

Vector Behavior and the Transmission of Anther-smut Infection in *Silene alba*

SONIA M. ALTIZER¹

*Department of Ecology, Evolution and Behavior, University of Minnesota, 100 Ecology Building,
1987 Upper Buford Circle, St. Paul 55108*

PETER H. THRALL AND JANIS ANTONOVICS

Department of Botany, Duke University, P.O. Box 90338, Durham, N. Carolina 27708

ABSTRACT.—Anther-smut disease caused by *Ustilago violacea* is transmitted by insects that visit diseased flowers and then deposit spores on healthy *Silene alba* plants. The transmission rate of this disease therefore depends on spore deposition by the vector species and preferences for infected vs. healthy hosts. We observed natural populations of *S. alba* to document the most abundant insect visitors, which include syrphid flies, andrenid bees, bumblebees and a variety of nocturnal moths. Using bumblebees and moths in flower preference studies, we showed that bumblebees preferentially visited healthy flowers, but the strength of this preference declined if the bees had prior exposure to diseased flowers. Nocturnal moths showed less discrimination with respect to disease status when plants were arranged in a field population of *S. alba*, and preferentially visited plants with more flowers. A laboratory analysis of spore deposition by bumblebees showed that whereas most spores were deposited on the first several flowers visited, flowers beyond the 15th visit may still receive enough spores to produce a new infection. Spore deposition was also influenced by changes in vector behavior associated with spacing in artificial arrays of *S. alba* plants. Models were fitted to deposition data to compare estimates for spore dispersal rates and the initial number of spores acquired by vectors. This study demonstrated several ways that vector behavior can influence rates of disease spread in natural populations. Preferences for healthy vs. infected hosts can cause disease dispersal to vary from that expected by randomly foraging insect vectors. Changes in visitation behavior associated with host density can affect the magnitude and distance of spore deposition in host populations.

INTRODUCTION

The population dynamics of infectious diseases are strongly influenced by the transmission mode by which healthy individuals are exposed to infection (Anderson and May, 1981; Getz and Pickering, 1983; Antonovics *et al.*, 1995). For arthropod-borne plant diseases, the foraging behavior of insect vectors may affect rates of disease spread (Real *et al.*, 1992; Roche, 1993; McElhany and Real, 1995). Disease dispersal in such systems is likely to depend on vector movement distances, the degree of discrimination towards healthy vs. infected hosts, and the rate at which infective propagules are acquired and dispersed to new hosts (Real *et al.*, 1992; Antonovics *et al.*, 1995).

A well-studied example of a vector-borne plant disease is the anther-smut infection of *Silene alba* caused by the fungus *Ustilago violacea*, in which infected hosts produce flowers with fungal spores rather than pollen (*e.g.*, Baker, 1947). Insects that visit infected flowers can deposit spores on healthy plants. Anther-smut infections have been described as sexually transmitted diseases of plants, and have been shown to share characteristics with both vector-mediated and venereally spread diseases of animals (Alexander and Antonovics, 1988; Thrall *et al.*, 1993; Lockhart *et al.*, 1996).

¹ Corresponding author

The *Silene-Ustilago* interaction has become a model system for studying the dynamics of host and pathogen populations (Alexander *et al.*, 1994; Antonovics, 1994; Antonovics and Thrall, 1994; Antonovics *et al.*, 1994; Thrall and Jarosz, 1994b; Thrall *et al.*, 1995; Thrall and Antonovics, 1995; Alexander *et al.* 1996). Previous work on this system has focused on general models of disease dynamics, the population biology of host and pathogen, and the effects of genetic composition of host populations (*i.e.*, variation in resistance) on disease dynamics. However, spore transfer by insects can also affect disease dynamics (Roche, 1993; Shykoff and Bucheli, 1995). Computer simulations based on insect foraging patterns have demonstrated that vector behavior can interact with disease incidence in affecting rates of disease spread (Real *et al.*, 1992). These simulations showed that vectors discriminating against infected plants can result in higher rates of transmission when the disease is common, but lower transmission rates when the disease is rare, compared with indiscriminating vectors.

The focus of the present study was to determine the potential effects of vector behavior on disease transmission in the *Silene alba-Ustilago violacea* system. This behavior includes not only preferences for healthy vs. infected hosts, but also rates of spore deposition and changes in vector movement associated with plant spacing. We first identified the insect species that are the most abundant visitors in local populations of *S. alba*. Various insects, including moths, syrphid flies, butterflies and several types of bees are known to visit the flowers of *S. alba* (Alexander, 1990; Roche, 1993). Studies of fluorescent dye transfer in experimental populations of *S. alba* indicate that nocturnal visitors disperse dye to more flowers than diurnal visitors (Shykoff and Bucheli, 1995). However, the relative abundance of diurnal and nocturnal visitors has not been formally investigated. We therefore conducted observations in natural populations of *S. alba* to document daily patterns of insect activity and to identify the most likely vectors for *U. violacea* spores.

The second objective of this study was to determine whether insect vectors discriminate between healthy and diseased flowers of *Silene alba*. Smutted flowers have dark centers due to extrusion of spores from the anthers, which could enable pollinators to identify infected plants. However, nectar rewards are available from flowers of both healthy and diseased plants (Roche, 1993; Shykoff and Bucheli, 1995). Since vectors must visit both healthy and diseased flowers to spread the infection, any biases with respect to host disease status may influence the rate at which the disease spreads (Real *et al.*, 1992).

Knowledge of how spore dispersal varies with distance is essential to modelling disease spread. The number of spores deposited on healthy hosts is likely to influence how infection probabilities vary with distance from diseased plants. Past work suggests that a threshold number of spores may be required to achieve infection, and that infection probability increases nonlinearly with spore deposition (Roche *et al.*, 1995). We therefore quantified the number of spores deposited sequentially by bumblebee vectors in the laboratory. In natural populations, spore dispersal will also depend on vector foraging patterns that may vary with plant spacing. Densely spaced patches of plants should attract higher numbers of vectors, whereas individual vectors may visit more flowers per plant in widely spaced patches. We therefore established field arrays of plants using three different interplant distances to measure how spore deposition varies with plant spacing. Models of spore deposition were then fitted to laboratory and field deposition data to characterize these spore dispersal patterns.

