ABSTRACT

CANNER, JUDITH ELENA. The Population Ecology of Ant-dispersed Plants in Space and Time. (Under the direction of Drs. Kevin Gross and Robert R Dunn.)

Myrmecochory, or seed dispersal by ants, is both common in nature and well-studied empirically. Throughout eastern North American temperate deciduous forests, the ants disperse up to 70% of understory plant species. In general, a single ant species complex, *Aphaenogaster rudis,* disperses a majority of seeds. Two of the factors that determine the benefits of ants to myrmecochores (ant-dispersed plants) are the temporal coupling of ant and plant phenology and the influence of ant dispersal on the spatial population dynamics of the plants. The coupling of the fruiting season and the peak foraging period of ants suggests that ants will disperse a majority of the seeds away from the parent plant. The distance ants disperse seeds away from the parent affects the population spread rate of the plant and the spatial dynamics of the plant population in general, including density-dependent effects. I examine the effects of ant dispersal and ant abundance on the population dynamics of ant-dispersed plants in temperate deciduous forests.

First, I focus on the behavior of ants towards seeds after initial dispersal of seeds into the ant nest. I developed a new tracking technique for small seeds in order to follow the fate of seeds once dispersed into *A. rudis* nests. The technique allowed me to recover 63% of the seeds after dispersal into the nest and provided a better alternative to comparable tracking techniques. I found that *A. rudis* redispersed >90% of seeds an average distance of 51.5 cm.

When I incorporated redispersal into a model of plant population spatial dynamics, I found that redispersal increases the rate of population spread by 22.5%. In addition, the redispersal of seeds by *A. rudis* increases the dispersal distance away from the parent plant by 24%. Therefore, redispersal has a significant effect on the spatial population dynamics of myrmecochores.

Second, I developed a model that allowed me to answer two questions about the effect of climate change on myrmecochory: will myrmecochory decouple with warming and how do researchers use experiments to predict population dynamics under future climate change. I focused on three climate-sensitive aspects of phenology that may affect the persistence of myrmecochory and consequently, the population dynamics of myrmecochores: the timing of the fruiting season of myrmecochores, the length of the ant foraging season, and the density of the keystone disperser, *A. rudis*. I found that the density of *A. rudis* has the greatest effect on the persistence of local myrmecochores. In contrast, the lengthening of the ant foraging season counteracts the potential decoupling of the fruiting season with the peak of the ant foraging season. In addition, I show the potential for myrmecochore populations to persist at low levels of ant dispersal through increased vegetative reproduction. I then conducted a simulation study to compare the predictions of warming experiments to the population dynamics of myrmecochores under gradual warming over the next century. My study shows the disparity between short-term press warming experiments and gradual warming, as the predictions of press experiments do not track the myrmecochore population dynamics under

gradual warming. Therefore, researchers must consider press warming experiments in association with observational studies and climate gradient studies in order to create useful models and to predict the persistence of species interactions under climate change.

The Population Ecology of Ant-dispersed Plants in Space and Time

by
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APPROVED BY:

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DEDICATION

To my family and friends.

BIOGRAPHY

I grew up with a large family (three brothers and three sisters) in Pine Grove Mills, PA, the fifth child of James and Alicia Canner. I spent my youth roaming around in the woods, singing, and playing field hockey. Though I always enjoyed math, it was not until my junior year of high school that I fell in love with math. My love of math, and the encouragement of my little sister, lead me to major in mathematics education at Shippensburg University. I became very involved in both research in education and curriculum development and research in mathematics and I soon dreamt of pursuing higher education. At the encouragement of my professors at Ship, I decided not to delay that pursuit and to enter directly into graduate school after graduation. Through a series of Google searches, I stumbled upon the Biomathematics Graduate Program at North Carolina State University. Though at first I thought I would pursue biomedical research, a summer internship at Shaver's Creek Environmental Center sealed my fate as a mathematical ecologist as I discovered that I loved the outdoors too much to be confined to a computer or a lab.

Once at NCSU, I began to discuss my research interests with Dr. Kevin Gross and Dr. Nick Haddad, who offered me the opportunity to get my feet wet (literally) surveying populations of a rare butterfly on a military base. In conjunction with the fieldwork, I also conducted a small model analysis project for a count-based model of insect population indexes. Thus began my pursuit of a research program that combined both empirical and theoretical study. After several courses with Dr. Rob Dunn, I became keenly interested in the world of ants and

mutualisms. The study of myrmecochory allowed me to pursue both empirical and theoretical venues of research, the perfect study system for an aspiring mathematical ecologist. In addition to scholarly pursuits, I also began to pursue opportunities to develop as a teacher and I applied for and was subsequently accepted into the Preparing the Professoriate Program at NCSU, under the mentorship of Dr. Pam Arroway. Each new endeavor shaped me as an educator and scholar and prepared me to accept a new role as an Assistant Professor in the Department of Mathematics and Statistics at California State University, Monterey Bay. I hope to continue my research as a mathematical ecologist, but also to pursue again research in curriculum development and to eventually create math and statistics courses that broadly appeal to the biological sciences.

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"Go to the ant, you sluggard! Consider her ways and be wise…"

Proverbs 6:6

CHAPTER 1

Cryptic seed movement by a keystone ant augments dispersal of common wildflowers[1]

1.1 Introduction

Much of ecology seeks to understand interactions among species and their consequences. Evidence continues to grow that facilitation, and more specifically mutualism, increases biological diversity and shapes the structure of ecological communities (Stachowicz 2001; Bruno et al. 2003; Lengyel et al. 2009). In particular, ant-seed dispersal mutualisms (myrmecochory) are both geographically widespread and ecologically important (Giladi 2006; Lengyel et al. 2010). Myrmecochorous seeds have a small, lipid-rich appendage called an elaiosome that ants remove and consume after dispersal. Elaiosomes have evolved tens of times in the monocots (Dunn et al. 2007) and over a hundred times in the angiosperms more generally (Lengyel et al. 2009; Lengyel et al. 2010). Over 11,000 species and 77 families of angiosperms participate in myrmecochorous relationships across a variety of ecosystems that span arid, tropical, and temperate regions (Giladi 2006; Lengyel et al. 2010). To date, our understanding of the benefits of myrmecochory to plants (reviewed in Giladi 2006) focus on dispersal distance of the seed away from its parent (e.g., Andersen 1988), reduction in seed predation due to dispersal (e.g., Culver and Beattie 1978; Heithaus 1981) and movement of the seed to a favorable germination site (e.g., Beattie and Culver 1983; Hanzawa et al. 1988).

[1] This chapter forms the basis for the manuscript of the same title, co-authored with Robert R Dunn, Itamar Giladi, and Kevin Gross. The manuscript is currently in preparation for submission to the journal *Acta Oecologia*.

However, despite the numerous advances in our understanding of myrmecochory, there are still key observations relevant to each mechanism that are missing, such as the fate of seeds once in the nest.

The work that we report here began as a study to assess the benefits of seed burial in nests by ants to understory herbs in temperate forests. To our surprise, we found very few of the seeds during nest excavation that we previously observed *A. rudis* take into nests. However, we did find seeds in the litter near the nest. Removal of seeds from the nest (redispersal) is a relatively common observation in other systems, such as European temperate deciduous forests (e.g., Kjellsson 1985; Gorb et al. 2000; Gorb and Gorb 2003) but, to the best of our knowledge, redispersal has only been roughly quantified once before in eastern North American forests (Heithaus 1986). Redispersal could have several interesting consequences for the ecology and evolution of ant-seed interactions. First, redispersal may increase the mean and variance of dispersal distances away from the parent as well as increase the maximum distance dispersed, with consequent effects on the shape of a dispersal kernel and the population spread rate of the plant. Second, frequent redispersal could alter our perception of the proposed benefits (and costs) of myrmecochory that are frequently associated with being dispersed into an ant nest. Based on our observations, we decided to explore further the redispersal of seeds by ants and the effect that it may have on the spatial population dynamics of plants.

Our study focuses specifically on the ant species complex *Aphaenogaster rudis*. These ants are widespread, common where they occur, the most frequently cited seed-dispersing species in eastern North America (Culver and Beattie 1978; Beattie and Culver 1981; Heithaus 1981; Gaddy 1986; Heithaus and Humes 2003; Giladi 2004; Zelikova et al. 2008) and considered the keystone seed disperser of myrmecochores in eastern North America (Ness et al. 2009). The average primary dispersal distance of seeds by *A. rudis* ranges from 50 to 100 cm (Culver and Beattie 1978; Gomez and Espadaler 1998; Kalisz et al. 1999; Giladi 2004; Zelikova et al. 2008). In temperate deciduous forests in eastern North America, ants, in particular *A. rudis*, disperse the seeds of 20% to 70% of the total herbaceous flora (Pudlo et al. 1980; Handel et al. 1981; Beattie and Culver 1981; Gaddy 1986) depending on the geographic location. Consequently, if *A. rudis* tends to avoid disposal of seeds inside its nests the effects may be relevant to a broad suite of plant species across a large geographic region.

To understand better the effects of redispersal on myrmecochore populations in eastern North America, we conducted both an empirical and mathematical exploration of redispersal. First, we documented redispersal frequency and distance by *A. rudis* using a novel seed-tagging technique for small seeds (Canner and Spence, in revision; Chapter 2). Second, we modeled the consequences of redispersal on population spread rate for two local myrmecochores *Hexastylis arifolia* and *Asarum canadense*. Although the ranges of *H. arifolia* and *A. canadense* are not actively expanding, their ability to spread to new, suitable habitats may play a role in their future survival given the potential threats to their current ranges (such as

climate change and habitat destruction). We discuss the effects of redispersal on our current understanding of the benefits of myrmecochory to plants based on our empirical and model results.

1.2 Methods and Materials

1.2.1 Study Location

We conducted our study in Lake Raleigh Woods, a mixed pine-hardwood forest located on Centennial Campus, North Carolina State University, Raleigh, NC. The Lake Raleigh Woods understory includes many myrmecochorous plant species, but to measure redispersal, we use the seeds from a species in the family Aristolochiaceae (Birthwort), *Asarum canadense* L.

1.2.2 Study Species

A. canadense (Canadian wild ginger) is a small, evergreen, herbaceous perennial, common in deciduous and occasionally mixed forests in eastern North America (Heithaus 1986; Offer 1992; Cain and Damman 1997). Reproduction occurs through seed production, with approximately 10-30 seeds per reproductive plant (Offer 1992; Cain and Damman 1997). Flowering begins in late March and early April and fruiting occurs late May and early June in North Carolina (Smith et al. 1989; personal observation). We collected *A. canadense* seeds for the study just prior to full fruit dehiscence. Seeds were promptly stored at -18° C to prevent decomposition and oxidation of the elaiosome. The seeds of *A. canadense* are about

3-5mm in length, narrowly ovate, with an elaiosome running the entire length of the seed, and a dry weight between 4-5mg (personal observation).

We focused our study on *A. rudis* because of its importance to understory seed dispersal in eastern North America. *Aphaenogaster rudis* nests are small and temporary (Culver and Beattie 1978; Smallwood 1982a,b) and are found in logs, under rocks, in the leaf litter, or below ground in temperate deciduous forests (Talbot 1951). *Aphaenogaster rudis* are omnivorous, indiscriminant foragers. They forage individually with modest recruitment when they find a food cache. *Aphaenogaster rudis* has been called a keystone mutualist (Zelikova et al. 2008; Ness et al. 2009) on which many understory herb species exclusively or nearly exclusively depend for seed dispersal.

1.2.3 Seed Preparation and Detection

We developed a new technique to mark and recover small seeds in the leaf litter using Coded Wire Tags (CWTs) (Northwest Marine Technologies, Inc., Shaw Island, WA). Though Canner and Spence (in review; Chapter 2) discuss the technique in detail, we will provide the pertinent details here as well. The CWT is a small (diameter 0.25 mm, length 1.1mm, weight 0.4mg) magnetized stainless steel wire originally designed to tag fish. We injected the tags into the top of the seeds with a Single Shot Tag Injector (NMT, Inc.). After the injection of the tags, we marked the seeds with yellow enamel paint to increase visibility within the leaf litter after initial detection. Preliminary studies showed that *A. rudis* and other ant species had no preference for tagged versus untagged seeds, nor did the paint or tag influence

redispersal from the nest after elaiosome consumption (Canner and Spence, in review; Chapter 2). At the conclusion of each trial, we used the Handheld Wand Detector (NMT, Inc.) to detect the tagged seeds in the nest area and surrounding leaf litter and could detect marked seeds artificially buried up to several centimeters in the leaf litter or soil. Though similar techniques already exist (Forget and Wenny 2005), to the best of our knowledge, we are the first to develop such a technique for such small seeds.

