The diversity-enhancing effects of plant pathogens: Moving beyond the Janzen-Connell hypothesis to explore how plant species abundance drives the accumulation of pathogen species and the effects of a specialist fungal pathogen on diversity in temperate forests of Eastern North America.

by

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ABSTRACT

The diversity-enhancing effects of plant pathogens:
Moving beyond the Janzen-Connell hypothesis to explore how plant species abundance drives the accumulation of pathogen species and the effects of a specialist fungal pathogen on diversity in temperate forests of Eastern North America.

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Chair: John H. Vandermeer

Plant enemies (pathogens and herbivores) have been hypothesized to maintain plant species diversity by causing greater impacts on common species than rare species thus preventing competitive exclusion. While the role of plant enemies in producing distance-or density dependent mortality in seeds and seedlings is well-documented, the exclusive focus on these effects provides limited understanding of the effects of enemies on plant communities. Understanding the role of plant enemies in maintaining diversity requires a broader approach that considers how abundance of a plant species determines the composition of the community of enemies that attack it as well as relates the demographic effects of enemies on the growth of their host plant populations to the diversity of the entire plant community.

The accumulation of plant enemies is hypothesized to be a passive, sampling process which predicts more common plant species, i.e. larger in individual size and more wide-spread, are attacked by more enemy species than smaller-statured and more

geographically-restricted species. I test this hypothesis in a comparative analysis of 490 plant species and find that, as predicted, the richness of fungal pathogens infecting a plant species increases with plant species' individual size and geographic distribution.

To test the hypothesis that plant enemies maintain plant diversity by limiting the growth of populations of competitively superior plant species through density-dependent disease or herbivory, I investigated the interactions between the host-specific fungal pathogen, *Puccinia podophyllii* and its host Mayapple (*Podophyllum peltatum*) as well as the effects of this interaction upon the species abundance and composition of the entire plant community in the forests of Washtenaw County, Michigan. I find that Mayapple is strong competitor reducing plant species seedling survival and the diversity of the plant community. Next, I find that the pathogen decreases host population growth by causing strong distance-dependent seedling survival and by reducing growth and reproductive rates. Furthermore, I show that rates of disease transmission and levels of disease severity increase with Mayapple density. The results strongly support the hypothesis that plant enemies maintain plant species diversity and provide the most complete demonstration, to date, of their diversity-enhancing role.

Chapter 1

Fungal pathogen species richness: Why do some plant species have more pathogens than others?

Abstract: Why are some plant species infected by more pathogen species than others? This question is of broad practical and theoretical interest, but to date a thorough analysis has not been published. Variation among plant species in the number of associated herbivore and pathogen species is predicted to fit a species-area relationship in which the area occupied by a plant species is a function of individual size and the area the species occupies. Using published estimates of geographic range, individual size, fungal-pathogen species richness and phylogenetic relationships for 490 plant species from the United States and controlling for sampling intensity and phylogenetic effects, I find that number of pathogens found on a plant species increases with metrics of plant species' area and/or habitat diversity. In non-phyogenetic tests, log geographic range size, log height, leaf-longevity, sampling intensity, and the interaction between sampling intensity and plant species' growth form were all significant and accounted for 71% of the variation in pathogen species richness among plant species, primarily driven by positive correlations with range size, individual size and sampling intensity which combined explained 52% of the variation. Analysis of the phylogenetic independent contrasts yielded similar results, with range size, individual size and sampling intensity accounting for 53% of the variation in pathogen species richness.

Introduction

Patterns in the diversity of ecological communities and mechanisms that generate these patterns are primary issues in ecology and evolution. Understanding the processes that determine the composition parasite and pathogen communities in wild plant populations is important because plant enemies can reduce the growth of host populations (Jarosz and Davelos 1995, Gilbert 2002) and structure plant communities (Dobson and Crawley 1994, Clay 1997). Within plant communities, the impact of plant enemies can vary among plant species (Kilronomos 2002, McCarthy-Neumann and Kobe 2008). This variation is, in part, due to differences in the diversity and abundance of the enemy communities among species. Parasites and pathogens form communities that can be organized into three nested hierarchical levels. The community of pathogens infecting individual hosts (infracommunity) is drawn from the community of pathogens infecting the host population (component community) (Holmes and Price 1986). Pathogen component communities are a subset of all the pathogen species infecting a host species across its geographic range. The diversity of plant enemies at the species level defines the species pool from which the infra- and component communities are drawn.

Several hypotheses predict that variation among plant species in the number of associated herbivore and pathogen species fits a species-area relationship in which the area occupied by a plant species is a function of individual size and the area the species occupies (i.e. size of geographic range). The hypotheses are based on the analogies between host plants and islands (Janzen 1968) which extends the theory of island biogeography (MacArthur and Wilson 1963, 1967) to host species and suggest that number of phytophagous species found on a plant species is driven by passive sampling

(Colemann 1981), habitat heterogeneity (Willams 1943, Lawton and Schroder 1977), and/or energy (Wright 1983) embodied by the plant species. Based on these hypotheses, larger individual plants should accumulate and support more enemies than smaller one because they are morphologically more complex, passively sample more enemies, and support large populations of enemies thus reducing extinction rates. Similarly, broadly distributed plant species should support a greater numbers of enemies than more restricted host species due to the same mechanisms except that habitat heterogenetity increases as populations occupy an increased range of abiotic conditions and biotic communities.

While species-area relationships in insect herbivores are well documented (e.g. Kennedy and Southwood 1984), relatively little is known about the role of host species abundance in determining pathogen species richness (p.s.r.(Morand 2000)). Although previous studies have shown that plants with smaller geographic ranges are infected by fewer total species of fungal pathogens compared to hosts with larger ranges (Clay 1995, Yarwood 1962,) and that increasing plant size also is associated with increasing p.s.r. (Yarwood 1962, Strong and Levin 1975, 1979), the validity of these results is not clear because the effects of sampling bias and phylogenetic relationships among host species on the p.s.r. were not considered.

Unequal sampling of pathogen species among plant species can drive the species-area pattern. Because the number of fungal pathogen species occurring on a plant species is estimated by using compilations of published reports, the number of reported pathogen species may be proportional to sampling intensity, as species that occupy a larger geographic range are studied more intensely (Connor and McCoy 1979).

Furthermore, testing and accounting for phylogenetic signal is critical in comparative analyses to account for the similarity (i.e. non-independence) in characteristics among closely-related species (Harvey et al. 1995). Indeed, several re-analyses of data thought to support broad ecological trends have failed to support the original conclusions when the analyses accounted for phylogenetic effects (Mazer 1990, Kelly and Purvis 1993, Harvey et al. 1995, Kelly 1995, 1997).

Additionally, estimates of the strength phylogenetic signal of p.s.r. and measures of plant species abundance provide evidence concerning the processes that determine p.s.r. since the magnitude of the signal reflects the relative lability across plant species characteristics (Ackerly and Donoghue 1998, Ackerly and Reich 1999, Morales 2000). Measures of the phylogenetic signal compare the similarity of characteristics of closely related species to those of distantly-related species. If the accumulation of total number of pathogen species asymptotes quickly to correspond with the area occupied by a species and the size of these areas are not clade specific (Strong 1974c, b, a, Kelly and Southwood 1999, Mitchell and Power 2003, Torchin and Mitchell 2004), then p.s.r. should exhibit low levels of phylogenetic signal. Alternatively, if p.s.r. is determined by clade-specific effects such as shared resistance strategies, lineage age (Birks 1980, Kennedy and Southwood 1984), and taxonomic isolation (Connor et al. 1980, Strong et al. 1985), then p.s.r. should be similar among closely related species. However, tests for phylogenetic signal in the species/area patterns of plant pathogens have not been previously reported.

Here, I first test the phylogenetic signal of the data for plant species' geographic range, individual size and fungal pathogen species richness using current phylogenies for

490 species of plants occurring in the United States. I then test the prediction that the richness of fungal pathogens infecting plant species is a function of both plant species' individual size and geographic range while controlling for sampling intensity and phylogenetic effects.

Methods

Plant species selected were based on an initial host species list of 314 plant species used by Strong and Levin (1979). Taxonomy of the species was updated using current species lists (IPNI 2004, USDA 2004). Additional species were added in order to sample more plant families and genera and to add species within genera. Species were randomly selected from the pool of congeneric species for which geographic range and p.s.r. data was available. Exotic species, those species not native to North America, were excluded, with seven exceptions. Excluding the exotic species does not affect the results. Agricultural species were also excluded, as they are wide-spread and well-studied, and may inflate the species-area effect. Here, agricultural species are defined trees and shrubs used in commercial fruit production and annual crops. The final list includes 490 plant species from 45 orders, 71 families, and 194 genera.

The plants were sorted into four plant growth forms: gymnosperm trees (46 spp.), angiosperm trees (116 spp.), shrubs (158 spp.), and forbs (170 spp.). Plant growth forms were further decomposed into categorical variables of woodiness and leaf longevity (deciduous versus evergreen). Maximal height for each plant species as listed in the Flora of North America and/or in regional floras is used as an estimate of individual biomass for species.

The range of each plant species was estimated by its presence within each of the 49 continental U.S. states. The presence in a state was determined using plant distribution maps (USDA 2004). These maps are a compilation of state herbaria reports and have been used in a similar fashion by other authors (Mitchell and Power 2003). If a plant species was present in a state the area of that state (km^2) was added to its range estimate.

The p.s.r. for each host plant species was estimated using published sources complied by Farr and colleagues (1989 available on-line at http://nt.ars-grin.gov/fungaldatabases/). Fungal species were excluded if they had been reported only from dead tissue, since these may be saprotrophs rather than pathogens. Pathogen species found only on sub-species of host were included in the estimate of p.s.r. for the entire host species.

To estimate the effect of sampling intensity on the number of reported pathogen species, I collected data on the number of published papers on any topic since 1975 for each host species by searching for the species' latin binomial and synonyms in a Web of Science search. The log (the number of citations per species plus one) was used as an index of sampling effort or citation index, following Nunn and colleagues (2003).

Range size, height, and p.s.r. data were log-transformed to homogenize variance. A type III general linear model (GLM) was used to test the relationships among plant species' growth form, height, woodiness, deciduous habit, citation index, range size, and p.s.r.. To test if the effects of range size, height, and deciduous habit differed among plant growth forms, the interactions of these three characteristics with growth form were tested. The interaction between plant growth form and citation index was also tested, as

the effects of sampling intensity may differ among plant groups due to both changes in the interest in pathogens or the number of pathogens among the plant growth forms.

To conduct tests of the phylogenetic signal of the traits and phylogenetic comparative analysis, I constructed a phylogeny for the plant species using current published sources. The program Phylomatic (Webb and Donoghue 2005) was used to establish the relationships among plant orders and families. This is based upon a well-supported phylogeny (Stevens 2004) with time-based branch lengths from Wikstrom et al. (2001). More resolved trees within families and genera were grafted on to this backbone. The publications used to determine the relationships within families and genera are listed in table 1.5.

For species characteristics of range size, height, citation index, and p.s.r., I tested whether related species exhibit a significant tendency to resemble one another by comparing the fit of the phylogeny to the data, measured by the mean squared error (MSE), was compared to a distribution of MSE's generated by randomly reassigning the data across the tips of the phylogeny. The strength of the phylogenetic signal was estimated using the K-statistic. This statistic compares the observed fit of the data to the phylogeny to the analytical expectation based on the topology and branch lengths of the phylogeny, assuming a Brownian model of character evolution. If characteristics of closely related species are less similar to one another than predicted by the model and the phylogeny, the K will be less than one. If the variation among relatives is less than expected, then values of K will be greater than one. This statistic is comparable across traits and trees (see note in Table 1.3.) These tests were implemented using the PHYSIG.M program (Blomberg et al. 2003).

To control for the phylogenetic relationships among species, I calculated the standardized independent contrast of each quantitative character using the PDTREE module (Midford et al. 2002) in MESQUITE (Maddison and Maddison 2008). For each character, the branch lengths were transformed for statistical adequacy (Garland et al. 1992) and to reflect the significance of its phylogenetic signal (Blomberg et al. 2003). The relationship between the standardized contrasts of the independent variables and p.s.r was analyzed using a linear regression through the origin (Felsenstein 1985).

Results

The general linear model that included plant species' range size, height, deciduous habit, citation index, and the interaction of citation index and plant functional group was highly predictive of p.s.r. (adj. $r^2 = 0.711$) and all predictors were significant (see table 1.1). Range size and the citation index explained the most variation (partial $r^2 = 0.21$ and 0.23 respectively) followed by plant height and the citation index by functional group interaction. Evergreen species have significantly more pathogen species even if confers are removed from the analysis ($F_{1, 425}=11.6$, $p<0.05$). A significant, positive relationship exists between both plant species' range size and p.s.r and height and p.s.r.. The main effects of woodiness and functional group and the interactions of functional group with range size and height were not significant (see table 1.4). In this interaction, the slope of the relationship between citation index and p.s.r. is most steep for the Angiosperm and Gymnosperm trees and decreases for shrubs and herbs respectively. The citation index explains more variation in p.s.r. among the trees ($r^2 = 0.5$ and 0.56 for angiosperm trees and conifers respectively) than among shrubs ($r^2 = 0.27$) or herbs ($r^2 = 0.23$).

Table 1.1. Results of non-phylogenetic GLM analysis showing effect of plant functional group, height, geographic range size, leaf-longevity, sampling intensity and the interaction between sampling intensity and functional group on pathogen species richness. r^2= 0.711, p<0.001. See text for transformations used.

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	105.430	7	15.061	172.741	.000	.715
Intercept	7.399	1	7.399	84.862	.000	.150
Functional Group * Citation Index	3.721	3	1.240	14.227	.000	.081
Geographic Range	11.381	1	11.381	130.533	.000	.213
Height	3.665	1	3.665	42.034	.000	.080
Citation Index	12.530	1	12.530	143.711	.000	.230
Deciduous	.569	1	.569	6.522	.011	.013
Error	42.026	482	.087			
Total	716.779	490				
Corrected Total	147.456	489				

Table 1.2. Results of phylogenetic analysis of effects of plant height, geographic range size, and sampling intensity on pathogen species richness. Linear regression through the origin of the independent contrasts, p<0.0001, r^2 = 0.534.

	Coefficients				
	Unstandardized		Standardized	t	Sig.
Standardized Contrast	B	Std. Error	Beta		
Geographic Range	0.78	0.08	0.33	9.77	1.06E-20
Height	0.08	0.01	0.27	8.42	4.18E-16
Citation Index	0.10	0.01	0.38	10.71	3.66E-24

Table1.3: Summary of the fit of data for plant species characteristics to the phylogeny and the strength and significance of phylogenetic signal.

Traits	MSE_{obs}	MSE_{star}	MSE_{tree}	Obs M/M	K	P-value
Geographic range	0.15	0.14	0.26	0.56	0.08	0.258
Pathogen species richness	0.32	0.30	0.42	0.77	0.11	<0.001
Citation Index	0.84	0.70	0.96	0.88	0.13	<0.001
Height	0.42	0.37	0.18	2.32	0.34	<0.001

Table note: All tests of phylogenetic signal were done on a tree with time-based branch lengths. MSE_{obs} refers to the mean squared error of the tip data, calculated using the phylogenetically corrected mean. MSE_{star} is the M.S.E. of the tip data, calculated using a star phylogeny (assuming no phylogenetic signal). MSE_{tree} is the M.S.E. calculated using the variance-covariance matrix of the tree. Obs. M/M is equal to MSE_{obs} / MSE_{tree} . Larger values of this ratio imply more phylogenetic signal. K is the ratio of Obs. M/M divided by the expected MSE_{obs} / MSE_{tree}. The expected MSE_{obs} / MSE_{tree} is the expected ratio of mean squared errors given the tree and a Brownian model of evolution and is equal to 6.75 for the tree. The P-value refers to the randomization test of the null hypothesis of no phylogenetic signal.

In the phylogenetic analysis, range size, height, citation index, and p.s.r. all exhibited less signal than expected under a Brownian model of evolution. All traits, except range size, exhibited a significant phylogenetic signal in the randomization test (see Table 1.3).

The regression of the independent contrasts supports the same general trends in the GLM analysis. The standardized contrasts for plant species' range size, height, and citation index are highly significant predictors of the p.s.r. contrasts ($p<0.000001$) and combined account for 53% of the variation. As in the GLM analysis, range size (Figure 1.1) and the citation index each exhibited a larger effect size than plant height (Figure 1.2 and Table 1.2).

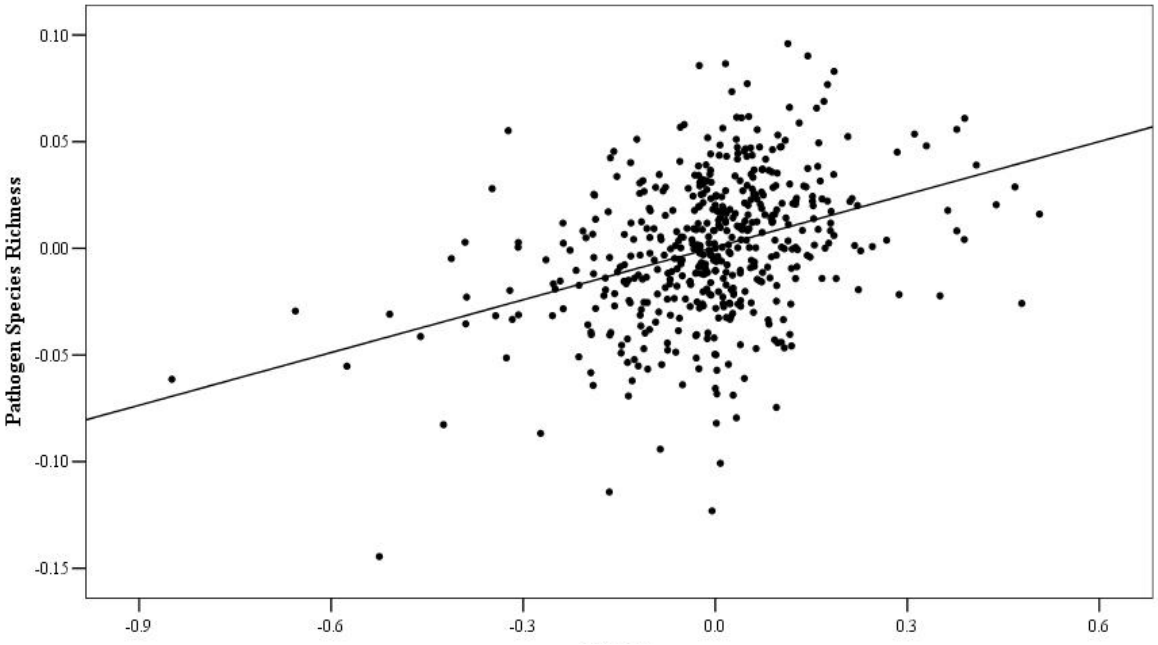

Figure 1.1. The relationship between the standardized contrasts of plant species' height and pathogen species richness. The partial regression plot from the analysis of the P.I.C.'s of p.s.r., height, and geographic range size, $r^2=0.134$, $p<0.0001$.

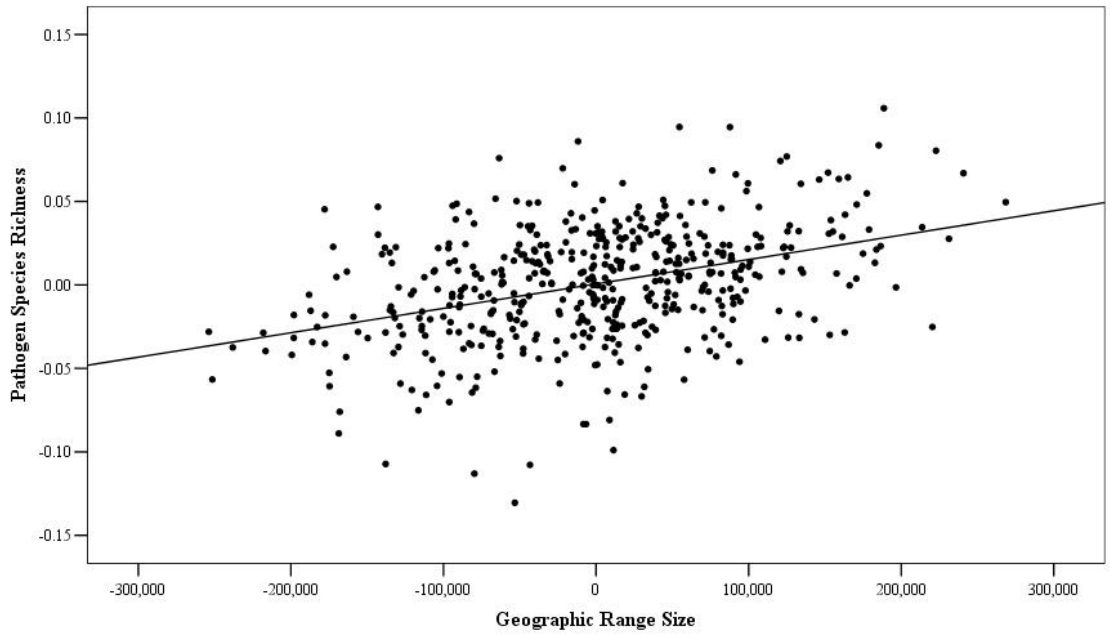

Figure 1.2: The relationship between the standardized contrasts of plant species' geographic range and pathogen species richness. The partial regression plot from the analysis of the P.I.C.'s of p.s.r., height, and geographic range size, r^2=0.15, p<0.0001.

Discussion

The results provide clear support for the two predictions that larger, more long-lived plant species are infected by higher numbers of pathogen species than smaller, relatively short-lived plant species and that p.s.r. increases with plant species' geographic range size.

Furthermore, this is the first study to show that the effect of sampling intensity of the number of reported pathogen species differs among plant functional groups and that

the sampling intensity exhibits significant phylogenetic signal. These results suggest that the effects of sampling bias should be analyzed using phylogenetic comparative methods and previous approaches to control for the variation in sampling intensity that utilize the residuals of regression between a citiation index and the number of reported pathogen or parasite species (e.g. Busch et al. 2004, Ezenwa et al. 2006) may be inadequate. The similarity among close relatives in sampling intensity is not surprising as close relatives of species of large academic interest or economic value also receive greater attention by virtue of being close relation per-se or by sharing a trait of interest or value.

The significant interaction between functional group and citation index is the result of a lower per citation probability of a new pathogen species being reported for herbs and shrubs than for trees. This analysis can not determine to what degree this pattern is due to smaller plants being infected by fewer pathogen species compared to larger plants or that the pathogens of economically important timber trees are more likely to be documented. Including this interaction in the model, however, assumes that the pattern is driven by sampling alone and diminishes the differences in p.s.r. among plant growth forms and the strength of the relationship between p.s.r. and traits that differ among plant growth forms such as plant height and woodiness. Other studies have used only the main effect of a citation index to control for sampling bias (e.g. Busch et al. 2004). This approach may bias the results because it inflates the differences among plant functional groups and traits that covary with plant functional group. After removing this interaction from the GLM analysis, the results reflect this effect: woodiness is significantly associated with an increase in p.s.r ($p= 0.017$, $F_{1, 484}=113$) and plant height

explains much more of the variation in p.s.r. ($F_{1, 484}$=134) than it does when the interaction is included ($F_{1, 482}$=42).

Pathogen species richness also exhibited a significant tendency to be similar among closely related plant species. However, p.s.r. was less similar among related plant species than expected by under Brownian motion evolution ($K_{p.s.r.}$=0.11). The significance and relative strength of the phylogenetic signals across the plant species characteristics suggest that the similarity among close relatives in p.s.r. is driven by the effects of more phylogenetically constrained characteristics of plant height and citation index on p.s.r..

Citation index, p.s.r., and geographic range size all exhibited low levels of phylogenetic signal (K<0.15). Rapid evolution, deviations from the Brownian model of evolution, and measurement error can decrease similarity among closely related species (Gittleman et al. 1996, Blomberg et al. 2003). For these species characteristics, departures from the Brownian model and rapid rates of change are likely causes of the low levels of phylogenetic signal. Citation index, range size, and p.s.r. are species-level characteristics that are products of an interaction between traits and the environment and may change rapidly thus reducing the similarity among related species. For example, while traits, such as temperature tolerance, generation time, and seed dispersal distances, are known to be correlated with range size (Morin and Chuine 2006), the size of the area occupied by a species also depends upon phylogenetically-independent factors such as geographic barriers or environmental conditions. The low phylogenetic signal of species-level characteristics may also stem from how the characteristics are "inherited" at speciation events. The Brownian model assumes that the attributes of sister species are exactly inherited. However, since most speciation events occur in allopatry, incipient

species will often have different range sizes. Considering the strong relationship between range size and p.s.r. and the dependence of the incidence of individual pathogen species on climatic conditions (Agrios 1997), it is likely that the dynamics of p.s.r. within a species through time and across speciation events deviates from the Brownian model in a manner similar to range size.

The low level of phylogenetic signal for p.s.r. is consistent with the hypothesis that p.s.r. changes within ecological time scales and at rates proportional to the geographic range of the host species (Strong 1974c, b, a, Kelly and Southwood 1999, Mitchell and Power 2003, Torchin and Mitchell 2004) and is not strongly affected by clade-specific effects such as resistance strategies, the age of the host lineage (Birks 1980, Kennedy and Southwood 1984), or taxonomic isolation (Strong et al. 1985). This result is not surprising given that host switching among distantly related host taxa is common in fungal plant pathogens (Roy 2001, Jackson 2004) and that, in exotic plant species, the degree of enemy-release, which is assumed to be inversely related to p.s.r., decreases with range size and only over residence times spanning 50 to 200 years (Hawkes 2007).