METHODS

NATURAL HISTORY OF THE STUDY ORGANISMS

The white campion, *Silene alba* (Miller) Krause, [= *S. latifolia* Poir.; = *S. pratensis* (Spreng) Gren.& Godr.; = *Lychnis alba* Miller; = *Melandrium album* (Miller) Garcke], is a

dioecious perennial commonly found in highly disturbed habitats (McNeill, 1977). Female flowers have long white stigmas and a large calyx that swells around the developing ovaries. Male flowers are somewhat smaller, and male plants produce more flowers per unit time than female plants. Male plants also bloom longer throughout the flowering season, probably because the female plants halt flower production once they have set fruit. *Silene alba* flowers open before dusk and remain open through the following morning. The peak flowering time ranges from late May to mid-July, although flowering can occur throughout the entire summer.

The anther smut disease *Ustilago violacea* (Pers) Fuckel (= *Microbotryum violaceum* (Pers) G. Deml & Oberw.) is an obligate fungal pathogen on a wide range of hosts within the Caryophyllaceae, including *S. alba* (e.g., Zillig, 1921; Alexander and Antonovics, 1988; Thrall *et al.*, 1993). Floral infection occurs when insects that visit diseased flowers deposit fungal teliospores on healthy plants (e.g., Alexander, 1987; Alexander and Antonovics, 1988, Jennersten, 1983, 1988). By comparing vegetative vs. floral infection rates, Roche *et al.* (1995) concluded that although vegetative plants can become infected close to inoculum sources, most new infections are due to spore deposition by insect visitors. Heterokaryotic infection hyphae then spread systemically throughout the plant, causing flowers on diseased branches to produce *U. violacea* spores (Baker, 1947). It has been shown that diseased plants tend to flower more profusely than healthy plants (Jennersten, 1988; Alexander and Maltby, 1991). Once the infection grows into the root stock, it can overwinter and cause the host to produce spore-bearing flowers in subsequent seasons.

STUDY SITES

Studies were carried out in the vicinity of the Mountain Lake Biological Station (University of Virginia) in Giles Co., Virginia, an area where both healthy and diseased *Silene alba* populations flower prominently along roadsides and fields (Antonovics *et al.*, 1994; Thrall and Antonovics, 1995). Observations of insect visitors were made between mid-June and early-August during the summers of 1992 and 1993. These observations were conducted in three separate populations of *S. alba* located within 16 km of each other. One of these populations was situated along a steep bank parallel to railroad tracks and the New River, along Route 682, Giles County. The other two populations were located in fields used for grazing cattle along Route 601, Giles County. Studies involving flower preference and spore carryover were performed on the Biological Station grounds and in nearby open areas during the summers of 1992 and 1993.

NOCTURNAL AND DIURNAL INSECT VISITORS

To identify potential disease vectors, we conducted observations in 1-m² patches of *Silene alba* in three different roadside populations (mean flower density = 58.22 flowers/m²; sd = 16.69; N = 32 observations). We recorded the number and species of insects observed within these patches during hour-long intervals throughout the day. In 1992, two diurnal observations were made every other day between 16–23 June, for a total of eight observations. In 1993, both diurnal and nocturnal observations were conducted every 2 wk between 14 June and 9 August, for a total of 10 diurnal and 14 nocturnal observations. Diurnal observations were conducted between 0930–1800, and nocturnal observations were conducted between 2000–2330.

In 1993, a weatherproof video recorder (Sony Fieldcam WCMS 6, with LED infrared illumination and infrared-sensitive remote camera) was placed in two of the three observational areas to record nocturnal visitors. The number and type of visitors observed in the 1m² field of view of the camera were noted for each hour-long observation period. Record-

ings were obtained on 21, 23 and 24 July for each hour between 2000–0600. Video recorder data were averaged with nocturnal and diurnal observations from 1993 to determine changes in the abundance of pollinators across a 24-h period.

FLOWER PREFERENCES OF VISITORS

Preferences in bumblebees.—Flower choice experiments were conducted using wild-caught bumblebees (*Bombus impatiens* and *B. affinis*) in an enclosed artificial array of *Silene alba* flowers. Bumblebees of both species were similar in size and appearance, and both were observed visiting *S. alba* flowers in the field. All bees were brought into the laboratory following capture and placed in small screened enclosures. Each bee was identified to species (Mitchell, 1962) and marked with a small numbered label. The bees were fed by adding 30% honey-water to *S. alba* flowers arranged in eppendorf tube racks. Bumblebees were provided with either only healthy or only diseased flowers for 48 h before the choice tests to train them to visit these two flower types.

Flower preferences of bees previously exposed to healthy or diseased flowers were tested using a “bee board” based on the design of Dukas and Real (1991). The board was enclosed in a 1.2-m × 0.8-m × 0.35-m wooden box fitted with a plexiglas cover. A two-dimensional grid of holes, 1 cm in diam and 6 cm apart were drilled in the bottom of the box (14 rows by 10 columns, for a total of 140 holes). Each hole was fitted with a small plastic tube to which water and a single flower could be added. Thirty-five healthy and 35 diseased flowers were randomly assigned to numbered positions on the bee board. Flowers for all bee board studies were collected from potted plants in mosquito-net cages (to protect from insect visitation). Bumblebees were chilled before each trial to sedate them and were individually released into the center of the bee board to forage. As a bee visited the flowers, we recorded the position, flower type (healthy vs. diseased) and visit type (land or probe). A visit was recorded as a probe when the bee inserted its proboscis into the flower, or as a land whenever the bee physically contacted a flower but did not attempt to feed. Individual bees visited 10–25 flowers before being removed from the arena. Bees occasionally visited the same flower more than once (*e.g.*, 19 out of 83 probes were repeat visits). Flowers that had been visited were replaced by fresh flowers following each bumblebee trial. Preference data was recorded using seven bees previously exposed only to healthy flowers, and seven bees previously exposed only to diseased flowers.

Preferences in nocturnal moths.—Moth flower preference was evaluated using the infrared-sensitive video recorder in an open field populated with patches of healthy *Silene alba*. Four potted plants (healthy male, healthy female, diseased male and diseased female) were arranged at random in a 1-m² area so that they could be easily distinguished in the camera’s field of view. Diseased female plants were identified by the presence of undeveloped ovaries at the base of each flower. The number of flowers on each plant was recorded. A different set of plants was used for each taping interval. The video recordings were taped continuously from 2100–2300 on 30 July, 5 August and 6 August 1993. The tapes were then viewed in the laboratory, and for each individual moth visitor the following information was recorded: type of moth (family), type of plant visited, number of flowers visited per plant and time spent on each flower.