1.2.4 Data Collection and Experimental Design

Initial dispersal distance

We collected data with Jane Zelikova for the initial dispersal distance of *H. arifolia* seeds by *A. rudis*. Zelikova et al. (2008) have previously published the data for the initial dispersal distances; therefore, we reiterate the methods briefly. In the summer of 2007, we placed a 25x25m grid with seed depots at 5m intervals at 7 different sites in Great Smokey Mountain National Park, TN. We then placed three *H. arifolia* seeds on a 4x6cm white index card at each depot. We observed each depot for 2 hours and recorded the radial distance from the depot to the final location of the ant-dispersed seed.

Redispersal distance

We fed 20 *A. rudis* colonies between late May and early July 2008, to coincide with peak fruiting time and peak ant activity. Nests were located with baits one to two days prior to feeding. In a similar manner to other studies of seed fate within the nest (see Culver and Beattie 1980; Hanzawa et al. 1988; Hughes and Westoby 1992; Heithaus et al. 2005), we

placed up to 50 marked *A. canadense* seeds near the nest entrance and observed the seeds' removal by *A. rudis* to be sure all seeds went into the same nest. Feedings occurred between 0900 and 1400 hours, when daily ant activity peaked, until the colony removed all 50 seeds or until 30 minutes had passed since the last removal. The number of seeds fed to each nest is in line with average number of seeds a single *A. rudis* colony may consume in a day (Heithaus et al. 2005) and the number of seeds that may be naturally available near a colony (Gonzalez 1972; Heithaus et al. 2005). After approximately 7 days, we excavated each nest and scanned all the leaf litter surrounding the nest within a 150 cm radius. We assume redispersal would not be greater than typical foraging distances of *A. rudis* colonies, therefore a 150 cm radius would be sufficient to recover most seeds because previous studies all reported an average seed dispersal distance by *A. rudis* of less than 100 cm (e.g., Culver and Beattie 1978; Pudlo et al. 1980; Giladi 2004; Zelikova et al. 2008).

We began the excavation at the outer edge of the 150 cm circle and systematically scanned the area with the detector until we reached the nest entrance. We marked detections with a flag, and then removed the leaf litter within a 2.5 cm radius of a detection and searched for the seed or tag within the litter. If we found the seed(s) or tag(s), we then recorded the radial distance and direction from the seed location to the original nest entrance. In addition to the surrounding leaf litter, we also excavated the nest in 1-2cm layers. We scanned each layer and recorded the depth of each recovered seed or tag. Excavation continued as long as we found ants or nest cavities within the log and/or leaf litter. We collected each colony and recorded the total number of workers, brood and alates. If a colony moved (n=3; a common

occurrence for *A. rudis*; see Culver and Beattie 1978; Smallwood 1982a,b), we marked the nest location of any colony that contained tagged seeds, but we measured redispersal distance from the original location of the nest. We justify measurement from the original location because any movement of the seeds away from the nest is a redispersal event from the location of seeds after initial dispersal. We recorded seeds found in the nest as a radial distance of 0 cm. We conducted all statistical analysis of the data with R Statistical Software (R Development Core Team 2009).

1.2.5 Modeling Spatial Population Dynamics

Redispersal has the potential to augment both a population's rate of spatial spread and its ability to reach a new, more suitable habitat. To compare population spread rate both with and without redispersal, we used a spatially explicit model of stage-structured population dynamics (Neubert and Caswell 2000). The model is a discrete-time, continuous-space model that uses an integro-difference equation to incorporate movement at each demographic transition. To build such a model for our study system, two components are necessary. First, the model requires the annual demographic transitions among stages for the myrmecochore. Second, the model requires specifying the dispersal kernels that define the movement of the myrmecochore at each demographic transition. We can then use the model to calculate population spread rate and compare spread rate under different dispersal scenarios for both *A. canadense* and *H. arifolia*. We describe the model in brief here and provide details in Appendix I.

Demography of Asarum canadense

We estimate the annual transitions (e.g., survival, growth, and fecundity) for *A. canadense* by using the average population projection matrix for multiple years (1990-1995) between 2 plots from Cain and Damman's (1997) demographic study of the same species in late successional forest habitat. Life history stages were classified by Cain and Damman (1997) as seedling, yearling, lateral shoot (reproductive), and mature ramets (reproductive) (Table A1.1, Appendix II). Dispersal can only occur between the lateral shoot and seedling stages and the mature ramets and seedling stages.

Demography of Hexastylis arifolia

We estimated the annual transitions for *H. arifolia*, a closely related species to *A. canadense*, by using the average population projection matrix for three populations from Giladi's (2004) demographic study of the same species (Table A1.2, Appendix II). Giladi (2004) classified life history stages by leaf size and reproductive ability. The stages are seedlings (stage 1), sub-adult (stage 2), non-reproductive adult (stage 3), reproductive adults with a small leaf size (stage 4), medium leaf size (stage 5), and large leaf size (stage 6), and dormant adult (stage 7). There is no known seed bank for *H. arifolia* (Giladi 2004). Dispersal can only occur between the reproductive adult (4-6) and seedling stage (1) and does not vary with stage.

Movement of seeds

To evaluate the effect of redispersal on plant population spread rate, we consider three dispersal scenarios to contrast the differences in spatial dynamics with the addition of each new dispersal event. The first scenario considers only autochorous dispersal (i.e., no ant dispersal) in which dispersal distances follow a half-Gaussian distribution with a mean of 0.53cm and a standard deviation of 0.44cm. We chose the parameters for the autochorous dispersal kernel based on the assumption that most seeds may disperse up to 2 cm from the parent plant in the absence of an ant disperser (personal observation; Zelikova 2008). The second scenario is primary dispersal, which incorporates dispersal to the nest by ants. The dispersal kernel for primary dispersal is the convolution of the autochorous dispersal kernel and the measured initial dispersal kernel of seed dispersal from the parent to the nest (Neubert and Parker 2004). We assume that a fraction p_1 of seeds undergo primary dispersal by ants and fix p_1 at 74%, which is the average removal rate for *A. rudis* when *A. rudis* is present (Ness et al. 2009). The third scenario we call secondary dispersal, which includes autochorous dispersal, initial dispersal and redispersal away from the nest. The dispersal kernel for secondary dispersal is the convolution of the primary dispersal kernel (autochorous and initial dispersal) and the measured dispersal kernel for redispersal from the nest, with the fraction p_2 fixed at 93% of seeds redispersed based on data presented herein. We assumed that dispersal isotropic (same in all directions) and we used a nonparametric method to fit the measured dispersal kernels to *A. rudis* initial dispersal distance data and redispersal distance

data (see Appendix I for details). Nonparametric methods allow us to avoid assumptions about the underlying distribution of the dispersal kernel (Clark et al. 2001; Lewis et al. 2006).

We also explored how changes in *A. rudis* density affect population spread rate by varying the removal rate, p_1, because the removal rate of seeds depends on both the presence and abundance of *A. rudis* (Zelikova et al. 2008; Ness et al. 2009). Our data only account for dispersal by *A. rudis*, so our overall invasion speed may be an underestimate because it does not account for dispersal by other ant species and other dispersal vectors (e.g., Vellend et al. 2003).

1.3 Results

1.3.1 Dispersal Frequency and Distances

Initial dispersal distance

We observed 303 dispersal events (out of 600 total seeds placed in the depots) and we recorded 146 unique distances that were dispersal events by *A. rudis* (mean= 73.85cm, sd=42.64cm). There was no effect of elevation on the dispersal distances at each site (Zelikova et al. 2008). We used all distances measured to the nest to fit the initial ant dispersal kernel in our model. Since *H. arifolia* and *A. canadense* have the same size and weight of seeds and given the similarity of dispersal distances to other studies of seed dispersal, we use this data to quantify our initial dispersal kernel for both species.

Redispersal distance

We fed 20 colonies 864 seeds and recovered 539 (63.3%) total seeds and tags from the excavations. All recovered seeds were intact and without elaiosomes. The recovery rate for individual nests ranged from 26.5% to 98% of seeds fed to the colony. The pooled overall mean redispersal distance for individual seeds dispersed from all nests, including those found in the nest, is 51.5 cm (n=539, se=1.4) with a maximum observed distance of 148 cm (Fig. 1.1). We found redispersed seeds within the leaf litter surrounding the nest, not in obvious middens. We recovered seeds in the nest in 9 out of 20 nests. Thirty-seven seeds and loose tags were found within the nests (6.8% of all recovered), a result comparable with the observations of Heithaus (1986). The proportion of seeds redispersed for individual nests ranged from 73.5% to 100% of seeds found. We found no seeds in the nests deeper than 2 cm in the ground, most (64% of seeds found in nests) within a log or leaf litter. We recovered 48 (8.9% of total seeds and tags) tags unattached to seeds. In rare cases, we found a marked seed without a tag in the excavated leaf litter, but always with an unattached tag in the excavated litter. Therefore, it is likely that some tags came loose from their seeds during excavation. We used the data collected herein to fit the kernel for redispersal distances by *A. rudis* in our model.

1.3.2 Variability of Redispersal Distances for Individual Nests

The distribution of redispersal distances for each nest was highly variable between nests (Fig. 1.2). The mean redispersal distances for individual nests ranged from 28.3 to 106.1 cm and the median redispersal distances for individual nests ranged from 11.8 cm to 111.8 cm.

There was a statistically significant difference between the median redispersal distances of nests (Kruskal-Wallis Test, χ^2=145.62, df=19, p<.0001). One possible explanation for this difference is the influence of two colonies with exceptionally high median redispersal distances (top two nests in Fig. 1.2 had median redispersal distances of 97.5 cm and 111.75 cm respectively). The colony could not be located for one nest (possibly moved and took the seeds with the colony dispersing them further), and the other nest had a very low recovery rate (29%). Removal of the two nests from the analysis did not change the statistical significance of the nest effect (Kruskal-Wallis Test, χ^2=94.70, df=17, p<.0001). Other possible sources of variation, such as the colony size, proportion of seeds recovered at each nest and nest type (log, leaf litter and soil) were not significant as predictors of median redispersal distance.

1.3.3 Population Spread Rate

Our model results found that redispersal increases the invasion speed of *H. arifolia* and *A. canadense* by 22.5% compared to the calculated spread rate without redispersal (Table 1.1). The higher spread rate for *A. canadense* compared to *H. arifolia* is due to the higher reproduction and growth rates of *A. canadense* (see Appendix II). The spread rate for primary dispersal is much smaller than previously calculated spread rates for *H. arifolia* (see Giladi 2004). The difference is most likely due to our focus on dispersal by only *A. rudis*, the previous study's artificial inflation of fecundity needed to satisfy the condition that the population growth rate is positive (Giladi 2004). In addition, we consider movement in two dimensions, which provides a more conservative estimate of population spread rate than

models that consider movement only in one dimension (Lewis et al. 2006). We found that invasion speed increases as removal rate increases under both dispersal scenarios (Fig. 1.3). Zelikova et al. (2008) showed that removal rate varies linearly with *A. rudis* abundance. We can then infer that an increase in *A. rudis* abundance increases population spread rate. Redispersal appears to especially affect spread rate when initial ant dispersal rate is low (<0.20), but not zero. The results underscore the importance of *A. rudis* to the spread of myrmecochore populations.

1.3.4 Cumulative Dispersal Distance

Redispersal also affects the shape of the composite dispersal kernel. In our model, we assumed a nonparametric dispersal kernel for both initial ant dispersal and redispersal. In order to compare the shapes of the composite dispersal kernels for primary dispersal and secondary dispersal, we assumed a parametric dispersal kernel, the Gamma distribution (as in Giladi 2004) for both initial ant dispersal and redispersal. We simulated dispersal in two dimensions, with the radial distance selected from the appropriate gamma distribution and the direction from a uniform distribution on (0, 2π) at each dispersal step, with probability of initial ant dispersal, $p_1 = 0.74$ (Ness et al. 2009) and probability of redispersal, $p_2 = 0.93$ (data herein). The addition of redispersal to the composite dispersal kernel increased the mean distance of dispersal of the seed away from the parent by 24% and increased the variance (Table 1.1). The effect of redispersal on the location and spread of the dispersal

kernel shows that the cumulative dispersal distance from the parent plant is greater and more variable than previously thought.