Levels of similarity in the number of pathogen and parasite species infecting closely related host species are lower among plant species than animal species where parasite diversity is often phylogenetically constrained (Poulin 1997, Morand and Poulin 1998, Nunn et al. 2003, but see Ezenwa et al. 2006). This suggests that different evolutionary and ecological processes operate between plant-pathogen and animal-parasite interactions to determine the number of pathogens or parasites infecting a host species. This is, in part, due to the fact that, in animal hosts, parasite diversity is

influenced by phylogenetically constrained characteristics of the host such as feeding behavior, social structure, and migration patterns (Adamson and Caira 1994). Indeed, these factors coupled with the complexity of animal immune systems may reduce the incidence of host switching and create more constrained, coevolutionary relationships thereby limiting the pool of potential colonizing species to pathogens and parasites that infect closely related host taxa.

The difference in relative effect of phylogeny on p.s.r. between plants and animal may also be a result of body size in modular organisms being more evolutionary labile than in unitary organisms. The value of K for plant height, reported here, is similar to values reported by other studies (0.38 for *Acer* (Ackerly and Donoghue 1998), and 0.53 for *Tithonia* in (Morales 2000) as calculated in Blomberg et al 2003). These K values for plant height contrast with the values for animal body size that are on average not significantly different from one (Blomberg et al. 2003).

These results agree with the predictions based on the analogy between plant species and oceanic islands (Janzen 1968, 1973) which extends the theory of island biogeography (MacArthur and Wilson 1963, 1967) to host species. Three complementary explanations have been proposed for species-area relationships among different-sized islands (Connor and McCoy 1979). The habitat-heterogeneity hypothesis proposes that species richness increases with island size because larger areas encompass a greater variety of habitats (Willams 1943, Lawton and Schroder 1977). On the other hand, the equilibrium theory of island biogeography suggests that it is the area-per se or species-energy (Wright 1983) that affect p.s.r. through changes in the extinction and colonization rates of pathogen species (Southwood 1960, Southwood and Kennedy

1983). Finally, as host-island size increases, the rate of pathogen lineage diversification within host species may increase due to greater opportunities for isolation of pathogen populations in allopatry and peripatry (Mayr 1954, Rosenzweig 1995).

The increase in p.s.r. with increasing plant species height and leaf longevity may be explained by the effects of increased area per se supporting larger pathogen populations and by increased morphological complexity providing wider variety of niches (Strong and Levin 1979). Yet, the height of plant species is correlated with other life history traits that may also affect p.s.r.. Specifically, smaller plants have shorter generation times and devote more energy to seed production and dispersal (Harper and White 1974). Consequently, smaller plants are less predictable in their location and local abundance and may favor pathogens with greater dispersal abilities. Thus, the diversity of pathogen species on shorter lived plant species may decrease by either increasing competition among pathogen species or excluding dispersal-limited pathogens from the pool of potential colonist species. This hypothesis has empirical support. Annual grasses have been shown to favor pathogens with shorter generation times and greater dispersal ability (Clay and Kover 1996) and species of native annual grasses in the United States are attacked by fewer fungal pathogen species than perennial grasses (Clay 1995).

Compared to the effect of plant height, the geographic range of host plant species has a larger effect on p.s.r.. As is the case with most species-area patterns, habitat heterogeneity and area co-vary with plant species' range size. However, the habitat heterogeneity and area-per se hypotheses differ in the mechanisms by which the species-area pattern is generated and the predictions of the relationship between component community pathogen diversity and p.s.r. (Stevens 1986). Disentangling the relative roles

of these hypotheses is critical to our interpretation of the ecological and evolutionary significance of the richness of pathogen species at the level of host species, especially in light of recent studies that use the p.s.r. of a plant species as a measure of the impact of pathogens on the host species (i.e. Mitchell and Power 2003, Busch et al. 2004). Using pathogen species richness as a metric of disease impact, however, assumes that the mechanisms of the area per se hypothesis are largely responsible for this species-area pattern.

The habitat-heterogeneity hypothesis predicts that the species-area pattern is the result of plants with larger ranges occupying an increased range of abiotic conditions and biotic communities and that these factors determine the composition of the pathogen component community. Consequently, the high levels of p.s.r. of more widely-distributed host species are driven by turnover in the pathogen community (beta diversity) across the host's geographic range, and thus the pathogen species richness at the population and individual level does not depend on the pathogen species richness at the species level. On the other hand, the area per se hypothesis predicts that a direct relationship exists between p.s.r. and the richness and abundance of pathogens in host populations because larger host-islands have lower extinction rates and thus higher p.s.r. because they support larger populations of each pathogen species.

The results of previous studies clearly support the prediction of the habitat-heterogeneity hypothesis. In general, species-area patterns for phytophagous species appear to be largely driven by turnover in species composition among individual plants and plant population. In a study of polypore fungi occurring on tropical trees, fungal richness per species increased with tree species' local abundance, but the richness per

individual did not (Gilbert et al. 2002). In the species-area pattern in the richness of insect herbivores found on plant species (Southwood 1960, 1961, Strong 1974a, Lawton and Schroder 1977, Kennedy and Southwood 1984, Brandle and Brandl 2001), studies have shown that local richness and abundance of insect herbivores on tree species are not related to the size of the plant species' range (Claridge and Wilson 1976, 1978, Futuyma and Gould 1979, Karban and Ricklefs 1983). Furthermore, the only direct test of the mechanisms that produce the species-area pattern in the richness of phytophagous insects among plant species found that turnover in insect species among host populations was responsible for the pattern (Stevens 1986).

In summary, the results of this study demonstrate that the number of pathogen species infecting a plant species is a positive function of individual size and geographic range suggesting that accumulation of pathogens at the species-level is driven by habitat (host species) size and/or heterogeneity. Relating the composition of the pathogen community at species-level to component and infracommunities requires further study. However, in light of the effects of plant species range and growth form on p.s.r and evidence suggesting pathogen species turnover generates species-area relations, pathogen species richness may not be a valid measure of plant species' disease burden, as noted by Torchin and Mitchell (2004).

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Table 1.4: Results of non-phylogenetic GLM analysis of pathogen species richness including additional factors. (r^2= 0.710)

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial r^2 Squared
Corrected Model	106.210	17	6.248	71.493	.000	.720
Intercept	4.674	1	4.674	53.481	.000	.102
Functional Group * Citation Index	1.800	3	.600	6.866	.000	.042
Log(Range)	6.806	1	6.806	77.883	.000	.142
Log(Height+1)	1.910	1	1.910	21.861	.000	.044
Citation Index	7.506	1	7.506	85.890	.000	.154
Deciduous	.587	1	.587	6.720	.010	.014
Woody	1.44E-005	1	<0.001	.000	.990	.000
Fgroup * Log(Range)	.123	3	.041	.468	.705	.003
Fgroup * Log(Ht+1)	.275	3	.092	1.049	.370	.007
Fgroup	.123	3	.041	.471	.703	.003
Error	41.247	472	.087			
Total	716.779	490				
Corrected Total	147.456	489				

Table 1.5: Literature used to resolve phylogeny within families and genera.

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Chapter 2

Specialist plant pathogen limits growth of Mayapple populations through negative effects on multiple host demographic rates

Abstract

Host-specific plant pathogens may act to maintain species diversity in plant communities by causing density-dependent population growth. While previous studies have focused on the effects of pathogens on seed and seedling survival, disease can also restrict the growth of host populations by reducing growth and fecundity of mature plants. Here, I show that the specialist pathogen, *Puccinia podophyllii*, reduces host population growth by causing strong distance-dependent seedling survival and by reducing the vegetative growth and reproductive effort of its host, Mayapple (*Podophyllum peltatum*). The negative effects of the pathogen on multiple demographic processes suggest that measuring only seed and seedling mortality may provide a poor estimate of the strength of pathogen-induced reductions in host population growth. While previous studies on the role of pathogens in structuring and maintaining diversity in temperate forests have focused exclusively on tree species, the host plant in this study is an understory herb suggesting that plant enemies play a role in maintaining diversity throughout plant communities in temperate forests.

Introduction

Understanding the processes that maintain plant diversity is an important goal in ecology and critical to our ability to preserve biodiversity. Specialist plant enemies may maintain plant diversity by limiting the population growth of competitively superior plant species through density-dependent disease or herbivory (Gillett 1962). Gillett's "theory of pest pressure" stipulates that the enemy is host specific, negatively affects the growth and survival of the host, and exhibit density-dependent transmission, i.e. the effects of enemies increase with increasing host abundance. The combination of these conditions creates a feedback loop where low impacts of enemies allow host populations to increase from low abundances but the effects of enemies impose limits on host growth and survival as host density increases. Plant pathogens are likely to meet these assumptions as they are often host-specific and reproduce rapidly compared to their host allowing the pathogen population to respond rapidly to changes in host density. If so, pathogen may act to enhance diversity in plant communities. Indeed, the empirical evidence of the role of pathogens in structuring plant communities has been growing (Packer and Clay 2000, Kilronomos 2002, Bell et al. 2006, Petermann et al. 2006, Yamazaki et al. 2009).

However, previous studies have not attempted integrate the effects of pathogens with the population dynamics of the host and the diversity of the plant community as proposed by Gillett. Most studies have tested the Janzen-Connell hypothesis, a special, and explicitly spatial, case of the Gillett's theory, which focuses on pathogen-induced seed and seedling mortality. Janzen and Connell proposed that tropical tree diversity is maintained by serial replacement driven by distance or density-dependent disease or herbivory. After an adult plant dies it is likely to be replaced by a different species (serial

replacement) because plant enemies cause density- or distance-dependent mortality in con-specific seeds and seedlings close to the parent plant, leading to greater recruitment success of other plant species (Janzen 1970, Connell 1971).

Rates of disease-induced mortality in just one or two early life stages provide a poor estimate of the effects of disease on host demography since estimates of lifetime fitness and population dynamics cannot be extrapolated from survival of a single life stage (McPeek and Peckarsky 1998). Furthermore, population growth of long-lived plants may not to be limited by seed and seedling mortality (Silvertown et al. 1993). In many cases, high seedling mortality at high densities may be inevitable due to self-thinning and thus the disease-induced mortality "may represent only a harvest by the pest or pathogen of the plant population's sustainable yield" (Harper 1990). Indeed, in a review of the role of pathogens in plant species coexistence, Freckleton and Lewis (2006) concluded that connecting the density-dependent effects of pathogens across host life history stages to the long-term host population dynamics is a major issue that has yet to be addressed.

The growth of the host-plant population depends upon multiple demographic processes, all of which can be negatively affected by pathogens. Although the growth of populations is defined by the change in the number of individuals over time, this is more complex in plants because population growth also depends upon vegetative growth within individuals (Harper and White 1974). Recruitment depends upon seed production and seedling survival. Fecundity in plants often increases with size and thus is a function of vegetative growth. Within each individual, vegetative growth results in the accumulation of modular or structural units, i.e. leaves with axillary buds, or shoots.

Vegetative growth can affect seed production in a variety of ways. Many perennial plant species begin flowering and producing seed only after reaching a critical size. The growth rate of the population of shoots determines the length of this non-flowering period. Once the plant begins flowering, plant size is often a better predictor of total seed output. The plant size is determined by both the number and size of the shoots, each of which effects seed production. For most species, the number of shoots sets the maximum seed production, while the size of structural unit, reflecting its resource status, can determine the sexual status of each unit and thus flowering effort. The resources available to each unit can also affect fruit set and seed set through effects on floral or ovule abortion rates (Harper and White 1974).

The costs of infection may be manifested in any of these demographic processes, but may be more subtle in mature plants than in seedlings were infection often results in mortality. In mature perennial plants with stored resources, disease-induced mortality is rare. Instead, the effects of infection may be manifested through decreases in vegetative growth rates and seed production. While the effects of plant-pathogens on the population dynamics of long-lived plant species is difficult to estimate over short time periods, the negative effects of a pathogen on multiple demographic processes can be measured to estimate the ability of a pathogen to regulate host populations.

Here, I examine how a host-specific pathogen may limit the abundance of its host through negative effects on multiple demographic processes. Using experiments and field data, I test the hypotheses that the host specific pathogen, *Puccinia podophylii*, reduces population growth rates of the clonal understory herb, *Podophyllum peltatum*. Specifically, I address how the pathogen affects seedling survival and rates of vegetative

growth and sexual reproduction in the host. Previous studies of the diversity-enhancing effects of disease in temperate forests have focused exclusively in tree species. Yet understory herbs constitute most of the plant diversity in these forests (Whigham 2004). Thus, in addition to exploring the mechanisms by which pathogens regulate populations of their hosts, this study may also contribute to the understanding of how pervasive the effects of host-specific pathogens are in structuring plant diversity within temperate forests.

Methods:

Study System*:* Mayapple (*Podophyllum peltatum*, Berberidaceae) is the only species of the genus *Podophyllum* (s.s.). Mayapple is a colonial, rhizomatous, understory herb and is broadly distributed over the eastern half of the United States wherever deciduous forests occur. It occurs from sea level to at least 1400 meters in the southern Appalachian Mountains (Sohn and Policansky 1977).

Mayapple is one of the first plants to emerge in the spring in the forests of the upper Midwestern region. Shoots emerge in early to mid-April, continue to expand until late May, and can persist until August or September. Mayapple colonies consist of one to ten's of thousands of stems that can cover up to nearly a hectare (personal observation). A single colony may be composed of typically one, but occasionally more, genets (Parker 1988). The annual above ground shoots are easily recognized by their lobed, peltate leaves. The shoots range from 5 to 60 cm in height and are dimorphic. Vegetative shoots consist of a petiole and single leaf and are morphologically leaves. The flowering shoots are true shoots and typically have two leaves that are each of similar size to the leaves found on vegetative shoots of the same height.

The flowers are large (3-5 cm in diameter), white, and do not produce nectar. The onset and duration of anthesis generally begins in early May and lasts approximately 2.5 weeks (Rust and Roth 1981) Mayapple is self-incompatible and insect pollinated (Swanson and Sohmer 1976, Rust and Roth 1981). The per flower probability of producing fruit (fruit set) is pollen-limited (Laverty and Plowright 1988, Whisler and Snow 1992)and generally rare (Lu 1996).

The fruits of Mayapple are the largest in the understory plant community of the eastern deciduous forests, reaching 4.5 cm in diameter and can produce up to 50 seeds per fruit (Rust and Roth 1981). Each seed is surrounded by a sugary aril that offers a rich reward to dispersing animals. Fruit removal rates are high and are positively correlated with fruit abundance within a colony (personal obs.). Eastern box turtles (*Terrapene carolina*) are the only identified dispersal agent (Rust and Roth 1981), but fruits are also consumed by variety of mammals (e.g. rodents, raccoons, white-tailed deer) that likely disperse the seeds.

New colonies (genets) are typically initiated from seed. Seedlings of Mayapple exhibit slow growth and low survival. Mayapple seedling survival has been observed to be low, with about one quarter of seeds germinating and less than 2% surviving one year after emergence (Rust and Roth 1981). Seedlings persist as solitary shoots for a period of at least five years, after which rhizome growth begins (Holm 1899)

As seedling survival appears nearly zero within Mayapple colonies, colony growth is thought to be driven primarily by vegetative reproduction. Growth and reproduction in Mayapple has been shown to be carbon rather than nutrient limited (Benner and Watson 1989, De Kroon et al. 1991). Mayapples maintain a long-lived

rhizome system made up of physically and physiologically connected rhizome segments. Rhizome segments may remain connected for 7-10 years. I refer to the interconnected segments as a rhizome system. The aerial shoots are produced at the tips of the rhizome system in undamaged rhizome segments. The annually produced segments are composed of a series of scale leaves that are compressed at the new shoot bud, a region that is, for convenience, called a node. Each terminal node can produce from 1 to 4 new rhizome segments in a year developing from lateral meristems (i.e. sympodial growth). New rhizome segments range from 0.5 to 20 cm in length. If the new rhizome segments are of a sufficient size (usually greater than 2cm in length), they will produce a shoot in the following year (Geber et al. 1997).

Similar to growth in many perennial plants, Mayapple exhibits organ preformation and extensive resource storage and transport, thus the demographic response of the plant to current conditions is modified by historical conditions experienced by the rhizome system. Investments in both vegetative and sexual reproduction are affected by the resource status of the rhizome system. The resource status is a function of stored resource (length of previous rhizome segments) and the shoots ability to acquire resources (leaf area) and resource costs due to fruit production or shoot damage. How these costs are manifested demographically, however, depends upon the timing relative to developmental decisions and seasonal resource transport.

The patterns of seasonal carbon assimilation and transport in Mayapple suggest that loss of leaf tissue, even as late as mid-summer can reduce rhizome segment growth. Mayapple continues to assimilate carbon late into the growing season (Taylor and Pearcy 1976) when the assimilate appears to be used to support rhizome growth. Landa and

colleagues (1992) found early in the growing season (late April) most of the assimilate (60%) remained in the shoot, while 27% was stored in older rhizome segments. During flowering, 77% was stored in old rhizome segments. The stored assimilate is not extensively remobilized until the following spring. During mid-June however, the majority of the assimilate (61%) moved into the expanding new rhizome segment.

However, the effects of infection on other demographic processes can not occur until following growing seasons due to organ preformation. In preformation, irreversible organ differentiation occurs months to years before the organ is fully developed. The environmental conditions that affect the plant's resource allocation to these organs may change in the intervening time period. Thus past conditions can have strong effects on the demography, thereby constraining the plants ability to respond to current conditions. In Mayapple, the number of potential new rhizome buds and the shoot type for the following year is determined as a new rhizome is expanding (Geber et al. 97). The shoot type is determined in mid to late June (Lu 1996) and is concurrent with fruit development. In the following year, a subset of the rhizome buds will grow into rhizome segments. The branching process is initiated just after flowering, but before fruiting.

Despite the complexity of the demographic processes in Mayapple, the timing of shoot senescence is an indicator of current and future demographic performance. The timing of shoot senescence appears to reflect the current and future sexual status of the rhizome system. Fruiting shoots senesce later than non-fruiting, sexual shoots and both senesce after vegetative shoots. Furthermore, shoot senesce is delayed in shoots that produce larger new rhizome segments, which are more likely to become sexual (Benner and Watson 1989). Shoot senescence also reflects the rates of vegetative reproduction.

Shoots that produce more than one new rhizome senesce significantly later than those than shoots producing a single rhizome (Benner and Watson 1989, De Kroon et al. 1991). The differences in endogenous shoot senescence among shoots with differing demographic potential reflect the greater resource requirements for both vegetative and sexual reproduction. These requirements may not be met if senescence is accelerated by other factors, like herbivory or environmental stress. Drought, herbivory (Benner and Watson 1989), and disease (Sohn and Policansky 1977, Parker 1988) have been suggested as exogenous factors that accelerate senescence in Mayapple.

Herbivore damage on Mayapple is rare. A few species of lepidopteran larvae (*Chorisincura rosaceana, Clepsis melaleucana* (Faeth 1978), *Papaipema cerina* (Geber et al. 1997)) are able to feed and develop on the plant but they are either absent or uncommon in the populations studied. The toxicity of Mayapple tissues appears to limit the diversity and abundance of herbivores with a lignan, podophyllin-podophyllotoxin, that interferes with cell division and is produced through out its tissues (Moraes et al. 2000).

The protection provided by these toxins is not complete. Mayapple is commonly infected by the non-systemic, host-specific fungal pathogen, *Puccinia podophylii* Schwein., that occurs throughout range of the host. Infection occurs during two discrete generations of the pathogen in each growing season. Infection is initiated via contact with spore-contaminated soil during shoot emergence in the early spring (Whetzel et al. 1925). Orange lesions (aecia) develop within a few weeks producing aecidiospores. The lesions occur on the leaves of a small portion, typically less than 10%, of the shoots in a colony and have an average area of one to two centimeters.

The aecidiospores are the only dispersing form of the pathogen. The aecidiospores are dispersed by two mechanisms. The spores are mechanically ejected from the aecidium a distance of about 1 cm (Dodge 1924). The spores are then aerially dispersed over short distances (most spores travel less than two meters). The aecidiospores that germinate on host tissue produce telia on the underside of the leaf and cause a yellow spot on the leaf at the point of infection. The telia are visible by early June and produce the overwintering teliospores that return to the soil. Aecial infection within a colony results in telia infection in nearly all shoots. These infections can occupy up to 100% of the leaf area. Figure 2.1 summarizes the phenology of Mayapple and disease development through the growing season.

Genetic immunity to the infections caused by this pathogen is not known to occur in Mayapple (Parker 1989). Instead, the primary host defense appears to be physical. The pre-emergent shoots are protected by a whorl of bud scales that protect the shoot from contact with the rust spores in the soil. These bud scales are frequently infected by the pathogen. However, the lesions that develop on the bud scales produce only teliospores that are incapable of re-infecting the shoots in the current growing season. If the bud scales are damaged, or if they are too far beneath the soil surface to prevent soil contact, aecial infection is significantly more likely to occur (Parker 1988). Bud scale size and number increases with shoot size. Parker (1988) hypothesized that the variation in the size of the bud determines the infection risk of shoots, as larger buds, associated with larger shoots, may protrude further above the soil surface, thus smaller shoots are more susceptible to infection.

Given the relative timing of infection and host growth and development (Figure 2.1), the predicted mechanism by which pathogen infection decreases the growth rate and

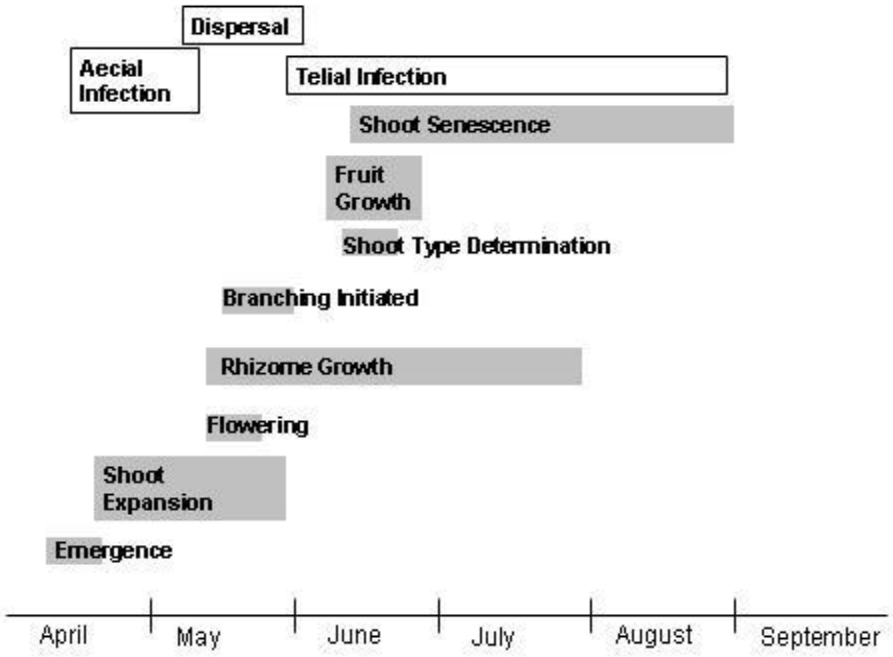

Figure 2.1: Phenology of Mayapple growth and development and disease development and transmission. Box width indicates the time period over which the process takes place. The development and transmission of *P. podophyllii* are in white boxes. Grey boxes indicate the growth and development of *P. peltatum* (Mayapple).

reproductive effort is as follows. Shoot size is correlated with both the resources stored in the rhizome system and the ability of the rhizome system to accumulate resources. As a consequence, larger shoots produce more and longer new rhizome segments than smaller shoots. The longer rhizomes of large shoots are more likely to produce flowering shoots in the following year. Infection by either aecial or telial forms of the pathogens causes a reduction in the resources available, by increasing leaf damage and decreasing shoot longevity, for the growth of new rhizome segments, thus diseased shoots should

grow shorter rhizomes and senesce earlier than healthy shoots. Shorter rhizomes are predicted to produce smaller shoots that will have decreased rates of rhizome branching and flowering. At the colony-level, increased disease severity is predicted to reduce shoot population growth rate, seed production through reductions in flowering effort, seedling survival, and increase the rate of shoot senescence.

This system is ideal for addressing the negative effects of disease on multiple host demographic rates. The growth and reproductive effort of Mayapple colonies is easily quantified as is the severity of the disease since all infected shoots are symptomatic and the individual infections vary little in size.

Study Sites*:* The study consisted of experiments and field observations and was conducted in three forested sites in Washtenaw County in southeast Michigan, Raddrick forest, the Newcomb tract, and Pinckney State Recreation area. The forests at the sites are typical second growth oak-hickory-maple forests, but differ in disturbance histories, soil types and plant community compositions.

Sampling Methods*:* In the field observations, I tested the relationships between disease severity and measures of within-colony vegetative growth and sexual reproduction across the three sites. In each site approximately 80 Mayapple colonies (259 in total) were mapped and measured each year from 2005 to 2008. In each year, the host plant and pathogen variables were sampled within each Mayapple colony. Each Mayapple colony was sampled twice per growing season. The first sampling period extended from early May until early June, during which I measured shoot density, shoot size (stem height and leaf radius (cm)), the proportion of flowering shoots (flowering effort), and the presence and the prevalence of the first generation of the infection.

Two sampling methods were used in the first sampling period. In colonies containing less than 200 shoots, I measured the number of shoots, numbers of flowering shoots, and pathogen incidence and prevalence using total counts. Shoot size was measured in a random subset of all stems. In larger colonies, these variables were sampled along two permanent transects running from north to south and east to west through the colony's center. Shoot density and the remaining measures were sampled using quadrats placed every 1 to 2 meters along these transects. If no infection was encountered in the quadrats, the presence and number of infected shoots were counted for the entire colony.