SPORE DEPOSITION BY VECTORS

Deposition by bumblebees.—We evaluated how spore deposition varies across flower visitation sequence for a single vector species in a controlled environment. Although several diurnal and nocturnal insects were common visitors of *Silene alba* (*see Results*), our laboratory investigation focused on spore dispersal by bumblebees since we frequently observed

them visiting *S. alba* and they will readily forage on artificial arrays of flowers. Individual bumblebees not used in the preference experiment were forced to visit diseased flowers by placing a jar over both the bee and a diseased flower until the bee was observed probing the flower. This procedure was repeated until the bee had probed one, two or three infected flowers (predetermined before trial). The jar was then removed and the bee was released to forage on an array of 80 healthy flowers in the center of the bee board (eight rows by 10 columns). Flower position and the type of visit (probe vs. land) was recorded sequentially for each flower the bee touched. Bees were removed from the bee board after visiting a maximum of 25 flowers. Fewer bees are represented in the multiple-diseased flower visits, as it was difficult to force bees to sequentially visit more than one diseased flower.

Flowers that had been visited were collected after each trial and placed into small labelled vials containing 1 ml of 45% ethanol. Following a previously established protocol for estimating spore deposition on flowers (Alexander and Antonovics, 1988; Roche *et al.*, 1995), vials were vortexed for 60 sec to remove any fungal spores from the flower. The flowers were then discarded, and the vials were stored for later spore counts. For each vial, spores were counted using a hemacytometer; counts were made for eight squares, each of volume 1×10^{-4} ml. The average of these counts was used to estimate the total number of spores per ml, which was considered to be an estimate of the number of spores deposited per flower.

Data were obtained for a total of 22 bee foraging runs, in which 13 bees were forced to visit one diseased flower, six bees were forced to visit two diseased flowers, and three bees were forced to visit three diseased flowers. Flowers that were visited more than once were not included in the data set for each bee.

Spore dispersal in linear arrays.—In nature, disease spread will depend not only on spore deposition rates, but also on the spatial arrangement of plants within populations. Roche *et al.* (1995) investigated disease dispersal in two-dimensional arrays of plants and found no directional bias in spore dispersal gradients. We therefore used linear arrays of plants to investigate the effect of plant spacing on spore dispersal from a disease source. We created linear arrays of *Silene alba* along several miles of a mowed powerline strip adjacent to Mountain Lake Biological Station. Twelve arrays were established in June of 1993; each array consisted of 30 potted plants separated by an infected plant (in the center of the array). Thus, each array contained two branches of 15 potted plants each. Array branches were randomly assigned to a spacing of either 0.5 m, 1.0 m, or 2.0 m between plants, so that each plant spacing was replicated eight times. Arrays were separated by a minimum distance of 50 m. We recorded plant sex and counted flowers on a weekly basis between 29 June–10 August 1993. Three wk after the plants began to flower, we collected one flower per plant on the following dates: 20 July, 27 July and 3 August 1993. These flowers were placed in vials and vortexed in ethanol as described above. Spore estimates for each flower were obtained using the hemacytometer technique described above. Plants were removed from arrays on 15 August and checked for transitions to infected status through 14 October 1993.

STATISTICAL ANALYSIS

Flower preference in bumblebees.—We used analysis of variance to investigate bee preference in two ways. First, we examined the effect of training type (*i.e.*, whether exposed to healthy or infected flowers) on the total number of visits by bees to healthy vs. diseased flowers. In a second analysis, we examined the effect of training type and status of flower visited (healthy vs. diseased) on the type of visit (land or probe; dependent variable = % flowers probed). In this case, the effect of individual bees was nested within training type, although

we did not test for preference differences between the two bumblebee species. Data on percent probes were arcsin-transformed for analysis and weighted by the number of visits for each bee.

Flower preference in moths.—Because portions of our videotapes of moth visitation were damaged, we could only evaluate moth activity for a total of 3.5 h over the 3 nights of observation. For this 3.5 h interval (2 h night 1, 1 h night 2, 0.5 h night 3), a total of 33 moths were observed visiting the four types of plants (healthy male, healthy female, diseased male, diseased female). Of the moths, three were sphingid moths, five were geometrids, and 25 were noctuids.

Twelve of these moths visited more than one plant in the array. The disease status of a visited plant did not influence whether or not the moth left the array or went to another plant in the array (contingency chi square = 0.01, d.f. = 1, $P = 0.954$) or, if it stayed, whether the next plant visited was diseased or healthy (contingency chi square = 2.28, d.f. = 1, $P = 0.131$). Therefore, the data for all separate moth visits to each plant were included as independent data points, and in the analyses, the number of moth visits refers to number of independent arrivals of a moth to a plant, whether or not that moth had previously visited another plant in the array. This yielded a total of 60 moth visitation events with an average of 2.7 flowers visited per plant.

We used analysis of variance to investigate the effects of night and plant type (sex and disease status) on the number of moth visits per hour. However, because we found that plants with more flowers received more visits per hour (slope of regression of visit per hour on the number of flowers per plant = 1.33; $P = 0.0005$), we also analyzed visitation rates on a per flower basis. We then investigated whether, given that a plant received a moth visit, the number of flowers visited subsequently on that plant was affected by sex or disease status.

Analysis of spore deposition.—A model developed to describe pollen dispersal was fitted to spore deposition data from laboratory studies and field arrays. Maximum likelihood estimates of the average initial number of spores acquired by bees from diseased flowers (S_0) and the mean fraction of spores remaining on bees that were deposited on visits to healthy flowers (λ) were obtained under the assumption of a constant deposition rate (Morris, 1993). The spore carryover function can be written as

$$S(n) = S_0\lambda(1 - \lambda)^{n-1} \quad (1)$$

where n is the n^{th} visit (following an initial visit to a diseased flower) and $S(n)$ is the number of spores deposited on the n^{th} visit. To obtain estimates for the linear arrays, we averaged deposition values across the three sampling dates but did not average across array replicates before analyzing the data. Both constant and variable deposition rate models were examined. Because we found no significant differences between them, we focus on the constant deposition model in the present paper.

