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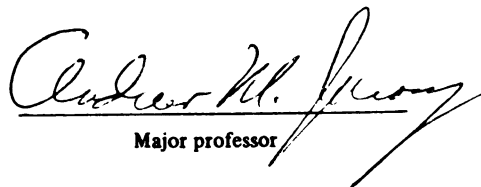
The geographic distribution of Silene latifolia and its
anther-smut pathogen Microbotryum violaceum in the eastern
United States

presented by

Emily J. Lyons

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THE GEOGRAPHIC DISTRIBUTION OF SILENE LATIFOLIA AND ITS ANTHERSMUT PATHOGEN MICROBOTRYUM VIOLACEUM IN THE EASTERN UNITED STATES

By

Emily J. Lyons

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ABSTRACT

THE GEOGRAPHIC DISTRIBUTION OF SILENE LATIFOLIA AND ITS ANTHR-SMUT PATHOGEN MICROBOTRYUM VIOLACEUM IN THE EASTERN UNITED STATES

By

Emily J. Lyons

The eastern United States was censused to map the geographic distribution of Silene latifolia and its obligate anther-smut pathogen Microbotryum violaceum. The smut fungus is found primarily in western Virginia though the natural range of S. latifolia extends throughout most of the eastern U.S.. Exceptions included three infected populations on Nantucket Island and one infected plant in northwestern New York. Northeastern states were characterized by large populations of S. latifolia that were randomly distributed across the landscape, while those in Virginia were smaller and more clumped. This may reduce the pathogen's ability to invade northern host populations.

A two year field experiment conducted at seven sites, composing two south-north transects in the eastern United States indicated that M. violaceum is able to infect and overwinter in S. latifolia plants at northern latitudes even though it is rare in these areas. Disease rarity at northern latitudes may be due to: 1) historical accident of disease introduction, 2) differences in plant population size and resistance, or 3) variation in pollinator foraging strategies.

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Chapter 1

REVIEW OF LITERATURE

The Pathosystem

Silene latifolia (Poiret) (= Silene alba [Miller] Krause) (Caryophyllaceae), white campion, is a short-lived, dioecious, perennial that is native to Europe though it has been found in the eastern United States since the mid-1800's (McNeill, 1977). Flowers of S. latifolia open in the evening and remain open until mid-morning the following day. The primary pollinators are bumble bees, butterflies, sphingid moths, and other Lepidoptera (Shykoff & Bucheli, 1995; Roche et al., 1995; Altizer, unpub.). S. latifolia grows primarily along roadsides and in other ruderal habitats.

Microbotryum violaceum (= Ustilago violacea Pers. [Deml & Oberwinkler]) (Ustilaginales, Basidiomycotina), the anther-smut pathogen of S. latifolia, is a basidiomycete fungus that is spread by vectors during the process that would normally be pollination, thus it is often analogized to a sexually transmitted disease. Diploid teliospores of the fungus are deposited on healthy, susceptible S. latifolia plants where they germinate and undergo meiosis. The resulting haploid sporidia of opposite mating type then conjugate and form an infection hypha that can penetrate the plant tissue. Mycelium grow throughout the adjacent stems and into the rootstock where the fungus perenniates. Newly diseased flowers appear after the fungus has grown into the host and

entered newly developing flower buds (Batcho & Audran, 1980), a period that ranges between three weeks and two months (Alexander, 1990b).

Successful infection by M. violaceum is manifested in the production of spore producing staminate flowers in both male and female plants. In males, the normal pollen producing stamens are replaced with spore sacs; in infected female flowers, the ovary becomes rudimentary and spore sacs develop on stamen-like structures. Plants often become systemically infected so that the following year all flowers are infected, resulting in complete sterility. Vertical transmission through the seed does not occur (Baker, 1947; E. Lyons, unpub.) though transmission can occur passively by spores falling on seedlings growing close to infected plants (Alexander and Maltby, 1991).

M. violaceum has been recorded on 21 species of the Caryophyllaceae in North America, including S. latifolia and S. virginica, and 92 species in Europe (Thrall et al., 1993). The distribution of M. violaceum among species in the Caryophyllaceae is related to the life span of its host species. More specifically, M. violaceum is more commonly found on perennial species than on annual species. This is not surprising given the life cycle of M. violaceum. Recall that fungus may take as long as two months from the time its spores are deposited, a large portion of the growing season, to invade newly developing flowers and cause spore production. Complete systemic infection usually occurs in the growing season following initial infection and the fungus is therefore reliant upon the perennial life history of its host plant to insure further transmission. Given this, it is plausible to suggest that, if the ability of M. violaceum to survive and persist is, in part,

dependent on the life history of its host, then the factors that influence host life histories may also affect the distribution of M. violaceum

History of the Pathosystem

M. violaceum occurs on S. latifolia and other members of the Caryophyllaceae in both North America and Europe. It is hypothesized that S. latifolia was introduced to North America in the mid-1800's when agricultural soil, used as ballast on European trade ships, was deposited on the shores in port areas. S. latifolia seeds contained in the soil germinated and the range of the plant subsequently spread throughout most of the eastern United States and Canada.

It is unclear how M. violaceum was introduced to North America though it infects both the introduced S. latifolia and the native fire-pink, Silene virginica. Two hypotheses have been explored experimentally (Antonovics et al., 1996): 1) The presence of infected S. virginica in close proximity to infected S. latifolia represents a host-shift from the endemic S. virginica to S. latifolia or vice versa; 2) The anther-smut of S. latifolia is related to isolates found in Europe and thus may have been introduced with S. latifolia. Results of cross-infection studies between the two Silene species reveal that the isolates of M. violaceum from both species are very host specific. Very little (1.5%) cross infection occurred when S. latifolia plants were inoculated with fungal isolates from S. virginica and vice versa (Antonovics et. al, 1996). These results do not support hypothesis 1 that the infection of both Silene species by M. violaceum is due to a host shift from one species to the other. With respect to hypothesis 2, electrophoretic studies indicate that M.

violaceum isolates from S. latifolia in the southeastern U.S. resemble isolates from an infected population in England (Antonovics, et al., in press). These data suggest that the anther-smut on S. latifolia in the U.S. may have been introduced from Europe with the host plant. The origin of M. violaceum isolates infecting S. virginica is still unclear.

Silene-Microbotryum Population Studies

The detrimental effect that pathogens often have on their hosts has led to the suggestion that disease-causing organisms can regulate host population size, affect genetic variability in host populations and influence species coexistence in communities (e.g. Burdon, 1987; May & Anderson, 1983 a,b; Alexander & Antonovics, 1988). Given the severe fitness effect, sterilization, that M. violaceum has on S. latifolia, it is likely that the fungus is influencing the population dynamics of its host. Since the mid-nineteen eighties, a great deal of research has been conducted on the Silene-Microbotryum host-pathogen system in an attempt to understand the population dynamics of both host and pathogen.

A deterministic model, by Alexander and Antonovics (1988) simulated the dynamics in the Silene-Microbotryum system and explored the effect of plant recruitment and disease spread on the fate of infected S. latifolia populations. The parameters for the model were estimated using demographic data from infected populations of S. latifolia in southwestern Virginia. Three outcomes were possible, depending on the initial model parameters. At low host recruitment rates and high infection rates, the outcome was total infection of the population resulting local extinction of both the host and pathogen. When infection was low and recruitment rates were high, the pathogen was expunged from the

plant population. When both recruitment and infection rates were high, the host and pathogen could co-exist at equilibrium and regulate each other's populations (Alexander & Antonovics, 1988). These results are highly dependent on the transmission mode of the pathogen and the probability of successful systemic infection of the host.