The second sampling period extended from June until early July, after the pathogen had dispersed and the telia had developed. I measured the presence and severity of the second generation of the disease, as well as fruit set, seed set, and the proportion of stems senescing. Again, in smaller colonies total samples were used. In large colonies, each sample consisted of 20 stems, where the number of infected, flowering, fruiting, and senesced was recorded. Fruiting stems were defined as a stem bearing a fruit greater than 14 mm in diameter, since nearly all fruits below this size contain no seeds. Fruit diameter, across the widest latitudinal section of the fruit was used as a proxy for seed set. The abundance of telial infections was estimated from the number of telia per leaf in 5 randomly selected stems in each sample. The samples were taken approximately every 3 meters along the permanent transects and along the edge of the colony. Disease severity was calculated as the average number of telia per leaf times the prevalence; one was added to allow log transformation. This measure is highly correlated with all other measures of disease abundance in a colony.

Colony-level vegetative growth rates: The effects of pathogen infection on host colony population growth rate were tested in the following ways. In colonies consisting of less than 200 shoots, the annual colony growth rate was calculated by dividing the number of shoots in the following year by the number of shoots in the current year. In larger colonies, annual colony growth rate was divided into annual rates of change in shoot density and in colony area. The two aspects of colony shoot population growth were measured and analyzed separately. Shoot density was measure in all large colonies, while colony area was measured in a subset of colonies.

Colony expansion was estimated using the areas of 59 colonies in 2006 and 2008. Colony area was estimated by approximating its shape as a sixteen to twenty four-sided polygon. The edges of the polygon were defined by the outer most pairs of shoots, separated by 30 to 100 cm, at the north, northeast, east, southeast, etc, edges of the colony. To ensure that each edge was measured in roughly the same location each year, permanent stakes were placed on the exterior of colony at the cardinal directions. The area of the polygon was measured, at each edge, using distance from the shoots to a permanent center stake and distance between the shoots. This results in a series of triangles and the area estimate is the sum of their areas. The average radius of the colony was calculated using the area. The annual rate of colony expansion was calculated by difference between the average radii in each year divided by the number of years between measurements.

I tested the predictions that rates annual change of average shoot density and colony radial expansion will decrease with increasing disease severity and average shoot

density and increase with average shoot height. In the analysis of colony expansion, I used the averages of these measures from 2005 to 2007 as the predictor variables.

Additionally, I tested the predictions that disease causes reductions in average shoot height and more rapid rates of shoot senescence as these variables are indicators of vigor and growth of rhizome system. I tested the prediction that infection will reduce the shoot size in the following year by comparing the annual rate of change in average shoot height to disease severity, shoot height, and shoot density. As maximum shoot height is likely physiologically constrained, the growth rate should decrease with increasing shoot height. The annual rate of change was calculated as the average height in 2008 divided by average height in 2007. I used the data for 2007 and 2008 because the shoot size was sampled more intensely in these years.

To test if increasing severity accelerates the rate of shoot senescence, I used the proportion of senesced shoots in the second sampling period as a measure of senescence rate. I tested the prediction that the rate of senescence will increase with increasing disease severity while controlling for other factors that are known to affect the response: average shoot density, flower effort, fruit set, year, and sampling date in the second sampling period in each year. Sampling date was calculated as number of days since fruits begin to develop (June 1st) for the second survey. The number of days was natural log transformed to reflect the non-linear dynamics of shoot senescence.

Colony-level reproductive effort: To test the prediction that infection results in decreased seed production through decreased flowering effort, I compared the measures of reproductive effort (flowering effort, fruit set, average fruit diameter and per-shoot seed production) across colonies that varied in disease severity. Flowering effort is

predicted to be consistent within a colony through time but should decrease with increasing shoot density, disease severity, and fruit set in the previous year. Fruit set is predicted to be unaffected by disease severity since fruit set is pollen-limited and the disease status of a colony should not affect pollinator activity. Furthermore, rates of floral abortion do not increase with disease severity (unpublished data). Instead, fruit and seed set are likely functions of the length of the period the flower is receptive to pollen; pollinator community and its activity level; as well as the presence and abundance of co-flowering species (Laverty and Plowright 1988, Laverty 1992). Fruit and seed set are also affected by pollinator behavior. Pollinators are likely to move between colonies separated by short distance and to colonies that have large numbers of flowers. In order to control for these effects, year, site, site by year interaction, log transformed flowers per colony and distances to neighboring colonies were used as predictors. The distance metric was calculated for each colony as the sum of 1/(distance from the center of the focal colony to the center of another colony)^2 across all colonies within a site. Additionally, as small amounts of fruit growth was observed across the second sampling period, sampling date was added to the analysis of fruit diameter and per shoot seed production. Increasing disease severity is predicted to decrease per shoot seed production through decreasing the flowering effort. This was tested by combining the unique predictor variables for the analyses of flower effort, fruit set, and seed set described above.

Data analysis: I analyzed the data for the rates of colony expansion and rate of average shoot height change using linear regression. For the both data sets, I used the log transformed rate, shoot density, and disease severity for each colony. For the analysis of

colony expansion, the Z-scores of the predictor variables were used to control for the co-linearity between disease severities and shoot heights among these colonies.

The remaining predicted effects of infection on colony growth and reproductive effort were analyzed using mixed-model ANOVA's. The proportional response variables were arcsine-square root transformed. The variables for disease severity, average shoot density, the rate responses were natural log transformed. In the model the data for each colony in each year are treated as a repeated measures type of response. To select the most appropriate covariance structure, I used the lowest Akaike's information criterion value between unstructured and compound symmetry covariance structures. The response and predictor variables are summarized in Tables 2.2 and 2.3**.**

Effects of disease on shoot-level demography*:* In order to test the proposed mechanisms by which infection reduces colony-level vegetative growth and reproductive rates, I tested the prediction that infection causes decreases in vegetative growth and reproductive rates at the shoot level. To estimate the effects of the disease on rhizome segment size, shoot size and reproductive status, and rhizome segment branching rates, I used a series of phenomenological models that relate; 1) shoot size, as a proxy for stored resource (previous rhizome size) and resource capture (leaf area and position), to branching rate; 2) ramet (rhizome) size to shoot size and reproductive status; 3) shoot size and disease severity to size of new rhizomes or to rhizome growth rate.

The prediction that infection reduces vegetative growth rates was tested by comparing rhizome branching rates and lengths among ramets connected to shoots that varied in size, reproductive status, and infection severity. In total, 318 shoots and rhizome systems were sampled. 278 ramets were measured in 2007 in three infected

colonies per site at two sites and the remaining shoots were sampled in 2008 at nine uninfected colonies in all three sites. In each infected colony, the ramets were selected to sample the variation in shoot size (estimated as product leaf radius and leaf number plus shoot height), shoot type (vegetative or flowering), and disease severity. The presence and size of aecial infection, and the number of telia per leaf were used as measures of disease severity. In August, after most of the rhizome growth has occurred, the number and length of the new rhizomes produced by each ramet was measured. Several alternative models were compared that explained branching rate and total rhizome length using maximum likelihood estimation and AIC values.

In the analysis of branching rate, the effect of the disease was not tested since the number of branches is determined before the shoot emerges in the spring and preliminary analysis showed that disease severity had no effect. Branching rate was modeled as a binomial process with a maximum of number of branches as functions of shoot size or height with the branching probability as functions of; shoot type, shoot size, shoot height, average and total leaf areas, again estimated by the leaf radii. The branching probability was modeled using a logistic link function of a constant (c) plus a coefficient (a) times the predictor variables and their log-transformed values.

Rhizome growth was modeled for only the shoots that produced a single new rhizome segment. Two and three branched systems were excluded since the total length of the new branched rhizome systems was highly variable, possibly due to growth rate asymmetries caused by apical dominance. Rhizome length was modeled as positive-linear, exponential, logistic functions of shoot size (see Table 2.1). The best predictive model of positive growth was selected based on the fit, measured by AIC values, to data

from shoots with no or very little infection (telia per leaf ranged from 0-34). In infected shoots, rhizome growth was decreased, proportionally or additively, by functions of disease severity (see Table 2.1). I selected functions for effects of disease on growth to determine 1) if the disease reduces rhizome growth and to what magnitude; 2) the relative contributions of each type of infection to the reduction in growth; and 3) if thresholds exist in the relationship between disease severity and growth reduction, i.e. rhizome growth is reduced only in severely infected shoots. Again, I selected the best fit model as the model with the lowest AIC value.

Increasing disease severity is predicted to reduce the size of new rhizome segments which will reduce shoot size in the following year. However, rhizome systems with long rhizome segments may have sufficient stored resources to produce large shoots even if rhizome segment growth is reduced to the previous year. To test the prediction that changes in rhizome segment growth rate in the previous year affect shoot size, I compared the effects of the length of the rhizome segment produced two years ago and

Table 2.1: Rhizome growth models tested to estimate effects of infection on shoot-level vegetative growth.

Rhizome length (rl):
rl (linear function) =a+b* shoot size
rl (logarithmic function) =a+b*log(shoot size)
rl (log-log relationship) =exp((log(shoot size)-a)/b)
rl (logistic function) =m/(1+exp(-(a+b*log(shoot size))
where m =maximum rhizome length

In diseased shoots, rhizome length is reduced by increasing disease severity (ds) where ds=c*telia/leaf+d*area of aecial infection (cm^2).
Disease-induced growth reduction may be additive or proportional:
Additive effects: rl-ds
Proportional effects:
Model 1: rl*1/(1-ds)
Model 2: rl* ((1+exp(-h))/(1+exp(-(h-ds)))
Model 3: rl*(h+(1-h)/((ds)^j+1))

the rhizome segment growth rate on shoot size in the current year. The data were collected by measuring rhizome segment lengths produced one and two years previous, shoot heights and leaf radii for 126 shoots across nine randomly selected colonies in the three sites. The rhizome segment growth rate was calculated as the length of the ultimate rhizome segment divided by the length of the penultimate rhizome segment. The data for shoot size, penultimate rhizome length and growth rate were log-transformed and analyzed in using linear regression. Additionally, the relative effects of ultimate and penultimate rhizome lengths were compared in a linear regression of their log-transformed values on shoot size.

Experiments:

Exclusion experiment: Effects of pathogen infection on Mayapple colony growth and sexual reproduction were also tested using a disease exclusion experiment replicated over six colonies that began in 2005 and continued for four growing seasons. The paired-design controls for effects of host genotype and local environmental variables. One half of the colony was randomly assigned to a treatment of disease exclusion and in the remaining half the pathogen was allowed to spread. The pathogen was excluded through biweekly application of a protectant fungicide (Daconil: Chlorothalonil). Fungicide was applied using a backpack sprayer at rate recommended by the manufacture. The fungicide limits the development of the teliospores and is not taken up by the plant. Disease severity in the treatment plots averaged 90 percent lower than the paired control plots. The effects of the disease on the host-plant's growth were measured by average shoot density in plots that contained over 100 shoots (four of the six paired plots, shoot density was not measured in the two small colonies), average shoot height,

average shoot leaf radius, and proportion of senesced shoots. The effects of the treatments on reproductive effort were measured using flower effort, fruit set and seed set. From 2005 to 2008, the response variables were measured in each treatment pair in each year using the same methods described above in the field study. The proportional response variables were Arcsine-square root transformed. The data were analyzed using mixed model ANOVA with fixed effects of treatment and year and the random effect of colony (treatment-pairs). In the model the data for each colony in each year are treated as a repeated measures type of response. To select the most appropriate covariance structure, I used the lowest Akaike's information criterion (AIC) value among unstructured, compound symmetry, heterogeneous compound symmetry, and scaled identity covariance structures.

Effects on seedling survival: The distance and density dependent effects of infection on seedling survival were addressed in two separate experiments. The first compared the survival of seeds and seedlings to different treatments of distance from infected colony. The second experiment tested the relative importance of several causes of seedling mortality.

To test the effects of distance from infected host colony on seedling survival, ten fruits (approximately 60 seeds) were placed at six distance treatments in the fall of 2004. At each colony, the fruits were placed within the colony at the center (distance=0) and edge (distance=1meter) of the colony, and every 10 meters along a linear transect 40 meters long, leading away from the edge of focal colonies. This was replicated across 7, randomly selected, infected colonies in two sites. Seedling survival was measured in each replicate by counting the number of seedlings emerging in the spring of each year

from 2005 to 2008. The probability of a seedling surviving three years for each replicate were regressed against distance and ln(distance+1) to infected Mayapple colony and ln(initial seedling density).

The first experiment, however, can not discern the cause of seedling mortality. Higher mortality of the seedlings in the treatments located inside Mayapple colonies could stem from increased exposure to the pathogen and/or competition from mature plants. It also can not determine the sources of infection that could contribute to seedling mortality. As the host defense is primarily through the bud scales preventing contact with spores in the soil, and seedlings do not produce scale leaves, seedling mortality may be primarily driven by infection from the soil, or other negative soil feedbacks that build up inside Mayapple colonies. These effects would be more spatially restricted and would suggest that dispersal to areas immediately adjacent to the colony is sufficient to escape the effect. Alternatively, infection by the second generation of the pathogen may be the cause of increased mortality. This effect would diminish gradually with distance as a function of pathogen dispersal

To determine the mechanisms of seedling mortality, I used a factorial experiment that varied the presence or absence of three factors: soil-borne pathogen spores, aerially dispersed spores, and shading from the adults. All combinations of treatments were tested, with the exceptions of two treatment combinations, soil (+), shade (+), aerially dispersed spores (-) and soil (-), shade (+), aerially dispersed spores (-), that were not possible since only infected colonies were used. An equal volume of seeds was planted into small pots that contained soil from the interior or exterior of the Mayapple colony. The presence of aerial dispersed spores was determined by the location of the replicate

(pot). Seedlings planted inside the colony were exposed to the dispersing spores. The seedlings planted seven meters away from the edge of the colony were exposed to the dispersing spores from primary infections of the seedlings. Light competition with mature plants was controlled by pinning the nearby shoots to the ground with metal wickets. Each treatment combination was replicated three times per colony in seven colonies across two sites. Seedling survival was measured as the number of seedlings emerging in the second year divided by the maximum number of seedlings observed in the first year. The effects of the treatments were analyzed using an ANOVA with ln(initial seedling density) as a covariate. After removing one outlier, the main effects of the treatments and seedling density and the interactions of the soil-borne and aerial pathogen treatments and of the seedling density and aerial pathogen treatment were analyzed.

Results:

Seedling survival: Increasing distance from infected Mayapple colonies increases seedling survival twenty fold in experiment one (Figure 2.2). The survival of seedlings in infected colonies is not significantly different from zero (mean =0.025 95% C.I. -0.003-0.054). The logarithm of distance (+1) fits the data better than distance ($r^2_{\ln(\text{distance})}$ =0.425 vs r^2_{distance} =0.312) ,and the effect of distance is not significant among the 10 to 40 meter treatments ($F_{3,22}$ =1.34, p>0.28). The average survival rate among the treatments outside Mayapple colonies is 0.52 (95% C.I. 0.38-0.65). While seedlings at high densities tended to have lower survival than seedlings at lower densities, the trend was not significant (t=-1.3, p>0.2).

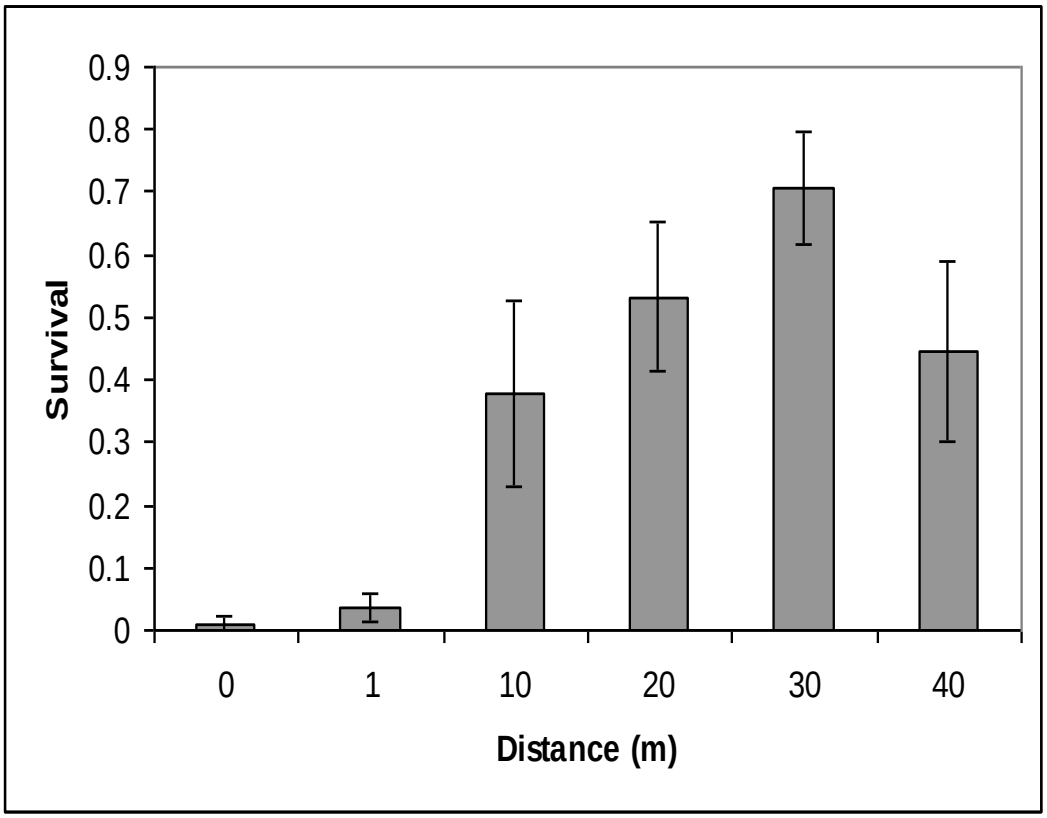

Figure 2.2: Relationship between proportions of seedlings surviving four years and distance from infected Mayapple colonies. Distances of zero are the seedlings located at the center of the colony. All other distances are measured from colony edge. Error bars indicate standard error of the mean.

Clearly, seedlings growing within an infected Mayapple colony have almost no chance of surviving, but what is the cause of this mortality? The results of the factorial experiment show that the aerially-dispersed pathogen spores dispersed from infected mature shoots (Generation 2) are the primary cause of seedling mortality (Figure 2.3). All of the treatments located inside infected Mayapple colonies and exposed to aerially dispersed spores had similarly low seedling survival (mean= 2.8% std.error=0.6).

The rates of pathogen induced-mortality clearly depend upon the distance from infected colonies rather than the density of seedlings. Seedling density had a significant negative effect on seedling survival but only in the absence of the aerially dispersed spores from the mature plants where nearly all the seedlings died (density by location

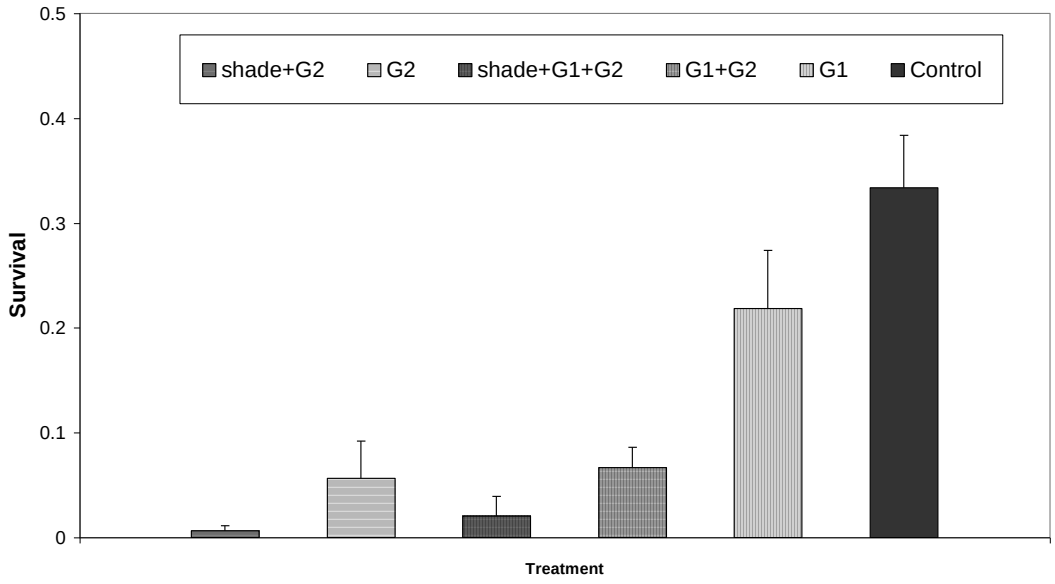

Figure 2.3: Seedling survival across treatment combinations of shade, soil-borne pathogens, and aerially-dispersed pathogens. The legend indicated the treatment combinations where shade= shading by mature shoots, G1= soil-borne pathogens, and G2=aerially-dispersed pathogens. Error bars indicate standard error of the mean

interaction, $F_{1,161}$ =42.8, p<0.001). Seedlings exposed to infected soil had significantly lower survival (mean=21.8%, 95% C.I.=15.6 to 28%) than the control but significantly higher than the other treatments (soil by location interaction, $F_{1,161}$ =19.25, p<0.001) indicating the mortality caused by disease transmission among seedlings, while significant, is not sufficient to cause the high levels of mortality observed inside Mayapple colonies (Figure 2.4). Shading from mature plants had a negative but non-significant effect on seedling survival. The difference in the significance of seedling density may be due to the differences in the design of the experiment. Where seedling density was significant, the seedlings were planted into small pots which could have increased competition or increased stress due to poor drainage.

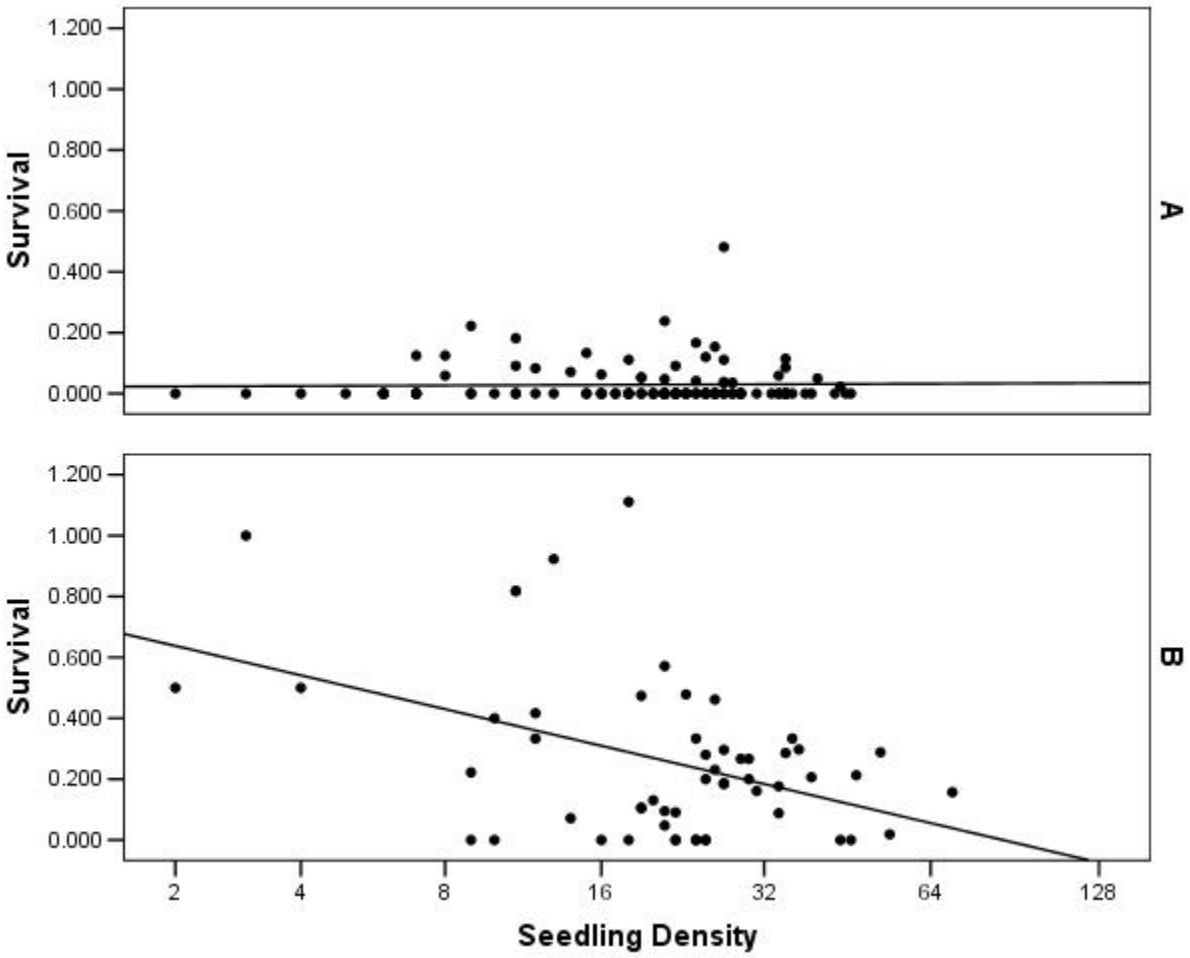

Figure 2.4: Effects of seedling density on seedling survival with and without disease transmission from infected mature plants. Seedling density is the initial number of seedlings per 80 cm^2. Seedling survival is the proportion of seedlings surviving one year. (A) Relationship between seedling survival and seedling density in treatments exposed to aerially dispersed spores from infected adults ($r^2<0.001$, $p>0.5$). (B) Relationship between seedling survival and seedling density in treatments without aerially dispersed spores from infected adults ($r^2=0.19$, $p<0.05$). Note that x-axis, seedling density is on log-scale.

