probably recent introductions. Previous studies have shown that the anther-smut fungus on *S. latifolia* is a host-race distinct from that found on *S. virginica* (Antonovics et al., 1996) and *S. caroliniana* (Perlin et al., 1996). This study confirms that the disease on *S. caroliniana* and *S. virginica* could not have come from the introduced *S. latifolia*, because disease was present on the native species well before the spread of *S. latifolia* into regions of sympatry.

Several other introduced and native species of *Silene* occur in the eastern United States. With the exception of *S. vulgaris*, we have not seen anther smut on any of the introduced species. In Virginia, we have frequently observed *S. noctiflora* L. and *S. antirrhina* L. sympatric with diseased *S. latifolia*. However,



Figs. 5–7. The difference in geographic range in the first temporal quartile of collections, top, and in the fourth quartile, bottom. The quartiles consist of an equal number of collections, rounded to the year. The dots represent occupied counties, and the size of the dots reflects the number of collections made in that county. The ranges were created in ArcView by making contour lines using inverse distance weighting (12 nearest neighbors, power of two). The shaded areas are bounded by the halfway distance between the contours representing zero and one plant per county, but with a regional weighting such that most isolated points are excluded. This method has some bias because it overestimates the range near borders and near large counties. In all maps due north is toward the top of the figure. Fig. 5 (above). *Silene latifolia*. The first quartile (a) contains collections made before 1930, and the fourth quartile (b) contains collections made from 1958 to the present.

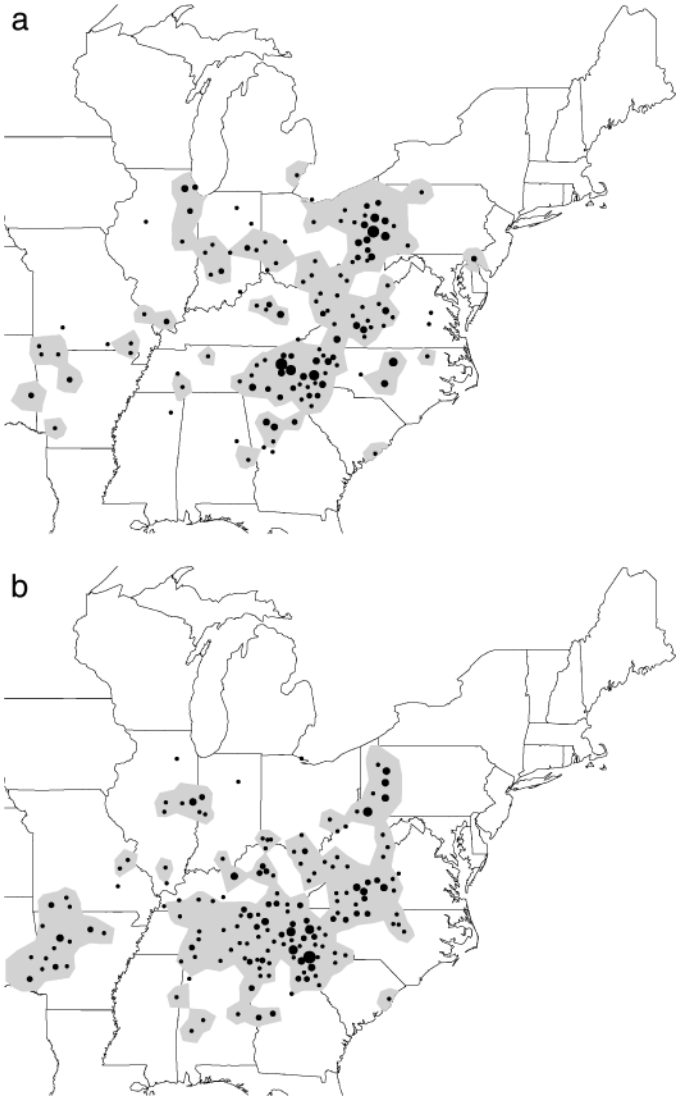


Fig. 6. *Silene virginica*. The first quartile (a) contains collections made before 1934, and the fourth quartile (b) contains collections made from 1970 to the present.

these species are annuals and disease persistence on them is unlikely (Thrall et al., 1993). Moreover, the flowers of *S. antirrhina* are small and inconspicuous, and this species is almost certainly highly self-pollinated and/or partially cleistogamous. *Silene vulgaris* (Moench) Garcke is perennial, and while populations are generally free of anther smut, we have found a population with diseased plants of *S. vulgaris* growing with heavily diseased *S. latifolia*. This represents a recent and local host-shift (Antonovics et al., 2002) and a similar host-shift has been observed in Europe (Hood et al., 2003).