As mentioned above, M. violaceum is a vector transmitted disease and therefore successful transmission from infected to healthy, susceptible hosts is highly dependent on pollinator behavior. Hand pollination studies performed in populations of S. latifolia indicate that the populations are pollinator limited. Plants that were hand pollinated produced approximately twice the number of flowers with maturing fruits and seeds as those that were naturally vector pollinated (Alexander, 1987). Inadequate pollinator service influences not only plant fecundity but successful transmission of M. violaceum as well.

Investigations of pollinator visitation patterns in S. latifolia populations reveal that pollinators typically deposit pollen and/or spores on plants that are in close proximity to the host plant (Roche et al, 1995; S. Altizer, unpublished). Vectors prefer plants with large floral displays and preferentially visit males over females and healthy plants over infected plants (Shykoff & Bucheli, 1995). The pollinator preference of males over females or infected plants corresponds with the production of higher quality nectar in males. Relative to females and infected plants, males consistently produce nectar with a higher sugar concentration (Shykoff & Bucheli, 1995). Also related to the preferential visitation of male plants by pollinators is the fact that males almost always produce many more flowers than females. Thus they are usually the plants with the largest floral displays

as well as the highest quality nectar. These factors may put males at a higher risk of infection by M. violaceum. This is consistent with the findings of Thrall and Jarosz (1994a) that males that begin flowering earlier, produce more flowers and remain in flower longer are two times more likely to become infected than females with shorter flowering periods and fewer flowers overall. Regardless of sex, plants that produce more flowers are, in general, more likely to become infected.

Pollinator behavior is not the only factor influencing the transmission of M. violaceum in populations of S. latifolia. The pollinator must not only visit an infected plant and pick up spores, but it must also deposit those spores on a healthy, susceptible host. An experimental inoculation study performed by Alexander et al. (1993) indicates that S. latifolia plants vary in their susceptibility to isolates of M. violaceum. This variation in susceptibility is not only present, but heritable and consistent in both the greenhouse and field environments (Alexander & Antonovics, 1995). Given that heritable variation in resistance exists in natural populations of S. latifolia, it is essential to understand how the host genetics influence the population dynamics of both plant and pathogen.

In order to further investigate the transmission dynamics of M. violaceum in the context of host resistance, Thrall and Jarosz (1994a,b) established experimental populations of S. latifolia containing both resistant and susceptible plants with different total densities. In addition, the frequency and density of plants infected with M. violaceum was also varied. The actual numbers of transitions from healthy to infected in S. latifolia were compared to predictions generated from computer simulations and it is clear that

susceptible and resistant populations have very different dynamics. Host and pathogen are able to coexist in susceptible populations while the fungus is expunged from resistant populations (Thrall & Jarosz, 1994b).

However, knowledge of population resistance structure alone is not adequate to explain the natural population variation in disease levels. More long-term, across-season processes are also important. These include: host recruitment, recovery from infection and over-wintering mortality (Thrall & Jarosz, 1994a). Variation in host recruitment and adult mortality may alter population age structure and subsequently affect disease spread (Burdon, 1987). First year plants are more likely to delay flowering until later in the growing season relative to older plants. Since time spent in flower is positively correlated with the probability of infection by M. violaceum, then pathogen transmission dynamics should vary with the relative abundance of first year and older plants (Thrall & Jarosz, 1994a). Additionally, the pathogen is expected to be rapidly lost from plant populations where host overwintering mortality is high (Thrall & Jarosz, 1994b). Even if M. violaceum is not lost, these across-season processes reduce the frequency of infected plants in the population at the start of the next growing season thereby decreasing the transmission rates within the next growing season (Thrall & Jarosz, 1994a).

What is clear from both of the above studies (Thrall & Jarosz, 1994a,b) is that in the Silene-Microbotryum system, host and pathogen within population dynamics are unstable at the scale of the population. The combined effects of plant resistance, pollinator avoidance of infected individuals, and the occasional year where overwintering mortality is high, lead to the conclusion that M. violaceum cannot persist indefinitely within a single

plant population. This suggests that stable coexistence of host and pathogen may be reliant upon long distance pollinator dispersal to affect new colonizations to counteract local extinction events of both the host and pathogen. This possibility requires the investigation of the Silene-Microbotryum system in a metapopulation context.

Silene-Microbotryum Metapopulation Studies

The concept of the metapopulation, defined here as a system of interconnected populations, has recently been used to address population processes on a larger scale (Hanski, 1991a,b). For example, the problem of increasing habitat fragmentation has prompted researchers to investigate systems in the context of the metapopulation where genetic structure is influenced by colonization and extinction processes (Hanski et al., 1994; Antonovics et al., 1994). The idea of a metapopulation is particularly amenable to the Silene-Microbotryum system because the host-pathogen dynamics at the population level are unstable and coexistence may be reliant upon continual colonization of the pathogen or resistant or susceptible plants. Metapopulation simulation studies support this idea showing that coexistence of host and pathogen can occur even when within-population dynamics lead to local extinction (Antonovics, et al., 1994).

In order to better determine the applicability of the metapopulation concept to the Silene-Microbotryum system, a roadside census of approximately ninety miles of roadside in southwestern Virginia was initiated in 1988. This study recorded every S. latifolia population that was encountered and counted the number of healthy and infected individuals in order to determine when colonizations or extinctions occurred for either the

plant or pathogen (Antonovics, et al., 1994). The data from the first three years of this study reveal that there is a high turnover rate of populations. In addition, the colonization rate of the fungus exceeds its extinction rate thereby suggesting that the disease is increasing in that area of Virginia (Antonovics, et al., 1994).

More generally, populations are more likely to be infected if they are large though among infected populations, smaller populations tend to have a higher percentage of disease. Additionally, colonization and establishment of both the host and pathogen is dependent upon their proximity to the nearest source population as most new populations and infections are found close to existing ones. However, some long-distance dispersal may occur because new populations do arise a considerable distance (> 1 mile) from any existing populations. It is plausible, from these data, to suggest that populations of S. latifolia and M. violaceum are interconnected even over long distances and are characterized by some long distance dispersal of pollen, seed and spores (Antonovics, et al., 1994).

For the purpose of further exploring the consequences of interconnected populations on disease spread, Thrall and Antonovics (1996) established an experimental metapopulation consisting of replicate sets of experimental populations separated by increasing distances (5, 10, 20, 40 and 80m). The results of this study indicate that disease spread is greater within the more isolated populations. This suggests that vectors may forage differently in small isolated populations than they do in those that are large resulting in the larger, or more closely distributed populations receiving a disproportionate

number of pollinator visits (Thrall & Antonovics, 1996). This phenomenon could have profound implications for the spread of M. violaceum on a larger geographic scale.

The transmission and subsequent establishment of M. violaceum in regions that are far from a source population may be hindered by the movements of vectors. If the distribution of S. latifolia across a landscape is characterized by small populations that are far apart, the data presented above (Thrall & Antonovics, 1996) suggest it is likely that pollinators will forage less selectively within a population and seldom move among populations. This may limit the spread of M. violaceum to localized populations and ultimately result in local extinction of both host and pathogen. Conversely, if populations of S. latifolia are large and continuously or randomly distributed across a landscape, it is possible that pollinators are more likely to move among populations and M. violaceum may be more easily transmitted among populations to invade new areas.