Shoot-level demography*:* If a seedling survives to begin sexual and vegetative reproduction, the pathogen can reduce the rates of vegetative growth and reproductive effort. In healthy shoots, the length of the new rhizome segment was best fit by the logarithmic function of shoot size: rhizome length=-30+9.3*log (shoot size) [Akaike's information criterion (AIC) =300] and was significantly better than the alternative models tested (likelihood ratio test, $p<0.05$). Infection of the shoot in either generation of the disease cause large reductions in rhizome growth. Increasing disease severity in both the aecial and telial infection significantly decreases the total length of new rhizome segments ($p<0.005$). The proportion disease effects models were significantly better than the linear model ($p<0.5$ log-likelihood test) but were equivalent fits to the data (AIC = 974, 976, and 976, for models one two and three respectively). All of the proportional disease effects models predict reductions in growth of nearly 50% at the highest disease severity levels of both aecial and telial infections. The form of the relationship between increasing infection levels and the proportional reduction is unclear as it varied among the three proportional models. The relationship was linear models in 1 and 2 (see Table 2.1) indicating that even low to moderate rates of infection can cause reduced rhizome growth. The parameter estimates for the third model indicate that sharp thresholds exist in the relationship between disease severity and growth so that growth is reduced by an equivalent proportion above a certain disease severity level. These threshold values are estimated to be around the average severity of an aecial infection (~2cm) and ~500 telial infections per leaf.

The results of the study testing how shoot size depends upon rhizome growth in previous years demonstrate that disease-induced reductions in rhizome segment length

reduce shoot size in the following year. The size of the shoot produced by the rhizome system is primarily a function of the length of the rhizome segment produced the previous year (linear regression of ln(rhizome length$_{t-2}$), ln(rhizome length$_{t-1}$), and ln(shoot size); r^2=0.52, $F_{2,124}$ =70, p<0.001). The length of the ultimate segment was a significant predictor of shoot size (t=7.2. p<0.001) while the length of the penultimate segment was not significant (t=0.64, p>0.5). Reductions in growth rate of the rhizome significantly reduce shoot size as well (linear regression of ln(rhizome length$_{t-2}$), ln(rhizome growth rate), and ln(shoot size); r^2=0.48, $F_{2,124}$ =60, p<0.001, $t_{\ln(\text{rhizome growth rate})}$=10.6, p<0.001).

By decreasing shoot size the pathogen can reduce the colony population growth rate, since reductions in shoot size dramatically reduce branching rates. Decreasing shoot size significantly reduces branching rates by both reducing the maximum number of branches and decreasing the probability that the branches will initiate growth. The models in which the branching probability as a function of log(shoot size) and log(shoot height) and the maximum number of branches as a step function of shoot size were equivalently good fits to the data (AIC's 514 and 512 respectively) and significantly better than the alternative models tested (p<0.05, likelihood ratio test). The production of additional rhizome segments only occurs in large-sized shoots. The step function setting the maximum number of branches indicates that the maximum number of new rhizome segments is zero for shoots with size values less than 23.5 cm; one for shoot sizes from 23.5 to 44.5; two for shoot sizes from 44.5 to 92.5; and three for shoots sizes larger than 92.5. The parameter estimates and predictions for the two branching probability models were similar for both predictors. In both models, branching

probability increases rapidly from 10 to 20 cm in shoot height and then increases more slowly as size increases.

Colony-level vegetative growth rates*:* These shoot-effects of the disease on branching rate and reproductive effort are clearly evident at the colony-level in the results of the disease exclusion experiment and the field survey. While the uninfected colonies grow rapidly (averaged growth rate =1.24), moderate to high levels of disease severity (average telia per leaf from 54 to 403) grew slowly or not at all (average growth rate=1.1, n=45, see Figure 2.5) and disease severity is significantly, negatively correlated with colony shoot-population growth rates ($F=11.1$, $p<0.001$). Growth rates were not affected by shoot height or density ($p>0.1$, see Table 2.2).

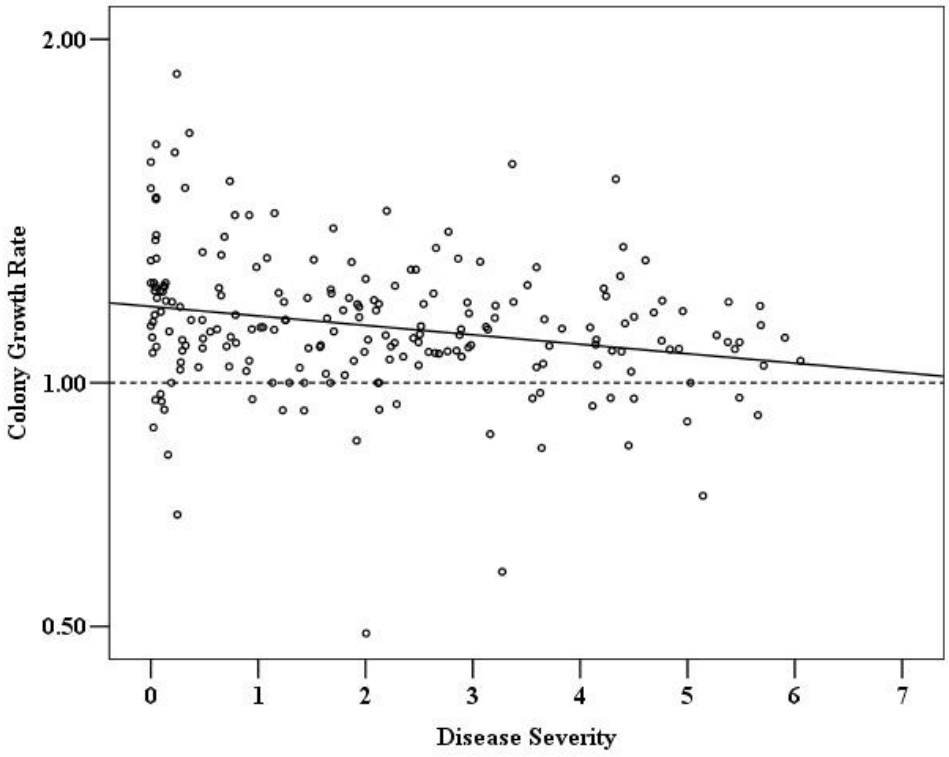

Fig 2.5: Colony growth rate decreases with increasing disease severity. Each circle represents one colony in one year. The average number of new shoots produced per shoot in each colony per year is depicted on the y-axis. The dashed horizontal line indicates no change in shoot numbers between years. The x-axis, disease severity, = ln(average number of infections (telial lesions) per host leaf plus one) for each colony in each year. r^2 =0.05

Table 2.2: Field survey results of effects of disease on colony-level growth rates. Numeric values with predictors are F-values from each ANOVA. Significance indicated by n.s. = p>0.1, * (p<0.1), ** (p<0.5). * (p<0.001) and direction of significant effects are indicated as positive (pos.) or negative (neg.). See text for transformations used. DS = disease severity**

Response	Predictors	Sample size
Annual shoot population growth rate	DS_t: 11.1 neg. *** Shoot $height_t$: 2.3 n.s. Shoot $density_t$: 0.4 n.s.	N=149
Annual rate of change in average shoot density	DS_t: 0.02 n.s. Shoot ht_t: 1.0 n.s. Shoot $density_t$: 0.7 n.s.	N=108
Annual Colony expansion rate	DS_t: n.s. Shoot $height_t$: pos.** Shoot $density_t$:n.s.	N=59
Annual rate of change in average shoot height	DS_t: neg. *** Shoot $height_t$: neg. *** Shoot $density_t$: n.s.	N=245
Shoot senesce rate	DS_t: 42.1 pos. *** Shoot $density_t$: 5.4 pos. ** Flowering effort: 2.1 n.s. Fruit set:3.8 neg. * Sampling date(year):28.6 pos. *** Year: 5.9 ***	N=191

The effects of disease severity on growth rates in larger colonies were less clear. Disease severity was negatively, but not significantly, correlated with annual rates of colony expansion and change in shoot density (see Table 2.2). The negative effects of the disease on shoot density are more clearly seen in the results of the disease exclusion experiment. In the final year of the experiment, shoot density per square meter in the treatment plots averaged 11 shoots greater than the average density in the paired controls (exclusion, $p<0.05$, see Figure 2.6). the average shoot density in the fungicide treatments increased over time from 32.8 to 51.3 shoots per meter, yet was not significant (exclusion by time interaction, $p=0.105$)

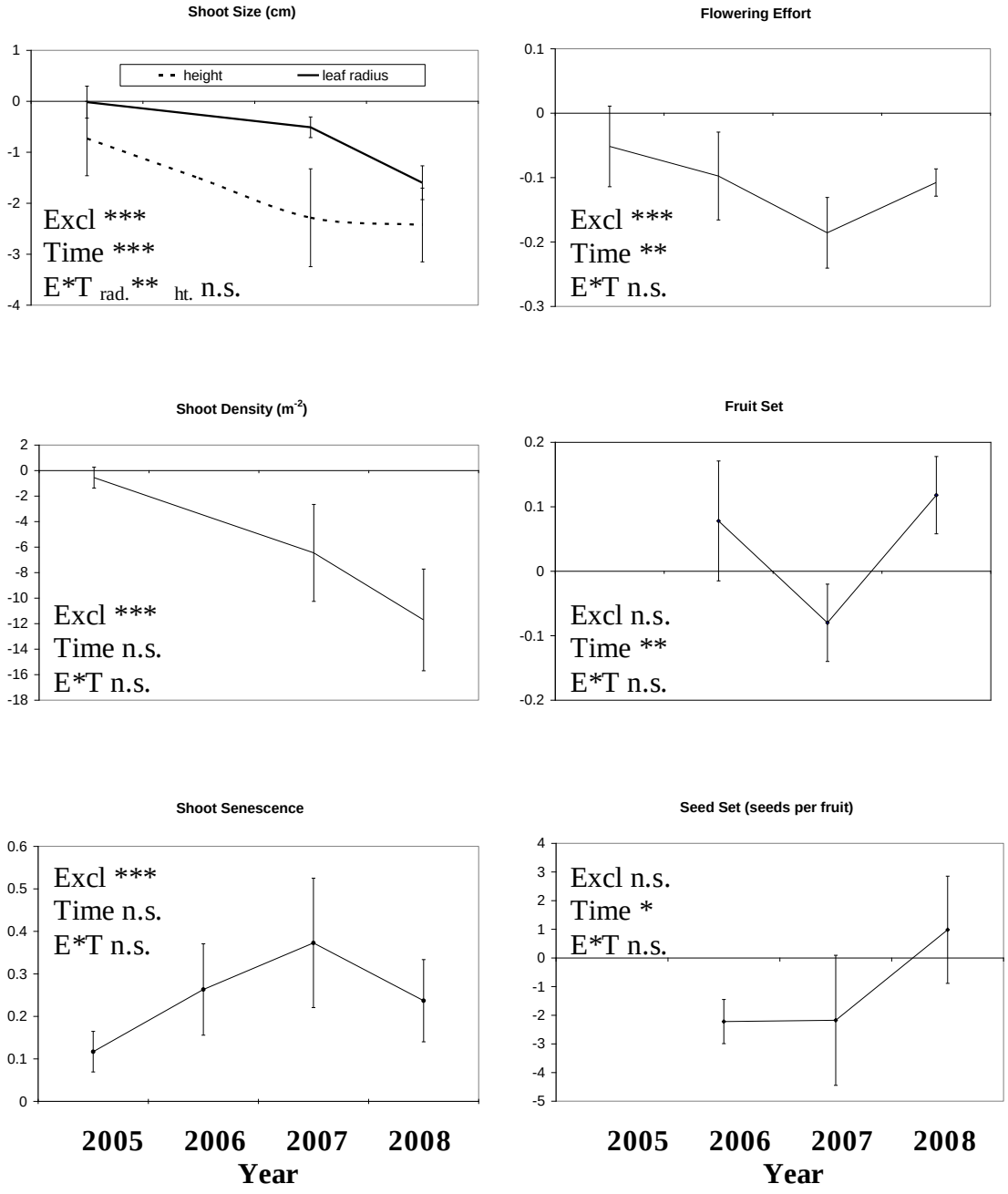

Figure 2.6: Effects of disease on Mayapple growth and reproductive effort in a four-year disease exclusion experiment. Y-axes are the average difference between paired diseased-control and fungicide treatments (diseased minus fungicide). Negative values result from lower response measures in diseased plots compared to fungicide plots. Units for shoot senescence, flowering effort, and fruit set are the difference in the proportional response. Within each graph, significant effects are indicated with asterisks ($p>0.1$=n.s., $p<0.1$=*, $p<0.05$=**, $p<0.01$=***). Error bars indicate standard error of the mean.

Disease reduces Mayapple colony growth rates through increasing the rate of shoot senescence and by decreasing shoot size. In the fungicide experiment, excluding the disease resulted in significant increases in shoot size. By the final year, Mayapple shoots in the sprayed plots averaged 2.6 cm taller in height and 1.7 cm larger in average leaf radius than the shoots in the diseased controls (exclusion, $p<0.01$). The difference in shoot size between the treatments increased over time. At the beginning of the experiment the differences between treatments in both shoot heights and leaf radii were less than a centimeter. This trend was significant for leaf radius (year by exclusion, $p<0.05$) but not for shoot height (year by exclusion, $p>0.1$) (see Figure 2.6). Furthermore, in the field survey, more severely diseased colonies had significantly lower rates of change in shoot height ($p<0.001$, see Table 2.3). Based on parameter estimates from this regression, the annual rate of change in shoot height can be arrested or decrease in any colony regardless of average shoot height by disease severity levels commonly recorded in the field (~150 telia per leaf).

The rate of shoot senescence is also significantly accelerated by the disease. The fungicide treatment dramatically slowed shoot senescence rate (exclusion, $p<0.001$, see Figure 2.6). Over all years, the percent of wilted stems in treatment plots averaged around 10% compared to 34% in the diseased controls. In the field survey, increasing disease severity was correlated with increasing rates of senescence in Mayapple colonies. In fact, disease severity explained most of variation in colony senescence rates (disease severity $F=42.1$, all other predictors $F< 6.0$ with the exception of sampling date $F=28.6$, see Table 2.2)

Colony-level reproductive effort: As predicted, the negative effects of the disease on reproductive effort were driven by decreases in flowering effort. In the disease exclusion experiment, excluding the disease nearly doubled flowering effort (exclusion, $p<0.005$). While flowering effort declined in the control plot throughout the course of the experiment to an average of 11.5% shoots flowering, flowering effort increased in the sprayed plots to an average of nearly 20%. There were no significant effects of the fungicide treatment on fruit set or seed set (see Figure 2.6). Similarly, in the field survey, severity diseased colonies had significantly lower flowering effort in the following year ($p<0.001$). In contrast, fruit and seed set were driven primarily by the effects of the distance to neighboring colonies, site, and year and were not significantly affected by the disease. The negative effects of disease severity on flower effort ultimately reduces per shoot rate of seed production ($p<0.005$, see Table 2.3)

Table 2.3: Field survey results of effects of disease on colony-level reproductive effort. Numeric values with predictors are F-values from ANOVA's. Significance indicated by n.s. = p>0.1, * (p<0.1), ** (p<0.5). * (p<0.001) and direction of significant effects are indicated as positive (pos.) or negative (neg.). DS=disease severity See text for transformations used.**

Response	Predictors	Sample size
Flowering effort	DS_{t-1}: 59.0 neg. *** Shoot density $_{t-1}$:0.1 n.s. Flowering effort $_{t-1}$: 418.6 pos. *** Fruit set $_{t-1}$: 12.2 pos. ***	N=225
Fruit set	DS_t: 2.0 n.s. Shoot density $_t$: 0.02 n.s. Flowers per colony: 18.5 neg. *** Distance metric: 2.8 pos. * Year: 96.2 *** Site: 9.0 *** Site*year: 9.6 ***	N=193
Seed set	DS_t: 1.4 n.s. Shoot density $_t$: 9.5 neg.** Flowers per colony: 7.3 pos. ** Distance metric: 12.2 pos. *** Year: 63.2*** Site: 2.4 * Site by year: 7.0 *** Sampling date: 19.9 pos. ***	N=204
Per shoot seed production	DS_{t-1}: 9.9 neg. *** Shoot density $_t$: 32.3 neg. *** Flowers per colony: 7.3 pos. ** Fruit set$_{t-1}$: 0.8 n.s. Distance metric: 9.2 pos. ** Year: 52.9*** Site: 5.3 ** Site by year: 3.9 ** Sampling date: 7.3 pos. ***	N=202

Discussion:

The results of this study clearly demonstrate that *Puccinia podophyii* limits host population growth, not only by causing strong distance-dependent seedling survival, but also by reducing the growth and reproductive effort of Mayapple colonies. The disease-driven effects on colony growth and reproductive effort appear to be caused by the same mechanism. Infection reduces the size of the new rhizome segments, resulting in smaller shoots in the following year. Smaller shoots are both less likely to flower, to produce

additional rhizome segments and, furthermore, may be more susceptible to infection (Parker 1988). This suggests a negative feedback that may result in rhizome segment mortality. Field observations of severely infected colonies are consistent with this hypothesis. The central area of these colonies consisted of only low densities of small shoots with high prevalence of aecial infection.

When pathogens do not cause rapid mortality in their hosts, the more subtle effects on demography may be underestimated without controlled experiments and careful observation. One previous study examined the demographic effects of *P. podophylli* on Mayapple and concluded that these effects were negligible (Parker 1988). This conclusion was based on the assumption that aecial infection is the most harmful, since plants are damaged early in the growing season, and that telial infection has limited impact since it occurs closer to normal shoot senescence. Based on evidence that aecial infection was common only in small shoots, and the assumption that large shoots were primarily responsible for sexual and vegetative reproduction, Parker (1988) concluded that while the rust has the potential to cause serious epidemics with in colonies, this potential remains unrealized due to the effectiveness of the host physical defense, and that "the overall demographic impact of pathogen attack …is negligible."

In contrast, the evidence presented here indicates that disease can strongly reduce host population growth. I experimentally tested the effects of on Mayapple seedling mortality, and mature plant growth, and fecundity. These effects are supported by field observations across multiple years and multiple host populations. Furthermore, the analysis of the field survey controlled for factors, such as shoot density, that may both reduce demographic rates of the plant and increase the severity of disease. I also used

experiments on shoot level growth to test how these effects are created. The multiple negative effects of the pathogen on host survival, growth and reproduction suggests that specialist plant enemies limit growth of host populations in a variety of ways as was originally envisioned in Gillett's theory of pest pressure (1962).

Furthermore, the pathogen is host-specific, only infecting Mayapple. Narrow host specificity is central in theories concerning the role of pathogens in plant species coexistence (Gillett 1962, Janzen 1970, Connell 1971). Yet, previous studies often find that soil pathogens drive these effects (e.g. Packer and Clay 2000, Kilronomos 2002, Bell et al. 2006, Petermann et al. 2008). However, the host specificities of soil-borne pathogens are rarely tested and are, in general, low (Agrios 1997). While generalist pathogen can still promote coexistence under some conditions (Webb et al. 2006), pathogens that infect single host species will have greater diversity enhancing effect (Gilbert 2005).

The magnitude of the distance dependent effects of the disease on seedling survival found here strongly supports the prediction of the Janzen-Connell hypothesis that pathogens cause con-specific recruitment to occur only away from the seed source, i.e. parent plant. While seedling survival was not significantly different from zero in and one meter away from infected colonies, over 70 percent of seedlings survived over four years at distances 30 meters away from infected colonies suggesting that distance-dependent seedling survival is likely important in determining the spatial distribution of Mayapple colonies. In Mayapple, rhizome growth begins after approximately five years (Holm 1899), so seedling survival over four years is essential for the seedlings to accumulate resource stores that will diminish their mortality rates.

Previous studies have primarily focused on whether or not the distance- and density-dependent effects occur, and have not considered the form of the relationship relating distance and/or density to seedling mortality. However, in order to generate the recruitment curves envisioned by Janzen (1970) where establishment only occurs far from the seed source despite most seeds dispersing near the seed source, over-compensating density or distance dependence is required (Freckleton and Lewis 2006). If mortality only increases proportionally with distance or density then the density of surviving seedlings may be high close to the parent plant even if mortality is high. In contrast, over-compensating distance or density dependence will leave virtually no survivors near the parent plant, generating competitor-free space for other plant species. This is exactly the pattern observed in this study.

However, competition among plant species in the understory may deviate from the model proposed by the Janzen-Connell hypothesis. In this hypothesis, the rare species advantage assumes that the composition of the plant community is driven by biotic interactions among seedlings that determine the relative abundance of species in the seedling or regeneration layer. The relative abundance of a species under an adult plant determines the probability that a seedling of that species will replace the adult when it dies. Heterospecific seedlings are predicted to increase in relative abundance due to mortality among conspecific seedlings caused by distance and/or density dependent disease or herbivory. Thus seedlings of a given species are predicted to have relatively higher recruitment under heterospecific adults compared to adults of their own species. However in the case of understory plants, such as Mayapple, the seedlings of other plant species may not experience a relative recruitment advantage under mature understory

plants because competition between heterospecific seedlings and mature plants can be strong (reviewed in Royo and Carson 2006) and may determine which plant species survive (see Chapter 4).

In this plant-pathogen interaction density-dependent disease transmission among seedlings is not sufficient to cause the overcompensating distance-dependent seedling mortality; rather disease transmission from infected mature plants drives the high rates of seedling mortality. This is clearly seen in the over three-fold increase in seedling survival when exposed to infection from the soil alone compared to seedlings only exposed to transmission from infected mature plants. This is similar to the patterns of distance- rather than density-dependent seedling mortality in other species caused by damp-off diseases (*Pythium* spp.) where the root system of adult tree sustains the pathogen population (e.g. Augsburger 1984, Packer and Clay 2000, Bell et al. 2006). In systems where disease transmission occurs between adults and seedlings, distance from adult is often a better predictor of seedling survival than seedling density (e.g. Packer and Clay 2000). The disease-induced seedling mortality declines with distance because the majority of the pathogen propagules originate from infected adults. The infection risk of seedlings will then be largely determined by the number of propagules produced on the adult and their dispersal. The size of the propagule pool will be determined by the number of infected units, the number of infected leaves for example. The number and size of these units are often much larger in the adult. In Mayapple, the adult leaves are several orders of magnitude larger than seedling leaves and support aecial infections that are many times larger in size than infections found on seedlings. Consequently, the effects of plant density on disease severity and plant population growth may be better

understood by incorporating variation in the number and size of infected units into the concept of host density rather than considering only the density of individual plants.

That rates of seedling mortality are determined by the infection status of mature plants also suggests that to understand the effects of specialist plant enemies on plant population dynamics, the processes of that determine the frequency and intensity of the interaction between mature plants and their enemies need to be understood (see Chapter 3). These processes have been largely ignored by studies of Janzen-Connell effects but would depend upon the relative dispersal patterns of host and enemy species. Theoretical models have begun to explore the consequences of enemy dispersal patterns on the spatial recruitment pattern of plant species (Nathan and Casagrandi 2004) and on plant community diversity (Adler and Muller-Landau 2005) and suggest that the relative dispersal abilities of plant and enemy are important in determining both.

The negative effects of this pathogen on Mayapple population growth rates demonstrate that pathogen-driven density dependence may be more widespread in temperate forests than previously thought. While pathogens have been shown to cause negative feedbacks in perennial herbs in grasslands (Petermann et al. 2008), in temperate forests the action of pathogens has only been demonstrated for tree species (Packer and Clay 2000, Nakashizuka 2001, Yamazaki et al. 2009). The focus on tree species is likely due to the fact that the Janzen-Connell hypothesis was originally proposed to explain the astounding diversity of tropical trees. But in temperate forests, by focusing exclusively on the trees we may be missing the forest. In contrast to tropical forests, in the deciduous forests of Eastern North America most of the plant diversity is made up of herbaceous plants. Across several forest types in the upper mid-west, 55-75 percent of forest plants

species are herbs (Curtis 1992). Similarly, in forests from Vermont to Virginia, herbaceous species make up 60-86% of all plant species (Siccama et al. 1970, Ramsey et al. 1993). Many species are perennial and most perennial species are clonal (Klimes et al. 1997). While previous studies have found that pathogens negatively affect the growth and survival in three herbaceous understory plant species (Parker 1985, Schnee and Waller 1986, Parker 1987, 1988), the role of disease in structuring the understory plant community has not been considered. Instead, habitat heterogeneity and deer herbivory have been suggested as the major drivers of community composition in the understory (reviewed in Whigham 2004)

Large disease-induced reductions in demographic rates of mature plants (e.g. colony growth and reproductive effort) suggests that studies focusing on only early life stages, i.e. seed and seedling mortality, may not provide a complete measure of the effects of specialist pathogens on plant population dynamics. Indeed, if focusing on the demographic effects of pathogens in a few life stages of a host species is a poor estimate of pathogen effects on host demography, then comparisons of effects of pathogens on seed and seedling mortality among species may reflect variation among species in where demographic effects of infection are manifested rather than differences in impact of pathogens on population growth. Ultimately, theories of coexistence should, by proposing mechanisms that determine the dynamics of plant species' populations, explain the static relative abundance of species. However, efforts to connect strength of negative feedbacks to plant abundance have found mixed results. Previous studies on the relative strength of density dependent mortality or plant-soil feedbacks among plant species have found no correlation with species abundance (Harms et al. 2000, Peters 2003, McCarthy-

Neumann and Kobe 2008); negative correlations, suggesting that common species are less affected by pests (He et al. 1997, Hubbell et al. 2001, Kilronomos 2002, Ahumada et al. 2004); and positive relationships where the dominant species are more negatively affected (Webb and Peart 1999, Wills and al. 2006). However, each study estimated the strength of negative feedbacks within just one or two, typically early, life stages of the plant species. The clear question is do these mixed results indicated a weak relationship between plant species relative abundance and the strength of pest-induced negative feedbacks or is seed and seedling growth and survival a poor comparative measure of the demographic effects of pathogens in long-lived plant species. The latter possibility seems likely because variation among these results may be explained by variation across plant life forms or among plant-pathogen interactions in where the demographic effects occur.