Two other native species of *Silene* occur in the eastern United States. *Silene stellata* (L.) Ait. is quite common, but we have never found anther smut on this species. *Silene ovata* Pursh. is a much rarer species restricted to relatively few forest glade sites in the southern Appalachians. Although it has not been observed diseased in nature, a few plants did become accidentally diseased with anther smut when growing in pots near diseased *S. latifolia* in a greenhouse (L. Moyle, University of California, Davis, personal communication). This suggests that *S. ovata* populations may be very susceptible to anther smut but remain disease free because they are spatially and temporally isolated.

Host distribution—Although we gathered the herbarium data primarily to assess disease incidence, this data also provides interesting information about host distribution. Above all, it points to the potential usefulness of herbarium data in establishing the current and historical ranges of plant species and in indicating long-term changes in distributions that are useful indicators of habitat change, species decline (Meagher

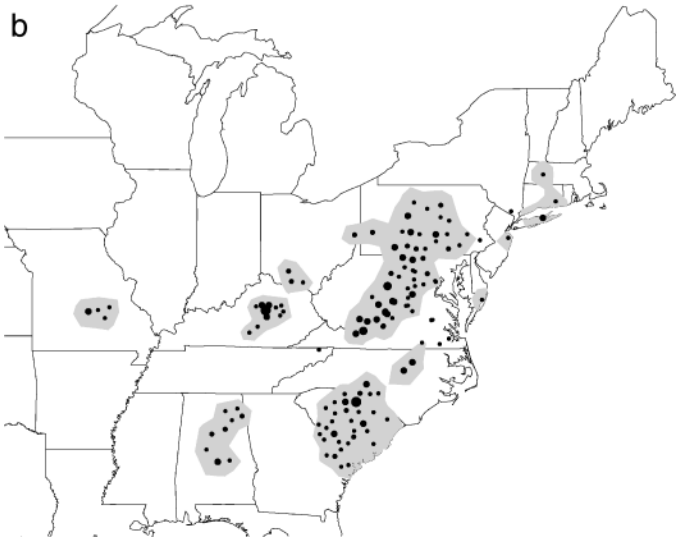


Fig. 7. *Silene caroliniana*. The first quartile (a) contains collections made before 1907, and the fourth quartile (b) contains collections made from 1955 to the present.

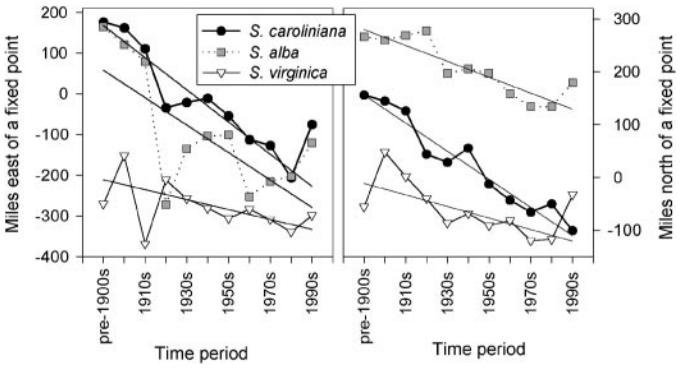


Fig. 8. The average latitude and longitude of *Silene latifolia*, *S. caroliniana*, and *S. virginica* over 10-yr intervals, expressed as miles north and east of a fixed point (specifically Charlottesville, Virginia; 1 mile = 1.6093 km). All trendlines are linear and weighted according to the number of specimens per decade.

et al., 1978; Kiang et al., 1979), or invasions (Forcella et al., 1986; Forcella and Harvey, 1988). Such long-term studies have been routine in many European countries with a strong tradition of recording and collection (Preston et al., 2002), but they are much rarer in the United States.