While the above speculations seem plausible, it is necessary to recall that the transmission of M. violaceum is frequency dependent and therefore reliant upon the probability of a vector visiting an infected plant and subsequently landing on a healthy, susceptible host (Thrall & Jarosz, 1994b). This fact may reduce the advantage of large populations for fungal transmission. For example, consider one infected plant in a population of one thousand plants. The frequency of the infected plant in that population is 0.001 and thus the probability that a vector will land on that plant is relatively low. Conversely, in a population of ten plants, one infected plant has a frequency of 0.1, and the probability of vector contact is greatly increased. It is therefore necessary to consider

the importance of both population size and distribution when investigating the spread of M. violaceum on a geographic scale and beyond the level of the metapopulation.

Silene-Microbotryum: A Model System

As evidenced in the review of literature above, there has been a great deal of research conducted on the Silene-Microbotryum host-pathogen system. One reason for this is the ease of use. The plant and fungus in this system are easily manipulated in both the field and greenhouse. In addition, the fact that the transmission of M. violaceum is frequency dependent and reliant upon vectors makes this system useful for understanding the dynamics of other vector transmitted diseases that are less manipulable. The systemic and sterilizing effects of the fungus on the host in conjunction with the mode of transmission makes it possible to liken the Silene-Microbotryum system to a sexually transmitted disease.

This analogy can be taken one step further in the context of metapopulation dynamics. For example, May and Anderson (1990) assert, based on the levels of sequence divergence between simian and human lentiviruses, that HIV, the virus that causes AIDS (Acquired Immune Deficiency Syndrome) has persisted in human populations for over a century. If this is indeed the case, they then assert that the persistence of the virus can be explained by the spatial models that underly sexual contacts within and among rural villages. In a metapopulation context, the villages are individual populations where the virus might be present. Any among village sexual contact potentially serves to maintain the virus in the overall geographic region. Increases in inter-village contact may account

for the recent increase in prevalence and spread of HIV. This is not unlike the way in which M. violaceum is maintained in populations of S. latifolia.

In addition to the applicability of the Silene-Microbotryum system to the understanding of sexually transmitted diseases, research on the system contributes to an emerging body of literature on the influence of metapopulation dynamics in plant-pathogen interactions (Frank, 1992; Burdon & Jarosz, 1992). For example, in the Linum marginale-Melampsora lini host-pathogen system, Burdon and Jarosz determined that the resistance structure of individual host populations is likely to be influenced by differences in the rates of local extinction of both host and pathogen populations. Given this, the dynamics of both the host and pathogen may best be understood in the context of a metapopulation characterized by frequent colonization and extinction events. (Burdon & Jarosz, 1992). Additionally, metapopulations have been recognized as important in influencing the coevolution of hosts and pathogens (Frank, 1992). The study of the Silene-Microbotryum system not only contributes to this emerging body of knowledge but provides an example of a situation where the concept of the metapopulation is particularly useful for the understanding of the dynamics of both host and pathogen.

Chapter 2

DESCRIPTIVE AND EXPERIMENTAL STUDIES

Introduction

The relationships of a species with its environment are reflected in the distribution of its abundance in both time and space. For almost a century, however, when ecologists have studied abundance, they have nearly always studied either population dynamics, (ie, fluctuations in the numbers of individuals in a single local population over time) or species distributions. The history of population dynamics extends from the theoretical contributions of Pearl (1925) and Verhulst (1938) and the classical empirical studies of Elton (1924, 1942), to recent attempts to understand the complex fluctuations within populations revealed in long time series (May 1974, 1987, Pimm and Redfearn 1988, 1989, Antonovics et al. 1994)

In contrast, when ecologists have studied species distributions, they have focused primarily on territoriality, foraging movements, habitat selection, and other processes that influence the spatial dispersion of individual organisms within populations or among habitats (e.g., Skellam 1951, Fretwell 1972). When biogeographers have studied distributions, they have been concerned primarily with the influence of contemporary processes and historical events on the size, location, and limits of geographic ranges of species as they appear on maps (e.g. Root 1988b, Meyers and Giller 1989, and Hengeveld 1990).

Similarly, biogeographers have equally rarely studied the abundance and distribution of individuals within a geographic range. There have been some notable exceptions (e.g. Whittaker 1967, MacArthur 1972, Hengeveld and Haeck 1981, 1982, Bock and Ricklefs 1983, Bock, 1984, Brown 1984, Shoener 1987, 1990, Root 1988 a,b Maurer 1994). These large scale geographic studies focused primarily on the influence of abiotic factors such as rainfall and temperature on species distributions. In particular, MacArthur refers directly to what he calls the “environmental control of community structure” or the concept that the members of an ecological community are determined by their ability to survive and reproduce in a particular environment (MacArthur, 1972).

Still largely missing, however, is any concerted theoretical or empirical research on the magnitude and pattern of interspecific geographic variation in local population density, particularly in the context of host-pathogen interactions. Host-pathogen interactions are particularly interesting because pathogen distributions are likely to be strongly affected by both abiotic factors (temperature, rainfall, and humidity) and biotic factors (host resistance and availability).

A few of the host-pathogen geographic distribution studies that have been done, investigated either powdery mildew resistance in wild barley (Nevo et. al, 1979, 1983, 1985a, 1985b, 1986) or crown rust resistance in wild oat species (Wahl, 1970). Because the host and pathogen were investigated separately in these studies, they relied heavily on correlations between host resistance and ecological factors to determine pathogen distribution. In this paper we discuss both a descriptive and empirical approach to studying the distribution and abundance of Silene latifolia (=Silene alba) and its anther-

smut pathogen Microbotryum violaceum (= Ustilago violacea) in the eastern United States. Anecdotal observations made by Dr. Janis Antonovics and others indicated the possibility that the range of M. violaceum was restricted to only a small fraction of S. latifolia's range. The Silene-Microbotryum host-pathogen system is particularly interesting because we can readily identify a series of highly probable abiotic and biotic reasons why the pathogen might be limited to only certain parts of the range of its host.

Below I provide a description of the Silene-Microbotryum system, as some knowledge of the life histories of these organisms will make our hypotheses more easily understood. Silene latifolia is a short-lived dioecious perennial found along roadsides and in other ruderal habitats. The plant was introduced from Europe in the mid-1800's and has since spread throughout eastern North America (McNeill, 1977). The presence of the plant provided suitable open niches for the colonization of M. violaceum and not surprisingly, the anther-smut is present in populations of S. latifolia in North America. The pathogen has most likely migrated from Europe, since it is not closely related to M. violaceum found on other Silene species that are native to the United States (Antonovics et al. 1996).

Prior to the initiation of our study, M. violaceum had only been reported in northwestern Virginia in the region surrounding Mountain Lake Biological Station where it is common, and once by Janis Antonovics and Stephen Tonsor (pers. comm.) in 1988 in southwestern Michigan near the W.K. Kellogg Biological Station (KBS), though it is no longer present in this area.

The Silene-Microbotryum plant-pathogen system is characterized by the production of teliospores on anther-like structures in male flowers. In female plants, M. violaceum causes the production of anther-like structures. In addition, the ovary becomes rudimentary and sterile. The teliospores are transmitted to new hosts by insect pollinators (Baker, 1947; Hassan and MacDonald, 1971; Lee, 1981; Jennersten, 1983, 1988; Alexander & Antonovics 1988; Thrall et al., 1993b). Newly diseased flowers appear after the fungus has grown into the host and entered newly developing flower buds (Batcho & Audran, 1980), a period that ranges between three weeks and two months (Alexander, 1990b). The fungus over-winters in the root crown of the plant resulting in systemic infection and complete sterility of the plant.