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Chapter 3
Density-dependent disease transmission of a specialist plant pathogen

Abstract:

While specialist pathogens are proposed to maintain plant diversity through density-dependent effects, because plant pathogens are difficult to detect, much less quantify, the relationships between host density and disease transmission rates have not been measured in field studies. Here, I test whether the transmission and severity of host specific, non-systemic, foliar pathogen, *Puccinia podophyii*, increase with the density of its host, *Podophyllum peltatum*. The persistence and spread of non-systemic plant pathogens that infect deciduous host tissues depend upon transmission within host patches, among host patches, and between growing seasons via an environmental reservoir. Within host patches, transmission rates increase by orders of magnitude across the range of host shoot densities found in the field. I find that spores of the pathogen are long-lived in the soil, suggesting that density of spores in the environmental reservoir and thus the risk of infection is a cumulative function of host density. The persistence of spores in the soil also appears to insulate the growth of the pathogen population within a host patch from density-independent effects that can cause local extinction in other foliar pathogens. To examine disease transmission among host patches, I use the pathogen's dispersal kernel to define a spatial scale at which host density determines transmission and find that probability and severity of infection in patches increase with measures of host density.

Introduction:

Specialist plant enemies may act to enhance plant diversity by preventing competitive exclusion (Gillett 1962, Janzen 1970, Connell 1971). The diversity-enhancing effects of enemies require that the negative effects of enemies increase with increasing host abundance**.** The negative effects of plant pathogens on host growth and survival increase with host abundance if the transmission of the pathogen among hosts increases with the density of the individual hosts. However, since individuals of a plant species may vary in size by orders of magnitude, density of individuals is likely a poor predictor of pathogen development. Rather, the density of susceptible units, e.g. leaves in the case of foliar pathogens, drives transmission (Burdon and Chilvers 1982). The direct effects of host density are due to two related and reinforcing factors. As host density increases so does the number of plants available to intercept dispersing propagules. Simultaneously, increasing host density decreases the distance over which spatially-dispersing inoculum must travel in order to spread between plants and reduces the probability that the inoculum will be lost (Burdon and Chilvers 1982). Higher pathogen transmission rates are equivalent to increased pathogen population growth rate and result in increased disease prevalence (proportion of infected hosts or susceptible units) and severity (the number of pathogens per host or susceptible unit of the host).

The simple logic of density-dependent disease development has led many to assume it to be true (Burdon and Chilvers 1982, Gilbert 2002, 2005), based on numerous studies that find a positive correlation between plant species densities and disease prevalence and severity, although exceptions occur (e.g. Lively et al. 1995, Dill-Macky and Roelfs 2000). In fact, a negative relationship between host density and disease is

generally found for heteroecious rusts and vector-transmitted pathogens (reviewed in Gilbert 2002) . Yet, the pattern of density-dependent disease abundance is a poor indicator of the transmission process. Many studies find this pattern for a host-species infected by a generalist pathogen, thus densities of other host species are likely to affect transmission (e.g. Augsburger 1984, Burdon et al. 1992, Helander et al. 1994, Bell et al. 2006). Most importantly, the absence of this pattern says little about the dependence of transmission on host density. The lack of pattern may only reflect the incorrect spatial and temporal scale of the sampled host densities. The spatial scale of pathogen dispersal will define the scale at which the host density will affect transmission (Burdon 1993). The number of pathogen generations determines the temporal scale in which the increased transmission efficiency at high host densities causes increases in disease severity.

Consequently, tests of the role of pathogens in plant species coexistence must consider the life history of the pathogen and demonstrate how the negative effects of infection depend upon host density. To do so, one must account for the spatial heterogeneity of host distribution, the effects of density-independent factors on transmission, or the seasonality of the interaction that involves an additional transmission event, from environmental reservoir to host.

The spatial distribution of hosts often exhibits a clumped distribution resulting from the aggregation of susceptible units within individual plants and/or the aggregation of individual host plants. The immobility and patchy distribution of plants makes the dispersal of plant pathogens critical in determining the dynamics of disease (Burdon 1993, Thrall and Burdon 1999) because the spatial scale of dispersal of the pathogen

determines the scale at which host density can affect transmission. The scale of dispersal of plant pathogens ranges from 5-100cm for many soil-borne pathogen, through several meters for wind- and splash-dispersed pathogens, to tens or hundreds of meters for wind or vector-borne pathogen (Agrios 1997). For pathogens with short range dispersal, new infections result from primarily within-patch transmission and thus depend upon the density of susceptible units within the patch. Within-patch transmission will determine the severity of disease as the numbers of propagules produced within a host patch is much larger than the numbers of spores that arrive from outside the patch. On the other hand, disease transmission among plant patches will determine the incidence of the disease at larger spatial scales. Among-patch transmission depends upon both the dispersal characteristics of pathogen propagules and the fecundity of the pathogen within source patches that, in turn, depends upon the size of the source patch (Burdon and Chilvers 1982).

However, density-dependent transmission is not sufficient to generate increased disease impacts on host growth and survival at high host densities. Density-independent factors, such as climatic conditions, can reduce transmission and limit the growth of the pathogen population or even cause local extinction of the pathogen. Text books full of examples of these effects and it is why the environment is included in the classic "disease triangle" model of what determines the spread of plant pathogens (Agrios 1997). Both inter-annual and site factors can affect the climatic conditions. As studies of the relationship between host density and disease severity in natural systems are often done in one site and over short time spans, it's impossible to determine how the relationship might change in other years and at other locations. In fact, where plant-pathogen

interactions have been studied over multiple years and multiple host populations, local pathogen extinction is common (Burdon and Jarosz 1992, Burdon et al. 1995, Ericson et al. 1999). The regulatory effects of pathogens on host populations will be greatest if the interaction is consistent through time, as climatically-driven reductions in disease incidence or transmission may allow host populations to escape the effects of disease (Burdon 1991).

For non-systemic pathogenic organisms, year to year persistence depends critically on off-host survival and re-infection of the host in the following growing season (Jarosz 2002). For specialist pathogens of perennial plants, overwintering spores are typically dispersed within the vicinity of the host. While density-dependent disease transmission has been primarily conceptualized as occurring among hosts, due to the seasonality, transmission of the pathogen to and from an environmental reservoir are critical in determining if the effects of host density on pathogen transmission are carried over to the following year. Increasing disease transmission with increasing host density will lead to an increase in disease severity over time if the probability of infection in the following year depends upon densities of overwintering spores produced in the previous year, that is, if infection risk at the onset of the growing season is a function of overwintering spore densities in the environmental reservoir. The density of spores in the environmental reservoir also depends upon longevity of the pathogen's overwintering spores. If overwintering spores survive multiple years, the density of these spores will reflect the transmission rates of several years and thus the effect of host density may be cumulative. On the other hand, if the probability of infection in the following year is instead determined by density independent factors such as climate or disturbance, then

while within season transmission may depend on host densities, disease incidence and severity will be independent of host density.

The transmission of spores from an environmental reservoir at the beginning of the growing season may also determine the frequency and intensity of the disease. For pathogens that must re-infect hosts each season from an environmental reservoir, this transmission event may also be dependent on host density. Host density may affect the density of infected host through a sampling effect in the transmission of the pathogen from the environment to the host. If the density of spores in the environment, in part, determines the probability of infection per host individual, then for a given probability of infection, the probability that infection occurs per unit area increases as the densities of individuals or shoots (units of sampling) increase. What determines the initial density of infected plants is thus a binomial process, like flipping a coin. As one continues to flip a coin, the chances of having no tails, analogous here to no infection, quickly diminishes. Thus as host densities increase, at a given probability of infection, the density of infected hosts will increase. The density of infected hosts can determine the severity of disease since the density of pathogen propagules at the onset of host to host transmission will be a function of the number of and distance to infected plants.

However, since plant pathogens are diverse and often difficult to detect, much less quantify, the relationship between host density and disease transmission and severity are rarely examined. Previous studies have tested for negative effects of pathogens on the growth and survival of host plants but have not addressed the transmission dynamics of the pathogen species (e.g. Packer and Clay 2000, Kilronomos 2002, Bell et al. 2006).

Consequently, few studies have considered the natural history of the pathogen that determines if and how these effects are manifested.

To test the relationship between host density and disease transmission, I have conducted several experiments and a four year observational study of the interaction of Mayapple rust, *Puccinia podophyllii*, and its host, *Podophyllum peltatum*. I demonstrate the dependence of transmission on host densities both within and among host patches and that the persistence and severity of this disease is largely determined by the density-dependent sampling of environmental reservoirs. Furthermore, I show that, while density-independent factors affect disease transmission, the incidence and severity of disease within host patches is not affected by these perturbations owing to long-term survival of overwintering spores, and thus severity increases over time in a host patch.

Methods:

Study System: Mayapple (*Podophyllum peltatum*, Berberidaceae) is the only species of the genus *Podophyllum* (s.s.). Mayapple is widely distributed over the eastern half of the United States wherever deciduous forests occur. Mayapple is one of the first plants to emerge in the spring. Shoots emerge in early to mid-April and can persist until August or September. Mayapple colonies consist of one to ten's of thousands of stems that can cover up to nearly a hectare. A single colony is typically composed of one but occasionally more genets (Parker 1988). The annual above ground shoots are easily recognized by the lobed, peltate leaves and are dimorphic. Vegetative shoots consist of a petiole and single leaf and are morphologically leaves. The flowering shoots are true shoots and typically have two leaves that are each of similar size to the leaves found on vegetative shoots of the same height.

Herbivore damage on Mayapple is rare. A few species of lepidopteran larvae are able to feed and develop on Mayapple (*Chorisincura rosaceana, Clepsis melaleucana* (Faeth 1978), *Papaipema cerina* (Geber et al. 1997), but they are either absent or exceedling uncommon in the Mayapple populations studied. The toxicity of Mayapple tissues limits diversity and abundance of herbivores of Mayapple. The anti-mitotic toxin, Podophyllin, is produced throughout its tissues (Moraes et al. 2000).

The protection provided by these toxins is not complete. Mayapple is commonly infected by the non-systemic, host-specific pathogen, *Puccinia podophylii* Schwein., throughout its range. Infection occurs during two discrete generations of the pathogen in each growing season. Primary infection occurs via contact with spore-contaminated soil during shoot emergence in the early spring (Whetzel et al. 1925). Orange lesions (aecia, referred to here as primary infections) develop within a few weeks producing aecidiospores. The lesions occur on the leaves of a small portion, typically less than 10%, of the shoots in a colony and have an average area of one to two centimeters.

The aecidiospores are the only known dispersing form of the pathogen. The aecidiospores are dispersed by two mechanisms. The spores are mechanically ejected from the aecidium a distance of about 1 cm (Dodge 1924). The spores are then aerially dispersed over short distances. The aecidiospores that germinate on host tissue produce telia (secondary infections) on the underside of the leaf and cause a yellow spot on the leaf at the point of infection. The telia are visible by early June and produce the overwintering teliospores that return to the soil. 80 to 90 percent of the shoots in colonies with average rates of aecial infection are infected by the telia which can occupy up to 100% of the leaf area.

Genetic resistance to infection by this pathogen is not known to occur in Mayapple (Parker 1989). Instead, the primary host defense appears to be physical. The pre-emergent shoots are protected by a whorl of bud scales that protect the shoot from contact with the rust spores in the soil. These bud scales are frequently infected by the pathogen. However, the lesions that develop on the bud scales produce only teliospores that are incapable of re-infecting the shoots in the current growing season. If the bud scales are damaged, or if they are too far beneath the soil surface to prevent soil contact, aecial infection is significantly more likely to occur (Parker 1988). Bud scale size and number increases with shoot size. Parker hypothesized that the variation in the size of the bud determines the infection risk of shoots, as larger buds, associated with larger shoots, may protrude further above the soil surface, thus smaller shoots are more susceptible to infection.

This study system is ideal for addressing the effects of host density on transmission of disease. The presence and severity of the disease is easily quantified since all infected shoots are symptomatic and the individual infections vary little in size.

Study Sites: The study was conducted three forested sites in Washtenaw County in southeast Michigan, Raddrick forest, the Newcomb tract, and Pinckney State Recreation area. These forests were typical second growth oak-hickory-maple forests, but differed in disturbance histories, soil types and plant community compositions.

Sampling Methods: The study consisted of experiments and field observations. In the field observations, I tested the relationships between disease severity and measures of host densities across the three sites. In each site approximately 80 Mayapple colonies (259 in total) were measured each year from 2005 to 2008. The locations of all colonies

in each site were mapped. In each year, the host plant and pathogen variables were sampled within each Mayapple colony. Each colony was sampled twice per growing season. The first sampling period extended from early May until early June, and measured shoot density, shoot size (stem height and leaf radius (cm)), and the presence and the prevalence of primary infections. The sampling methods have been described previously (Chapter 2). Table 3.1 provides a summary of the numbers of colonies at each site and the frequency of disease infection, colonization, and extinction.

Colony area was estimated by approximating its shape as a sixteen to twenty four-sided polygon using methods detailed previously (Chapter 2). I estimated the edge to area ratio for each colony by using colony area and the circumference of a circle of that area.

Among Colony Transmission and Severity:

For negative effects of disease to increase with host density at larger spatial

Table 3.1. Percentage of *Podophyllum peltatum* colonies infected by *Puccinia podophyllii* and frequency of new infections and extinctions. All frequencies are expressed as the percent of total colonies at each site and year. The number of colonies sampled is shown in parentheses.

Sites		2005	2006	2007	2008
Newcomb	% of colonies infected	78 (58)	60 (77)	61 (80)	60 (80)
	% new infections	-	3.5	5.2	3.8
	% extinctions	-	1.7	1.3	5.0
Pinckney	% of colonies infected	33 (57)	39 (69)	45 (76)	42 (77)
	% new infections	-	14	8.8	4
	% extinctions	-	3.5	1.5	6.7
Raddrick	% of colonies infected	68 (58)	68 (93)	66 (94)	67 (95)
	% new infections	-	3.4	5.5	6.4
	% extinctions	-	4.5	5.5	5.3
All sites	% of colonies infected	61 (208)	57 (239)	58 (250)	57 (252)
	% new infections	-	6.4	6.4	4.8
	% extinctions	-	1.7	1.3	5.0

scales, disease transmission among colonies must increase with host density. Host density can affect transmission among colonies in three ways. In an infected colony from which the pathogen disperses, the maximum number of dispersing pathogen spores will be proportional to the number of shoots. Additionally, if pathogen dispersal rates decrease with distance, then healthy colonies near to diseased colonies will receive more dispersing pathogen propagules and become infected, thus initiating within-colony transmission, more rapidly than isolated colonies. In an uninfected colony, the number of stems in a colony will determine number of times the environmental reservoir (pathogen spores in the soil) is sampled each year. These effects of host density should only be visible in the early stages of disease establishment within a colony since, once within-colony transmission is established, pathogen spores are produced within the colony at rates much higher than the colonization rates.

To test the prediction that pathogen dispersal rates decrease with distance from infected colonies, I fit dispersal curves relating the number of colonizing telial infections in colonies without aecial infection to the observed pathogen population sizes in all other colonies and distances among colonies. I used the data from 2005 and 2006. The pathogen dispersal was estimated in the field using the number of pathogen lesions that occurred in colonies that had no primary infection, thus all secondary infections arise from dispersal into the colony. The dispersal curve for the disease was estimated using a power function relating the colonization rate in uninfected colonies (average number of colonizing telia per leaf) to the dispersal source, calculated as the sum, over all colonies within a site, of total telia in the infected colony divided by the dispersal distance.

Density-dependent disease transmission among colonies leads to increased probability of disease establishment and increased disease severity within a colony. I tested this prediction by relating the likelihood of disease establishment and disease severity to size of the colony and Mayapple density at larger spatial scales in colonies with less than 500 stems (N=178). Colonies in this size class vary in disease status and thus reflect the variation in disease transmission. The likelihood of disease establishment was calculated as the average presence (1) or absence (0) of the primary infection across the years the colony was sampled. Disease severity within each colony was estimated in the same manner described above and averaged across the years as well. The density of hosts surrounding the colony (neighborhood density) was estimated using a metric based on the dispersal rates of the disease. Transmission can only occur if the disease is present in neighboring colonies, so the density metric for each colony was calculated as the sum of shoot population size within colony/(distance to colony)$^{1.9}$ over all colonies infected with the first generation in a site. The effects of distance and colony size on the likelihood of disease establishment were analyzed using a logistic regression using the natural log-transformed values for the neighborhood density metric and the number of stems in the focal colony. The effect of these density measures on disease severity was analyzed using linear regression of the log-transformed values of focal colony size, neighborhood density, and disease severity.

Within-Colony Transmission and Severity

Within-season transmission dynamics:

To test for effects of host density on disease transmission**,** the annual rate of increase for pathogen population in each colony was estimated in each year from 2005 to

2008. This is equivalent to the realized population growth rate of the pathogen and represents the number of secondary infections produced by a single primary infection. The rate was calculated as the estimated numbers of secondary (telial) infections divided by the primary (aecial) infections. The number of secondary infections per colony was estimated as the product of the average number of secondary infections per leaf, the average number of leaves per shoot, and the number of shoots in the colony. In colonies of less than 200 shoots the all primary infections were counted. In larger colonies,I estimated the number of primary infections as the frequency of primary infections times the number of shoots in the colony. The within-season transmission rate was only calculated for colonies with rates of primary infection greater than five percent to minimize the effect of measurement error inflating the transmission rate estimate.

Rust severity in the telial stage is predicted to be the product of host density and per-shoot probability of infection from the soil. Severity is also predicted to be affected by host density through density-dependent transmission from the primary infections to the secondary infections, thus severity should be reduced in small colonies, where most of the spores fail to reach a host. I tested this prediction across sites and years using the data collected in the field from colonies infected with the aecia and consisting of more than 5 shoots. I analyzed the relationship between the log transformed measures of prevalence of the aecial stage, as an estimate of per shoot probability of primary infection, and shoot densities, and colony edge-area ratios to disease severity, estimated as the average telia per host leaf.

Between-season transmission dynamics

Within-season transmission must affect transmission from the environmental reservoir for disease severity to be a function of host density. Thus, a host colony's probability of infection from the soil is predicted to increase with increasing overwintering spore densities in the previous year. The number of overwintering spores produced per unit area in the previous year are predicted to determine the per shoot probability of primary infection. The density of overwintering spores in the previous year is the product of number of secondary infections per host leaf and density of leaves. I tested this prediction in one observational study and two experiments. In the observational study, the prediction was tested using a linear regression of the log transformed spore density per host leaf, leaf density, and probability of infection from the soil using data from colonies infected from the soil in that year. I used the lower limit of one percent infection probability so as to remove several large outliers that in preliminary analyses significantly affected the results.

The experiments tested the prediction by manipulating the density of overwintering spores and measuring the response as the per shoot probability of primary infection in the following year. In the first experiment, I applied the following treatments; removal of overwintering spores, doubling of overwintering spores, and a control. Each treatment was replicated once per colony at two colonies at three sites (n=6). All colonies had equivalently high disease severities. The treatment plots were one meter squared in area. I removed the overwintering spores by removing all shoots from the plot. These shoots and their spores were placed in the addition treatment. In the control plots, the stems were clipped and left in the plot. The manipulations were carried out in late July when the teliospores were mature, but still attached to the leaves of the

shoots. In the following spring, I measured the prevalence of primary infection across all shoots within the plots. The effects of the treatments on the arcsin-square root transformed prevalences were analyzed using an ANOVA.

In the second experiment, I applied fungicide treatments to test the dependence of probability of infection from the soil-borne spore on overwintering spore densities. I utilized a paired plot design where in each replicate colony half of the colony was randomly assigned fungicide or control treatments. Over four years, protectant fungicide (Daconil: Chlorothalonil) was sprayed biweekly during the period when the pathogen was dispersing (June through early July). Fungicide was applied using a backpack sprayer at the rate recommended by the manufacturer. The fungicide treatment reduced overwintering spore densities by approximately ninety percent compared to the paired controls. The prevalence of primary infection was recorded in the final year of the experiment. The effects of the fungicide treatment on primary infection rates were analyzed using a paired sample t-test.

Density-dependent disease transmission predicts a positive feedback where disease severity increases over time, if host density is sufficiently high. This feedback should be limited by the maximum spores that can fit on a host leaf, thus the rate of change in per host severity should decrease with increasing disease severity. This progression of disease may also be interrupted by density independent factors, e.g. weather. These effects may override or alter the relationship between host abundance and density. Comparing changes in severity within colonies across several years gives an indication of the dynamics of the disease over longer time periods. To test the predictions that disease severity increases over time and at a rate that slows with

increasing severity, I compared the annual rate of change in disease severity with the disease severity in the previous year across colonies that had primary infections in all years. For each colony in each year, the disease severity was measured as the average number of secondary infections per leaf. The rate was calculated as the disease severity in the current year divided by the disease severity in the previous year. With the exceptions listed above, all data were analyzed using a repeated measures mixed-model ANOVA. All rates and host densities were log-transformed prior to analysis. To test for density-independent effects, I added the site and year and interactions of site by year, site by density, and year by density as factors.

Results:

Among Colony Transmission and Severity:

Density-dependence of among-colony transmission was, in part, driven by the dispersal rate of the pathogen which decreased rapidly with distance. Among uninfected colonies (without primary infection), colonies located further than 20 meters from an infected colony frequently had no telial infection (pathogen dispersal) while colonies a few meters away from infected colonies exhibited rates of infection up to 100 telia per leaf. The dispersal kernel estimated for the pathogen was $1.88*10^{-3}$ * distance (meters)$^{-1.9}$ ($r^2=0.64$, $p<0.01$) suggesting that most dispersal occurs over distances less than 10 meters. In fact, the estimated dispersal kernel likely overestimates dispersal rates at distances less than 10 meters since the distance between colonies was measured from the centers of the colonies. Compared to this approximation of dispersal distance, the actual distance traveled by the pathogen may be much less, from the edge of the infected colony to the uninfected colony rather than from the center.

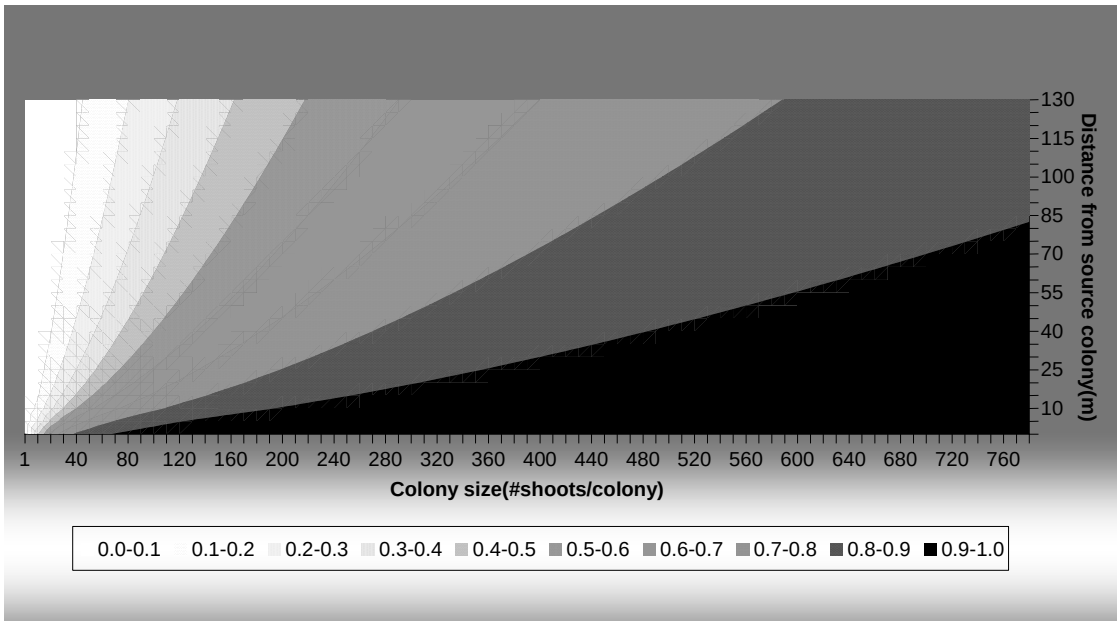

Figure 3.1 Relationships among colony size, spatial isolation, and rates of among-colony transmission. The shading and values in the legend indicate the likelihood of primary infection within a host colony. The x-axis, colony size, is the number of shoots in the colony. The y-axis, distance, is the distance from the focal colony to an infected colony with 1000 shoots.

The likelihood of disease establishment in small colonies significantly increased with increasing host density (r^2=0.69, $p<0.05$). Both measures of density, focal colony size and neighborhood density, were significant predictors of the probability of primary infection within focal colonies in the logistic regression (Table 3.2, $p<0.05$). The parameters estimated for this model indicate that these two density measures have strong effects on rates of disease transmission among Mayapple colonies (Figure 3.1). To add in interpretation, I converted the neighborhood density index values to the distance (meters) from the colony to an infected colony of 1000 stems, the typical size of a large colony. The most isolated colonies are on average 100 meters away from a large infected colony. The colonies with the highest neighborhood density values are approximately 3

meters from a large infected colony. For small colonies (less than 10 shoots), the colonies three meters away from an infected colony are 40 times more likely to be infected than colonies 100 meters away. As shoot numbers within a colony increase, the likelihood of infection becomes almost certain (>99%) in colonies with greater than 600 stems. This corresponds to the likelihoods of infection of over ninety nine percent observed in the field for colonies of this size, which were not used in the analysis.