We used regression analysis to examine the effect of plant spacing on patterns of spore dispersal in the linear arrays. One was added to each spore estimate value to avoid problems associated with taking the log of zero. To test whether regression lines of spore deposition across plant position have different slopes or intercepts based on interplant spacing, we created three dummy variables to indicate spacing class. We fitted four models that assumed (1) separate slopes and intercepts for each spacing (general case); (2) same slope but different intercepts (parallel regression lines); (3) same intercept but different slopes (concurrent regression lines) and (4) same intercept and slope (coincident regression lines). Model 1 (general case) was used as the alternative model for the construction of F-statistics for comparing the relative adequacy of the four models in describing the observed depo-

TABLE 1.—Insect visitors observed in natural populations of *Silene alba* across 2 yr (1992, 1993). Visitation rates are presented as mean number of visitors per hour per m² (mean flower density = 58.22 flowers/m²; data for diurnal visitors averaged across 17 observation periods; data for nocturnal visitors averaged across 14 observation periods; all data averaged across three different sites)

Diurnal flower visitors				
Common name	Family	Species	Visitation rate	Notes
Syrphid fly	Syrphidae	<i>Metasyrphus americanus</i>	5.39	Eats pollen on male flowers
Mining bee	Andrenidae	<i>Andrena</i> sp.	2.70	Eats pollen on male flowers
Bumblebees	Apidae	<i>Bombus fervidus</i> , <i>B. affinis</i> , <i>B. impatiens</i> , <i>B. bimaculatus</i>	1.97	Collects pollen, nectar
Honey bee	Apidae	<i>Apis mellifera</i>	0.82	Collects pollen, nectar
Cabbage butterfly	Pieridae	<i>Pieris</i> sp.	0.45	Drinks nectar
Silver-spotted skipper	Hesperiidae	<i>Epargyreus clarus</i>	0.40	Drinks nectar
*Unknown flies			0.30	One observed eating smut spores
Green metallic bee	Halictidae	<i>Agapostemon</i> sp., <i>Augochlora</i> sp.	0.27	Eats pollen, nectar
Clearwing moth	Sphingidae	<i>Hemaris thysbe</i>	0.12	Hovers to feed on nectar
Great spangled fritillary	Nymphalidae	<i>Speyeria cybele</i>	0.06	Drinks nectar
Spicebush swallowtail	Papilionidae	<i>Papilio troilus</i>	0.06	Drinks nectar
Nocturnal flower visitors				
Common looper moth	Noctuidae	<i>Autographa precatonis</i>	1.57	Lands on flowers to feed
Laurel sphinx	Sphingidae	<i>Sphinx kalmiae</i>	0.79	Hovers while feeding
Spanworm moth	Geometridae	<i>Itame pustularia</i>	0.71	Abundant but few on flowers
American ear moth	Noctuidae	<i>Amphipoea americana</i>	0.50	Lands on flowers
Saw-wing moth	Geometridae	<i>Euchlaena serrata</i>	0.29	Lands on flowers
White-lined sphinx	Sphingidae	<i>Hyles lineata</i>	0.21	Hovers while feeding

sition patterns. For example, if Model 4 (no differences in slopes or intercepts) provides as good a fit to the data as the alternative model (1), then no significant effects of plant spacing on spore deposition can be resolved. However, if models that assume the same slope or intercept for all regression lines are not adequate (*i.e.*, large F-value) we can infer that either the magnitude or decay of spore dispersal varies with interplant distance.

RESULTS

OBSERVATIONS OF NOCTURNAL AND DIURNAL POLLINATORS

A wide variety of insects were observed visiting *Silene alba* in the study areas during 1992 and 1993 (Table 1). Diurnal visitors were mainly bees, lepidopterans and flies; nocturnal visitors were mainly noctuid, geometrid and sphingid moths. Syrphid flies and andrenid bees, although the most abundant diurnal visitors, were generally observed eating pollen from male flowers and spent prolonged intervals of time perched on individual flowers. The most abundant nocturnal visitors were noctuid moths, hawkmoths and geometrid moths. Almost all hawkmoths observed in patches visited *Silene* flowers, while only a subset of the other moth species observed were recorded on flowers. Overall, the number of

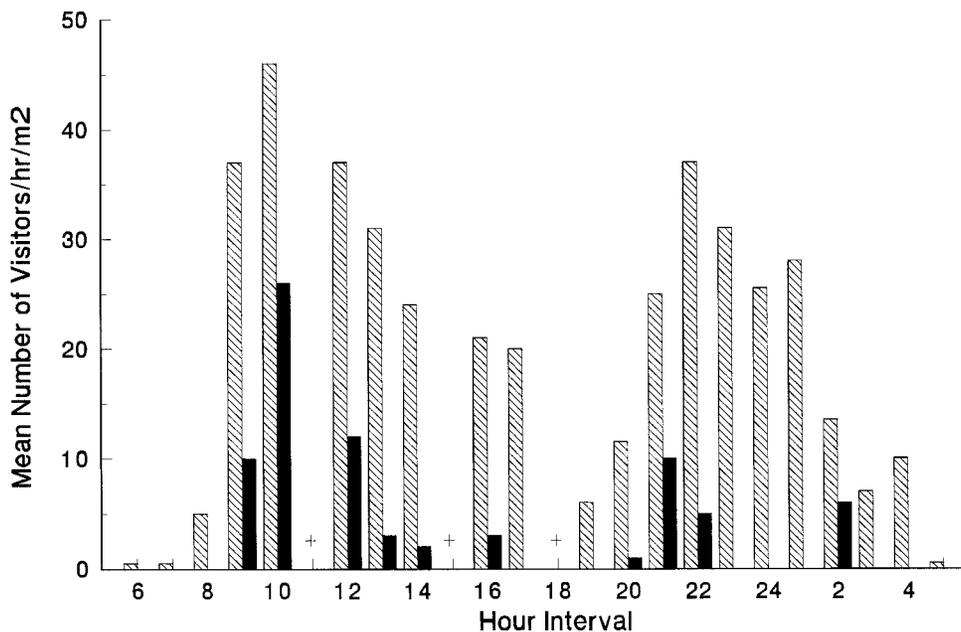


FIG. 1.—Frequency of all insect visitors over a 24-h period as averaged from 1993 field observations combined with data from videotaped nocturnal visits. Means were determined by averaging observations from hour-long time intervals on different days and sites (a total of 40 observation intervals at three different sites were obtained). Diagonal hatched bars represent the mean number of visitors/m²/h; solid bars represent the subset of those visitors observed on flowers of *Silene alba*. Time intervals are shown as the 1 h period following each tick on the x-axis. No data was available for the hour-long intervals following 1100, 1500 and 1800 (indicated with a '+'). Only one observation was available for the intervals following 700, 800, 1700 and 1900

nocturnal visitors was lower than the number of diurnal visitors (Table 1). The distribution of all pollinator activity throughout the 24-h period (Fig. 1) shows that the peak times for pollinator activity were between 0900–1200 and between 2100–2400.

FLOWER PREFERENCES OF VISITORS

Bumblebee preferences.—Overall, bumblebees showed a strong preference for healthy flowers of *Silene alba* when presented with a random array of equal numbers of diseased and healthy flowers (Fig. 2). Bumblebees previously exposed to only healthy flowers showed a stronger preference for healthy flowers (Fig. 2a; % total visits to diseased flowers = 22.9) than did bumblebees exposed to only infected flowers (Fig. 2b; % total visits to diseased flowers = 39.3). No bumblebees trained on healthy flowers probed diseased flowers, whereas bees exposed only to diseased flowers did probe infected flowers (Fig. 2b; % visits to diseased flowers that were probes = 10.0).