We propose five hypotheses for the rarity of M. violaceum in northern latitudes. They are:

1) **The distribution of M. violaceum is the result of an historical accident i.e., infection foci initiated at MLBS and KBS have not yet spread to other parts of S. latifolia's range.** The introduction of S. latifolia from Europe occurred only recently in the early 1800's and there is evidence to suggest that the fungus had a restricted introduction and then spread from that point. This is further corroborated by the fact that it is not the same anther-smut that infects members of the Caryophyllaceae (e.g. Silene virginica) native to the United States (Antonovics et al., 1996).

2) **There is a lack of pollinator service such that pollinators are not visiting infected plants with a high enough frequency to insure fungal transmission within and among metapopulations.** Unpublished work of Ms. Sonia Altizer and others

indicates that bumblebees can discriminate between healthy and infected S. latifolia flowers, and preferentially visit those that are healthy. The way in which pollinators forage has implications for fungal transmission both within and among S. latifolia populations. For example, it has been suggested by Altizer and others that in small plant populations bumblebees are less discriminatory in the flowers that they visit than they are in large populations, often foraging on all of the flowers in a small population (Altizer et al., unpublished). In contrast, hawk moths, the primary nocturnal pollinator of S. latifolia, are trapliners and regularly move from population to population carrying spores and pollen. The relative abundance of these two types of pollinators as well as S. latifolia population structure is likely to influence pollinator foraging behavior and subsequently, transmission of M. violaceum.

3) High levels of resistance among northern S. latifolia populations prevent the successful infection and subsequent persistence of M. violaceum at northern latitudes. Highly resistant plants of S. latifolia are known to occur in southwestern Virginia (Alexander et al. 1993). In addition, computer simulations indicate that in populations where resistance is high, M. violaceum is readily expunged (Thrall and Jarosz 1994b). Thus, the rarity of infected populations at northern latitudes might be due to high levels of resistance in S. latifolia prohibiting the persistence of the fungus.

4) The inability of the pathogen to establish perennial infection limits further transmission. There are three sub-hypotheses within this hypothesis. 1) Within the Caryophyllaceae, the distribution of M. violaceum is closely related to the life span of its host species with the proportion of perennial species on which anther-smuts have been

reported being five times higher than the proportion of annuals (Thrall et. al, 1993b). At northern latitudes, the life history of S. latifolia may change from perennial to annual habit due to harsh over-wintering conditions. It may be that the temperatures in the north are too cold during the winter for the plants to survive to flower again in the spring. 2) The growing season at northern latitudes is shorter than in more southern regions resulting in a shorter and perhaps insufficient amount of time for the fungus to grow from its point of deposition on a flower down into the root crown where it must be to over-winter. 3) It is possible that even if the fungus does successfully infect a plant and reach the root crown, it is unable to survive the cold winter temperatures in the north.

5) Plant population structure in areas outside Virginia is not conducive to invasion by the pathogen. By this we mean to suggest that the conditions are not optimal for pollinator movement of spores and transmission is subsequently reduced as a result. The transmission of M. violaceum is frequency dependent, and thus reliant on the probability of a pollinator landing on an infected flower and depositing spores on to healthy, susceptible flowers (Thrall and Jarosz, 1994b). Therefore, there are two problems associated with fungal invasion into a region: 1) The frequency of disease in the colonized population. In larger populations, the frequency of disease is low and therefore less spread is expected. 2) The movement of the fungus to another population must occur before it becomes locally extinct. The facility of movement of the pathogen may be determined by the dispersion (uniform, random, or clumped) of the neighboring populations (A.M. Jarosz, P.H. Thrall, and E.J. Lyons, unpublished). It is realistic to expect that the size and distribution of S. latifolia populations would be important in

determining pollinator foraging behavior and fungal transmission. We investigated these five hypotheses using a combination of the roadside census and field transplant studies described below.

Methods

Roadside Census Data Collection

In the summer of 1993, 1994 and 1995 twelve states in the eastern United States were censused for the presence of Silene latifolia and Microbotryum violaceum. 1993: Virginia, Tennessee, Ohio, and Michigan; 1994: New York, Connecticut, Vermont, New Hampshire, Massachusetts, New Jersey, and Maryland; 1995: Pennsylvania. In addition, data were gathered on population sizes and distances between populations to test our hypothesis regarding the role of metapopulation structure in the successful colonization of M. violaceum. States were censused according to the following protocol.

To expedite the process of covering large areas (i.e., states) in a reasonable amount of time, the following census protocol was developed. A starting point for a slow segment was haphazardly chosen on the map (De Lorme's detailed topographic atlases were used) and biased by concentrating on disturbed or open farm areas where S. latifolia was likely to be present. From that starting point we drove slowly (20 mph) until we found S. latifolia plants or until 5 miles elapsed. If S. latifolia plants were found within 5 miles then we drove 0.3 miles to the end of the population and estimated the number of plants present. Three tenths of a mile was chosen as the distance over which the population size would be estimated. This was the size of the largest population of S. latifolia that we found in our first week of censusing. Population sizes were put into the

following categories : 1-10, 10-100, 100-1000 or >100. The presence or absence of infected plants was also recorded for each population. If no S. latifolia plants were found within 5 miles of the starting point or no nearest neighbor could be found within 5 miles of the previous population, we drove 5 miles at the speed limit without looking for plants and then resumed the slow census according to the rules above.

Roadside Census Analysis

The information gained from the census is plotted on a United States map divided by county (Figure 1). Counties shaded in red indicate that S. latifolia populations are present but no M. violaceum was found. Counties shaded in blue denote the presence of infected plants, while counties shaded in yellow are those in which censuses were done but S. latifolia was not found. For the purpose of analysis the map is divided into three regions based on latitude. Region 1 includes those populations south of 39°N. Region 2 includes those populations between 39°N and 42°N. Region 3 includes populations located at latitudes greater than 42°N. Counties that straddle a latitudinal boundary were considered to be in the more southerly region. These regions were defined in an attempt to make distinctions that might be more ecologically meaningful in terms of fungal growth and establishment than arbitrary state boundaries. Factors used in making this decision include temperature, rainfall and overall weather conditions.

The data are categorized by the distance (in miles) of populations from the starting point of a slow segment (0-1, 1-2, 2-3, 3-4, 4-5, >5) and by population size (number of plants) (1-10, 10-100, 100-1000, >1000). The mean (weighted) distances are calculated using 0.5, 1.5, 2.5, 3.5, 4.5, and 5.5 to weight each of the above distance categories

respectively. The mean (weighted) population sizes were calculated using 5, 50, 500, and 1500 to weight each of the size categories. These weights are somewhat arbitrary though with the exception of 1,500 they represent the mid-point of each category.

To further explore the differences between the three regions with respect to population size and distance between populations, a non-parametric, one-way analysis of variance (ANOVA) was performed using the NPAR1WAY procedure in SAS (SAS Institute, 1995). The rank sums resulting from this procedure were then used to hand calculate post-data pair-wise comparisons among the three regions with respect to both size and distance (Conover, 1988).