The increasing likelihood of disease establishment at higher host densities within the colony and in the surrounding area results in increasing disease severity in small colonies ($F=78.9$, $p<0.0001$, $r^2=0.46$). Both increasing focal colony size and neighborhood density were significantly correlated with increasing disease severity in colonies containing less than 500 shoots ($T=6.7_{\ln(\text{colony size})}$ and $T=9.2_{\ln(\text{neighborhood density})}$ $p<0.0001$, see Table 3.2).

Within-Colony Transmission and Severity

Within-season transmission dynamics:

The rate of disease transmission within a colony from primary to secondary infections increases significantly with increasing host density ($p<0.001$, see Table 3.2). The density of host leaves appears to be the primary determinate of the rate of disease transmission as it explains more variation than site, year, colony area, and the interaction terms combined (see Figure 3.2). As predicted, disease transmission was significantly lower in colonies with high edge to area ratios ($p<0.05$, see Figure 3.3) indicating that the rate at which spores are lost to areas outside the colony increases with decreasing colony area. The lowest observed transmission rates were less than 20 telial infections per aecial infection and occurred in colonies consisting of 5 or fewer shoots. On average, the

transmission rate increased to approximately 3000 secondary infections per primary infection in colonies with leaf densities greater than the average across colonies of 45 leaves per square meter. These two measures of host density were the only significant predictors ($p<0.05$) of the pathogen transmission rate indicating that within-colony disease transmission depends on host density and was largely unaffected by density-independent factors associated with year or site. Transmission, however, varied slightly in some sites in some years, but did not alter the relationship between host density and disease transmission (see Table 3.2, year and site by year interaction $p<0.1$, density by year and density by site interactions not significant).

Table 3.2: Summary of effects of host density on disease transmission and severity within and among Mayapple colonies and the dynamics of severity within colonies over time. Significance of the factors and covariates are as n.s. (not significant), p<0.1 (*), p<0.05 (**), and p<0.001 (***). F-values are listed for the predictors of within colony transmission. See text for transformations used.

Transmission	Response Measure	Predictors	Sample size
Among colonies			
	Annual probability of primary infection	Logistic regression: r^2=0.69, $F_{3,177}$ =297.5 Neighborhood density: *** Colony population size:***	N=180
	Disease Severity	Constant: t=4.4 *** Neighborhood density: t=6.7 *** Colony population size: t=9.2 ***	N=177
Within colonies			
Within season: primary to secondary infection	Transmission	Site: 1.2 n.s. Year: 2.2 * Leaf density: 38.5 *** Colony area: 4.0** Site by year: 2.1 * Leaf density (site): 0.7 n.s. Leaf density (year): 1.0 n.s.	N=160
	Disease Severity	Site:1.6 n.s. Year: 3.0 ** Shoot density: 21.2 *** Prevalence of primary infection : 119.1 *** Colony area: 6.7 ** Site by year: 0.7 n.s. Prevalence (year): 2.6 * Shoot density (year) 0.5 n.s. Prevalence (site): 0.8 n.s. Shoot density (site): 0.7 n.s.	N=160
Between season: Overwinter: secondary $_t$ to primary $_{t+1}$ infection	Prevalence of primary infection $_t$	Site: 0.6 n.s. Year: 1.1 n.s. Disease severity $_{t-1}$: 105.1 *** Leaf density $_{t-1}$: 1.7 n.s. Site by year: 8.3 *** Disease severity $_{t-1}$ (year): 1.0 n.s. Leaf density $_{t-1}$(year): 1.8 n.s. Disease severity $_{t-1}$ (site): 0.7 n.s. Leaf density $_{t-1}$(site): 0.1 n.s.	N=147
Disease progress: secondary $_t$ to secondary $_{t+1}$ infection	Annual Rate Change of Severity of Secondary Infection	Site: n.s. Year: 20.1*** Disease Severity $_{t-1}$: 20.1*** Site by year: 3.0 ** Disease severity $_{t-1}$ (year): 3.0 ** Disease severity $_{t-1}$ (site): 0.4 n.s.	N=117

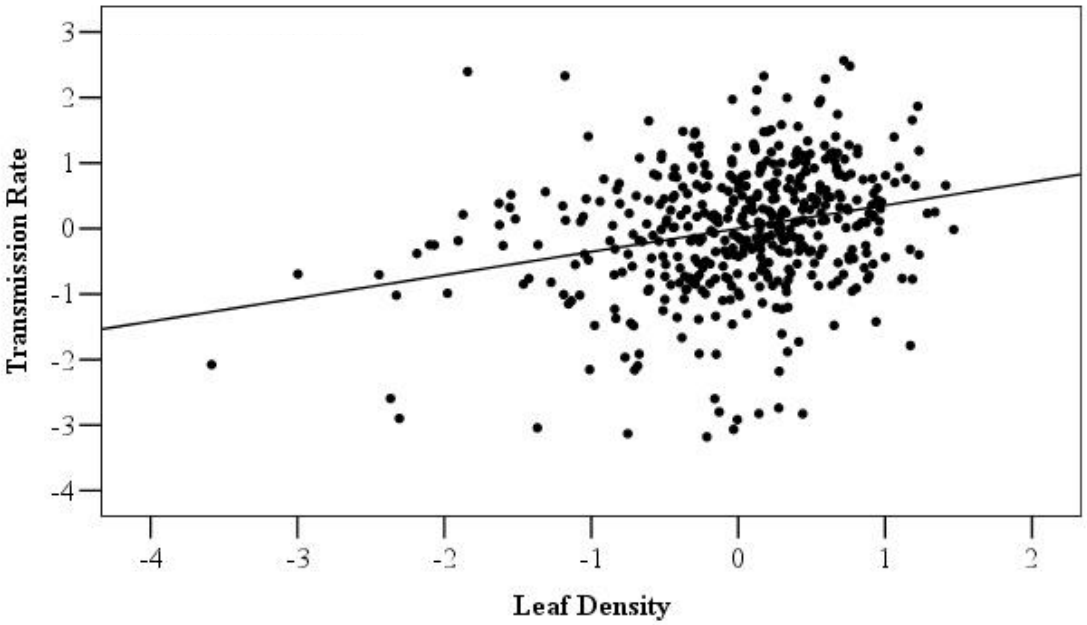

Figure 3.2. Relationship between within-season transmission within host colonies and host density. Relationship is the partial regression plot from regression of log-transformed transmission rate, leaf density and edge-area ratio (r^2=0.07, p<0.005).

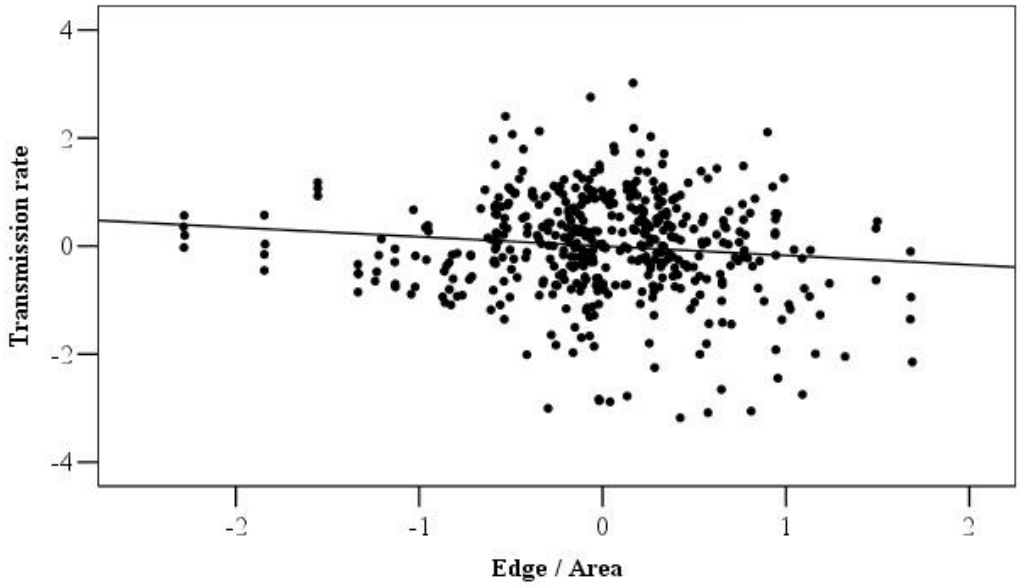

Figure 3.3. Relationship between within-season transmission and colony size. Colony size is measured as the edge to area ratio (X-axis). Note that small colonies have large edge to area values. Relationship is the partial regression plot from regression of log-transformed transmission rate, leaf density and edge-area ratio (r^2=0.01, $p<0.05$).

I found strong support for the prediction that Mayapple shoot density determines the severity of the telial form of disease. Both shoot density and the prevalence of aecial infection were positively correlated with disease severity (telia per leaf) and explained most of the variation in severity ($F_{\text{Shoot Density}}$=21.2, $F_{\text{Prevalence}}$=119.1 $p<0.0001$, see Table 3.2). The coefficient estimates for the log-transformed shoot density (0.42, std. error =0.14) and the probability of primary infection (0.47 std. error =0.07) terms suggest

severity is a function of the square root of the density of infected shoots, which is proportional to the average nearest-neighbor distance among infected shoots.

The increased loss of spores to areas outside the colony in smaller-sized colonies was also evident in the results. Disease severity in colonies occupying small areas was significantly less than in larger colonies ($F=6.732$, $p<0.05$). The partial regression of the effect of colony size on disease severity suggests that the relationship is not linear as disease severity is only negatively correlated with colony area in the colonies less than 7 meters in diameter (relationship is similar to that shown in Figure 3.3). Annual variation in climatic conditions had a small but significant effect on disease severity (year, $F=3.03$, $p<0.05$). In 2005 severity was lower than all other years probably due to reduced within-season transmission in that year (prevalence*2005 interaction, $t=-2.6$, $p<0.05$).

Between-season transmission dynamics

The rates of primary infection varied among colonies, ranging from zero to sixty-nine percent (mean= 8.7%). Contrary to the prediction that these rates are a function of the density of overwintering spores in the previous year where spore density is product of the densities of spores per leaf and leaves per area, only the density of spores per leaf (disease severity in the previous year) was a significant predictor of the probability of infection from the soil in the following year ($F=104.5$, $p<0.001$, see Table3.2) and the density of leaves was not significant ($p>0.1$). At low severities, small changes in severity lead to large increases in the rates of infection from the soil in the following year. As disease severity in the previous year changes from 1 to 100, the probability of infection increases from less than one to seven percent and increases linearly and more slowly as disease severity increases further (Figure 3.4). The probability of infection from the soil

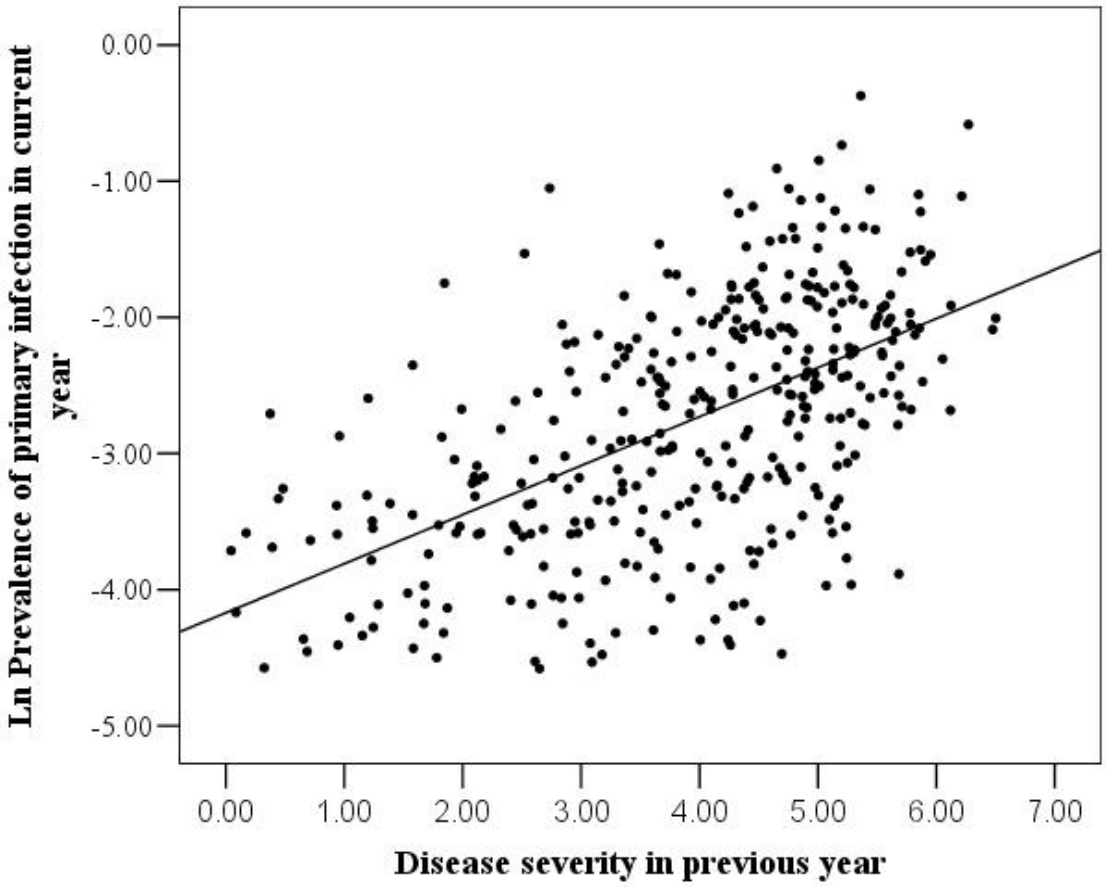

Figure 3.4: Relationship between severity of telial infection in previous year and prevalence of primary infection within Mayapple colonies. Each dot represents one colony in one year. Disease severity, the x-axis, is equal to ln (average telia per leaf +1) in the previous year. Prevalence of primary infection $_t$ = exp(-4.17 + 0.33* disease severity$_{t-1}$), r^2=0.31, p<0.001.

was also affected by environmental factors in some years and at some sites (location*year F=7.98 p<0.001), with two sites in 2007 exhibiting a one to two percent increase in the infection rate.

The prediction that the infection probability will depend on the density of overwintering spores in the previous year was based on the hypothesis that overwintering spores survive only one year. That only the density of spores per leaf predicts the probability of infection from the soil suggests that shoots in the following year only sample the spore bank in the immediate vicinity of the previous shoot or that the spores live more than one year. While the first hypothesis is possible since the length of new rhizome segments are approximately equal to the radius of a leaf, the results of the two experiments testing how primary infection depends on the density of overwintering spores demonstrate that spores live longer than one year and the probability of infection depends on the inputs of overwinter spores over several years. Adding and removing the overwintering spores had no significant effect on the probability a shoot the following year was infected ($F_{2,15}$=0.7, p>0.5), suggesting that the spores survive longer than one year in the soil. In fact, further analysis revealed that the risk of infection was best explained by the colony in which the treatments were located ($F_{5,10}$=4.2, p<0.05, see Figure 3.5) and within colonies no consistent treatment effects were evident, suggesting that density of spores in the soil does determine the probability of infection, but the density is a product of the multiple years of inputs of overwintering spores in a colony.

The spore bank may decay over time but spores are persistent over multiple years. In the four-year fungicide experiment, the primary infection was present in all but one fungicide treatment plot in the final year. Four years of fungicide treatment did result in a small but significant reduction in the probability of primary infection (mean treatment effect =-1.6% std. error= 0.5%, p<0.0001).

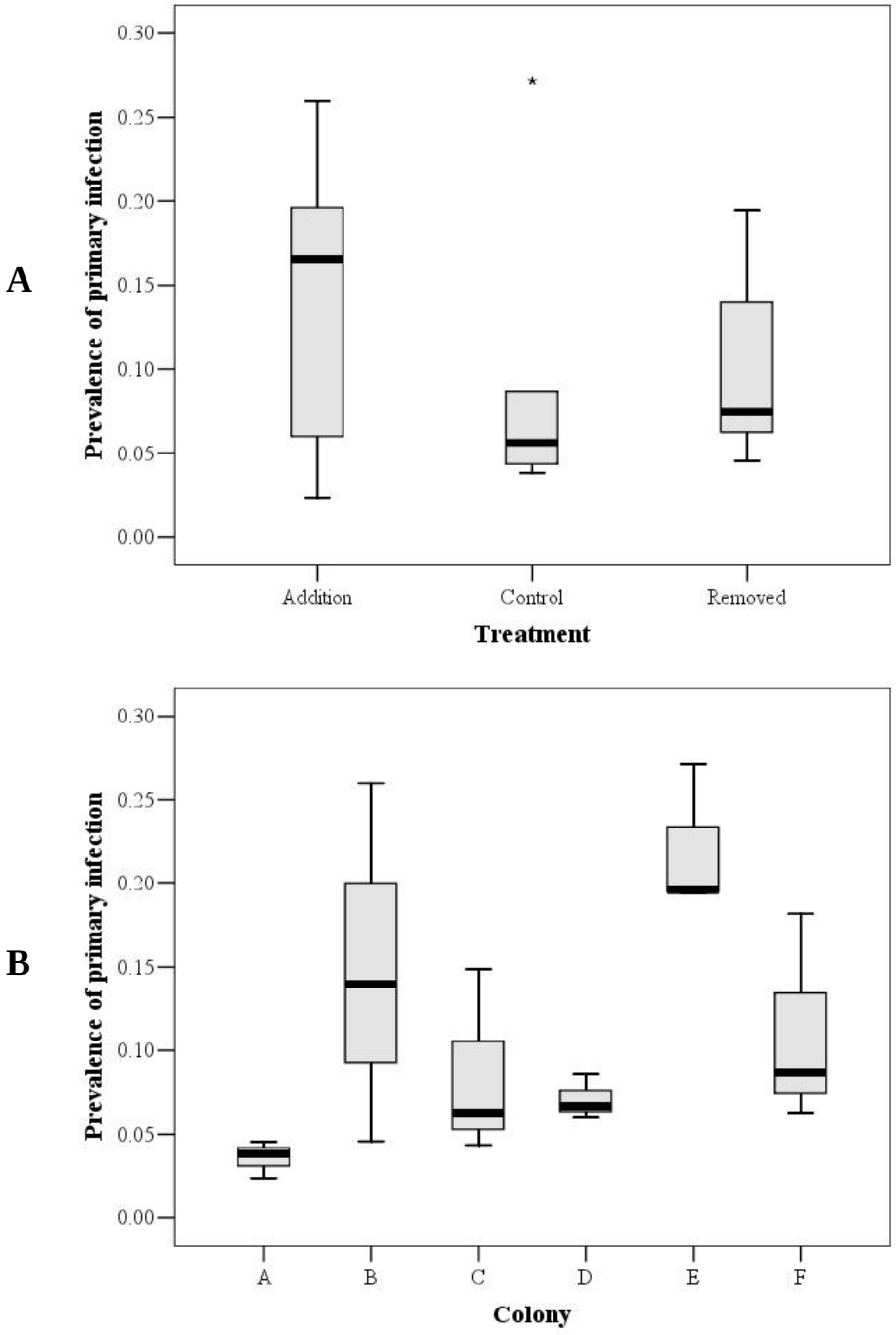

Figure 3.5: Rates of primary infection are more similar within Mayapple colonies than among levels of teliaspore inputs in the previous year. Box plots indicating the median, quartiles, and extreme values of prevalence of infection from the soil among factors A) prevalence of infection from the soil among treatments of doubling teliaspores (Addition), removing teliaspores (Remove), and control; $p>0,5$. B) prevalence of infection among Mayapple colonies used in the experiment; $p<0.05$.

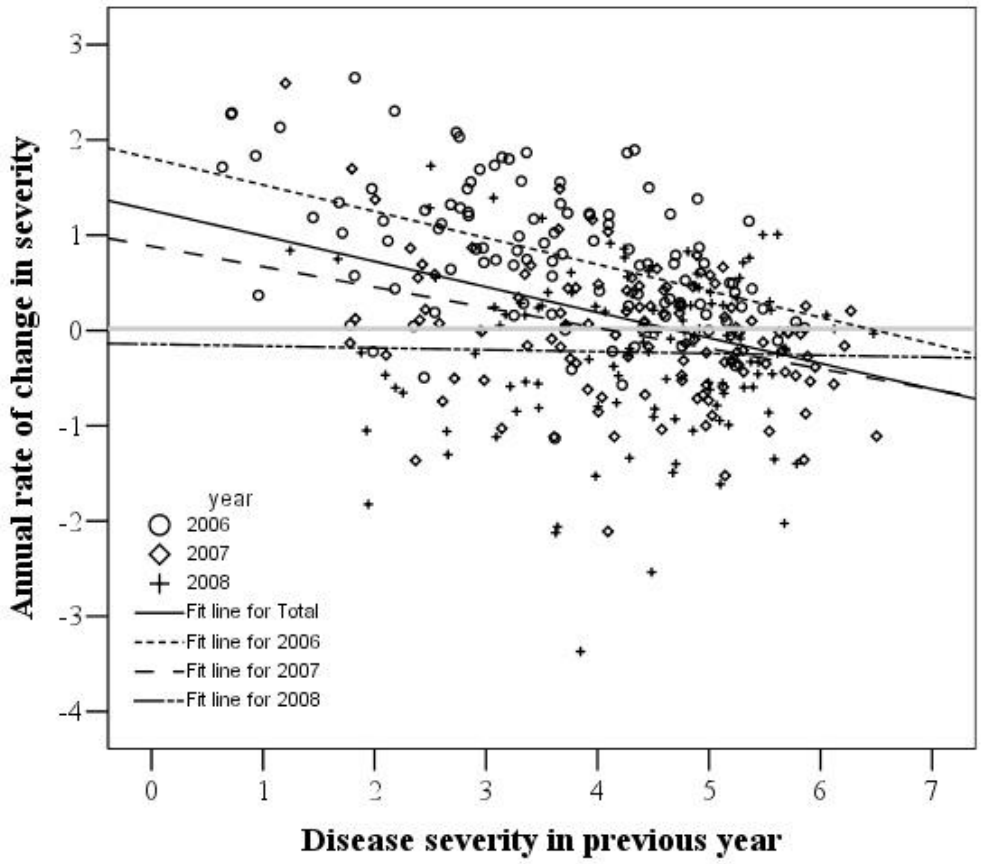

Figure 3.6: Effects of severity of telial infection in previous year and annual variation on annual rate of change in disease severity within Mayapple colonies. Symbols represent data from a colony in each year. Disease severity, the x-axis, is equal to ln (average telia per leaf +1) in the previous year. Annual rate of change in severity, the y-axis, is equal to ln (average telial lesions per leaf $_t$ / average telial lesions per leaf $_{t-1}$), i.e. rate of pathogen population growth per host leaf. The horizontal grey line indicates no change in severity. Regression lines show the relationships for all years (solid line, r^2=0.14, $p<0.05$) and for each year (dashed lines). Rate of severity change in 2006 = 1.8 - 0.3* disease severity$_{t-1}$, r^2=0.23, $p<0.001$. Rate of severity change in 2007 = 0.99 - 0.18* disease severity$_{t-1}$, r^2=0.13, $p<0.05$. Rate of severity change in 2008 is not significantly correlated with disease severity$_{t-1}$.

Disease severity in a colony significantly increases across years with average rate of increase across all colonies and all years of 1.7 telia per leaf in the current year per telia per leaf in the previous year. The annual rate of increase in disease severity is highest in colonies with low disease severity in the previous year (Table 3.2: Disease

progress and Figure 3.6), with the exception of 2008 (diseases severity $_{t-1}$ by year interaction, $p<0.05$) The density-independent effects (i.e. annual variation in climate) had significant effects on the rate of severity increase, with larger increases in severity from 2005 to 2006 (mean rate =2.9) and 2006 to 2007 (mean rate=1.25), and in 2007 to 2008 (mean rate =1.08). Differences in rates among years were not consistent across sites (site*year interaction, $F=3.03$, $p<0.05$). While the growth rate of severity decreases as disease severity increases, the parameter estimates from the model predict that the disease severity will increase over time to the high levels of disease severity.

Discussion:

The results of this study demonstrate that disease transmission is density-dependent and that transmission increases with host density not only within host colonies but among host colonies as well. This study is unique in that it tests how host density affects the transmission dynamics that determine the frequency and severity of the disease in the field. Previous studies of the role of plant pathogens in regulating host populations and maintaining plant species diversity have been unable to directly measure disease transmission, due to the difficulty in identifying, much less quantifying the pathogens responsible for the observed effects. Instead, they test correlations between host density and disease levels or host mortality caused by pathogens that are often not host specific (e.g. Augsburger 1984, Burdon et al. 1992, Helander et al. 1994, Bell et al. 2006) and thus it is difficult to determine causality or predict the dynamics of disease spread and consequences to host populations and plant communities.

It is clear that host density is critical in determining the transmission among colonies and the severity of disease in newly infected colonies. The dispersal-limitation

in the pathogen allows plants to escape the disease temporally and experience higher growth and seedling survival (see chapter 2). The length of this disease-free period depends upon the size (number of shoots) of the uninfected colony and abundance of host shoots within a neighborhood defined by the dispersal distance of the pathogen and is decades longer in most isolated colonies compared to the least isolated colonies, based on estimates of colony age using shoot population growth rate estimated previously (Chapter 2). The consequences of host dispersal and escape from pathogens on host growth and population dynamics has not been explored empirically, despite the critical importance of this process in Janzen-Connell processes. Spatial escape and elevated recruitment rates may contribute to the clumped distribution of mature plants and contribute to coexistence by aggregating con-specific plants (Hibbs 1982, Pacala 1986, Silvertown and Law 1987, Pacala et al. 1993).