1.4 Discussion

Here, we have shown that ants redisperse the seeds of the understory herb, *A. canadense,* out of their nests and into the surrounding leaf litter. Redispersal from the nest has been noted before (Heithaus 1986) for *Sanguinaria canadensis*, but we provide the first documentation of the extent of redispersal and its consequences for spatial population dynamics in eastern North American forests (for examples in European forests see Gorb and Gorb 2003). Overall, the data indicate that *A. rudis* redisperses a majority of seeds from the nest at distances comparable to measures of primary dispersal (see Culver and Beattie 1978; Gomez and Espadaler 1998; Kalisz et al. 1999; Giladi 2004; Zelikova et al. 2008). Redispersal increases the mean dispersal distance of seeds by *A. rudis* by 24%. Integrating the redispersal data with a demographic model suggests that redispersal may increase the speed of population spread for *H. arifolia* and *A. canadense* by 22.5%. The sensitivity of population spread rate to changes in removal rate and *A. rudis* abundance serves to highlight the importance of *A. rudis* to the spread of local understory herbs. *Aphaenogaster rudis* is the primary seed disperser of myrmecochorous seeds in eastern North American forests, and thus we suspect that redispersal is a widespread phenomenon.

1.4.1 Ant Dispersal and Migration Rates

Although redispersal occurs in many myrmecochorous relationships worldwide, our study is the first to model redispersal and its effects on population spread rate explicitly. Even with redispersal, the population spread rate that we calculate is small and falls short of the post-glaciation migration rates necessary to account for current myrmecochore ranges in eastern North America (Cain et al. 1998; Vellend et al. 2003). The discrepancy may occur due to the omission from our model of rare, long-distance dispersal events by other ant species or vertebrates that may account for dispersal at a continental scale (Vellend et al. 2003; Myers et al. 2004). If non-standard dispersal events are common, omission of long distance dispersal may inflate the perceived increase in population spread rate due to the addition of redispersal in our model. Even if redispersal does not account for population spread at a continental scale, it does affect local dispersal distance of seeds, which may affect the local plant population fitness and the benefits plants receive from myrmecochory more generally (see Section 1.4.2).

1.4.2 Plant Benefits from Dispersal

Redispersal changes our understanding of potential explanations for the evolution of myrmecochory based on the benefits offered to plants, at least in eastern North American temperate forests. There are three prominent hypotheses for plant benefits from myrmecochory- the predator-avoidance hypothesis, the directed-dispersal hypothesis, and the distance-dispersal hypothesis (reviewed in Giladi 2006). We consider each in turn.

Directed dispersal

The directed dispersal hypothesis argues that the chief advantage of myrmecochory is that ants disperse seeds to locations where plant fitness is higher than it would be if seeds were dispersed randomly, a so-called "site effect." For example, dispersal into ant nests may provide a nutrient-rich environment that increases plant fitness and survivorship (Culver and Beattie 1978; Beattie and Culver 1983; Hanzawa et al. 1988; Giladi 2006), an effect often cited as a primary benefit to plants in European temperate forests (Culver and Beattie 1980; Gorb et al. 2000; Gorb and Gorb 2003) and for the few myrmecochore species in the western North American meadows (Beattie and Culver 1983; Hanzawa et al. 1988). The seed dispersing ant species in the above habitats (generally *Formica* spp.) have nutrient-rich, long-term (often many years) nest sites (Culver and Beattie 1980; Smallwood 1982a; Gorb et al. 2000; Gorb and Gorb 2003). In addition, redispersal in some European forests places the seeds in middens at territory borders, which are often nutrient-rich and beneficial to germination (Gorb et al. 2000).

In direct contrast to the European temperate forest system the benefits from directed dispersal into ant nests do not necessarily apply to eastern North American temperate forests in the same manner for the simple reason that seeds do not end up inside nests or even in middens. The keystone disperser in eastern North American forests, *A. rudis*, has temporary (20 days) nest locations (Culver and Beattie 1978; Smallwood 1982a,b) and redisperses a majority (~93%) of seeds outside the nest into the surrounding leaf litter (data herein). Therefore, any benefit that nutrient enrichment or burial within the nest provides is unlikely to apply to the

majority of dispersed seeds in the eastern North American system. Instead, the possible benefits of directed dispersal would have to arise from the non-random placement of seeds outside of nests in favorable locations. Perhaps seeds tend to be redispersed to sunnier areas on the forest floor near the nest (Smallwood 1982b) or scattered throughout the litter layer (Gonzalez 1972). Either location may be favorable for germinating understory herbs, but such tendencies are, for now, pure speculation. In practice, however, the final locations of the seed appear to be similar to the initial location of the seed at dehiscence (within the leaf litter), with the exception that the seed is no longer near the parent or siblings.

Predator avoidance

In the predator avoidance hypothesis, both initial dispersal and subsequent burial of seeds by ants reduce the ability of predators to locate and obtain seeds (Culver and Beattie 1978; Heithaus 1981; Giladi 2006). In our study, however, the high rate of redispersal indicates that seeds do not experience safety from predators (or potential safety) through burial in *A. rudis* nests. In the context of predation, the only potential selective advantage of myrmecochory when seeds ants redisperse seeds is the reduction of the seed density in the area around the nest (lower density diminishes predation; Heithaus 1981). In other words, it is the dispersal distance by ants, which decreases the density of seeds located near the parent, which leads to lower predation rates by rodents (Heithaus 1981). Other possible predator avoidance benefits, such as reduced rodent detection due to elaiosome removal by ants post-dispersal (Heithaus 1981), are ultimately the consequence of the evolution of elaiosomes, not a driver, since such benefits occur only if the elaiosome is present in the first place.

Distance dispersal

The distance dispersal hypothesis proposes that seed dispersal reduces competition between parents and offspring, as well as among siblings (Andersen 1988; Giladi 2006). Support for the hypothesis is common in studies of primary dispersal in temperate forests (reviewed in Giladi 2006). In these studies, understory herbs experience reduced competition and density-dependent effects at relatively short distances from the parent (and each other) due to ant dispersal (Heithaus 1986; Higashi et al. 1989; Kalisz et al. 1999; Gorb and Gorb 2003; Giladi 2006). The redispersal of seeds outside the nest increases the mean, variance and maximum possible dispersal distance of the seed from the parent plant. Consequently, redispersal, like primary dispersal, decreases the density of seeds around the parent. It also decreases the density of seeds within and around ant nests, and thus seedling density. Increased distances from the parent and reduced seedling density are also effects of redispersal in European temperate forests (Gorb et al. 2000). Several studies have shown that an increase in local seed density decreases the survival and growth of seedlings and adults of *H. arifolia* (Gonzalez 1972; Giladi 2004; Zelikova 2008) as well as for other temperate forest myrmecochores (Culver and Beattie 1980; Heithaus 1986; Higashi et al. 1989; Kalisz et al. 1999). Therefore, the increase in dispersal distance and potential decrease in seedling density due to redispersal ought to augment myrmecochore population fitness. As we previously stated, some studies suggest both predator avoidance and directed dispersal as drivers of the origin of myrmecochory in eastern North American. In light of redispersal, though, those benefits are contingent on dispersal away from the parent in the first place

(dispersal distance). Therefore, the documentation of redispersal supports the proposal that dispersal for distance is a driver of the evolution and origin of myrmecochory in the eastern temperate forests of North America.

1.4.3 Evolution and Distance Dispersal

The dispersal distance hypothesis also relates the evolution of myrmecochory to the evolution of dispersal morphologies in forests more generally. Givnish et al. (2005) argued that both the evolution of fleshy fruits in monocots in forest understory and the loss of fruits in open habitats indicate a necessary adaptation to a more effective dispersal vector in a new environment. Elaiosome production, a less costly means of dispersal than a full fruit, is often associated with forest habitats in temperate regions and is thought to have resulted from a loss of passive (e.g., wind) dispersal (Dunn et al. 2007). In addition, both Dunn et al. (2007) and Lengyel et al. (2010) suggest that elaiosomes in monocots and angiosperms respectively arose in conjunction with increased abundance of ants, which is to say, once ants were dependably present, the benefits of reduced density due to ant-dispersal lead to selection for elaiosome production. It is conceivable that myrmecochory arose in forests as the least costly dispersal mode, driven by the necessity of a dispersal vector and the availability of ants to provide that dispersal vector (such as *A. rudis*, although current evidence for direct coevolution is lacking). All other possible benefits of myrmecochory for temperate forest understory may be secondary to the simple need of dispersal away from the parent.

1.4.4 Other Consequences of Redispersal

We must address two additional questions in light of redispersal by *A. rudis*. The first question is how redispersal affects the germination rates of myrmecochores and ultimately their population fitness. Several studies show that simply the handling of the seeds by ants has a positive effect on germination rates (Culver and Beattie 1978; Culver and Beattie 1980) and redispersal is beneficial in European systems (Gorb et al. 2000). Concrete evidence, though, for the benefits redispersal to germination for the eastern North American system does not yet exist. The second question that remains is why *A. rudis* expends the energy to remove the seeds from the nest. In lab colonies, seeds fed to the colonies and remain in the nest often grow a fungus (personal observation). Smallwood (1982a) noted that ants, such as *A. rudis*, might frequently relocate nests to avoid the accumulation of waste and fungus in the nest. Redispersal may simply be the result of the ants cleaning out their nests to avoid such accumulation, which in lab colony nests proved fatal to the ants (personal observation). In addition, the possible effects of such a fungus on germination and survival of seedlings is unknown. Despite more than a century of study of myrmecochory (Sernander 1906), there is still much to learn.

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Table 1.1 Comparison each dispersal scenario on the spatial dynamics of *H. arifolia* and *A. canadense*, including the population spread rate and the mean and standard deviation of cumulative dispersal distance.

Movement	***H. arifolia* spread rate (cm/yr)**	***A. canadense* spread rate (cm/yr)**	**Mean cumulative distance (cm)**	**Standard deviation (cm)**
Autochorous dispersal	0.17	0.60	0.50	0.81
Primary Dispersal	1.89	6.10	54.75	48.77
Secondary Dispersal	2.32	7.47	67.85	59.87

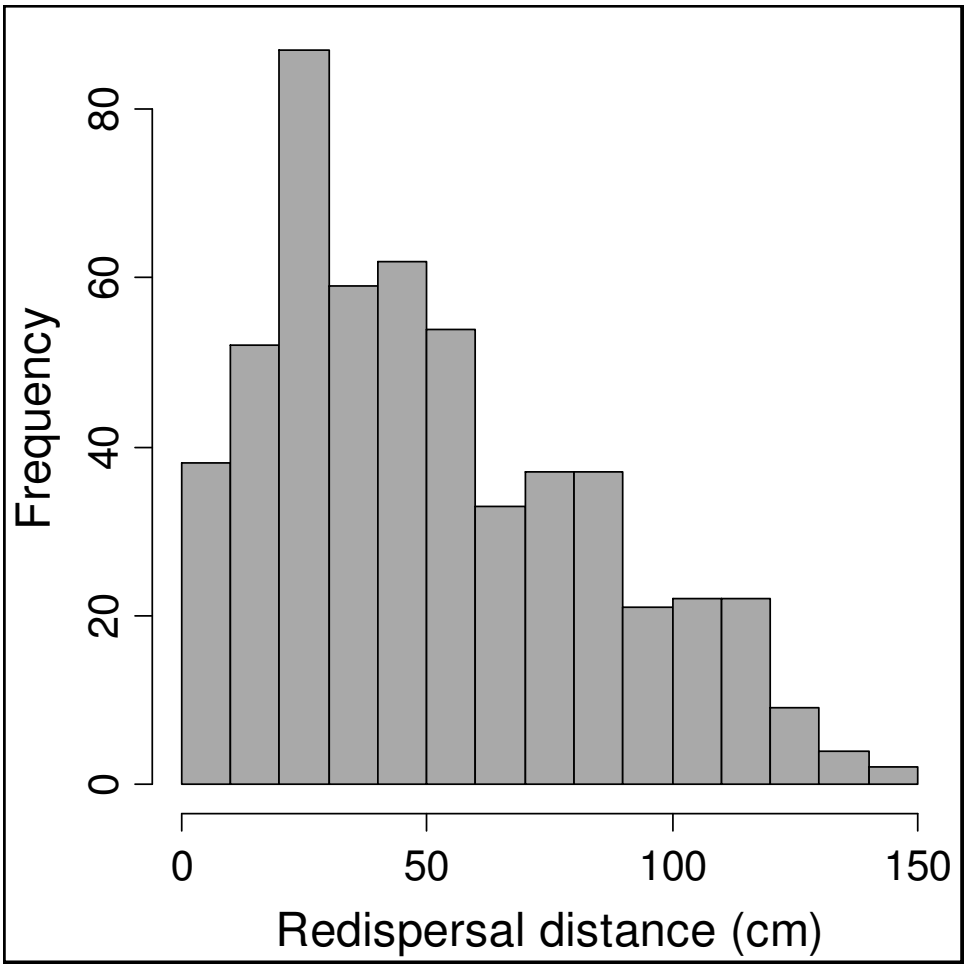

Figure 1.1

Figure 1.1: Histogram of redispersal distances for all sampled nests. Data include seeds that remained in the nest (measured as 0 cm).