There was a marginally significant effect of training type on the status of the visited flowers ($F = 4.03$, $P = 0.068$), with bees trained only on diseased flowers being somewhat more likely to visit diseased flowers. In our second analysis of variance (with bee nested within training type), we found a significant effect of bees ($F = 3.95$, $P = 0.015$), such that individual bees varied greatly in the percent probes of infected flowers. We also found a

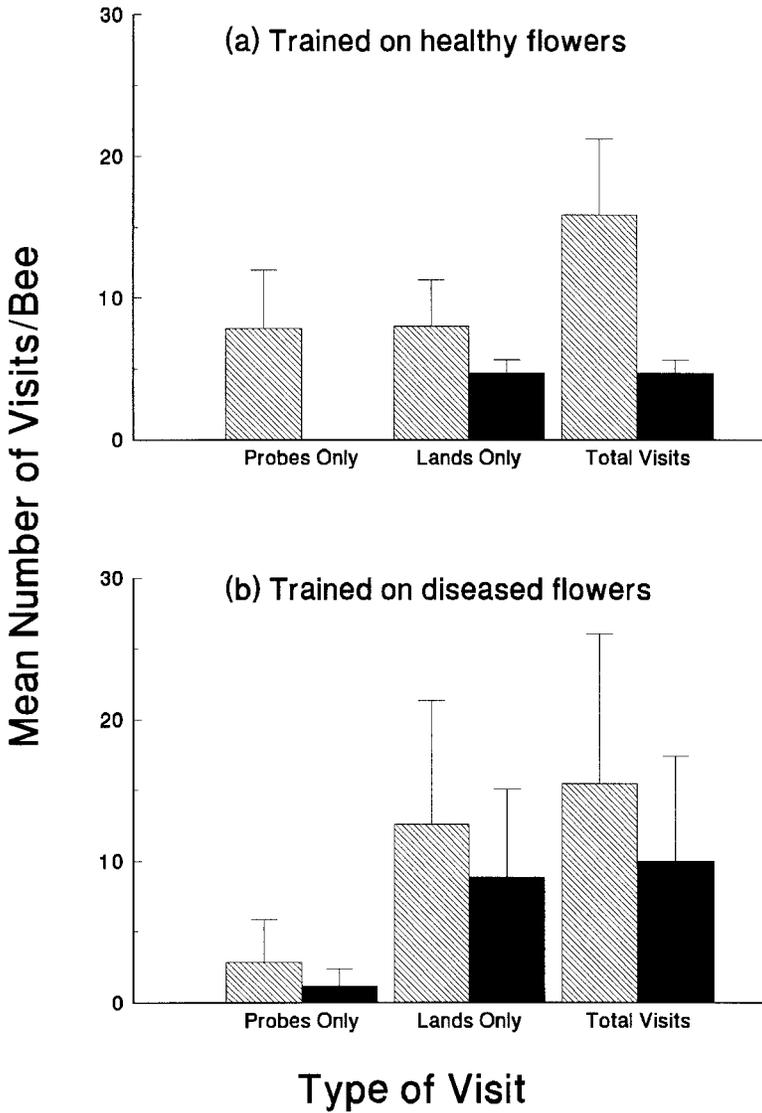


FIG. 2.—Flower preference of bumblebees, shown as the mean number of flowers visited per bee, together with 95% confidence intervals. Diagonal hatched bars represent visits to healthy flowers, and solid bars represent visits to infected flowers; (a) bumblebees trained only on healthy flowers ($n = 7$); (b) bumblebees trained only on infected flowers ($n = 7$)

significant effect of disease status ($F = 47.58, P = 0.0001$), such that bees were much more likely to probe healthy flowers over infected flowers regardless of training type. There was also a significant interaction between training type and status of flower visited ($F = 20.65, P = 0.0008$), indicating that whether or not bees probed diseased flowers depended on whether they were trained on healthy or diseased flowers.

TABLE 2.—Effect of night, sex and disease status on number of visits per flower per hour. Results of analysis of variance for the general linear model (visits per flower per hour = night sex status sex*status, weighted by length of observation period per night) are shown

Source	df	Mean square	F Value	Pr > F
Night	2	0.912	15.40	0.004
Sex	1	0.057	0.96	0.365
Disease status	1	0.854	14.41	0.009
Sex*Status	1	0.428	7.22	0.036
Error	6	0.059		

Moth preferences.—Male plants received more moth visits per hour than female plants (6.00 vs. 2.57), and this difference was significant (ANOVA model: visits = night sex status sex*status; $F = 11.75$, $df = 1,6$; $P = 0.014$). Healthy and diseased plants did not differ significantly in their number of visits per hour (5.00 vs. 3.57; ANOVA model as above; $F = 2.04$, $df = 1,6$; $P = 0.203$) and there was no significant interaction of sex and disease status. However, if the number of flowers per plant is taken into account, then the number of visits per flower per hour differed among nights. There were significantly more visits per flower per hour on healthy plants than on diseased plants (0.99 and 0.49 visits per flower per hour on healthy and diseased plants, respectively; Table 2). This effect was greater in female than

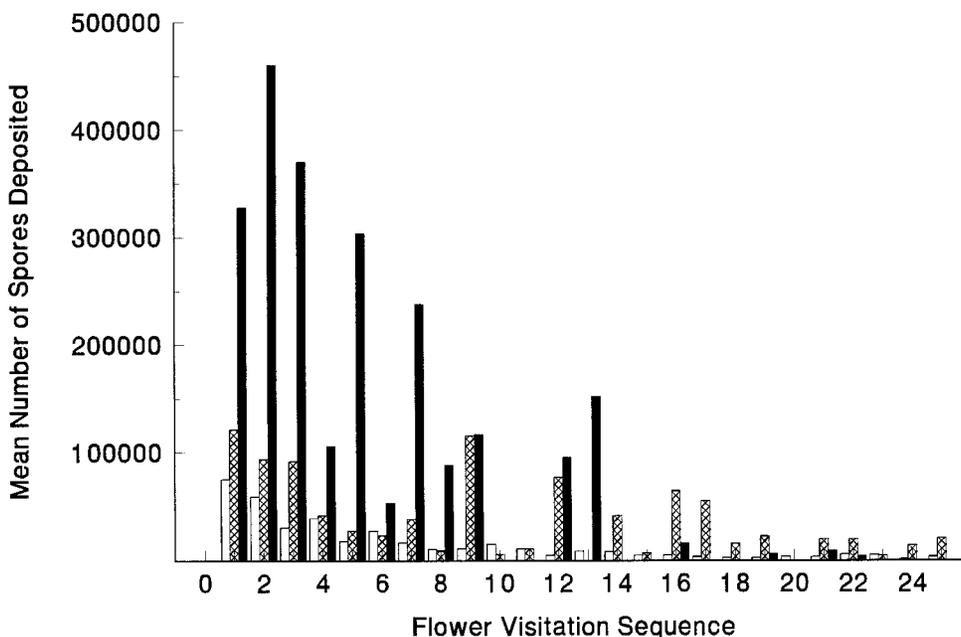


FIG. 3.—Comparison of the mean number of spores deposited by bumblebees that visited one (open bars; $n = 13$), two (cross-hatched bars; $n = 6$), and three (solid bars; $n = 3$) diseased flowers. Spore deposition for each sequential flower visit was calculated as the mean number of spores deposited (averaged across individual bees)