These results also demonstrate that once the infection becomes established within a colony, shoot and leaf densities within the colony and colony size determine the rates of transmission. The rate of transmission (i.e. growth of the pathogen population) determines how severity changes over time. The limited number of generations of the pathogen per season makes the build up of disease severity slow. The slow increase in severity coupled with the variation in when colonies are infected causes infection status to vary among similar-sized colonies, in terms of numbers of shoots per colony or area occupied by the colony, and among colonies with similar shoot densities. Thus host density and how long the host patch has been infected likely jointly determine disease severity. In other plant-pathogen interactions rates of transmission can not be easily observed, making experimental tests of density dependence difficult. Instead,

experiments often compare disease severity or mortality among host-density treatments. If disease severity is determined by transmission from hosts outside the density treatment plots, e.g. from adults to seedlings where the majority of the inoculum is produced by the adults, then severity in the treatment plots will be a function of the disease fecundity or infection rates in host that are the source of the inoculum. For slow growing pathogen, age of infection may determine these rates. The dependence of disease severity on age of infection may explain the variation among host trees in the effects of pathogens on seedlings found in *Platypodium elegans* (Augsburger and Kelly 1984) and *Prunus serotina* (Packer and Clay 2000). In the latter study, the levels of disease-induced seedling mortality were lower around smaller trees compared to larger adult trees.

In contrast to other studies that have found frequent pathogen extinctions within host populations, local extinction only occurs early in disease establishment within a Mayapple colony. While abiotic factors have significant effects on disease transmission, the incidence of disease does not exhibit the large year to year variation commonly found in non-systemic foliar pathogens (Jarosz and Davelos 1995). The strong relationship between severity in the previous year and the risk of infection in the following year suggests that, despite the limited number of generations of the pathogen in a single season, the increased transmission in one season does lead to progressive increases in the severity of the disease. Furthermore, disease severity clearly increases in colonies once they are infected. These results, in conjunction with the multiple negative effects on host demographic rates caused by the disease (Chapter 2), strongly support the hypothesis that density-dependent transmission results in regulation on the host population.

The progressive increases in disease severity within Mayapple colonies over time appear to be primarily driven by the accumulation of pathogen spores in the environmental reservoir. The longevity of spores in the soil has not been determined. While a disease exclusion experiment produced significant differences in the prevalence of primary infection, the primary infection was still present after 4 years of fungicide treatments. The less than 2% difference in disease prevalence between paired fungicide and diseased plots could be due to reduction in the size of the spore bank in the soil in the treatment plots. On the other hand, the small difference in rates of infection between treatments could be driven by decreased susceptibility to infection of shoots in the fungicide treatment and unrelated to the size of the spore bank. The shoots in the treatment plots were significantly larger and shoot size is correlated with bud scale size. As the bud scale is the primary physical defense against infection from the soil, the differences between treatments in the prevalence of primary infection could be due to increased bud scale size in the treatment plot compared to the diseased plots.

While environmental reservoirs are critical for the persistence of disease in host populations (Jarosz 2002), the role of host density in the transmission of disease from an environmental reservoir has not been considered in previous studies. If the density of spores in the environment, in part, determines the probability of infection per host individual, then for a given probability of infection, the probability that infection occurs per unit area increases as the densities of individuals or shoots (units of sampling) increase. That local extinction of the pathogen only occurs in host colonies with less than 600 shoots strongly suggests that this sampling process is important not only for the persistence of the disease but also in determining the density of primary infections and

thus severity of disease. It is likely that the same process operates in many plant-pathogen interactions. Soil-borne pathogens, particularly ooycetes that cause 'damping off' disease, cause density dependent seedling mortality in temperate (Shibata and Nakashizuka 1995, Packer and Clay 2000, 2003, Yamazaki et al. 2009) and tropical tree species (reviewed in Gilbert 2005). In these systems, the seedlings are sampling an environmental reservoir of latent pathogens in the soil. As seedling densities increase, the probability that a primary infection (from the soil) will occur increases. The incidence and prevalence of primary infection will determine the severity of infection resulting from host to host transmission.

While many theoretical models predict pathogen extinction at low host densities (e.g. May 1990), the longevity of the spores of this pathogen in the soil may stabilize the pathogen population, buffering it against year-to-year variability in pathogen transmission due to changes in host density or climatic conditions (Hochberg 1989). Several empirical and theoretical studies also suggest that the persistent environmental reservoir of the pathogen can allow the pathogen to drive hosts to local extinction (de Castro and Bolker 2005). Future studies will be required to examine whether the longevity of spores in the soil is sufficient to decouple host density and disease severity (i.e. negative impact on hosts) and produce continued negative effects on the host despite reductions in host density.

This study also emphasizes the importance of understanding pathogen life-history features in order to understand pathogen persistence and patterns of disease incidence (Thrall and Burdon 1997, Burdon and Thrall 1999, Thrall and Burdon 1999). Without this knowledge, it is difficult to determine the spatial scales at which host density should

be manipulated to control the disease and measured to explore its effect. Similarly, knowledge of the frequency of pathogen reproduction is necessary to predict the rate at which increased host density results in increased disease severity. For example, pathogens that exhibit epidemic spread with multiple generations per growing season may result in higher severity in high host densities compared to low densities within a single growing season. However, for pathogens with lower reproductive rates density-dependent transmission should determine rates of severity increase over multiple growing seasons, but the relationship between host density and disease severity in a single point in time may be weak or non-existent because severity also depends upon the age of infection.

Thus the observed relationship between host density and disease levels at a given spatial and temporal scale provides limited evidence as to the density dependence of disease transmission. In plant-pathogen interactions in natural systems, transmission has not been measured directly previously. Instead studies explore the predicted outcomes of density-dependent disease transmission (e.g. increasing host mortality or infection levels with increasing host density) (reviewed in Burdon and Chilvers 1982 and Gilbert 2002). As these studies frequently occur over small spatial and temporal scales, support for the hypothesized density-dependent transmission will only be if the pathogen disperses over short distances and reproduces rapidly relative to the spatial and temporal scales of the study. If, however, the transmission of the pathogen depends upon host densities but at larger spatial scale and/or occurs slowly, then these approaches may not find the patterns predicted by density-dependent transmission despite the density-dependence of the process.

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Chapter 4

The indirect effects of a specialist pathogen on plant community composition

Abstract.

Specialist pathogens are thought to maintain plant community diversity through density-dependent effects on their host species, thereby preventing competitive exclusion. Thus the effects of pathogens that infect competitively superior plant species are predicted to play a larger role in structuring the plant community than pathogens that infect less competitive host species. To estimate the effects of a host-specific pathogen, *Puccinia podophyllii*, on the plant community, I test the competitive effects of its host, *Podophyllum peltatum*, on resource availability, plant species growth and survival, and the diversity and productivity of the plant community. The presence of the host plant significantly reduces light availability in the forest understory. Under these low light conditions, the seedling survival of most, but not all, plant species is reduced. Estimates of growth and survival of the seedlings of other plant species in competition with the host suggest that only a subset of species can reach reproductive size/age. In areas occupied by the host, the abundance and diversity of the plant community is reduced, leading to shifts in plant community composition compared to the surrounding understory. The pathogen, then, may maintain species diversity by regulating the frequency and intensity of competition between the host and other plant species.

Introduction:

Specialist plant pests, particularly pathogens, are proposed as important drivers of plant community composition and are hypothesized to maintain plant diversity by causing density dependent population growth of their host and thus preventing competitive exclusion by providing an advantage to species when they are rare (Gillett 1962, Janzen 1970, Connell 1971). Specialist pathogens may alter plant community composition through their effects on the abundance and spatial distribution of host plants. However, specialist pathogens can also have indirect effects on plant community composition and diversity by altering the frequency of competition between host and non-host species (Bever 2003). That is, the effects of the pathogen on the plant community depend upon the specific effects of the host plant species on other plant species in the community (Alexander and Holt 1998). Interactions among plant species vary in direction, from facilitation to competition, and magnitude. Thus pathogens that infect competitively superior plant species are predicted to play a larger role in structuring the plant community than those that infect less competitive host species. Consequently, estimating the effects of pathogens on plant community composition involves testing how the host plant species affects the abundance and diversity of other plant species.

In this study, I examine the indirect effects of the specialist pathogen, *Puccinia podophyllii* (Mayapple rust) on plant diversity, by testing the effects of its host, Mayapple, a clonal understory herb, on the plant community. Mayapple is a native understory herb that forms large patches of closed canopy and could affect the regeneration of other species. In addition, it is commonly infected by a fungal pathogen

that affects its growth and survival (Chapter 2). The pathogen reduces both the frequency and intensity of interactions between the host and other plant species. The frequency of the interactions is reduced through strong distance-dependent, disease-induced reductions in seedling survival that limits recruitment of new Mayapple colonies and reductions in rates of clonal spread. The intensity of competitive interactions is reduced by the pathogen through reductions in shoot density and height and increases in rates of shoot senescence (Chapter 2). Both host and pathogen are native to forests in the Eastern United States and the pathogen is common in populations of Mayapple.

The effects of native specialist plant pathogens on plant community composition are not well understood. Indeed, the strongest evidence that plant pathogens can alter plant community composition comes from pathogens with often broad host ranges introduced into previously unexposed natural communities. The classic examples are Chestnut Blight (*Cryphonetria parasitica*), Dutch elm disease (*Ophiostoma ulmi*), Sudden Oak Death (*Phytophthora ramorum*), and *Phytophthora cinnimomi* that caused a dramatic reduction in host species' abundances and changes in the plant community (Parker and Leopold 1983, Stephenson 1986, Weste and Marks 1987, Maloney et al. 2005). Empirical tests of the effects of pathogens on plant community composition and host competitive ability have utilized communities of non-native, short-lived hosts, primarily weeds and agricultural species, where the effects of the pathogen and the community dynamics are relatively rapid (reviewed in Alexander and Holt 1998).

In communities of native long-lived plants and native pathogens, we have only indirect evidence of the role of pests in structuring plant communities. In forest systems, previous studies concerning the role of native pathogens on plant communities have

focused on the pathogen-induced reductions in the growth and survival of the host species, and rarely test the indirect effects on the other plant species, although there are notable exceptions (e.g. Petermann et al. 2008). This is despite the fact that the critical prediction of the hypotheses that specialist plant enemies act to maintain plant diversity is that the survival of other, non-host plant species is increased indirectly by the host-specific pests (see Gilbert 2002, Gilbert 2005, Freckleton and Lewis 2006 for reviews).

Furthermore, in forests, the question of the effects of pathogens on plant community composition has focused almost exclusively on tree species. This approach is based on the assumption that plants that occur only in the understory do not affect tree and shrub regeneration and diversity (Barnes et al. 1998, Beatty 2003). Yet recently, the effects of understory vegetation on tree and shrub regeneration have gained empirical support (Nilsson and Wardle 2005, Royo and Carson 2006). The effect of understory species on tree regeneration is greatest if the understory species form dense stands, termed "recalcitrant understory layers" (Royo and Carson 2006) or "low canopies" (Schnitzer et al. 2000). Like the focal species in this study, species that form low canopies are often clonal and long-lived.

While seed dispersal into these low canopies from the surrounding area allows new seedling cohorts to establish, these low canopy-forming species can affect forest dynamics by creating low light conditions and inhibiting the growth and survival of seeds and seedlings of many plant species (George and Bazzaz 1999). These effects may be particularly strong for understory species that also emerge early in the spring as the growth and survival of seedlings of most species in temperate deciduous forests depend

upon carbon fixed before canopy closure (Chazdon 1988, DePamphilis and Neufeld 1989, Seiwa 1998).

Often the understory layer acts as a selective filter, differentially reducing growth and survival among plant species. For example, understory layers that cast a dense shade can selectively reduce the survival of shade intolerant or small-seeded species more than the survival of shade tolerant or large-seeded species. Over time, the different mortality rates among species will alter the composition of the plant community. Even the composition of canopy trees can be altered by understory layers. Recruitment into the canopy often requires a gap-forming disturbance. In post-disturbance competition, taller saplings in a advanced regeneration layer may have a competitive advantage over smaller seedlings (Connell 1989). Under these low canopies, for a seedling to survive to reach the advanced regeneration layer, in the absence of disturbance, it must be able to survive long enough to grow into the higher light levels above the low canopy. For many species, areas occupied by low-canopy species are sink populations because the growth rate of the species under the low-light conditions is not sufficiently large enough to out weigh the higher mortality rates. Thus, it is exceedingly unlikely that an individual seedling will survive to the height of the low canopy and escape the low light conditions (George and Bazzaz 1999). Consequently, the regeneration dynamics and plant community composition may be significantly altered in areas where this layer exists compared to those areas without it.

In order to estimate the effect of Mayapple on the plant community and thus the indirect effect of the pathogen on the plant community, I first measure the effects of Mayapple on light availability. I then experimentally test the prediction that Mayapple

has negative effects on the growth and survival of several common plant species and use these data to project which species will be able to persist in competition with Mayapple. To test that the effects of Mayapple on the plant community are consistent across sites and to determine how Mayapple shoot density affects the magnitude of competition, I conducted field surveys to compare the plant community diversity and abundance in the surrounding understory to areas where Mayapple was present.

Methods:

Study Sites: The study was conducted three forested sites in Washtenaw County in southeast Michigan, Raddrick forest, the Newcomb tract, and Pinckney State Recreation area. These forests were typical second growth oak-hickory-maple forests, but differed in disturbance histories, soil types and plant community compositions. The seedling survival experiment, described below, was conducted at the Newcomb tract site.

Study System: Mayapple (*Podophyllum peltatum*, Berberidaceae) is the only species of the genus *Podophyllum* (s.s.). Mayapple is widely distributed over the eastern half of the United States wherever deciduous forests occur. Mayapple is one of the first plants to emerge in the spring. Shoots emerge in early to mid-April and can persist until August or September. Mayapple colonies consist of 1 to ten's of thousands of stems that can cover up to nearly a hectare. A single colony may be composed of typically one but occasionally more genets (Parker 1988). The annual above ground shoots are easily recognized by the lobed, peltate leaves and are dimorphic. Vegetative shoots consist of a petiole and single leaf and are morphologically leaves. The flowering shoots are true shoots and typically have two leaves that are each similar size to the leaves found on vegetative shoots of the same height.

However, herbivore damage on Mayapple is rare. A few species of lepidopteran larvae (*Chorisincura rosaceana, Clepsis melaleucana* (Faeth 1978), *Papaipema cerina* (Geber et al. 1997)) are able to feed and develop on the plant but they are either absent or uncommon in the populations studied. The toxicity of Mayapple tissues appears to limit the diversity and abundance of herbivores with a lignan, podophyllin-podophyllotoxin, that interferes with cell division and is produced through out its tissues (Moraes et al. 2000).

Mayapple is, however, commonly infected by the non-systemic, host-specific pathogen, *Puccinia podophylii* Schwein., throughout its range. Infection occurs during two discrete generations of the pathogen in each growing season. Primary infection occurs via contact with spore-contaminated soil during shoot emergence in the early spring (Whetzel et al 1925). Orange lesions (aecia) develop within a few weeks producing aecidiospores. The aecidiospores are then aerially dispersed over short distances. The aecidiospores that germinate on host tissue produce telia on the underside of the leaf and cause a yellow spot on the leaf at the point of infection. The telia are visible by early June and produce the overwintering teliospores that return to the soil. 80 to 90 percent of the shoots in colonies with average rates of aecial infection are infected by the telia which can occupy up to 100% of the leaf area.

Genetic resistance to infection by this pathogen is not known to occur in Mayapple (Parker 1989). Instead, the primary host defense appears to be physical. The pre-emergent shoots are protected by a whorl of bud scales that protect the shoot from contact with the rust spores in the soil. These bud scales are frequently infected by the

pathogen. However, the lesions that develop on the bud scales produce only teliospores that are incapable of re-infecting the shoots in the current growing season.

Light availability:

To quantify the degree to which Maypple competes for light, I used two measures; light availability and canopy density. To measure the light availability, I measured the flux of photosynthetically active radiation using a Licor photon flux meter. To determine the effect of Mayapple abundance on light availability on the forest floor, I calculated the light availability, relative to full sun, across plots that varied in Mayapple shoot density. I sampled light availability in 42 plots across two colonies and the surrounding understory within one site. The plots were 0.25 square meters in size. In each plot, I counted the number of Mayapple shoots and took a light reading at the center of the plot 5 centimeters above ground level. The data were analyzed using a linear regression of shoot density and the log-transformed light availability.

To compare how the canopy density in the understory differs in areas occupied by Mayapple verses the surrounding understory, I measured the leaf area index (leaf area per meter^2 per meter^2 of ground surface) of each area using a Licor LAI-2000 Plant Canopy Analyzer. I measured canopy density at 41 colonies at two sites. At each Mayapple colony, I sampled the leaf area index within the colony every meter along two, perpendicular transects running through the center of the colony. On the forest floor surrounding the colony, I took an equal number of samples spaced evenly along a circular transect located two meters outside the colony edge. All readings were taken at 5 to 10 cm above ground level. To test if Mayapple colonies form a denser canopy than the surrounding area, I compared the average leaf area index readings on the exterior and

interior of each colony using a paired sampled t-test. All light and canopy measurements were taken in mid-June, after tree canopy closure.

Seedling growth and survival:

I experimentally tested whether Mayapple has negative effects on seedling survival and if these effects differ among plant species. Seedlings of fifteen plant species were used and constitute a mixture of native and non-native species common to forests in Southeast Michigan. The shrub species used were the non-native species, *Ligustrum vulgare, Lonicera maackii, Rhamnus cathartica,* and the native species, *Cornus amomum, Lindera benzoin,* and *Sambucus racemosa*. The tree species used were *Carya ovata, Carya glabra, Betula alleghaniensis, Asimina triloba, Acer saccharum, Cercis canadensis,* and *Ulmus americana*. Virginia creeper (a woody vine), *Parthenocissus quinquefolia,* and St. Johnswort (an herbaceous, non-native perennial), *Hypericum perforatum,* were also used in the experiment.

To maximize the number of seedlings available for the experiment, the seeds were germinated in a greenhouse. Once the seedlings had developed leaves, the individual seedlings were transplanted into four by four by fifteen centimeter tall paper containers filled with Faffard potting soil mix. To minimize transplant mortality, after being planted into the paper pots, the seedling were left in the greenhouse for two weeks and then planted into the treatment plots in late-June.

For each species, half of the seedlings were planted into a treatment plot that contained established Mayapple plants and the other half into a treatment plot naturally lacking Mayapple. The treatment plots were located next to one another in an area of the forest with similar slope, aspect, soil texture, and canopy cover. In each treatment, the

seedlings were planted every 0.5 meter along parallel transects spaced 1 meter apart. The species identities of the seedlings were randomly assigned at each location along the transects. The following numbers of seedlings were planted in each treatment for each species: 60 of *Parthenocissus*; 50 of *Betula*, *Rhamnus*, *Carya ovata*, *Lonicera*, and *Ulmus*; 40 of *Lindera* and *Acer*; 30 of *Sambucus* and *Ligustrum*; 25 of *Asimina* and *Hypericum*; 15 of *Carya glabra*; and 10 of *Cercis*. To control for mortality associated with transplant stress, I removed the seedlings that died in the month following transplantation from the analysis, leaving a total of 938 seedlings. Mortality during this period was highest for the hickory seedlings and the two hickory (*Carya*) species had to be combined for the analysis. At the end of the following summer, I measured the survival and height of each seedling.

To test the hypothesis that average survival across species is lower in the presence of Mayapple I compared the numbers of surviving and dead seedlings of all species combined within each treatment using a χ^2 test. To test if the effect of Mayapple on seedling survival differed among species, I analyzed the survival of each species in each treatment using a χ^2 test for heterogeneity of effects.

The persistence of a species in competition with Mayapple depends upon its ability to survive and grow past the Mayapple canopy. Using a simple model, I combined the growth and survival data for each species in the Mayapple treatment to examine the species-specific responses to Mayapple in their recruitment through the Mayapple canopy. In the model, I assumed that the survival and growth rates in each treatment are constant over time. I estimated the annual growth rates of each species in each treatment by dividing the average final height of the surviving seedlings of each

species by their age (two years). The estimate of growth rate assumes a linear growth rate and is an overestimate since the seedlings were grown in the greenhouse prior to transplantation. For each species, the time required to reach the canopy is equal to the average canopy height divided by its estimated annual growth rate. I calculated the proportion of seedlings of each species surviving to canopy height as $e^{(\ln(\text{survival rate})*(\text{canopy height/growth rate})}$.

Plant community composition:

The effect of Mayapple on the plant community depends on whether the growth and survival of other species are affected negatively and if this effect varies among species. I investigated the effect of Mayapple on plant community composition by comparing the abundance, diversity, and composition of the plant communities inside and outside Mayapple colonies using paired samples. At each of the three sites, I measured plant community composition in a 0.25 m^2 quadrat. In each quadrat, all plant species were identified and their percent cover was recorded. For tree species, the densities of seedlings of each species were also recorded. I defined seedlings as all stems less than 0.5 meters tall. This definition includes individuals both above and below the Mayapple canopy. At each Mayapple colony, the plant community on the interior of the colony was sampled at four points (at the north, east, south, and west) on the edge of the colony. This gives a conservative measure of the changes in the plant community associated with the presence of Mayapple since the colony edge advances outward at rates of approximately five centimeters per year thus presumably each colony has expanded into this area of the forest floor over the past five to ten years (Chapter 2). The plant community in the area surrounding each colony was sampled three meters to the exterior

of the colony from each interior sample. Thus the sampled area at each colony consists of one square meter (4 quadrats) inside the colony and one square meter outside of it. I sampled 48 colonies in the Raddrick forest site, 26 colonies at the Newcomb site, and 23 colonies at the Pinckney site. For woody plant species, I conducted an additional survey to increase sample sizes using the same methods. In this survey, I sampled 65, 38, and 36 additional colonies in the Raddrick forest, Newcomb, and Pinckney sites respectively. To compare the diversity of the plant communities on the interior and exterior of Mayapple colonies, I calculated the total species richness and Shannon diversity and evenness indices for each community at each site.

To estimate the effect of Mayapple on the abundance of other species and species richness for all species and for woody and herbaceous species separately, I compared the cover and species densities (numbers of species per sample) at exterior and interior of each colony. I analyzed the data using a paired-samples t-test.

The magnitude of Mayapple competition and its effects on the plant community are likely to depend upon the density of Mayapple shoots and the level of shading caused by the forest canopy. To determine if the effect of Mayapple on the abundance of other species varies by site and increases with increasing Mayapple shoot density, I calculated the effect of the Mayapple colony as the difference in percent cover of other plant species between the interior and exterior samples. The effect size was calculated for all species and for woody and herbaceous species separately. Average shoot densities for each colony were estimated from quadrat samples. I analyzed the data using an ANOVA and included site as a factor, shoot density as a covariate, and their interaction.

To test whether the composition of the plant communities is altered by Mayapple, I compared the relative abundances in separate functional classes of plant species in each site. To isolate the effects on the understory, subcanopy, and canopy species, I divided the species into functional groups of canopy trees, subcanopy trees, woody understory plants, and herbs based on their growth forms. Subcanopy trees were defined as a species with an average adult height greater than two meters and less than ten meters. Woody understory plants were defined as species smaller than subcanopy trees. For canopy trees and subcanopy trees, I measured species abundances using the total number of stems recorded in the samples in each site. I limited the analysis to common species with more than ten individuals in the samples. The abundances of rare species were summed and included as a separate group. To estimate the abundances in the other functional groups, I used the summed percent cover of each species. This measure was used since many of the species exhibit colonial growth making counts of individuals impractical. I compared the composition of the plant community inside Mayapple colonies to the composition of the surrounding understory for each functional group at each site using χ^2 tests for independence**.**

Results:

Light availability is significantly negatively correlated with Mayapple shoot densities (r^2=0.44, t=-5.7, p<0.001). Light levels were reduced from an average of 2 to 3 percent of full sun on the forest floor outside the Mayapple canopy to less than 0.2 percent of full sun at Mayapple shoot densities of 60 shoots per square meter (Figure 4.1). The measurements of leaf area indices also demonstrate the ability of Mayapple to form a dense canopy distinct from the surround forest floor. Under the Mayapple the

L.A.I. was significantly higher than canopy densities in the surrounding understory (mean 7.18 versus mean 5.43 in surrounding understory, $t=17.8$ $p<0.0001$).