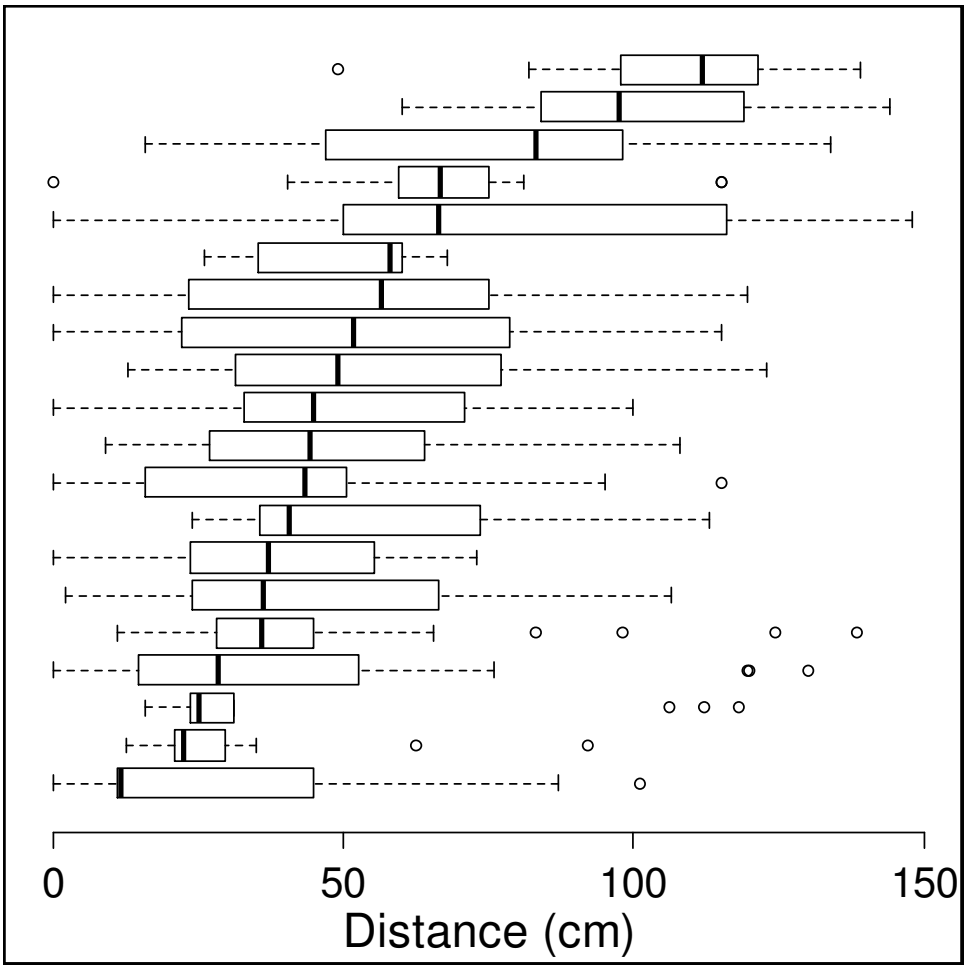

Figure 1.2

Figure 1.2: Box-plots for individual nest redispersal distances ordered bottom to top by the median redispersal distance (smallest to largest) for each nest. Redispersal distances include seeds that remained in the nest (0 cm).

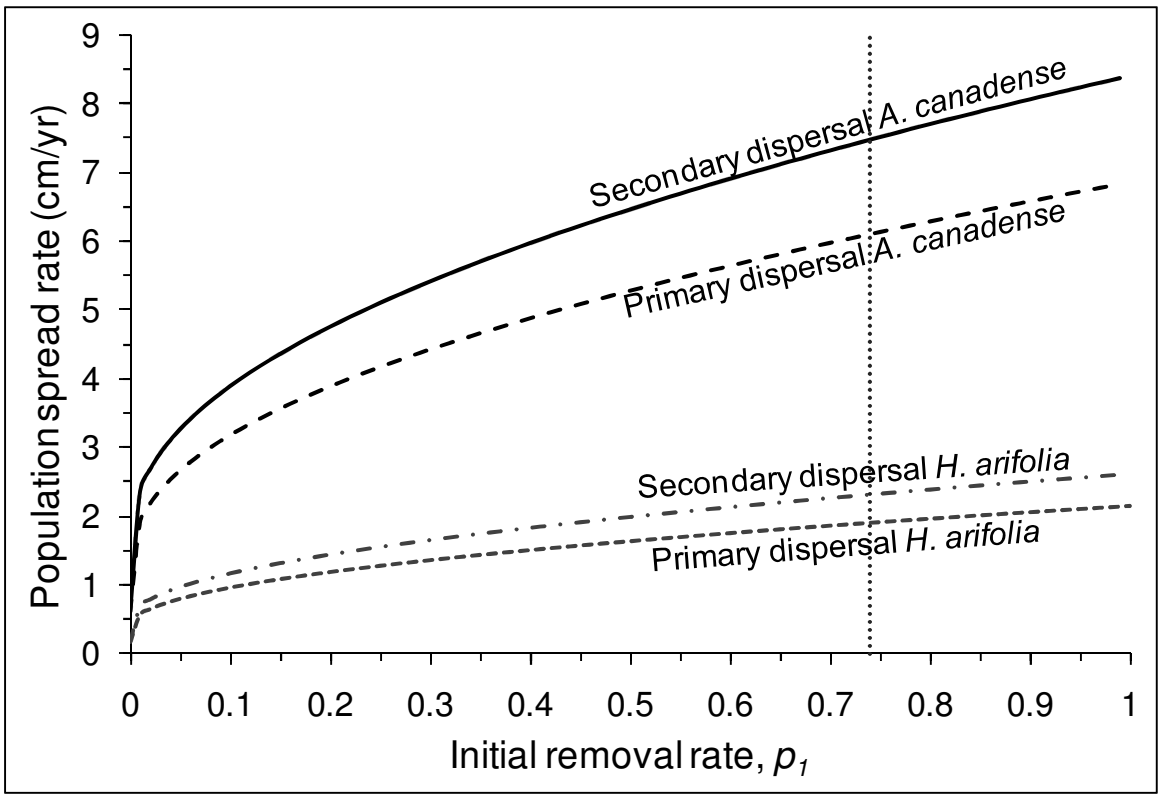

Figure 1.3

Figure 1.3: The invasion speed versus initial removal rate of seeds. The vertical line represents the reported results for population spread with an initial removal rate of 74%. Note that primary dispersal is the convolution of autochorous dispersal and initial ant dispersal and secondary dispersal in the convolution of primary dispersal and redispersal by ants.

CHAPTER 2

A new technique using metal tags to track small seeds short distances[2]

2.1 Introduction

Seed dispersal by ants, myrmecochory, is common in many lineages of plants across diverse geographic regions (Lengyel et al. 2009). In temperate deciduous forests in eastern North America, ants disperse 20% to 70% of the total herbaceous flora (Handel et al. 1981; Zelikova et al. 2008). Remarkably, dispersal of most of these species appears to be by a single species complex of ants, *Aphaenogaster rudis* (Ness et al. 2009). We first began our study of myrmecochory as we sought to observe the fate of seeds after initial dispersal to the nest by *A. rudis*. Our task presented us with several difficulties. We could observe the initial dispersal of seeds into the nest, but it was difficult to find the seeds within the nest as it appeared that ants redispersed the seeds into the surrounding litter. The small size of the seeds (length: 3-5mm, weight:4-5mg) of our plant species of interest, *Asarum canadense* combined with the thick leaf litter of the mixed hardwood forests, made it nearly impossible to find seeds in the leaf litter without aid. Other methods used to track ant-dispersed seeds were ineffective because of the potential burial of seeds underground and placement among the leaf litter (e.g., fluorescent dye and UV light; Bullock et al. 2006) or because they were expensive and highly regulated (e.g., radiolabels; Kalisz et al. 1999). Therefore, we adapted

[2] This chapter forms the basis for the manuscript of the same title, co-authored with Meredith Spence. The manuscript is currently in revision for submission to the journal *Ecological Research*.

a technique originally developed for mark-recapture studies of salmonid fishes in order to track the fate of ant-dispersed seeds after dispersal to the nest. Here we report on the details of this method, offer suggestions for improvement and briefly mention other contexts in which the new technique may prove useful.

2.2 Methods and Materials

2.2.1 Study site and species

We conducted our study in Lake Raleigh Woods, a mixed pine-hardwood forest located on Centennial Campus, North Carolina State University, Raleigh, NC. We tracked the dispersal of a myrmecochore in the family Aristolochiaceae (Birthwort), *Asarum canadense* L. The seeds are small (3-5mm) and ovate with a fleshy appendage, called an elaiosome, attached to the length of the seed.

2.2.2 Seed preparation

We used a small (0.25mm diameter; 1.1mm length; weight 0.4mg) magnetized stainless steel Coded Wire Tag (CWT) (Northwest Marine Technologies, Inc., Shaw Island, WA), to mark each seed (Fig. 2.1a). We loaded the CWT's into the Single Shot Tag Injector (NMT, Inc.), a syringe with a small hypodermic needle (Fig. 2.1b). We held the seeds with a pair of small forceps so the elaiosome faced down in order to prepare for injection. We inserted the injector tip into the broader side of the top of the seed, with care not to angle the injector too far up or down to prevent the tag from exiting the seed upon injection. After injecting the

CWT into the seed, we marked the tops of the seeds with yellow enamel paint to increase their visibility in the leaf litter after the initial detection by the Handheld Wand Detector (HWD; NMT, Inc.; Fig.2.1c).

2.2.3 Pilot study

We conducted a pilot study in the spring 2008, to test the limitations of the technique. We found that the HWD could locate seeds buried up to 4cm underground. We were also concerned that the addition of a tag to the seed might change the behavior of *A. rudis* towards the seeds. In preference studies, we provided both tagged and untagged seeds simultaneously and recorded the removal rate and behavior of *A. rudis* towards each seed. *A. rudis* showed no preference for tagged versus untagged seeds and removed both within equal time and in equal quantities. In addition, we conducted a study in the summer of 2007 that allowed us to observe the redispersal of untagged seeds within a barrier surrounding *A. rudis* nests in the field. The redispersal rates for tagged seeds are comparable to the redispersal rates of untagged seeds (~85%), given the restrictions of the barrier. Therefore, we are confident that the movement and treatment of seeds by ants is the same independent of the presence of the tag.

2.2.4 Field study

We fed 20 *A. rudis* colonies up to 50 seeds between late May to early July 2008, between the hours of 0900 and 1400. We observed the removal of the seeds to ensure that only a single *A. rudis* colony dispersed all seeds into the nest. We assumed redispersal distances would be

less than 150 cm, a distance well above the reported seed dispersal distances for *A. rudis*, which tends to be below one meter (Gomez and Espadaler 1998; Zelikova et al. 2008). After 7 days, we used the HWD to scan systematically for tagged seeds within a 150cm radius surrounding the nest. We began at 150cm from the nest, scanned the first 10cm of the outer perimeter, and marked detections with a flag. We continued in a like manner moving toward the nest in the center of the search area. We then excavated the leaf litter in a 2.5cm radius around each detection, placed the litter into a plastic bin, and searched the litter until we located the seed or, in some cases, loose tag. We then marked positive detections with a new flag and measured the distance from the nest entrance to the seed location (Fig. 2.2). We also excavated the nest in 1-2 cm layers until no ants or nest cavities remained in the nest site and no positive seed detections occurred. We scanned sites twice to ensure that we recovered as many seeds as possible. On average, a complete scan and excavation of a nest site (~7m^2) required five hours of search effort by two people, though the search effort varied with the condition of the site and the presence of magnetic materials embedded in the soil that led to false detections. We conducted all statistical analysis of the data with R Statistical Software (R Development Core Team 2009).