Field Experiment

In an attempt to test our hypotheses regarding the rarity of M. violaceum at latitudes above 39°N we initiated a two year field transplant experiment. Seven sites were chosen forming two south-north transects in the eastern United States. The western transects included: Dunbar Forest Experiment Station (Dunbar), Sault St. Marie, MI; W.K. Kellogg Biological Station (KBS), Hickory Corners, MI; and the Ohio Agricultural Research and Development Center (OARDC), Wooster, OH. While the eastern transect included: University of Maryland Agricultural Experiment Station (UMD), Beltsville, MD; University of Connecticut Agricultural Experiment Station (UCONN), South Haven, CT; and the University of Vermont Horticultural Farm (UVM), South Burlington, VT. Experimental populations at Mountain Lake Biological Station (MLBS), Pembroke, VA served as the southernmost location for both transects. The latitude and longitude of each site is given in Table 1.

Table 1: Latitude and longitude coordinates for the seven sites used in the field experiment.

Field site	Latitude	Longitude
DUNBAR	46°27' North	84°16' East
KBS	42°24' North	85°23' East
OARDC	40°46' North	81°56' East
MLBS	37°22' North	80°31' East
UMD	39°01' North	76°52' East
UCONN	41°32' North	72°54' East
UVM	44°28' North	73°08' East

At each site three 3m x 3m plots, at least 3m apart, were established for a total of twenty-one plots across the seven sites. Each plot contained 64 S. latifolia plants of three types, 24 native, 36 susceptible and 4 infected. The native plants were explants from the area surrounding the plots collected approximately 18 hrs. prior to planting. In the area around the Dunbar site, the natural density of S. latifolia was too low to accommodate the needs of the experiment. Plants from the northern lower peninsula of MI were used in this case. The native plants were used to determine if M. violaceum can successfully infect plants native to each of the seven regions.

The susceptible plants were the progeny of crosses between two known susceptible genotypes (lines 1 and 10) collected from populations at MLBS (Alexander et al., 1993). Seed were obtained from crosses performed at Duke University, germinated in conetainers in the greenhouse at Michigan State, and then transported to the seven sites for planting. These plants, which become readily infected upon inoculation, were used to assess the ability of M. violaceum to establish and persist in regions north of 39°N. Infected plants were dug up in May of 1994 from natural populations in the area of MLBS and potted in standard greenhouse potting mix for transport to the sites. These plants served as the natural inoculum source allowing for fungal transmission by vectors.

In early June of 1994, the plots at all seven sites were mown or roto-tilled in preparation for planting. Sixty healthy plants were randomly assigned to random positions in an 8 x 8 grid within each plot with 0.3 meters between each plant. The infected plants were placed in non-random focal points (positions 3,3; 3,6; 6,3; and 6,6 of the 8 x 8 grid population) to insure that no healthy plant would be more than two positions from an

inoculum source. Once the experimental populations were established, approximately one third of the flowering susceptible and native plants were hand inoculated using a Q-tip covered with spores from a bud of an infected plant in the plot. This was done in an attempt to eliminate the possibility that transmission of the fungus would not occur due to a lack of pollinator service.

The seven sites were censused in September of 1994 to determine if any transitions had occurred. The plants were recorded as either dead, alive, healthy, or infected. Healthy plants were recorded if empty capsules remained on the stems or healthy flowers were visible. Infected plants were recorded only if infected flowers were visible. Those plants that were still rosettes were recorded as alive.

Due to exceptionally high mortality in the populations in 1994 caused by drought and encroachment of grasses and other weeds, the dead plants were replaced in May 1995 with the appropriate type according to the original randomized plot maps for each site. In places where inoculum source plants were replaced, plants that had been inoculated in the greenhouse at Michigan State and flowered infected were used. This is not problematic as the plants were inoculated with a single fungal strain from the region near MLBS where the original plants were collected. In addition, Alexander and Oudemans (1994) have documented very little variation among strains of M. violaceum.

In an attempt to reduce mortality in the replanted populations, the replacement plants were planted in mid-late May rather than in June to give them time to establish prior to the germination of the grasses and other weedy vegetation. Upon re-establishment, approximately one third of the healthy, susceptible plants were again hand inoculated. The

plots were mown during the first week of August 1995 to cut back the encroaching grasses and weeds and simulate the disturbed roadside environment in which S. latifolia is commonly found.

The plots were censused in late August 1995 for the presence of transitions. I was unable to census both the UMD and the OARDC sites. The plants at the UMD site had succumbed to drought and those at the OARDC had been accidentally mown and their tags removed making an accurate census impossible. Therefore, these sites were not used in the statistical analyses presented below.

Field Experiment Analysis:

The data collected from the field experiment were used to test several different hypotheses. In order to determine if infection of S. latifolia by M. violaceum varied across the northeastern United States, the data were analyzed using a categorical analysis of variance in the SAS CATMOD procedure (SAS Institute, 1995). The analysis revealed no differences in the number of transitions from healthy to infected between plots within sites, so the data were pooled for comparisons among sites.

For the purpose of investigating the hypothesis that the native plants are more resistant to infection than the transplanted susceptible plants, a chi-square test was done comparing the final disease status of both plant types. Because plants from the region surrounding MLBS were used, chi-square comparisons were performed with and without the data from the MLBS plots. To test the hypothesis that disease transmission may be pollinator limited, a chi-square was again performed on the hand inoculated and vector

infected plants. All chi-square tests were done using the FREQ procedure in SAS (SAS Institute, 1995).

Finally, the above pollinator limitation hypothesis was examined with respect to latitude. For this analysis, the experiment sites were divided into three regions according to latitude and corresponding to the three regions used in the roadside census study.

MLBS was placed in region 1, KBS and UCONN in region 2, and UVM and DUNBAR in region 3. The results of this analysis are used to investigate the variation in fungal transmission and growth across the latitudinal gradient in the eastern U.S..

Results

Roadside Census

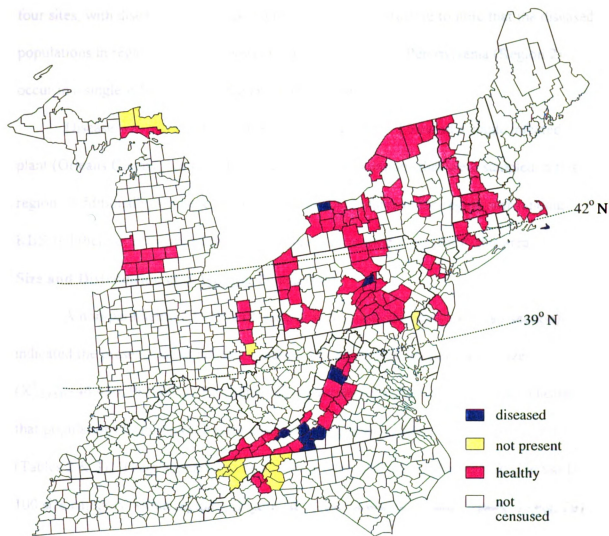
Distribution of S. latifolia and M. violaceum

Silene latifolia populations were common throughout the eastern United States north of the Virginia state line (Figure 1). Populations are either absent or very rare south of Virginia, since extensive efforts failed to detect any S. latifolia plants in Tennessee (Figure 1). On average, populations infected with M. violaceum were quite rare, being detected in only 21 of the 658 total populations censused over the three year period. However, plants infected with M. violaceum were relatively common in region 1, being detected in 16 of the 102 populations censused. The disease was very common in the area surrounding MLBS, where approximately 23% of the populations contained diseased individuals (Antonovics, Thrall, Jarosz and Taylor, unpublished data). Four infected populations were also found along the Blue Ridge Parkway in Virginia (represented by the clump of four counties shaded in blue in south central Virginia in Figure 1) (Patrick, Floyd, Roanoke and Franklin counties, Appendix 1), and two other infected populations were found near Front Royal, Virginia (represented by the one county shaded in blue in northern Virginia, Figure 1) (Rockingham County, Appendix 1).