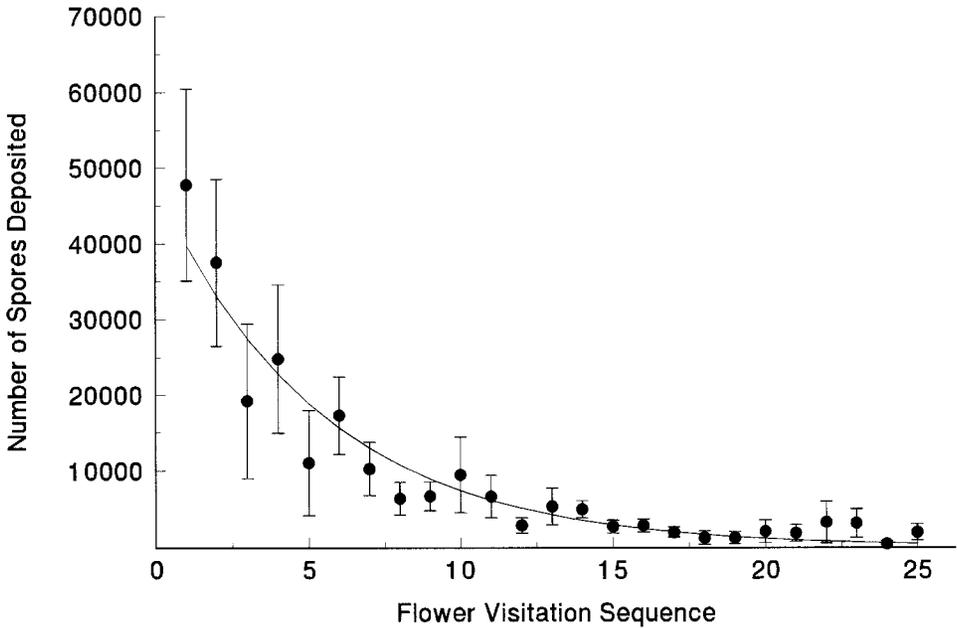


FIG. 4.—Mean number of spores deposited by bumblebees ($n = 13$) on successive visits to healthy flowers after visiting a single diseased flower, together with predicted deposition curve from model (see eqn (1) in the text). Actual means are shown as filled circles (bars represent standard errors); estimated values for predicted curve were: $S_0 = 2.34 \times 10^5$; $\lambda = 0.17$

in male plants (0.75 vs. 0.60 in males, and 1.22 vs 0.38 in females; significant sex*disease status interaction; Table 2) but there was no overall effect of sex.

Given that a plant was visited initially, the number of subsequent visits per flower on that same plant was significantly related with its flower number ($P = 0.0182$; general linear model: subsequent visits = night, flowers, with night as a class variable). Females tended to have lower rates of subsequent visits than males ($P = 0.0618$). This subsequent per flower visitation rate did not differ significantly between diseased and healthy plants (0.176 and 0.219 respectively, $P = 0.404$).

SPORE DEPOSITION BY VECTORS

Deposition by bumblebees.—Maximum likelihood estimates of the initial number of spores acquired on a bee's body ranged from 2.34×10^5 spores following a visit to a single diseased flower, to 1.86×10^6 spores following three successive visits to diseased flowers; estimates for the fraction of spores deposited with each visit ranged from 0.14 to 0.17. Bees visiting two and three diseased flowers deposited a greater number of spores when compared with bees visiting only one diseased flower (Fig. 3). Although the mean number of spores deposited on healthy flowers decreased with successive flower visits, most deposition values for these later visits were still higher than 1×10^3 spores per flower (Fig. 4). Flowers that were probed by bees had more spores deposited on them than flowers that were only touched or landed on. When lands were excluded from the average spore deposition curve, the mean number of spores deposited with each visit increased. However, because both

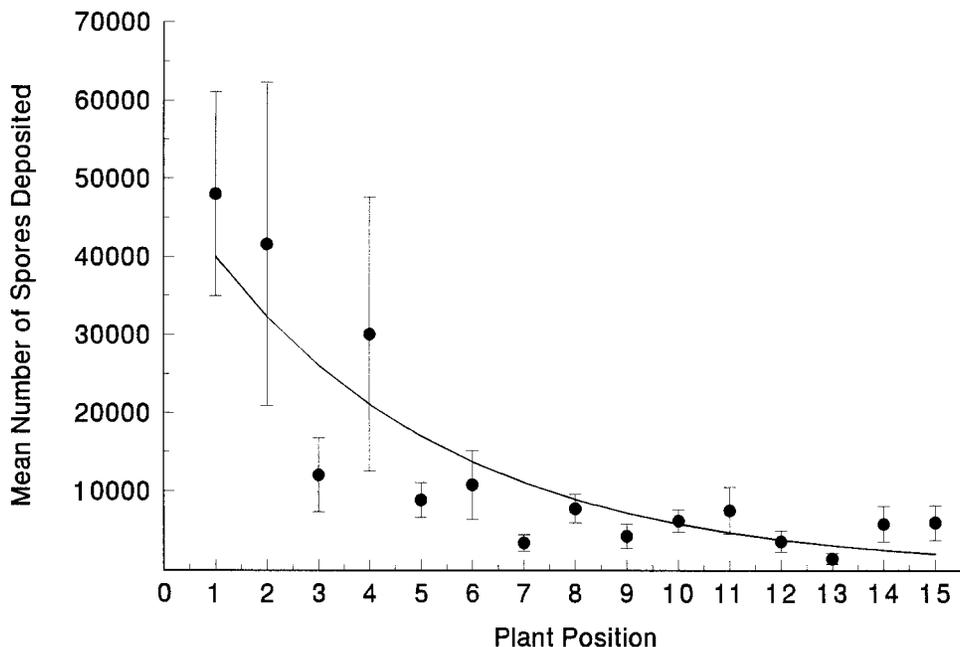


FIG. 5.—Mean number of spores deposited in linear arrays of potted plants (data combined for all three interplant distances). Solid line represents predicted deposition curve from model (*see* eqn (1) in text); actual means are shown as filled circles; bars represent standard errors. For predicted curve, $S_0 = 2.08 \times 10^5$; $\lambda = 0.19$

behaviors are involved in the deposition of spores, data for lands and probes were combined in the analysis.