The low light availability in Mayapple colonies is associated with significant reductions in seedling survival of some but not all plant species. Averaged across species seedling survival dropped from 70 percent in the control plot to 39 percent in the presence of Mayapple ($\chi^2=90.7$, $df=1$, $p<0.001$). However, the effect of Mayapple on

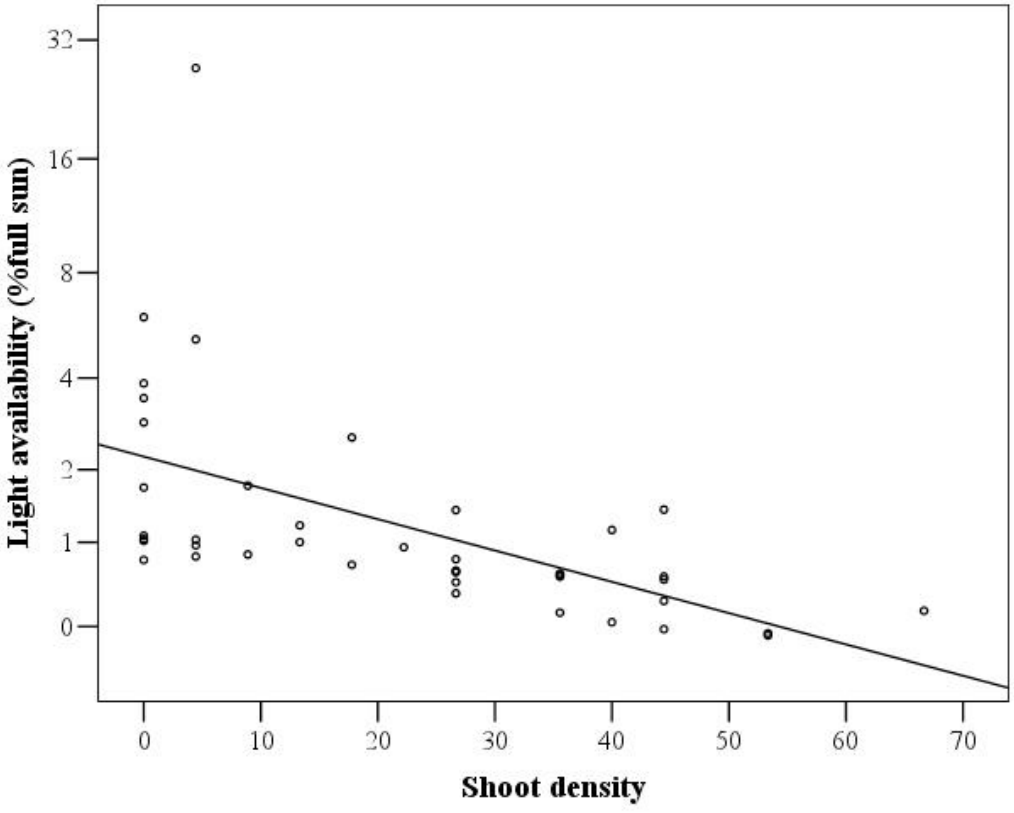

Figure 4.1: Reduction in light availability on the forest floor associated with increases in Mayapple shoot density. Each circle represents data from a single 0.25 m^2 plot. Light availability is measured as the amount of photosynthetically active radiation present relative to full sun and is presented on a log scale. Shoot density is the number of shoots per $meter^2$. % full sun = exp(0.75-0.034 * shoot density), $r^2=0.44$, $p<0.001$.

seedling survival differed significantly among plant (χ^2=46.3, df=15, p<0.005, Figure 4.2). The lowest rates of seedling survival in the Mayapple plot occurred for *Hypericum perforatum* and *Sambucus racemosa*, each which had just a single seedling survive the season in the Mayapple treatment (survival rates=4.2% and 3.1% respectively) compared to survival rates of 66.7% and 40.6%, respectively, in the control plot. In contrast, the survivorships of Hickory (*Carya spp*.), Pawpaw (*Asimina trioloba*), Sugar Maple (*Acer*

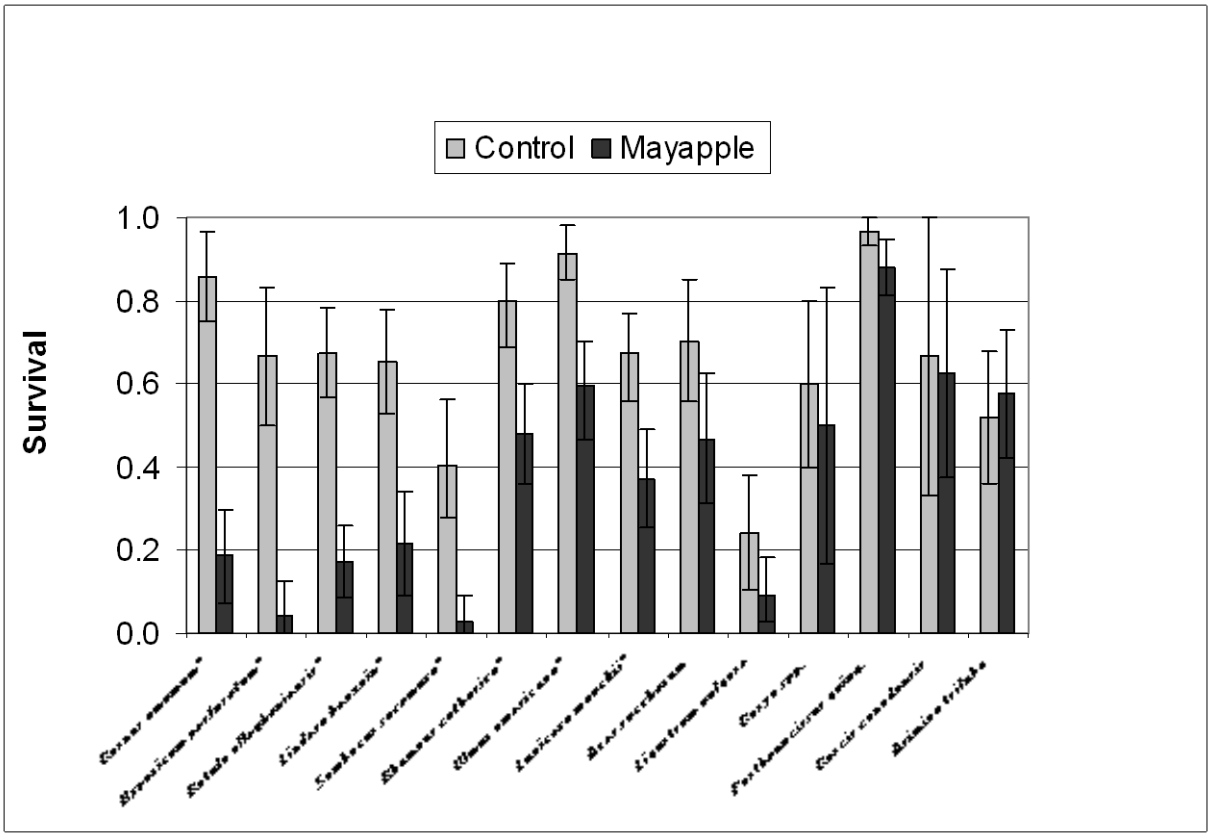

Figure 4.2: Proportions of seedlings surviving for each plant species in Mayapple and Mayapple-free plots. Significant differences between treatments (p<0.05) are noted with an asterisk after the species name. Error bars are equal to the 95% confidence intervals of annual survival rates of seedlings of each species. Species are ordered from left to right by the magnitude of the difference in seeding survival between control and Mayapple treatments.

saccharum), European Privet (*Ligustrum vulgare*) and Virginia Creeper (*Parthenocissus quinquefolia*) were not significantly lower in competition with Mayapple than in the control plot (Figure 4.2).

Seedling cohorts are constantly replenished by newly germinated seedlings under Mayapple canopies, so seedlings of all species are expected to be found with Mayapple. However, based on the growth and survival model that assumes mortality rates and seedling growth rates are constant over time, only three of the fifteen species, *Parthenocissus, Ulmus,* and *Cercis,* would survive to reach the height of the Mayapple canopy at rates greater than one percent of the initial seedling cohort, (Table 4.1). *Ulmus* seedlings would surpass the canopy in 5.6 years at which time 4.6% of the original cohort would survive. 6.5 % of the original cohort of *Cercis* seedlings would survive and it

Table 4.1 Percent survival to Mayapple canopy height of seedlings cohorts of 15 plant species in Mayapple and Mayapple free areas. The proportion of seedlings of each species surviving to canopy height = $e^{(\ln(\text{survival rate})*(\text{canopy height}/\text{growth rate})}$ where growth rate= average final height/2. Time to reach canopy height (41 cm) in years for each species in each treatment is noted in parentheses.

species	Mayapple	No Mayapple
Parthenocissus quinquefolia	11.95 (17)	85.98 (4)
Cercis canadensis	6.13 (6)	4.54 (8)
Ulmus americana	4.61 (6)	56.80 (6)
Carya spp.	0.45 (8)	0.45 (11)
Asimina triloba	0.42 (10)	0.29 (9)
Acer saccharum	0.27 (8)	3.20 (10)
Lonicera maackii	0.27 (6)	14.60 (5)
Rhamnus catharica	0.07 (10)	14.33 (9)
Cornus amomum	0.01 (6)	49.40 (5)
Lindera benzoin	0.00 (9)	2.56 (9)
Betula alleghaniensis	0.00 (10)	4.31 (8)
Hypericum perforatum	0.00 (6)	5.21 (7)
Ligustrum vulgare	0.00 (14)	0.00 (12)
Sambucus racemosa	0.00 (16)	0.00 (12)

would take 5.9 years for the seedlings to reach the canopy. *Parthenocissus* seedlings can exceed the canopy height (mean=41cm) in 16.5 years at which time 12% of the original seedling cohort would remain. While this species is a liana, increased stem length would increase its ability to forage for light, so I have used the same height threshold to standardize the comparison among species. The lower growth rates and/or higher mortality rates of the remaining species result in less than 1% survival of the original seedling cohort to the canopy height of Mayapple. In contrast, the richness of the seedling community reaching the same height in the control plot would be much higher. Ten of the fourteen species have greater than 1% of the original seedling cohort survive to reach 41 cm in height (Table 4.1).

Across the three sites, total species richness in Mayapple colonies was 83% of the number of species found outside the Mayapple colonies (Table 4.2). However, surprisingly, this did not led to a decrease in the Shannon diversity index, likely due to increases in evenness (Table 4.2). On average Mayapple reduced the percent cover of other plant species from 32% to 17% ($t=-9.0$, $df=96$, $p<0.001$, paired-samples t-test), reflecting decreases in the percent cover of both herbaceous and woody species ($t_{herbs}=-7.1$, $t_{woodyspp.}=-7.3$, $p<0.001$). The species densities were significantly lower in areas occupied by Mayapple compared to the surrounding understory (mean difference=-1.65, $t=-5.3$, $df=96$, $p<0.001$).

Table 4.2. The diversity of the understory plant community in areas occupied by Mayapple and in the surrounding understory.

Site	Mayapple Present/Absent	Plant species richness	Shannon diversity (H')	Shannon evenness (J)
Pinckney	Absent	51	2.5	0.64
	Present	41	2.4	0.66
Newcomb	Absent	70	3.1	0.72
	Present	59	3.0	0.73
Raddrick	Absent	52	2.7	0.68
	Present	44	2.8	0.74

While Mayapple reduced the abundance of other plant species, as measured by the paired differences between percent cover in plots inside and outside Mayapple colonies, the effect was greatest in sites with abundant ground cover in the surrounding understory (Site: $F_{2,91}=5.5$, $p<0.005$, Figure 4.3). In all sites, cover was reduced by approximately 50% but one site (Pinckney) had an average of 61% ground cover outside Mayapple colonies compared to averages of 30 and 20 percent cover in the other sites. Significant site differences in the effect of Mayapple on plant cover are driven by the differences among sites in the cover of woody plant species in the surrounding understory and not by site to site differences in herbaceous cover (Site $_{\text{woody species}}$, $F_{2,91}=4.7$, $p<0.05$; Site $_{\text{herbaceous species}}$ $F_{2,91}=0.99$, $p>0.3$).

Furthermore, the reduction in abundance of other plant species increased in colonies with higher average Mayapple shoot densities (Shoot density: $F_{1,91}=10.8$, $p<0.001$, Figure 4.3). Increasing Mayapple shoot density reduces the abundance of both woody and herbaceous species (Shoot density $_{\text{woody species}}$, $F_{1,91}=5.7$, $p<0.05$; Shoot density $_{\text{herbaceous species}}$, $F_{1,91}=5.2$, $p<0.05$). The effects of increasing shoot densities on plant abundance varied significantly among sites with significant relationships between average shoot density and the reduction in the abundance of other plant species in two of the three sites (Site*shoot density: $F_{2,91}=4.3$, $p<0.05$, Figure 4.3).

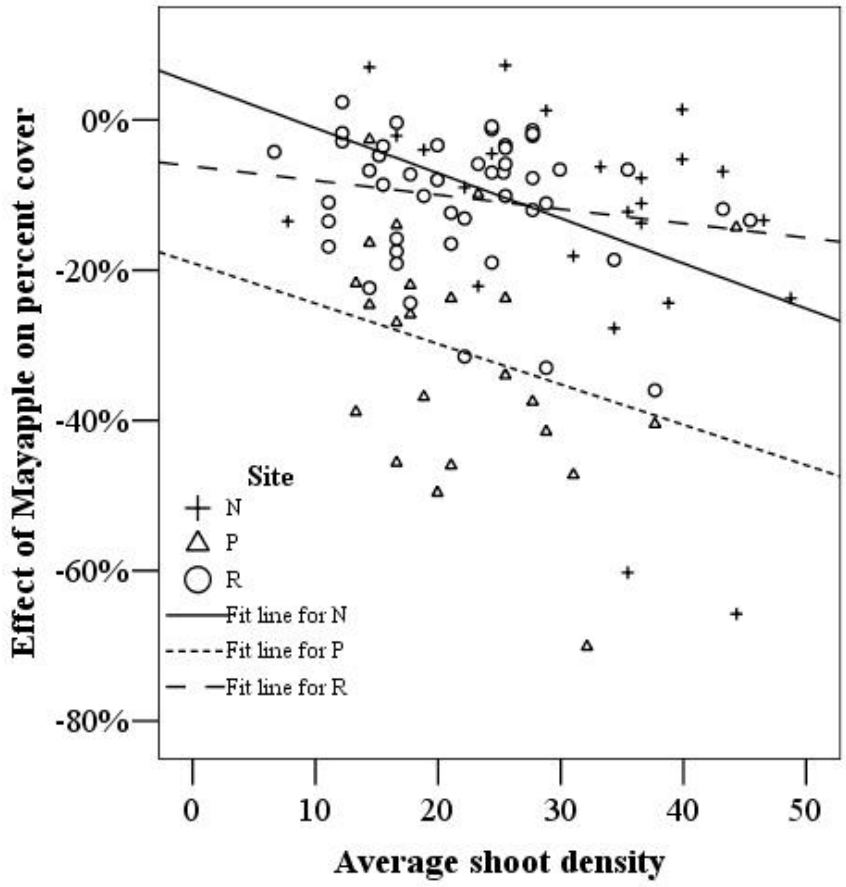

Figure 4.3: Effects of site and shoot density of Mayapple colonies on the abundance of other plant species. Symbols indicated data for each Mayapple colony in each site (N=Newcomb tract, P=Pinckney, R=Raddrick Forest). Effect of Mayapple on percent cover, the y-axis, is difference in % cover of other plant species inside a colony compared to samples taken three meters outside the colony. Shoot density is the average shoot per meter^2 for each colony. Relationship between shoot density and the reduction in % cover are significant ($p<0.05$) in the Newcomb ($r^2=0.15$) and Pinckney sites ($r^2=0.08$), but not significant in the Raddrick site.

The plant community composition under Mayapple was significantly different from the surrounding understory for all plant functional groups with the exception of seedlings of canopy trees and shrubs in one site. The frequencies of seedlings of canopy tree species in Mayapple colonies were significantly different from the surrounding forest in two of the three sites ($\chi^2_{pinckney}=14.6$, df=6, $p<0.05$; $\chi^2_{newcomb}=14.3$, df=7, $p<0.05$). The community composition of subcanopy tree seedlings was significantly altered by

Mayapple in all sites ($\chi^2_{pinckney}$=9.5, df=3, p<0.05; $\chi^2_{newcomb}$=11.5, df=6, p<0.05; $\chi^2_{raddrick}$=21.9, df=7, p<0.001). The frequencies of species within the shrub community were also significantly affected by Mayapple in the two sites where shrubs are abundant (**$\chi^2_{pinckney}$**=32.3, df=7, p<0.001; $\chi^2_{newcomb}$=27.9, df=5, p<0.001). In the herbaceous community, the relative abundances of perennial species that are able to grow to heights taller than the Mayapple canopy increased and the relative abundances of shorter-lived species, such as *Alliaria petiolata,* decreased in all sites ($\chi^2_{pinckney}$=21.1, df=10, p<0.05; $\chi^2_{newcomb}$=21.8, df=10, p<0.05; $\chi^2_{raddrick}$=25.8 df=10. p<0.01).

Discussion.

These results demonstrate that Mayapple is a strong competitor for light and reduces the abundance and diversity of the plant community. Under Mayapple canopies, the survival of seedlings of most plant species is reduced as demonstrated by the results of the seedling survival experiment. The growth and survival estimates for seedlings in competition with Mayapple suggest that only a small subset of species can persist with Mayapple. Differential seedling mortality among plant species under Mayapple is predicted to alter plant community composition in the seedling layer only if the effects of Mayapple are strong. If they are weak, the inputs of seeds from the surrounding forest and subsequent germination of seedlings each year will homogenize plant communities. Significant differences, compared to samples of the surrounding understory, in the abundances and frequencies of the seedlings of canopy, subcanopy, shrub, and herbaceous were observed in Mayapple colonies suggesting that the competitive effects of Mayapple are strong and general.

The effects of Mayapple on the plant community are likely driven by competition for light. Mayapple colonies are a distinct microhabitat with little light penetrating to the forest floor. Light levels below 1 percent of full sun frequently occur in Mayapple colonies, a point below the light compensation point for most forest trees (Barnes et al. 1998). The leaf area index measure within these colonies is not only distinctly higher than the surrounding understory but it is equivalent to levels found in dense pine plantations (Gower and Norman 1991). Temperate forests tend to be light limited (Finzi and Canham 2000, Ricard et al. 2003) and the growth and survival of seedlings of tree species in Eastern temperate forests is largely a function of light availability (Pacala et al. 1994, Pacala et al. 1996). Furthermore, the experiment that measured the effects of Mayapple on seedling survival was designed to measure the effects of above-ground interactions, including light competition, since the seedlings were all planted into the same potting soil.

The phenology of Mayapple likely contributes to its strong competitive effects. Mayapple shoots emerge and expand in mid-April, a month or more before the overstory canopy closure. In temperate deciduous forests, leaf expansion before canopy closure (early phenological avoidance) is a common trait of plant species in the understory. Over ninety percent of tree and shrub species studies in forests similar to those in this study exhibited early phenological avoidance as seedlings and saplings in the understory (Augsburger and Bartlett 2003, Augsburger et al. 2005). The growth and persistence of many plant species in the understory depends largely on carbon fixed during this period before canopy closure (Chazdon 1988, DePamphilis and Neufeld 1989, Seiwa 1998, Augsburger et al. 2005).

By reducing resource availability, Mayapple appears to have far-reaching effects on the plant community. In the seedling survival experiment, averaged across species seedling mortality rates doubled in Mayapple compared to the control from 30 to 61 percent but seedling survival of some species did not differ between the treatments suggesting that Mayapple reduces seedling density and alters community composition in the understory. While this experiment had limited replication, the results of the field surveys are consistent with the experimental results and sampled the effects of Mayapple in over 100 colonies across three sites suggesting that Mayapple's negative effects on the persistence of other plant species is widespread. The field survey found that seedling densities and the percent cover of other plant species were reduced by nearly 50% across sites that varied in species composition, succession stage, and edaphic factors. The field survey also demonstrates that there are significant shifts in the composition of the understory plant community under Mayapple colones compared to the surrounding understory. This result is consistent with the species-specific effects of Mayapple competition on seedling mortality observed in the experiment.

Mayapple may also affect the plant community through other mechanisms not tested in this study. The activity of small mammals and other herbivores may be altered in the presence of a dense understory, thereby increasing (Schreiner et al. 2000) or decreasing (George and Pacala 1999) rates of seed and seedling herbivory. Dense understory canopies can also reduce light quality (e.g. red:far red wavelengths) which can result in inhibition of germination and development (Messier et al. 1989, Horsley 1993, Mancinelli 1994) and intercept sun flecks which are critical for the growth and survival of some plants in the understory (Chazdon 1988, Lei et al. 2002).

The reduced diversity of species predicted in the seedling growth and survival model, presented here, suggests that only a subset of species in the plant community possesses the necessary traits to survive long enough to either reproduce in the low light condition or to grow through the low canopy in competition with established Mayapple colonies. This result also suggests that the changes in the understory plant community associated with Mayapple observed in the field survey are likely an underestimate of the effects this species has on the plant community because most seedlings found under Mayapple canopies will die before reaching a reproductive size or age.

The decreased richness of species able to reproduce within Maypple colonies is likely due to physiological trait-offs in plant traits that determine persistence in low-light environments. For tree species, ability to recruit through the low canopy of Mayapple is likely determined by the species-specific traits of seed crop size and resources available to seeds and seedlings. The numbers of seedlings of each species able to grow through the Mayapple canopy is the product of seed inputs rates and probability of surviving to reach the canopy. Consequently, the tree species possessing a combination of large seed crops, shade tolerance, and sufficient growth rates are predicted to dominate the advanced regeneration layer of seedlings above Mayapple colonies. Tree species able to reproduce colonially from root sprouts, such as *Fagus sylvatica* and *Sassafras albidum*, would also able to grow through the canopy. Indeed, the change in community composition of tree species in Mayapple colonies was driven by increases in the relative abundances of species that possess traits, such as vegetative reproduction, large seeds, large seed crops, or high shade tolerance, or combinations of traits that allow them to persist for longer time periods under the canopy of Mayapple. For example, the relative abundances of

Sassafras albidum seedlings were consistently higher in Mayapple colonies at sites where this tree species was sampled. Most of the "seedlings" of this species were really root sprouts that draw on the resources on the parent tree to grow through the low canopy. However, the trade-offs between the ability to persist in low light and maximum growth rates (Pacala et al. 1994) and between seed size and seed production decrease the likelihood that any one species exhibits the traits required recruit through the low canopy and reduces the number of species in the advanced regeneration layer.

The reduction in richness in the advanced regeneration layer community above Mayapple is also likely to carryover into the canopy. Due to decreased recruitment rates, the densities of saplings above the Mayapple canopy would be lower than in areas without Mayapple, allowing increased seedling growth and higher competitive ability, due to increased height, when a canopy disturbance occurs (Barden 1979, Canham 1988, Connell 1989).

Mayapple colonies may be a naturally occurring understory filter. In contrast, the formation of other "recalcitrant understory layers" has occurred over the past century and is suggested to be the outcome of anthropogenic deviations from the natural levels of overstory disturbance, fire frequency, and herbivory (Royo and Carson 2006). While Mayapple can persist in heavily disturbed forests, it is abundant in undisturbed forests as well. While the effects of altered fire regimes on this plant have not been studied, understory fires occurring the early spring and late fall do not produce any noticeable changes in Mayapple colonies (personal observation). On the other hand, Mayapple abundance may have increased with recent increases in densities of white-tailed deer, since it is unpalatable to grazers.

In light of Mayapple's strong negative effects of the diversity of the plant community, the specialist-pathogen, *Puccinia podophyllii*, maintains plant species diversity because my earlier experiments demonstrate that this disease is a major mechanism regulating the growth of Mayapple populations and is the only common antagonist of the Mayapple. Due to the strong competitive effects of Mayapple on the plant community, *Puccinia podophyllii* indirectly increases species diversity by reducing the competitive ability and by affecting the spatial distribution of its host and thus reduces both the frequency and intensity of competition between Mayapple and other plant species.

The pathogen affects the frequency of host to non-host competition through its effects on the spatial distribution of the host via distance-dependent seedling survival and reductions in vegetative growth rates which slow colony expansion (see chapter 2). The pathogen appears to be the primary factor driving the spatial distribution of the host. Distance-dependent seedling survival caused by the pathogen suggests that recruitment of new colonies only occurs at distances greater than ten meters away from infected colonies. By regulating the spatial distribution of its host, the pathogen affects the plant community in two possible ways. If Mayapple acts as a selective filter then the pathogen would act to maintain levels of habitat heterogeneity and by doing so facilitate plant species coexistence (Hibbs 1982, Pacala 1986, Silvertown and Law 1987, Pacala et al. 1993). Alternatively, for species that fare poorly in competition with the host, the pathogen may prevent competitive exclusion.

Within an infected host colony, the pathogen reduces the intensity of light competition causing decreases in Mayapple shoot density and canopy height and

increasing the rate of shoot senescence (Chapter 2). Severity of infection is the primary factor driving rates of shoot senescence in Mayapple colonies (Chapter 2). Mayapple shoots in severely disease colonies senesce completely by early-July, a month earlier than in uninfected colonies (unpublished data). By accelerating shoot senescence, infection increases light availability for other plant species within Mayapple colonies.

Infection also increases resource availability for other plant species by reducing Mayapple shoot density within colonies. Experimentally excluding the disease for three years led to a 55% increase in shoot density (shoots m^{-2}), in contrast to the density in the diseased-control plots which was unchanged. The density of shoots is strongly correlated with the reduction in light availability and the abundances of other plant species in Mayapple colonies, thus disease would have an indirect positive effect on the growth and survival of other plant species by reducing Mayapple shoot density.

The pathogen can also reduce the intensity of competition between Mayapple and other plant species by reducing the height of the Mayapple canopy. Infection by either generation (aecial or telial) of the pathogen also significantly reduces vegetative growth rates which results in decreases in shoot height the following growing season (Chapter 2). At the colony level, infection reduces the height of the Mayapple canopy over time. Among colonies, annual rates of change in Mayapple canopy height are significantly negatively correlated with canopy height and disease severity in the previous year (Chapter2). Parameter estimates from this model suggest that canopy height will increase in uninfected colonies to approximately 40 centimeters, a value similar to the canopy height in the seedling survival experiment, but if the colony is infected equilibrium canopy height is reduced with increasing levels of disease severity in the colony. At high

severity levels (colony averages of ~ 150 to 400 telial infections per leaf) the equilibrium canopy height is less 30 centimeters. Changes in canopy height may cause species-specific shifts in proportions of seedlings surviving to reach the canopy because the form of negative exponential relationship between canopy height and probability of seedling survival to that height depends on both seedling survival and growth rates of a species in competition with Mayapple. Thus the composition and diversity of the community of species able to survive and reproduce in competition with Mayapple may be indirectly determined by the level of infection in the host colony.

This study is the first study to link the effects of the pathogen on host demography and competitive ability (chapter 2) and the competitive effects of the host on other plant species. As such, the results provide the most complete and compelling evidence to date of the diversity-enhancing role of specialist plant enemies in plant communities. Previous studies of the role of plant enemies in the maintenance of diversity have focused on effects of plant enemies on the growth and survival of seeds and seedlings and rarely test how these effects impact the plant community. Clearly, a more comprehensive approach, like I've taken here, is required to better elucidate the role plant herbivores and pathogens play in the maintenance of species diversity.

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