2.3 Results

We recovered 63.3% (539 tagged seeds recovered of 864 total tagged seeds) of the seeds that were fed to *A. rudis* colonies, and the recovery rates ranged from 26.5% to 98% (mean=61.3%, se=4.5%) for the 20 individual nest sites. The low recovery rates tended to

occur at nest locations that contained metallic material in the soils, which lead to false detections. We calculated a nonparametric test for correlation (Spearman rank correlation) and found that there is not a significant relationship between recovery rate and mean redispersal distance ($S = 1611.21$, $p = 0.37$). Therefore, recovery rate did not affect the mean redispersal distance measured for each nest.

We found 37 of the 539 tagged seeds buried within the nests, none deeper than 2 cm in the ground. In addition, we found 48 tags unattached to seeds. Though we took precautions, we may have injected some tags too far into the seed and that exposed the tag after the ants removed the elaiosome. Of the 48 tags recovered, we found 41 outside of the nest. Therefore, it is possible that the tags fell out of the seed during redispersal, which may cause a slight underestimate of those individual redispersal distances.

2.4 Discussion

One of the chief difficulties in ecology is visualizing ecological processes and seed dispersal is often a difficult process to measure. The use of CWTs was an effective method of seed detection and retrieval in our study of short distance dispersal of *A. canadense* by *A. rudis* in mixed hardwood forests with dense leaf litter. There are two important factors to consider prior to the use of our tracking technique. First, one must consider the depth at which the dispersal vector deposits the seeds. If the depth is greater than 4cm, then decreased recovery is possible. Second, one must consider the size of the search area. We scanned an area of

~7m^2, which required an average of 5 hours of search effort between two people. For larger search areas, the technique may become impractical due to the small size of the wand detector. However, with sites slightly larger than or smaller than our established search area, the tracking technique may be a useful tool for seed recovery.

2.4.1 Comparison to other seed tracking techniques

The use of a tracking technique always depends on the question of interest (see Bullock et al. 2006) and the size and dispersal vector of the seed (Forget and Wenny 2005). Though internal labeling methods similar to our method exist (reviewed in Forget and Wenny 2005), they are not suitable for small seeds with a small dispersal vector. Therefore, the only other seed tracking technique comparable to the method we describe here is the use of radiolabels to mark and track seeds within the leaf litter (Kalisz et al. 1999). The radiolabel method offers the capability of searching large areas, but requires the use of expensive (~$2 per radiolabeled seed versus $0.36 per seed for Coded Wire Tags (http://www.nmt-inc.com); price quote for radiolabel from PerkinElmer 3/24/2010) and heavily regulated equipment (Bullock et al. 2006). In contrast, our technique is not regulated and less costly, especially since equipment is often available to rent ($415/month for Handheld Wand Detector) or borrow and recovered tags are reusable. Both methods may have false detections associated with search effort either because of the presence of metallic materials in our method or because of traces of the radioisotope coming off the radiolabeled seeds. In addition, the recovery rates for both techniques are similar, 63% for our study (within one year) and 63 and 73% for radiolabels (over two years; Kalisz et al. 1999). Therefore, for studies of short-

distance dispersal, we contend that the method we developed is a less expensive and a less regulated method than radiolabeling methods and a useful alternative to other tracking techniques.

2.4.2 Improvement of the technique

We offer several suggestions for improvement of the technique for future studies. First, we experienced a high volume of false detections if there were high concentrations of metallic materials in the soil, a common problem with magnetic tagging techniques for large seeds (Alverson and Diaz 1989). The frequency of false detections increased the search effort necessary to fully scan and excavate a site (upwards of 8-10 hours total for two people). More importantly, the presence of the metallic materials may decrease the recovery rate of tagged seeds, as indicated by the lower recovery rates at those sites. We recommend an initial scan of the potential search area to minimize the potential for false detections, to minimize search effort, and to increase recovery rate of tagged seeds.

Our second suggestion is to employ multiple wand detectors per site. The increase in searchers and detectors may allow for a larger search area and decrease the time necessary to search a site. Third, we did encounter some tag loss (8.9%). We could reduce this number with extra care in proper tag injection. We often observed exposed tags at the former location of the removed elaiosome. Therefore, it may be useful to seal the injection site, or even coat the tag prior to injection with a clear, odorless form of glue to minimize the possibility of tag loss.

2.4.3 Further applications

We suggest that the best use of the technique is for studies of short-distance dispersal, because of the restrictions on the search range possible with the required equipment. We do offer the following suggestions for other possible applications. The use of anticides on baits as a method of eradication requires the poison reaches the brood and queen. If researchers tagged baits, they could observe the location of the majority of the tags upon excavation of the nest after ant consumption of the bait to be sure the bait choice reached the necessary location. Tagged baits may also be useful to track which ant species exploit different types of bait. A scan of the area around the baits would detect which species took the baits by the presence of the tags in the nest. In addition, each tag has an individual code on it. The individual code on each tag would also allow researchers to determine which type of bait different ant species exploited or the original location of the bait. Other uses for the technique are up to the imagination of the user.

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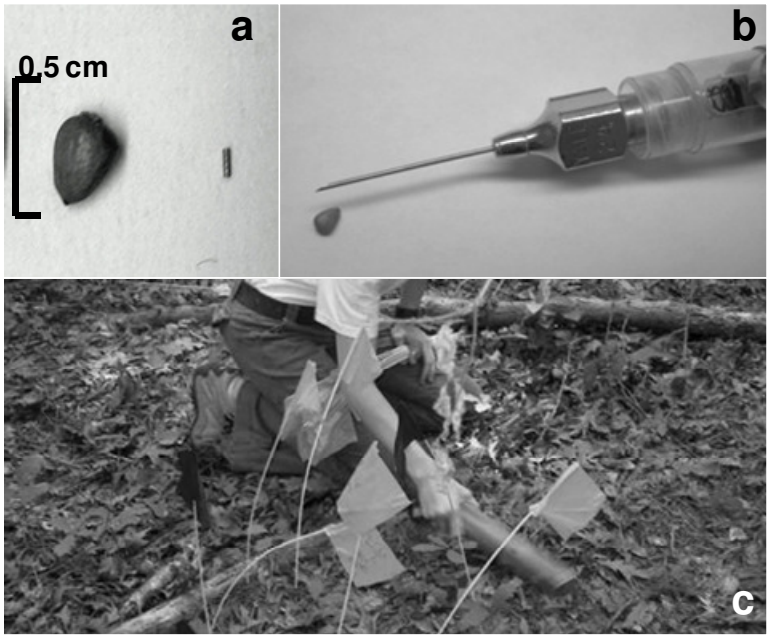

Figure 2.1

Figure 2.1: a) A seed and Coded Wire Tag with scale, b) the Single-Shot Tag Injector and seed, c) the Handheld Wand Detector in use during the search process.

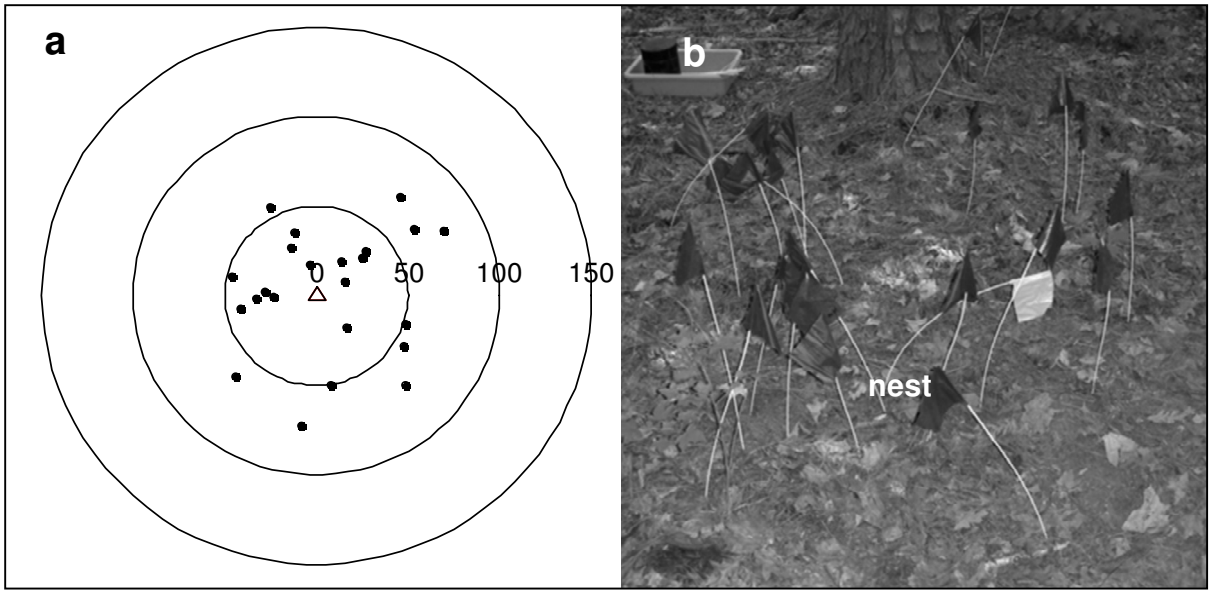

Figure 2.2

Figure 2.2: a) The locations of individual seeds (dark circles) found in relation to the nest (triangle) we fed the seeds, and b) the excavated nest site, dark flags denote positive seed detections, the light flag is the nest location. We measured the radial distance from the nest to the seed for all nests. For this nest, the mean redispersal distance was 44.67cm, with a standard deviation of 19.05cm

CHAPTER 3

How do we measure the response of species interactions to climate change? The use of models and experiments to study myrmecochory

3.1 Introduction

A key issue in the study of climate change is the assessment of not just the influence of warming on individual species, but on the interactions between species, especially those interactions that provide important services to each species (Tylianakis et al. 2008; Gilman et al. 2010; Walther 2010). Species interactions, and more specifically mutualisms, play an important role in the structuring of communities (Stachowicz 2001; Gilman et al. 2010). Climate change may lead to the spatial and temporal decoupling of mutualists (Harrington et al. 1999) and may reduce the interaction strength in mutualisms, especially with plants (Tylianakis et al. 2008). In particular, the impact of warming on plant phenology, specifically flowering and fruiting timing, may disrupt their relationship with pollinators and dispersers (Sparks et al. 2000; Memmott et al. 2007; Hegland et al. 2009; Gilman et al. 2010; Walther 2010). In addition, the effects of warming on the insect partners of plants may lead to changes in insect foraging phenology and abundance. There are four general approaches to the study of climate change on ecosystems and species interactions: climate gradient studies, long-term observations, climate manipulation experiments and modeling (Rustad 2008). We focus our study on the two latter approaches in order to examine the effects of climate change on myrmecochory, the dispersal of seeds by ants.

The use of experimental treatments that mimic either global warming or some other feature of global change (such as CO_2 enrichment) is a common approach to understanding the influence of species in isolation or in concert with their partners (reviewed in Rustad 2008; see also Sherry et al. 2007). Such experiments are often manipulation or press experiments, in which fixed temperature increases above ambient temperatures reflect future warming scenarios, generally based on IPCC (Intergovernmental Panel on Climate Change) projections (reviewed in Rustad 2008). The results from short-term experiments, though, may be transient, and may not reflect the change in the magnitude or direction of the response over time (Brooker et al. 2007; Rustad 2008). In a climate-controlled chamber that represents conditions likely to exist in 2100, a hundred years pass immediately, as soon as the treatment is applied. We therefore cannot observe all of the behavioral or physiological responses available to species or demographic and evolutionary shifts that may negate or add to the possible detrimental effects of climate change (Memmott et al. 2007; Rustad 2008; Hegland et al. 2009) and extrapolation of short-term experiments may lead to inaccurate predictions of future ecosystem responses (see Rustad 2008 for examples). Therefore, though short-term experiments provide insight into the current and, to some extent, future responses of mutualists to climate change, modeling is necessary to improve our understanding of the differences between short and long-term responses to warming (Classen and Langley 2005; Brooker et al. 2007; Rustad 2008).

There have been several theoretical approaches to studying the responses of mutualisms and more generally species interactions to perturbations such as climate change. For example, Brooker et al. (2007) modeled the future spatial distribution of mutualist species with climate change using bioclimatic envelope models. In addition, Memmott et al. (2007) used a simulation approach to project the future temporal overlap of plants and their pollinators. Neither approach considered (though both recognized) the possible display of behavioral or physiological plasticity of species under the pressures of climate change (Memmott et al. 2007; Walther 2010). In addition, Memmott et al. (2007) did not consider the effects of warming on pollinator abundance, which may play a role in the persistence of the interaction (Memmott et al. 2007), especially in specialized interactions which are more sensitive to partner loss (Gilman et al. 2010).