Our studies show the enormous potential of herbarium data in delimiting the distribution of species in the United States. Even though we obtained specimens from only 28 herbaria, the effectiveness of the coverage was very high. Thus, in the three most abundant species, we estimated that we had identified well over 80% of the counties in which the species were likely to occur. Adding between 500 and 1000 samples (if the specimens actually exist) would result in close to 100% coverage. Clearly these figures are very approximate, as they do not take into account ongoing changes in plant distribution or regional biases in herbarium collections. To examine this we compared our coverage with the coverage as indicated in published county level distribution maps for Pennsylvania, Virginia, North Carolina, and South Carolina. There was clear evidence of ''herbarium bias.'' For example, in Pennsylvania and South Carolina, our county coverage corresponded well (between 82.0 and 91.94%, averaged over all species except *S. rotundifolia*) with the published distribution because we sampled the major herbaria in these states. However, in Virginia and North Carolina our coverage was poor (between 51.0 and 54.7%). We were unaware at the time of the substantial collections at Longwood College in Virginia, and we inadvertently did not sample the University of North Carolina herbarium because we moved institutions halfway through the study.

Indeed, it would be interesting to quantify the incremental contribution of added samples within herbaria vs. added samples from different herbaria. Like species diversity in communities, we can envisage that the diversity of herbarium collections could be partitioned into categories analogous to those used by community ecologists, namely alpha (or within-herbarium) and beta (between herbarium) diversity. Although we have not done this, our overall analyses point to the fact that it is possible to *quantify* to a remarkable degree the adequacy of herbarium sampling in describing the distribution of any particular species. In contrast to the host species, our results for anther smut show that the effective sampling of the disease has been very *inadequate* because adding more specimens of the host still leads to a high probability of detecting counties where the disease has not yet been discovered (see Fig. 1, *M. violaceum* on *S. virginica*).

This study shows large changes in the distribution of the collections of three of the four species that we studied (in *S. rotundifolia* the number of collections was too few to discern any clear trends). The main question that arises is whether these changes represent collection bias or whether they truly reflect changes in plant distribution. Thus, the increase over time in the number of collections made toward the south and west is consistent not only with the idea that human disturbance and movement in these areas occurred later than in New England and the Mid-Atlantic states, but also with the fact that there has been a greater increase in academic institutions in these areas over the past century. Prather et al. (2003) also found that herbaria in the southeastern United States started collections later than did those in the northeast and that their period of peak collection was also much later. Their results were generally consistent with ours with regard to overall col-

lecting frequency, which peaked between 1930 and 1950 (even though we used different focal species and fewer herbaria in our study).

However, real changes in species abundance are likely to have taken place, as judged from the *relative* frequency of the collections of the species that we have studied. The decrease in the abundance of *S. caroliniana* collections relative to the number of collections of the other common species strongly suggests that the abundance of this species may be declining, especially in New England and the Mid-Atlantic. This is consistent with our own natural history observations that the populations are often highly fragmented, very small, and absent in spite of good locality data from herbarium records. Our studies also show clearly that *S. latifolia* was largely confined to New England in the earlier part of this century but then spread southwards. The first collection of *S. latifolia* further south than Pennsylvania or New Jersey was in 1924, and there were no collections from Kentucky until 1950. Given the large number of collections and the fact that flowering plants are very conspicuous, the earlier absence of *S. latifolia* in the southern and western United States is unlikely to be due to collecting bias. *Silene latifolia* is itself introduced into the United States from Europe (McNeill, 1977), and our studies indicate that this introduction was initially in the New England region (or perhaps Canada).

A number of previous studies have emphasized the importance of herbarium specimens in understanding not just species distributions but also other important aspects of plant biology. In this study, we have shown that herbarium specimens can be valuable in showing changes in both disease abundance and host distribution. We have also shown that it is possible to assess the completeness (or precision) of the coverage provided by such data and to approach the problem of collection bias (or accuracy) by focusing on relative rather than absolute abundances of collections. The advent of computer-based imaging and record keeping is likely to greatly increase accessibility and hence the usefulness of herbarium data for these types of study in the future.

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