Spore dispersal in arrays.—Spore counts were obtained for a total of 182 flowers from the 0.5-m arrays, 154 flowers from the 1.0-m arrays, and 179 flowers from the 2.0-m arrays. We were unable to collect flowers from every plant each week due to inconsistent flowering in some individuals. Maximum likelihood estimates for S_0 and λ across the three array spacings were similar to those obtained in the laboratory studies following a single visit to an infected flower. Most spore deposition was close to the source of infection, although considerable long-distance dispersal (up to 1×10^3 spores for plant 15) was observed for all arrays (Fig. 5). Deposition curves based on estimated values of S_0 and λ (Fig. 6) show that more deposition occurred in closely spaced arrays, particularly for plants close to the source of infection. In addition, the average spore dispersal distance increased with increasing interplant distance (Fig. 6).

When data for all three spacings were combined, the slope of the regression of spore deposition on plant position was $b = -0.107$ ($r^2 = 0.76$; $P < 0.001$). Comparison of F-statistics for Models 2–4 (to evaluate the effect of spacing on the slopes and intercepts of dispersal patterns) showed that models assuming the same intercept for each spacing (Models 3 and 4) were not adequate compared to Models 1 and 2 (Table 3). A similar comparison between Models 2 and 4 (analysis of covariance) indicated that the regression lines for each spacing have different intercepts but similar slopes ($F = 2.466$; $P < 0.01$). Thus, although regression intercepts were higher for smaller interplant distances, slopes did not signifi-

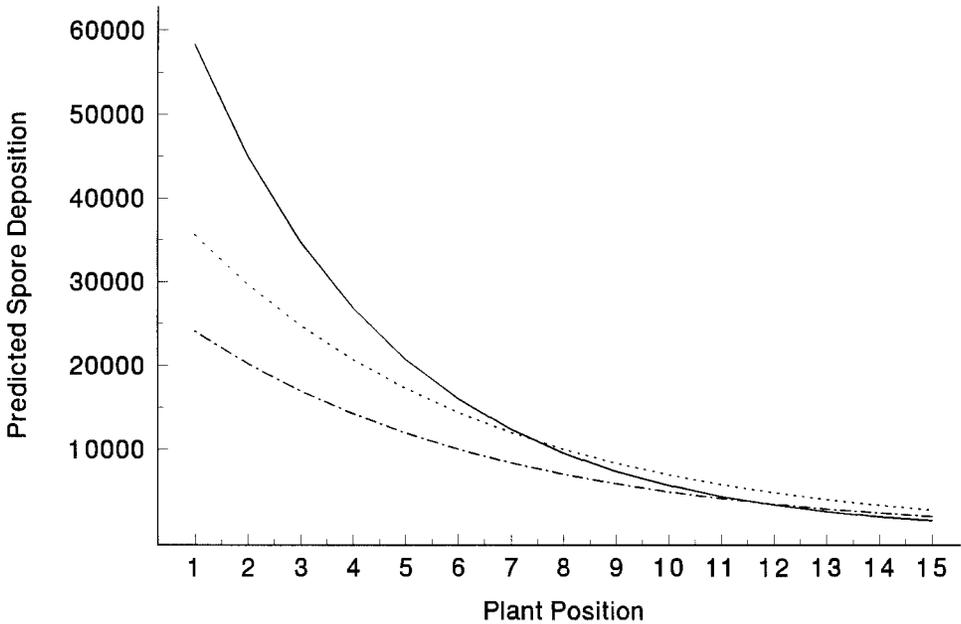


FIG. 6.—Predicted spore deposition curves for three different interplant spacings in linear arrays [see model; eqn (1) in text]. Solid line represents 0.5 m interplant distance ($S_0 = 2.56 \times 10^5$; $\lambda = 0.23$), dotted line represents 1.0 m interplant distance ($S_0 = 2.15 \times 10^5$; $\lambda = 0.17$), and dashed-dot line represents 2.0 m interplant distance ($S_0 = 1.50 \times 10^5$; $\lambda = 0.16$)

cantly vary with plant spacing. This suggests that the log of spore deposition in the arrays is best described by three parallel lines, with more spore deposition close to the disease source occurring in the densely spaced arrays.

Due to hot and dry weather conditions, only 13 out of 345 plants that flowered in the experimental arrays became infected. Of these, 10 were located very close to the disease source (in positions 1, 2 or 3). The three plants that became infected farther from the disease source were in arrays with larger interplant distances (1 and 2 m). Because of the low number of transitions to infected status, we could not examine the relationship between spore dispersal, infection probability and plant position in our linear arrays.

TABLE 3.—Regression analysis of \log_{10} spore deposition across plant position, testing similarity of slopes and intercepts of three different regression lines corresponding to plant spacing. Model 1 (assuming different slopes and intercepts for all three regression lines) is used as alternative model for F-tests. Model 2 assumes parallel regression lines (same slope but different intercepts); Model 3 assumes concurrent lines (same intercept but different slopes) and Model 4 assumes coincident (overlapping) lines. Comparisons indicate that regression lines have significantly different intercepts but similar slopes

Model	RSS	df	F value	Pr > F
1 (General)	663.504	259	—	—
2 (Parallel)	665.055	261	0.3033	NS
3 (Concurrent)	671.16	261	1.495	< 0.001
4 (Coincident)	677.62	263	1.378	< 0.001

DISCUSSION

Visitors of Silene alba in local populations.—The present study indicates that *Silene alba* has a wide array of visitors, the most common of which are bees, syrphid flies, noctuid moths and sphingid moths. A previous study of visitation activity in experimental populations of *S. alba* suggested that bumblebees were important diurnal visitors (Roche, 1993). We found that bumblebees were the third most common insect observed visiting *S. alba* flowers. Syrphid flies and andrenid bees, though commonly observed eating pollen from male flowers, spent prolonged intervals of time in individual flowers; they are therefore less likely to be important long-distance vectors for *Ustilago violacea* spores. *Silene alba* flowers are also commonly visited by a guild of nocturnal moths. Moth visitors were observed feeding in both dense and widely spaced populations of *S. alba*, while bumblebees were rarely observed visiting *S. alba* at low densities (S. Altizer, pers. observ.).