The theoretical study of climate change and species interactions is still in its infancy (Gilman et al. 2010; but see also Visser et al. 2006 for example), as is the integration of climate change experiments and models more generally (Classen and Langley 2005; Rustad 2008). To date, a majority of the theoretical explorations of the effects of climate change on insect-plant mutualisms are in the context of plant-pollinator interactions (Memmott et al. 2007; Tylianakis et al. 2008; Hegland et al. 2009; Walther 2010). Less attention has been paid to seed dispersal (Tylianakis et al. 2008). Myrmecochory, the dispersal of seeds by ants, is both geographically widespread and ecologically important (Giladi 2006; Lengyel et al. 2009; Lengyel et al. 2010), but has not received the same theoretical attention as plant-pollinator

interactions, both in general and in the context of climate change. Therefore, we focus our study on the impacts of climate change on myrmecochory.

Here, we study the dynamics of myrmecochory and consequently myrmecochore (plants dispersed by ants) population dynamics under climate change. In addition, we examine the possible disparity between the response of myrmecochore populations to gradual climate change and predictions based on the integration of experimental data and models of species interactions and climate change. We conduct our study in two parts. First, we develop a model of the daily interactions between ants and plants in which interactions depend on the timing of the fruit dehiscence and seasonal ant foraging and abundance. The model will reflect the changes in plant population dynamics because of changes in seed dispersal by ants that arise from the effects of warming. From our model, we will examine which temperature-sensitive characteristics of myrmecochory have the most influence on plant population dynamics and project the response of plant population dynamics over 100 years of gradual warming. Second, we simulate the dynamics of a press warming experiment and use the experimental results to inform two model methods of prediction of myrmecochore population dynamics under warming. Our focus on myrmecochory compliments a new press warming experiment termed the Future Life Project (FLP) in Duke Forest, NC, which we use as the basis of our simulated press warming experiment. We compare model projections and dynamics with gradual warming to the two projected plant population dynamics based on the outcomes of our simulated press warming experiment. We then discuss the use of press warming experiments to inform models in the study of climate change and species

interactions and the cautions and caveats of our simulation study of myrmecochory. Though we develop our model in the context of a specific biological system, myrmecochory, our results have elements that generalize to press experiments and their limits more generally.

3.2 Overview of climate change and myrmecochory

To model the response of myrmecochores to climate change and climate change experiments, a first step is to determine how increased temperatures affect the myrmecochore fruiting phenology and ant foraging phenology. In eastern North America, the species *Aphaenogaster rudis* disperses the majority of seeds of herbaceous understory plants in temperature deciduous forests (Ness et al. 2009), and dispersal varies with *A. rudis* abundance (Zelikova et al. 2008). In addition, the peak date of fruit dehiscence of myrmecochores coincides with the peak of *A. rudis* foraging (Oberrath and Böhning-Gaese 2002; Giladi 2006; Ness et al. 2009). Warming may affect the temporal coupling of the relationship between ants and plants due to temperature dependent changes in the dynamics of both fruiting phenology and foraging phenology. We use the temperature-dependent characteristics of plant phenology (first date of fruit dehiscence), ant phenology (foraging season length) and population dynamics (ant colony density) as guidelines for our development of a model of myrmecochory.

3.2.1 Warming and fruiting phenology

Examination of historical records yields evidence of shifting flowering and fruiting phenologies for a variety of plant species worldwide (Fitter et al 1995; Sparks et al. 2000; Abu-Asab et al. 2001; Menzel 2002; Badeck et al. 2004; Tryjankowski et al. 2006; Sherry et al. 2007). Though there are many possible triggers of flowering time (e.g., temperature, precipitation, CO_2 stores, and photoperiod), both experimental studies (e.g., Sherry et al. 2007) and observational studies (e.g., Tryjankowski et al. 2006) that control for precipitation and photoperiod indicate temperature as the main driver of flowering time (see also Sparks et al. 2000; Badeck et al. 2004). In general, warming advances spring flowering and fruiting time for spring bloomers (Fitter et al. 1995; Sparks et al. 2000; Fitter and Fitter 2002; Badeck et al. 2004; Tryjankowski et al. 2006; Sherry et al. 2007), including myrmecochores (Abu-Asab et al. 2001; Appendix III). Warming also advances the timing of fruit dehiscence, which will have consequences on the subsequent dispersal, germination, and survival of the seed (Giladi 2006; Tryjankowski et al. 2006; Kudo et al. 2008; Galloway and Burgess 2009). The advancement of myrmecochore fruiting phenology may alter the timing of the relationship with the ant foraging phenology, possibly altering the population dynamics of myrmecochores in eastern North America.

3.2.2 Warming and foraging phenology

Current predictions state that ants may be more susceptible to disturbance and climate change than other, non-social species (Chapman and Bourke 2001). In eastern North American temperate deciduous forests, *Aphaenogaster rudis* is the keystone disperser of up to 70% of

herbaceous understory plants (Ness et al. 2009). It ranges north into Ontario (Offer 1992), south into Georgia (Giladi 2004) and west into Missouri (Talbot 1951). Temperature explains the seasonal patterns of foraging phenology for *A. rudis* (Lynch et al. 1980; Kaspari et al. 2000; Dunn et al. 2007; Lessard et al. 2009) and *A. rudis* abundance and colony density varies with temperature and season (Herbers 1989; Zelikova et al. 2008). *A. rudis* requires a minimum temperature of 15˚C to develop (Southerland 1988) and forage (Lynch et al. 1980; Lessard et al. 2009) and has a maximum thermal tolerance estimated between 30˚C and 40˚C (Lynch et al. 1980; Southerland 1988; Kaspari et al. 2000; Lessard et al. 2009). In addition, *A. rudis* hibernates in the winter (Talbot 1951) and uses winter as a metabolic refuge (Kaspari et al. 2000). Warming may result in the loss of that metabolic refuge, cause an increase in the occurrence of temperatures above the maximum thermal tolerance of *A. rudis* and lead to diminished colony density and worker abundance, even to extinction.

3.2.3 Plant population dynamics and dispersal

We will focus on the deciduous forest understory myrmecochore, *Asarum canadense* to provide a biologically meaningful context for our study. *Asarum canadense* is widespread and common throughout eastern North America. The natural range of *A. canadense* is throughout eastern North America, from north in New Brunswick to south in North Carolina, and west into eastern Kansas (Heithaus 1986; Smith et al. 1989; Offer 1992; Cain and Damman 1997; Damman and Cain 1998). We chose *A. canadense* as our focal species because of the wealth of information available on its life history and demography (Baskin and Baskin 1986; Lobstein and Rockwood 1993; Cain and Damman 1997; Damman and

Cain 1998), fruiting phenology (Smith et al. 1989; Abu-Asab et al. 2001), and dispersal of seeds (Heithaus 1986; Smith et al. 1989; Offer 1992).

Life history of Asarum canadense

Asarum canadense blooms in the early spring. The flowers self-pollinate (Cain and Damman 1997) and the fruit capsules dehisce in mid-May in the southeast (personal observation for North Carolina) and early to mid-June in the north and mid-west (Smith et al. 1989; Offer 1992). The fruit is located the base of the plant and opens to reveal approximately 10-30 seeds, with a mean of 11 seeds per fruit (Baskin and Baskin 1996; Offer 1992; Lobstein and Rockwood 1993; Cain and Damman 1997). Each seed bears a fleshy appendage called an elaiosome, which attracts ants to disperse the seeds. The seeds exhibit temperature-dependent epicotyl dormancy (Baskin and Baskin 1986; Offer 1992; Lobstein and Rockwood 1993), in which radical dormancy is broken by high summer temperatures and shoot dormancy is broken by low winter temperatures (Baskin and Baskin 1986).

In the first year, the seedlings produce two cotyledons, but no true leaves (Baskin and Baskin 1986). In the second year seedlings develop into a one-leaf ramet. After transition to maturity, *A. canadense* produces either one or two leaves each year. Adults generally reproduce sexually as two-leaved ramets and produce, on average, a single fruit (Cain and Damman 1997). *A. canadense* also reproduces asexually (Baskin and Baskin 1986; Cain and Damman 1997; Damman and Cain 1998), through the production of lateral shoots. The lateral shoots do not grow rapidly and do not greatly contribute to sexual reproduction or

dispersal (Baskin and Baskin 1986; Cain and Damman 1998), but they do contribute to the natural density of ramets in temperate forests.

3.3 Methods I: Model development

We developed a model that allows us to observe the influence of the climate sensitive characteristics (fruiting phenology, ant foraging phenology and abundance) on the population dynamics of myrmecochores. We wanted to be able to base the parameterization of our model on available data when possible. Therefore, we first extended a model of the population demography of our focal myrmecochore, *A. canadense*, so that it depends on the fraction of seeds dispersed each year. We then modeled the fraction of seeds dispersed each year as a function of the fruiting phenology, ant foraging phenology and ant abundance, and we modeled each as a function of temperature. We then observed the overall effects of increases in average temperature (above current conditions) on the population density and dynamics of *A. canadense.* We parameterized our model with the best available data from studies across eastern North America, but we formulated our model with North Carolina temperate deciduous forests as our focal location to compliment the Future Life Project in Duke Forest, NC. We now provide the details of the model.

3.3.1 Demographic model of *A. canadense*

We adapt the density-independent population projection matrix model of *A. canadense* based on data collected by Cain and Damman (1997). Cain and Damman (1997) conducted a

demographic study of *A. canadense* populations near Ontario, Canada from 1989 up to and through 1995. We utilize the same stage structure for our population as Cain and Damman (1997), who consider four stages, seedling, n_0, yearling, n_1, lateral shoots, n_L, and one and two-leaf ramets (mature), n_2,where n_i is the density of each stage (number/m^2) (Fig. 3.1). We denote the vector of the stage-structured population as $\mathbf{n} = \begin{bmatrix} n_0 & n_1 & n_L & n_2 \end{bmatrix}^T$. We selected a population projection matrix, **A,** averaged among years and sites based on data from the long-term demographic study (Cain and Damman 1997; Table A1.1, Appendix II) such that the growth rate of the density-independent matrix was positive (i.e., $\lambda > 1$). The matrix model structure is

$$\mathbf{A} = \begin{bmatrix} 0 & 0 & \phi_L & \phi_2 \\ a_{01} & 0 & 0 & 0 \\ 0 & 0 & \sigma_{LL} & \sigma_{2L} \\ 0 & a_{12} & a_{L2} & a_{22} \end{bmatrix}$$

where a_{ij} represent the transition rates between stages, σ_{iL} is the reproduction of new lateral shoots and ϕ_i is the fecundity (seedling production). The model for population density within year *t*+1 is

$$\mathbf{n}(t+1) = \mathbf{A}\mathbf{n}(t) .$$

We modify the above matrix model to incorporate density-dependence at the seedling stage. The dispersal of seeds by ants can have direct effects on the germination and survival of seedlings, such as reduced density-dependent effects through dispersal distance, reduction in

predation and dispersal to a favorable germination site (reviewed in Giladi 2006). In addition, clumped seedlings in other perennial herbs have higher rates of mortality (Gonzalez 1972) and exhibit higher mortality due to density-dependent effects (Solbrig et al. 1988; Giladi 2004; Zelikova 2008). Therefore, we consider dispersal successful when ants disperse seeds to an unoccupied 'patch'. To incorporate the relationship between density and dispersal, we use the Ricker model for density-dependence, which we may interpret as the probability of dispersal to an unoccupied patch, where dispersal to a patch is a Poisson process.

To incorporate the effects of warming on fruiting phenology and foraging phenology on the fecundity of the mature and lateral shoot stages, we must continue to deconstruct fecundity as follows. The parameters that define fecundity are the germination and survival to the seedling stage of ant dispersed seeds, γ, the average number of seeds per fruit, σ, the proportion of individuals within stage *i* that reproduce, κ_i, and the fraction of seeds dispersed by ants within a given year, $\beta[\mathbf{n}, P]$. The term $\beta[\mathbf{n}, P]$ depends on the fruiting phenology and density of the plant ($\mathbf{n}$; plants/m^2) and the foraging phenology and density of the ants (P) within year *t* (see section 3.3.4). We therefore construct the fecundities for lateral shoots and mature one and two-leaved ramets as follows

$$\phi_2(\mathbf{n}, P) = \beta[\mathbf{n}, P]\gamma\kappa_2\sigma e^{-\omega N} \quad (1)$$

$$\phi_L(\mathbf{n}, P) = \beta[\mathbf{n}, P]\gamma\kappa_L\sigma e^{-\omega N} \quad (2)$$

where ω is strength of the density dependence and N is the total population size. We chose $\omega = 0.04$ such that the probability of dispersal to an unoccupied patch is close to zero when N is 100 plants/m^2 (based on Offer 1992).