The disease became increasingly rare with increasing latitude. In region 2, only four of the 169 populations that were censused contained infected plants. Three of these populations were found on Nantucket Island, while the fourth was located near Lewisburg, Pennsylvania (Figure 1). The amount of disease was surprisingly high at all

Figure 1: Geographic distribution of Silene latifolia and Microbotryum violaceum in the eastern United States.

Counties shaded in red indicate the presence of S. latifolia populations but absence of M. violaceum. Counties shaded in blue denote the presence of infected plants, while counties shaded in yellow are those in which censuses were done but S. latifolia was not found. The map is divided into three regions based on latitude. Region 1 includes those populations south of 39°N. Region 2 includes populations between 39°N and 42°N. Region 3 includes populations at latitudes north of 42°N. Counties that straddle a latitudinal boundary were considered to be in the more southerly region.



Source: U. S. Bureau of Census, 1983; Lyons, 1995.

Figure 1

four sites, with disease incidence averaging 30%. It is interesting to note that the diseased populations in region 1 and the diseased population found in Pennsylvania (Region 2) occur in a single valley of the Ridge and Valley system.

Disease was extremely rare in Region 3 (Figure 1). I located a single infected plant (Orleans County, New York) within the 387 populations that were sampled in this region. Additionally, we were unable to find infected plants in the region surrounding KBS and therefore concluded that *M. violaceum* is no longer present in this area.

Size and Distribution of S. latifolia Populations

A non-parametric ANOVA (described in the roadside census analysis section) indicated the presence of significant variation among regions for population size ($X^2_{(2,658)}=46.436$, $P<0.001$). Post-data pair-wise comparisons (Conover 1988) indicated that populations from region 2 were larger than populations from either region 1 or 3 (Tables 2 & 3). There appeared to be a relative over-abundance of populations in the 10-100 size category within region 2 (Figure 2d) when compared to either region 1 (Fig. 2b) or 3 (Fig. 2f)

There were also significant differences in the distribution of the populations among regions as indicated by the significant non-parametric ANOVA for distance from the start of a census to a population ($X^2_{(2,658)}=47.167$, $P<0.001$). The post-data pair-wise comparisons indicated that the distance to the population was significantly longer for region 1 (Table 2). Indeed, the average distance to a population was nearly a mile farther compared to either region 2 or 3 (Table 3). The distribution of populations in region 1 appeared rather clumped based on the simultaneously high frequency of populations < 1

Figure 2: Size and distribution of S. latifolia populations in the three regions of the eastern United States.

The frequency distributions of populations in the four size classes or five distance classes for region 1 (south of 39° N; **a** and **b**), region 2 (between 39° N and 42° N; **c** and **d**), region 3 (north of 42° N; **e** and **f**). See Figure 1 for areas sampled within each region. The census protocol is explained in the Materials and Methods section.

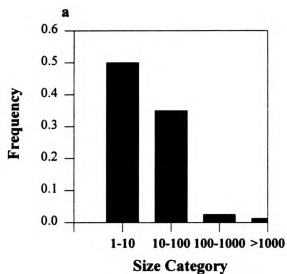
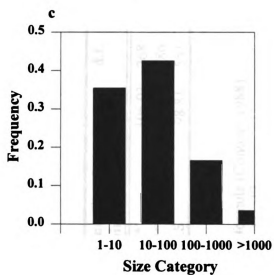
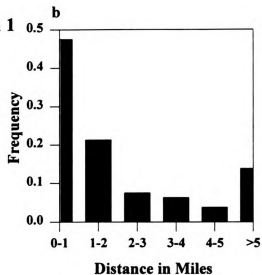
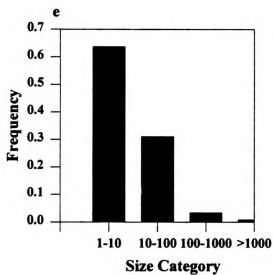
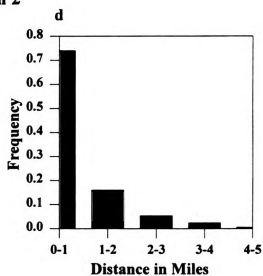
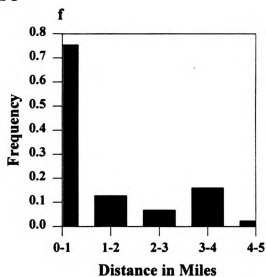
**Region 1****Region 2****Region 3**

Figure 2

Table 2: Non-parametric, post-data pairwise comparisons of S. latifolia distance of populations from census starting point and population size for the three regions.

Region Comparison	t	d.f.	Distance from Starting Point	t	d.f.	Population Size
Region 1 vs. Region 2	150.71	268	**a	106.91	268	**
Region 1 vs. Region 3	150.38	486	**	8.101	486	N.S.
Region 2 vs. Region 3	0.32	553	N.S.	98.81	553	**

** denotes significance at $p < 0.05$

a; all calculations of the test statistic were done using formula (Conover 1988):
 $|R_x/n_x - R_y/n_y| > t \alpha/2 (s^2 N-1-H/N-K)^{1/2} (1/n_x + 1/n_y)^{1/2}$

Table 3: Weighted mean distance to populations and population size of S. latifolia for three regions of the eastern United States.

Geographic Region	Mean Distance From Starting Point	Mean Population Size
Region 1	2.39 mi.	51.25 plants
Region 2	1.45 mi.	159.17 plants
Region 3	1.47 mi.	47.10 plants

mile and > 5 miles from the starting point (Fig. 2a). In contrast, populations in region 2 and 3 were characterized by populations that are <1 mile from the starting point and seem to be more random across the landscape (Figs. 2c & 2e).

Field Experiment

The results of the 1994 census indicated severe mortality of all three plant types. Overall plant mortality was above 45% at all of the sites with the exception of MLBS (40%) and Dunbar (2%) (Table 4). In general, the class that had the highest mortality was the susceptible class (50% or greater at all sites except Dunbar).

Despite high mortality, there were 6 transitions at MLBS, 7 at the OARDC, 2 at both KBS and Dunbar, and 1 at UVM (Table 4). Two of the transitions at MLBS were the result of hand inoculation as was the one transition at UVM. The remainder of the transitions were due to spore deposition on healthy flowers by pollinators. The low number of transitions that occurred during 1994 prohibited statistical analyses.

In 1995 transitions occurred at the five sites that were successfully censused (Dunbar, KBS, MLBS, UCONN and UVM). Specifically, there were 14 infections at Dunbar, 13 at UCONN, 12 at both KBS and MLBS and 10 at UVM (Table 5). In addition, mortality was greatly reduced to 30% or less at all sites except UMD and OARDC where it was 100% (Table 5). All of the infections that occurred in 1994 survived overwinter (Table 5). A non-parametric categorical ANOVA on the 1995 data revealed no differences in the number of transitions between plots within sites so the data were pooled for comparisons among sites. Again, there were no differences among sites with respect to the number of transitions from healthy to diseased.