There were two peaks in visitation rates for natural populations of *Silene alba*, with a greater absolute number of visitors during the diurnal peak than the nocturnal peak. However, data from Shykoff and Bucheli (1995) showed that nocturnal moths are more effective dye movers than are diurnal insects. Whether moths are more effective than bees as vectors for fungal spores has not yet been quantified, and may depend on plant density or the distance between plant patches, as well as morphological features of the insects. For example, bees may be better vectors in dense patches of mostly healthy plants, while moths may be better vectors in low density populations. Insects that fly long distances (such as sphingid moths and bumblebees) may be important vectors between patches of plants.

Flower preferences in bees and moths.—Flower preference studies showed that bumblebees preferentially visited healthy flowers of *Silene alba* as opposed to *Ustilago*-infected flowers. Preferences in bumblebees were maintained regardless of whether they were exposed to healthy or diseased flowers before being tested for preference. These data are consistent with findings by Roche (1993) for *S. alba* and by Jennersten (1988) for *Viscaria vulgaris*. If bumblebees visit flowers to collect nectar and pollen, they may actively avoid diseased flowers which on average have less nectar than healthy male flowers and contain no pollen.

The finding that bees discriminated against diseased flowers has interesting consequences for disease spread. For example, the simulation results of Real *et al.* (1992) suggested that although discriminating vectors have a lower probability of encountering disease, they also have a higher probability of spreading spores to healthy plants after contacting a diseased host. At low levels of disease, vectors that prefer healthy plants may spread disease more slowly than randomly foraging vectors. However, at higher levels of disease, the avoidance of diseased flowers can be compensated by the increased probability of spread to healthy plants following infected host contact.

Bees previously exposed to diseased flowers discriminated less against diseased flowers than bees trained only on healthy flowers. Diseased *Silene alba* plants often flower earlier in the season than healthy plants. This high proportion of disease may encourage insects to visit infected plants and thus enhance spore dispersal and disease spread early in the season. Jennersten (1988) found that significantly more *Ustilago* spores were deposited on healthy *Viscaria vulgaris* flowers during the early portion of the flowering season, when there were high levels of disease. Alexander (1990) also found a higher proportion of *S. alba* flowers with spores early in the flowering season.

The moth visitation study showed that moths made more visits to plants with more flowers. This observation is consistent with earlier work by Alexander (1987) and Thrall and Jarosz (1994a) who showed that plants with more flowers per unit time were more likely to become diseased. Thrall and Jarosz (1994a) also showed that male plants were more likely to become

infected in experimental populations of *Silene alba*. Although moths showed no preference for healthy plants in terms of the number of flowers visited per unit time, when visitation rates were divided by the number of flowers, moths discriminated in favor of healthy flowers over infected ones.

There have been relatively few studies of pollination behavior of night-flying moths (Richards, 1986). Odor seems to be the primary stimulus leading moths to approach flowers, and odor is also often a prerequisite for initiation of visual preferences for white flowers (Brantjes, 1978). Whether moths can discriminate between diseased and healthy flowers of *Silene* on the basis of odor is not known. Our findings indicate the need for more studies on moth behavior in relation to disease spread.

Patterns of spore deposition.—Our results show that even with limited flight distances between plants (e.g., as a result of close plant spacing), spore dispersal at substantial distances from a point source can occur. Large quantities of spores were deposited by bumblebees even after the 15th flower visit following a single visit to an infected flower. In addition, spore dispersal was greater for bees that were forced to visit more than one infected flower before visiting healthy flowers. These estimates for the mean number of spores deposited per visit are in agreement with previously measured levels of spore deposition in natural populations. For example, Alexander and Antonovics (1988) recorded healthy flowers with spore loads ranging from 5×10^2 to 2×10^5 , depending on the number of diseased plants in the immediate area. Similar magnitudes and distributions of spore deposition within an established array of plants have been reported by Roche *et al.* (1995).

A decrease in spore deposition with increasing visits suggests that if vector behavior changes with overall plant density, then the rates of disease spread will also be affected. For example, if plants are spaced farther apart, flower visitors would be expected to visit more flowers per plant than they would in high density plant populations (Levin and Kerster, 1974). In this case, vectors may visit multiple flowers on infected plants and thus acquire more spores on their bodies. However, if most of these spores are deposited on only a few plants, then the observed spore deposition function should drop off more steeply than in closely spaced populations.

Results from the linear arrays showed that spore deposition close to the source of infection was greater in the more densely spaced arrays. Although Figure 6 suggests that spore dispersal distance increases with interplant distance, our regression analysis of spore deposition with plant position did not detect significantly different slopes between the three plant spacings. These results suggest that vector behavior may not interact with plant density in the manner described above. Rather, increasing distances between plants may result in fewer insect visitors and lower magnitudes of spore deposition, or a greater loss of spores in flight or by deposition on alternate plant species.

Information related to spore loads and infection probabilities is needed to predict how variation in spore deposition will influence rates of disease spread. Previous data suggest that a threshold number of spores may be required in order to detect infection probabilities on *Silene alba* plants, and that high levels of spore deposition experience diminishing returns (Roche *et al.*, 1995). During the summer of 1993, we examined the effect of different sized spore loads on the per contact rate of infection by applying known quantities of spores onto flowers of healthy plants. A total of 960 plants were inoculated, but only 13 became infected due to a prolonged drought. While no plant receiving less than 1×10^3 spores became diseased, the present study suggests that even plants far from a disease source experience these spore loads. Thus, the actual likelihood of infection may drop off less steeply with the number of visits than the spore carryover results suggest.

A substantial number of plant diseases are mediated by insect vectors (*see* Mink, 1993,

for a review of pollen transmitted viral diseases). Patterns of vector behavior (*e.g.*, ability to discriminate between healthy and diseased hosts) and patterns of spore dispersal influence rates of disease spread in natural populations. The present study indicates that *Silene alba* is visited by many species of diurnal and nocturnal insects with diverse morphologies and behaviors. Determining the effect of vector behavior on disease spread in such systems requires evaluation of the relative contributions of different species to spore dispersal. This task is likely to be difficult, since not only do the numbers and kinds of visitors vary temporally between day and night, but their relative abundances may vary across years. Nevertheless, this study has identified some general patterns of host preferences, spore dispersal, and effects of plant spacing. Understanding such host preferences and spore deposition patterns is a necessary step towards understanding the relative importance of different types of insect vectors to the spread of disease within and among populations.

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