The reported average number of seeds per fruit is $\sigma = 11$ (Baskin and Baskin 1996; Offer 1992; Lobstein and Rockwood 1993; Cain and Damman 1997). In laboratory experiments, Lobstein and Rockwood (1993) found germination rates of 51.7% to 91.7% for seeds without elaiosomes. In contrast, Heithaus (1986) found a relationship of 0.178 new seedlings for every one ant-dispersed seed. To account for possibly less than ideal field conditions as compared to laboratory conditions and the observations of Heithaus (1986), we assume an intermediate value of $\gamma = 0.35$ as the rate of germination and survival to the seedling stage. Within a given year, approximately 10-16% of the adult population successfully reproduces (Cain and Damman 1997; Heithaus 1986). We chose $\kappa_2 = 0.10$, the value reported by Cain and Damman (1997), because it is from both a longer-term demographic study and the study we use to parameterize the rest of our demographic rates. We let $\kappa_L = 0.04$ also based on data from Cain and Damman (1997). We defined all other demographic parameters based on the study of *A. canadense* by Cain and Damman (1997) (Table A1.1, Appendix II). Therefore, our density-dependent population projection matrix is

$$\mathbf{A} = \begin{bmatrix} 0 & 0 & \phi_L & \phi_2 \\ a_{01} & 0 & 0 & 0 \\ 0 & 0 & \sigma_{LL} & \sigma_{2L} \\ 0 & a_{12} & a_{L2} & a_{22} \end{bmatrix} = \begin{bmatrix} 0 & 0 & 0.154\beta[\mathbf{n}, P]e^{-0.04N} & 0.385\beta[\mathbf{n}, P]e^{-0.04N} \\ 0.5 & 0 & 0 & 0 \\ 0 & 0 & 0.015 & 0.155 \\ 0 & 0.73 & 0.7025 & 0.8275 \end{bmatrix}$$

If we assume a fixed proportion of dispersed seeds each year, i.e., $\beta[\mathbf{n}, P] = b$, we can then solve $\mathbf{n} = \mathbf{An}$ for n_0, n_1, n_L and n_2 to determine the stage-specific population density at equilibrium, $\hat{n}_0, \hat{n}_1, \hat{n}_L$ and $\hat{n}_2$, as a function of the demographic parameters. If we then sum $\hat{n}_0$, $\hat{n}_1, \hat{n}_L$ and $\hat{n}_2$, we can find the total population density at equilibrium, *N*, as

$$N = \frac{1}{\omega} \ln \left[\frac{b\sigma\gamma a_{01} a_{12} \left(\sigma_{2L} \kappa_L + \kappa_2 \left(1 - \sigma_{LL} \right) \right)}{\left(1 - a_{22} \right)\left(1 - \sigma_{LL} \right) - \sigma_{2L} a_{L2}} \right] \tag{3}$$

The equilibrium is stable if the modulus of the dominant eigenvalue of the Jacobian matrix evaluated at the equilibrium, $\hat{n}_0, \hat{n}_1, \hat{n}_L$ and $\hat{n}_2$, or

$$\begin{bmatrix} -b\sigma\gamma e^{-N} & 0 & 0 & 0 \\ 0 & 1 & 0 & 0 \\ 0 & 0 & 1 & 0 \\ 0 & 0 & 0 & 1 \end{bmatrix} \bullet \begin{bmatrix} \kappa_L \hat{n}_L + \kappa_2 \hat{n}_2 & \kappa_L \hat{n}_L + \kappa_2 \hat{n}_2 & \left(\hat{n}_L + 1 \right) \kappa_L + \kappa_2 \hat{n}_2 & \kappa_L \hat{n}_L + \kappa_2 \left(\hat{n}_2 + 1 \right) \\ a_{01} & 0 & 0 & 0 \\ 0 & 0 & \sigma_{LL} & \sigma_{2L} \\ 0 & a_{12} & a_{L2} & a_{22} \end{bmatrix}$$

is less than unity. We can then derive the criterion for a positive equilibrium of population density as

$$\ln \left[\frac{b\sigma\gamma a_{01} a_{12} \left(\sigma_{2L} \kappa_L + \kappa_2 \left(1 - \sigma_{LL} \right) \right)}{\left(1 - a_{22} \right)\left(1 - \sigma_{LL} \right) - \sigma_{2L} a_{L2}} \right] > 0 \tag{4}$$

which we can use to evaluate the effect of varying parameters on the population density and the existence of a positive population density at equilibrium. If we rewrite our criterion in Eq. 4, we find

$$\frac{\left(1 - a_{22} \right)\left(1 - \sigma_{LL} \right) - \sigma_{2L} a_{L2}}{a_{01} a_{12} \left(\sigma_{2L} \kappa_L + \kappa_2 \left(1 - \sigma_{LL} \right) \right)} < b\sigma\gamma \tag{5}$$

We observe that there exists a minimum threshold that the density of dispersed seedlings, $b\sigma\gamma$, must exceed in order for the population to persist. An increase in reproduction, through κ_2 and κ_L, or increased survival rates from seedling to yearling to maturity will decrease the minimum threshold for seedling development such that the population maintains a positive population density at equilibrium. Finally, we observe that a diminished death rate at maturity, $1-a_{22}$, also decreases the minimum threshold of seedling development on the population density equilibrium.

3.3.2 Model of daily fruit dehiscence of *A. canadense*

To account for the possible advancement of myrmecochore fruiting seasons, we adopt logistic cumulative distribution function, first proposed by Kjellsson (1985) as typical of woodland herbaceous species, to determine the daily cumulative fruit dehiscence,

$$F(\tau) = \frac{1}{1+e^{-b_1(\tau-T_1)}} \tag{6}$$

where $F(\tau)$ is the cumulative proportion of fruits dehisced on day τ, b_1 is the scale parameter that controls the length of the fruiting season, and T_1 is the date of peak fruit dehiscence. We then determine the density of seeds available, N_τ, on a given day, τ, in year *t* as

$$N_\tau = \sigma\left[\kappa_2 n_2(t) + \kappa_L n_L(t)\right]\left(F(\tau+1) - F(\tau)\right) \tag{7}$$

where $\kappa_2 n_2(t) + \kappa_L n_L(t)$ is the density of reproductives within a year, *t*.

We fit our model of cumulative daily fruit dehiscence for the myrmecochore *A. canadense* to data from Smith et al. (1989). We used nonlinear least squares regression and found $b_1 = 0.335$ (se=0.081), which determines the length of the fruiting season for *A. canadense* (Fig. 3.2a). The start date for the fruiting period of *A. canadense* ranges from mid-May to mid-June depending on geographic location (Smith et al. 1989; Offer 1992; Abu-Asab et al. 2001). In our model, we modify the peak date of dehiscence, which causes an equivalent shift in the onset of fruit dehiscence. We construct our baseline based on the system in North Carolina and set our peak in early June, or $T_1 = 160$ (personal observation).

Temperature dependent parameters

Our model of fruit dehiscence allows us to advance the peak date of the fruiting season if we decrease the value of the parameter, T_1. We can rewrite our model of fruit dehiscence as

$$F(\tau) = \frac{1}{1 + e^{-b_1(\tau - T_1 + \tau_0(\theta))}} \tag{8}$$

where $\tau_0(\theta)$ is the number of days the peak advances and θ is the degree increase in temperature in a given year relative to current temperatures. In general, first flower dates advance 2-6 day per 1˚C, with an overall mean of 4 days, based on studies across temperate regions of the United States and the United Kingdom (Fitter et al. 1995; Spark et al. 2000; Abu-Asab et al. 2001; Fitter and Fitter 2002; Memmott et al. 2007). If we assume the more general average prediction of a 4-day advancement for every degree increase in temperature

(Fitter et al. 1995), then we must decrease T_1 by four days for every degree increase in temperature, or $\tau_0(\theta) = 4\theta$.

Stochastic extension

Historically, first bloom dates (and first fruit dehiscence dates) for spring-blooming species are highly variable from year to year (Fitter et al. 1995; Kudo et al. 2008). The distribution of the advance in first bloom date is approximately normal with a standard deviation equal to the mean (Sparks et al. 2000; Fitter and Fitter 2002). Therefore, we consider stochasticity in the advance of the peak date of fruit dehiscence through a simple Gaussian distribution,

$$\tau_0 \sim Normal(4\theta, 4).$$

where four is the standard deviation reported by Fitter et al. (1995). The value of 4θ is the number of days, on average, the first (or peak) fruit dehiscence day advances for a specific temperature increase, θ. If τ_0 takes on a negative value, then the result is a delay in the peak date of fruit dehiscence.

3.3.3 Model of daily seed dispersal

We now develop the function for $\beta[\mathbf{n}, P]$, the proportion of seeds dispersed over an entire year, which depends on both the fruiting phenology and ant foraging phenology. First, though, we must determine a model for daily seed dispersal. We adopt the Type II functional

response to model the depletion of seeds over the course of a single day as a function of the density of seeds available, N_τ and colony density, P_τ on a given day, τ, within a year, t

$$\frac{dS_\tau(\varsigma)}{d\varsigma} = -\frac{\alpha S_\tau(\varsigma) P_\tau}{1+\alpha h S_\tau(\varsigma)}, \quad S_\tau(0) = N_\tau \tag{9}$$

where $S_\tau(\varsigma)$ is the instantaneous density of available seeds (not yet dispersed) at time ς (days), h is the 'handling time', and α is the search efficiency. The solution to the ordinary differential equation is

$$S_\tau(\varsigma) = \frac{W\left(\alpha h N_\tau e^{\alpha h N_\tau - \alpha P_\tau \varsigma}\right)}{\alpha h} \tag{10}$$

where W is the Lambert (product log) function which is the solution to $z = W(z)e^{w(z)}$. Therefore, the density of seeds available (not dispersed) at the end of day τ is $S_\tau = S_\tau(1)$.

The Type II functional response is often used in the context of predator-prey models, but it has been applied to pollination and seed parasitism as well (DeAngelis and Holland 2006). We adapt it as a phenomenological model to account for the effects of saturation for a single colony (Heithaus et al. 2005). The model assumes the random distribution of seeds, a valid assumption since *A. rudis* do not exhibit behavioral responses (changes in speed or efficiency) to prey distribution (random, clumped or hyper-distributed) in general (Leonard and Herbers 1986) and exhibit low levels of recruitment (Lynch et al.1980).

As $S(\zeta)$ gets larger, then the functional response approaches $1/h$. Therefore, we can define h as one divided the maximum possible number of seeds a colony can disperse within a day with seeds place directly in front of the nest entrance. In a given day, a colony of *A. rudis* may disperse on average 50-80 seeds (Heithaus et al. 2005) when seed supplies are unlimited and there is no necessity to search. Therefore, if we assume the maximum number of seeds that a colony can disperse in a single day is 50 seeds (Heithaus et al. 2005), we can then determine $h = 0.02$.

We define α as the 'search efficiency' or 'attack rate' of an individual colony. Since we do not possess data to quantify α in the usual manner (non-linear least square regression), we choose α based on our knowledge of the behavior of the system. At low seed densities, the colony will disperse seeds at a rate proportional to the attack rate. If we observe that at high densities of *A. rudis* we have nearly complete dispersal, even for low densities of seeds (Zelikova et al. 2008), we choose a value of $\alpha = 3$ which allows for a dispersal rate of ~90-95% at low seed densities when $P_\tau = 1$ colony/m^2.

We assume that only seeds dispersed within 24 hours of dehiscence are viable for germination and predators destroy the remaining seeds (Heithaus 1986). We can now define the fraction of seeds dispersed by ants in year t, $\beta[\mathbf{n}, P]$, as

$$\beta[\mathbf{n}(t), P] = 1 - \frac{\sum_{\tau=0}^{365} S_\tau}{\sigma\left(\kappa_2 n_2(t) + \kappa_L n_L(t)\right)} \tag{11}$$

3.3.4 Ant foraging phenology and population dynamics

We know that both foraging phenology (Lynch et al. 1980; Dunn et al. 2007; Lessard et al. 2009) and colony density (Herbers 1989) of *A. rudis* vary with season and temperature. For simplicity, we assume that colony and foraging phenology is a function of seasonality, though certain parameters may depend on average temperatures. We assume forager density and colony density are directly proportional and therefore we simply model colony density over the foraging season. We adapt the logistic probability density function as a functional basis for a convenient phenomenological model of the seasonality of *A. rudis* colony density (Lynch et al. 1980; Dunn et al. 2007), such that

$$P(\tau) = \frac{4ae^{-b_2(\tau - T_2)}}{\left(1 + e^{-b_2(\tau - T_2)}\right)^2} \tag{12}$$

where $P(\tau)$ is colony density on day τ, T_2 is the date of the peak of density in a year, b_2 determines the length of the foraging season, and a is the density of *A. rudis* colonies at the peak of the foraging season. We assume that colony density does not depend on the presence or density of myrmecochores (Ness et al. 2009).