Table 4: Survival and infection of *S. latifolia* in 1994 from the seven field sites.

Field Site	% Mortality	# Survived to Fall	# Newly Infected in Fall
Dunbar	2%	188	2
KBS	62%	83	2
OARDC	46%	117	7
MLBS	40%	114	6
UMD	76%	47	0
UCONN	57%	82	0
UVM	20%	154	1

Table 5: Survival and infection of *S. latifolia* in 1995 from the seven field sites.

Field Site	% Mortality	# Survived to Fall	Newly Infected in Fall	Overwinter infections	Total # of Infections
Dunbar	4%	170	12	2	14
KBS	16%	161	10	2	12
OARDC	100%	0	0	0	0
MLBS	25%	143	6	6	12
UMD	100%	0	0	0	0
UCONN	9%	174	13	0	13
UVM	30%	133	10	1	11

The chi-square tests revealed a significant difference ($X^2_{(3,744)}=4.274$, $P<0.04$) between native and susceptible plants indicating that the susceptible plants were more likely to become infected than native plants. In addition, plants that were hand inoculated were more 15% more likely to become infected than plants that were subject to only natural vector transmission ($X^2_{(3,744)}=49.425$, $P<0.001$). This phenomenon did not follow any detectable latitudinal gradient ($X^2_{(3,744)}=0.12$, $P<0.73$).

Discussion

It is clear from the large scale distribution study that, as we suspected, the range of S. latifolia extends throughout the eastern United States. However, the range of M. violaceum is larger than we originally thought, extending from western Virginia to western New York and east to Nantucket Island. There appears to be no latitudinal trend in population size or distance between populations in the eastern U.S. though, in general, the three regions differ with respect to these parameters.

The results of our field experiment indicated that: 1) M. violaceum is able to infect and successfully overwinter in S. latifolia plants in areas above 39° N latitude. 2) Plants that were hand inoculated were more likely to become infected than plants that were subject to only natural vector transmission, and 3) The susceptible genotype plants were more likely to become infected than the plants that were native to each experimental site.

Recall our five hypotheses for the rarity of M. violaceum at latitudes above 39° N latitude: 1) The distribution of M. violaceum is the result of an historical accident. 2) There is a lack of pollinator service such that pollinators are not visiting infected plants with a high enough frequency to insure fungal transmission within and among metapopulations. 3) High levels of resistance among northern S. latifolia populations prevent the successful infection and subsequent persistence of M. violaceum at northern latitudes. 4) The inability of the pathogen to establish perennial infection limits further transmission. 5) The size and distribution of populations in areas outside Virginia is not conducive to invasion by the pathogen.

Our roadside census data provide partial support for hypothesis 1 that the distribution of M. violaceum is due to an historical accident. It appears that M. violaceum is spreading from a focus in one valley system in Virginia and from a second isolated focus on Nantucket Island. The fact that the infected population in Pennsylvania occurs in the same valley of the Ridge and Valley system as the northernmost, infected populations in Virginia suggests that M. violaceum may be spreading northward from Virginia.

In addition, the infected populations on Nantucket Island may represent another focus of introduction from which the fungus is now spreading. In the early nineteenth century when S. latifolia was introduced to the United States from soil used as ballast on trade ships. It is plausible that M. violaceum was introduced to Nantucket Island at this time and has since spread to infect 3 of the 5 populations of S. latifolia on the island. Further spread to mainland Massachusetts may be limited by the 30 miles between the mainland and Nantucket Island.

While these data suggest that historical accident explains much of the current distribution of M. violaceum, the presence of infected plants in New York (Figure 1) and Michigan (S. Tonsor and J. Antonovics pers. comm.) suggests that it does not explain all aspects of the distribution. Neither of these sites is near the presumed sites of disease introduction on Nantucket or in Virginia suggesting that long distance migration has occurred to these areas either from Europe or the disease foci in the United States. The Michigan population of M. violaceum is likely to have become extinct since the extensive searching of the past five years has failed to detect any infected plants (Figure 1 and A.M.

Jarosz, unpublished observations). The failure of this population to persist requires an explanation other than historical accident. It is possible that M. violaceum has been introduced in several areas in the eastern U.S. and throughout the last hundred years it has spread in some areas and gone extinct in others.

Our field experiments allowed us to further investigate factors we thought might be important in determining the distribution of M. violaceum. Hypothesis 2 asserts that a lack of pollinator service is an important factor in the Silene-Microbotryum host-pathogen system. We directly addressed this by comparing infections resulting from hand inoculations and those that were caused by pollinator spore deposition (i.e., all other infections). Hand inoculated plants were more likely to become infected than were those that were naturally infected. These data can be interpreted in a variety of ways.

First, it may be that the number of spores deposited on each flower was greater in the hand inoculated plants than the spore load typically deposited by pollinators thereby increasing the chance that successful infection will occur. It has also been suggested that pollinators are able to discriminate between healthy and infected S. latifolia plants and preferentially choose to land and probe on healthy flowers over infected ones (Altizer, et al., unpublished). Additionally, it has been shown in a study done by Helen Alexander that populations of S. latifolia are naturally pollen limited, that is, when hand pollinations were performed seed set increased dramatically (Alexander, 1987). Taking together the results of the pollen limitation study and our field experiment it is plausible to suggest that the transmission of M. violaceum is be limited by pollinator visitation. This may serve as a partial explanation of the patchy distribution of the fungus, as movement of spores, like

pollen between populations may be low. However, if the pattern of pollinator service at northern latitudes is similar to that in the region surrounding MLBS, then pollinator service may not be a major limitation.

Transmission of M. violaceum may be further limited by the foraging movements of its vectors. For example, though this was not explicitly tested, it may be that the trap-lining hawk moths that nocturnally pollinate S. latifolia and generally facilitate interpopulational movement of spores and pollen do not live in the areas above 39° N thereby reducing the probability that M. violaceum will successfully invade northern populations. However, the lack of a distinct latitudinal trend in the presence of disease argues against this idea.

Hypothesis 3 states that the rarity of M. violaceum at northern latitudes is due to high levels of resistance among northern S. latifolia populations. Comparisons of the infection of plants native to the northern regions and those of known susceptibility suggest that the northern plants may be somewhat resistant, as they were less likely to become infected than the susceptible plants. However, this difference in infection between native and susceptible plants may be driven by the data from the plots at MLBS where the disease is abundant. Recall that when the data were analysed with out the MLBS plots, the difference between natives and susceptibles was no longer present. This may be indicative of the fact that the pathogen has not yet reached the northern regions and thus resistant types are absent.

If nothern genotypes are somewhat resistant, the abundance of resistant genotypes and overall population structure, may or may not serve to prevent M. violaceum from

spreading and persisting in areas above 39°N (Burdon, 1987; Thrall & Jarosz, 1994b). It is also possible, given the low frequency of disease in Pennsylvania and New York, that the fungus was once prevalent in northern regions and due to a build up of resistant genotypes is now in the process of being expunged from populations in this area. A more complete answer to this question would require an in depth genetic study of the plant genotypes present in the northern regions. However, our field study does tell us that M. violaceum can successfully infect plants in the northeastern U.S., a fact we were not certain of prior to the initiation of this study.

Hypothesis 4 suggests that the rarity of M. violaceum in northern latitudes is due to poor over-wintering survival of either the plant or fungus prohibiting successful systemic infection thereby reducing fungal transmission and establishment. This has been suggested previously by Thrall, et al.(1993) in their investigation of disease susceptibility and variation in life history strategies within the Caryophyllaceae. While we were not able to test this hypothesis as rigorously as we had hoped due to high levels of growing season mortality in 1994 we do have some data that suggest that over-wintering is not problematic for S. latifolia in colder, northern climates. At the DUNBAR field site in Sault Saint Marie, MI, we had very low levels of growing season mortality (4%) and high over-wintering survival (98%). In addition, the fourteen plants that became infected during the summer of 1994 remained infected in the spring and summer of 1995. Similarly, at the UVM site in Burlington, VT, where growing season mortality was higher (30%), 9 of the 10 plants that became infected in 1994 were alive and systemically infected during both the spring and summer censuses of 1995. DUNBAR and UVM

represent our northern most sites where winter temperatures are regularly in the teens and below zero thereby providing evidence that both S. latifolia and M. violaceum can successfully over-winter and persist at northern latitudes.

Finally, hypothesis 5 attributes the rarity of M. violaceum above 39° N to the fact that the plant population structure in areas outside Virginia is not conducive to invasion by the pathogen. Our expectation upon the initiation of this study was that a metapopulation structure of small populations located in close proximity to each other would be necessary for successful establishment of M. violaceum in S. latifolia populations. This assertion is based on the fact that the transmission of M. violaceum is frequency dependent and thus more likely to be transmitted from small populations where its initial frequency would be high. Short distances between populations would facilitate colonization of new populations. Due to restricted pollinator movements, it is possible that the large population sizes in northern regions may be hinderance to fungal invasion in those areas.

The results of the roadside census study indicate that the three regions in the eastern U.S. represent a complex mosaic of S. latifolia populations of different sizes and distances between them. There are no clear trends with regard to either of the distributional characters. Disease is present in all three regions and population size and distance do not vary predictably along the latitudinal gradient of the eastern U.S. . However, it is possible that the band of large populations in region 2 may be a barrier to further transmission northward from Virginia. Even so, contrary to hypothesis 5, metapopulation structure is at best a partial explanation of the distribution of M. violaceum.

Chapter 3

CONCLUSIONS

While our descriptive, roadside census study is not exhaustive, it does provide information that is essential for determining the importance of population structure (size and distribution) in the overall distributions of S. latifolia and M. violaceum in North America. Additionally, we now know that the pathogen can be found in S. latifolia populations in Pennsylvania, New York and on Nantucket Island. Throughout the mosaic that represents the distribution of S. latifolia and the potential host material for M. violaceum, there is a great deal of variation in population size and distances between populations. There are mountain ranges, roads and highway systems that serve as barriers to pollinator flight and subsequently to both gene flow and fungal transmission. Our eastern United States census data point to the importance of studying not only more than one population but possibly more than one metapopulation in order to understand the dynamics of host-pathogen systems.

From our field experiments, we have determined that M. violaceum can infect and over-winter in S. latifolia plants that are native to northern regions. However, the possibility exists that plants in the northern regions may be more resistant to the fungus than the susceptible genotypes found in the area around MLBS as evidenced in the comparison of infected native versus susceptible plants. The differential infection success of hand inoculated versus natural vector transmission pollinator movements and foraging strategies may play an important role in understanding the distribution of M. violaceum.

In the context of both our roadside census and experimental studies, we think that the present distribution of M. violaceum is due to a combination of historical accident, population structure and distribution. If M. violaceum was introduced into the area surrounding MLBS, it may have been very difficult for it to move northward due to the ridges of the Ridge and Valley system which are characterized by shady, undisturbed, mountain regions which do not provide suitable habitat for S. latifolia. It may not be possible for M. violaceum reach the next valley and thus its movement is restricted to the valley in which it presently exists. This is exemplified by the infected populations in northern Virginia and Pennsylvania that are contained in the same valley.

In addition to geographical barriers, we suspect that population structure is also an integral factor in determining the distribution of M. violaceum. Patterns of resistance in S. latifolia populations can prevent the successful establishment of the fungus. In populations where resistance is high, the fungus will be readily expunged and any further movement from its focus of introduction will be inhibited (Thrall & Jarosz, 1994b).

Finally, the distribution and size of populations may affect the successful spread of M. violaceum from its focus of introduction. For example, it may be more difficult for the fungus to invade more northerly populations because in general they are larger and close together. My suggestion here is that the pollinators of S. latifolia may forage differently in large populations than they do in those that are smaller (Thrall & Antonovics, 1996). In small populations, a bee is likely to visit each flower in the population before moving to the next population. This increases the chances of landing on an infected flower and simultaneously increases among population spread. In large

populations that are close together, such as those in the northeastern U.S., pollinators are likely to view a few large populations as one contiguous population. The consequence of this is may be that they forage more randomly and seldom go beyond that large population. The probability of a pollinator landing on an infected plant in a large population is very low as is the among population transmission of M. violaceum. However, it is currently not known whether larger populations have more pollinators and if so, what the ratio of pollinators to flowers is. Obviously, this information would be useful in more closely examining among population disease spread.

In the future it would be interesting to more rigorously explore the genetics of northern S. latifolia populations in an attempt to characterize their resistance structure. This might provide a clearer picture of where the fungus has been and where it is going. In addition, it is essential to gain a better understanding of pollinator behavior and the role it plays in the transmission of M. violaceum within and among populations and metapopulations. Finally, it would be worthwhile to conduct a similar large scale census and genetic study in Europe where both S. latifolia and M. violaceum are native. This would allow us to make comparisons between regions in Europe where the host and pathogen have co-existed for a long time and the United States, where the association is relatively new thereby providing information on both long term and short term host-pathogen metapopulation dynamics.

APPENDIX

Appendix I: Counties in the eastern United States where S. latifolia is present. Presence of populations infected with M. violaceum is denoted with a (D)

Connecticut	New York	Bradford	Lee
Hartford	Cayuga	Butler	Madison
Litchfield	Clinton	Centre	Nelson
New Haven	Erie	Crawford	Patrick (D)
Delaware	Franklin	Dauphin	Roanoke (D)
New Castle	Genesee	Erie	Rockbridge
Maryland	Jefferson	Franklin	Rockingham (D)
Howard	Livingston	Juniata	Russel
Massachusetts	Madison	Lancaster	Scott
Barnstable	Montgomery	Lawrence	Shenandoah
Franklin	Monroe	Lebanon	Smith
Hampden	Oneida	Lycoming	Warren
Hampshire	Ontario	Mercer	Washington
Nantucket (D)	Orleans (D)	Mifflin	Vermont
Plymouth	Schoharie	Northumberland	Addison
Worcester	Seneca	Perry	Burlington
Michigan	St. Lawrence	Snyder	Chittenden
Allegan	Steuben	Tioga	Franklin
Barry	Tompkins	Union (D)	Rutland
Clahoun	Wyoming	Washington	
Eaton	Mitchell	West Moreland	
Ingham	North Carolina	York	
Kalamazoo	Burcombe	Virginia	
Mackinac	McDowell	Albemarle	
Ottawa	Mitchell	Amherst	
Vanburen	Ohio	Augusta	
New Hampshire	Athens	Bedford	
Cheshire	Coshocton	Bland	
Sullivan	Holmes	Craig	
New Jersey	Muskingum	Floyd (D)	
Atlantic	Wayne	Franklin (D)	
Burlington	Pennsylvania	Giles (D)	
Morris	Adams	Grayson	
Sussex	Berks	Greene	

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