We fit our model to data from Dunn et al. (2007) who measured a proxy of *A. rudis* density over several years in forests in North Carolina and Tennessee. Dunn et al. (2007) sampled eleven 30m transects each month over the course of two years with pitfall traps. We used

nonlinear least squares regression to fit our model to the relationship between date and foraging activity and found $T_2 = 176$ (se=4.866) and $b_2 = 0.035$ (se=0.003) (Fig. 3.2b). The reported densities of *A. rudis* colonies range from 0.38 colonies/m^2 (West Virginia; Smallwood 1982), 0.50 colonies/m^2 (Ohio; Morales and Heithaus 1998), and 1.55 colonies/m^2 (Missouri; Talbot 1957). We choose the intermediate value of $a = 1$ colony/m^2 for the baseline case of our model (based on personal observation), which also allows for almost complete dispersal of seeds, a response which we have observed throughout temperate deciduous forests in the southeast (Zelikova et al. 2008; Canner unpublished data), where *A. rudis* is common.

Temperature dependent parameters

The advantage of this model is that we can easily control the two parameters of foraging phenology that will most likely vary with warming: the length of the foraging season (b_2), and the peak colony density (a). We define the relationship between b_2 and the length of the foraging season as $L = 2.634/b_2$, where L is the distance between the inflection points of our adapted logistic distribution. Based on our current parameterization of b_2, we calculate $L = 75.255$ days. We can then write b_2 as

$$b_2 = \frac{2.634}{75.255 + \Delta L} \tag{13}$$

where ΔL is the change in the length of foraging season. We estimate the increase in foraging season length through a simple observation of the number of days each year that

have a mean daily temperature above 15˚C, the minimum temperature necessary to observe sufficient foraging (Lynch et al. 1980; Lessard et al. 2009). If we increase the mean daily temperature 1˚C, the length of the foraging season increases approximately 12 day (based on 30-year average daily temperatures for the Raleigh-Durham, NC area; http://www.erh.noaa.gov). Thus, we can define $\Delta L = 12\theta$ and

$$b_2(\theta) = \frac{2.634}{75.255 + 12\theta} \tag{14}$$

where θ is the temperature increase above current temperatures.

Finally, we estimate how the density of *A. rudis* colonies will increase or decrease as a response to warming. We observe that the relationship between mean monthly temperature and the percent occupancy by *A. rudis* along an elevational gradient is a downward concave quadratic relationship (unpublished data, RR Dunn). Similar patterns have been observed for *A. rudis* foraging relative to temperature (Lynch et al. 1980). We then assume a phenomenological model for the change in colony density as a function of temperature of the form,

$$a(\theta) = a_0 - \left[\rho\theta^2 + \upsilon\theta\right] \tag{15}$$

where a_0 is the initial density of *A. rudis* colonies without warming, θ is the temperature increase, and ρ and υ are constants that define the shape of the curve. If we assume that *A. rudis* goes extinct with warming of 6˚C in the southeast, but warming of 1˚C will only

slightly decrease colony density (from $a = 1$ to 0.9 colonies/m^2), then $\rho = 0.013$ and $\nu = 0.087$.

Stochastic extension

Events such as unusually warm days, unusually cold days and rain all contribute to variability in daily ant forager density (Lynch et al. 1980; Lessard et al. 2009). For instance, a reduced daily temperature or precipitation leads to lower levels of foraging (Lessard et al. 2009), while a raised daily temperature, especially in the late spring and early summer, causes increased levels of foraging. Therefore, we can incorporate stochasticity in daily ant foraging levels through the incorporation of noise,

$$P^*(\tau) \sim Normal\left(P(\tau), P(\tau)/3\right)$$

such that we now determine the colony density on day τ as

$$P_\sigma(\tau) = \min\left(0, \max\left(P^*(\tau), a\right)\right)$$

We chose the standard deviation of $P^*(\tau)$ to be proportional to the average daily density such that $P^*(\tau)$ would approach zero (such as on a rainy day) with low probability. In addition, the maximum of $P^*(\tau)$ would rarely approach three times the daily ant colony density, with a cap at a, the peak possible colony density.

3.4 Results I: Model analysis

We conducted all model analysis and simulations with R Statistical Software (R Development Core Team 2009).

3.4.1 Plant population dynamics

With our model, for the baseline case of all determined parameters, i.e., no warming, we determine a plant population density for *A. canadense* of 20.23 plants/m^2 at equilibrium, with 14.33 mature plants/m^2. If we incorporate stochasticity in the yearly fruiting dates, the expected (average) density at equilibrium does not change significantly (20.19 plants/m^2). If we incorporate stochasticity in daily foraging, the expected density at equilibrium is 19.06 plants/m^2, with 13.5 mature plants /m^2. With both modes of stochasticity, the plant population density is 19.08 plants/m^2 on average. The densities of *A. canadense* at equilibrium based on our model are comparable to reported natural densities of *A. canadense* (15 plants/m^2; Heithaus 1986; Offer 1992).

Vegetative reproduction and climate change

Pudlo et al. (1980) suggest that a decrease in ant dispersal negates the advantages of sexual reproduction for *S. canadensis*, and turns seed production into a poor energetic investment. Therefore, Pudlo et al. (1980) expect an increase in vegetative reproduction when ant densities are low. If we consider this same possibility for *A. canadense,* at low ant densities, a likely outcome of warming, *A. canadense* populations may persist due to increased

vegetative reproduction as a response to increased competition with offspring due to a lack of dispersal. Though we do not have the means to quantify the transient dynamics of increased vegetative reproduction with diminished dispersal, we can determine the necessary increase in vegetative reproduction, σ_{2L}, at low levels of seed dispersal, b, with the equilibrium criteria of our model such that our population persists (Fig. 3.3). We see that at lower levels of seed dispersal (i.e., lower ant colony density), the minimum threshold for vegetative reproduction must increase in order to maintain a positive equilibrium. Notice, though, that there is also an upper threshold on vegetative reproduction such that a stable equilibrium no longer exists. For values above that threshold, the population increases without bound as the population no longer responds to density-dependent effects, but acts as a density-independent population. We do not consider the density-dependent effects on vegetative reproduction in our model; therefore, the transition from a density-dependent dynamics to a density-independent dynamics at high levels of vegetative reproduction is an artifact of the model formulation. However, though *A. canadense* populations exhibit density-dependent characteristics, i.e., stable population density, Damman and Cain (1998) report little evidence of density dependence between adult ramets and lateral shoots.

Germination rate and climate change

Germination may also be sensitive to warming (De Frenne et al. 2009). *A. canadense* exhibits temperature-dependent epicotyl dormancy (Baskin and Baskin 1986; Lobstein and Rockwood 1993), and shoot dormancy breaks after cold stratification of 5˚C (Baskin and

Baskin 1986). Therefore, for extreme temperature increases, the seeds may not experience the low winter temperatures necessary to break shoot dormancy, thereby reducing germination and growth to the seedling stage. If we compare the germination rate, γ, and the fraction of seeds dispersed, b, we see that as seed dispersal diminishes the germination rate must increase to compensate, i.e., $b\gamma$ must be greater than some minimum threshold. If both germination rate decreases as well as seed dispersal, two possible consequences of warming, the plant population would no longer maintain a positive density at equilibrium. We note, however, that the requirement for dispersal in order to germinate may restrict the possible range of parameters that allow the population to persist.

3.4.2 Influence of temperature dependent parameters

We want to determine the magnitude of the influence of temperature-dependent variables- the length of the ant foraging season, the date of the peak of fruit dehiscence, and the ant colony density- on the population density at equilibrium. First, let us simply observe graphically the individual and additive effect of each climate-sensitive parameter (Fig. 3.4). In the absence of other changes, the increase in foraging season length increases the proportion of seeds dispersed within a year. We see some decline in dispersal with the advance of the peak date of fruit dehiscence at high increases in temperature above current conditions, but the decline in colony density with increased warming has the greatest influence on the proportion of seeds dispersed (Fig. 3.4a). If we observe the interaction of each pair of temperature dependent parameters (Fig. 3.4b), we see that the increase in

foraging season length counteracts the effect of an advanced fruiting season and diminishes the negative effects of lower colony density. Based on our model and assumptions, we may observe that the myrmecochory will not necessarily 'decouple' temporally due to warming, but the interaction will not persist if warming significantly reduces the colony density of *A. rudis* and no substitute disperser exists. If we consider the effect of all three temperature dependent parameters on the plant population density at equilibrium of *A. canadense* (Fig. 3.5), we observe a steady, but slow, decline for moderate warming (0-2.5˚C) and a steep decline to zero for high levels of warming (3-6˚C) which reflects the pattern of *A. rudis* colony density decline with temperature.

In our previous analysis, we made specific assumptions about the effects of warming on each parameter (τ_0, a, and ΔL). To evaluate the influence of each climate sensitive parameter on the population density of *A. canadense* in the absence of our underlying assumption, we simulated the population dynamics of *A. canadense* for six different values of each climate-sensitive parameter independent of warming, ($\tau_0 = \{0,4,8,12,16,20\}$, $a = \{0,0.2,0.4,0.6,0.8,1\}$, and $\Delta L = \{0,12,24,36,48,60\}$). We can then measure the response of the total population density at equilibrium for each combination of parameter values, in a similar manner to a full factorial experiment. We analyze our results as a factorial ANOVA and observe the Type III sum of squares decomposition for each main effect: peak date of fruit dehiscence, colony density and foraging season length (Fig. 3.6). The analysis of the sum of squares decomposition reveals the same general result as our previous analysis (Fig.

3.4); the colony density exerts the most influence on the population density at equilibrium for *A. canadense*, based on our model.

3.5 Methods II: Press warming experiments and predictions

In climate manipulation experiments, investigators administer warming as a press perturbation, with an automatic increase in temperature followed by a measure of the response (Sherry et al. 2007; Rustad 2008). In reality, though, climate change is not instantaneous, but gradual, and the response to press perturbations may reflect a transient response, not a long-term response (Brooker et al. 2007; Rustad 2008). We test two applications of press perturbation experiments as a means to inform models to predict the effects of warming on myrmecochory. We simulated the press warming experiment, called the 'Future Life Project' (FLP), currently happening in Duke Forest, NC (see Section 3.5.1). We then compared the predicted effects of warming on ant and plant dynamics based on the results of the press warming experiment to the simulated effects of long-term gradual warming.

3.5.1 FLP Field Experiment

We first provide a description of the press experiment we mimic in our simulation.

In Duke Forest, NC (and replicated in Harvard Forest, MA) the FLP constructed 12 warming chambers (area ~20 m^2). Three of the chambers are controls, with no warming, and the other nine chambers each have a randomly assigned target temperature increase above ambient

temperature from 1.5˚C to 5.5˚C, in 0.5˚C increments. Within each chamber, FLP will open four pitfalls for 48 hours once a month over the next three years to measure ant foraging and abundance. In addition to ant dynamics, FLP transplanted three reproductive *Hexastylis arifolia*, a local myrmecochore, in early spring 2010. FLP will record the dates of fruit dehiscence for each plant, if they reproduce, each year. Finally, FLP will measure the dispersal of seeds from depots within each chamber over 24 hours multiple times over the course of the fruiting season. In general, the response variables FLP will measure over the next three consecutive years include ant activity, population densities and colony sizes of focal species, such as *A. rudis*, ant community diversity and species composition, and rates of ant-mediated seed dispersal and predation.

3.5.2 Model simulations

We simulated the dynamics of the chambers for three consecutive years. To incorporate year-to-year variability in ant forager density, we created a vector for each year that represents the daily variability in ant forager density due to sensitivity to weather conditions. The vector remains the same for all chambers in a single year, though actual forager density depends on the warming treatment of the chamber we simulate and our assumed relationship between colony density and temperature. We selected two days, every 30 days, from our model of ant colony dynamics over the year, $P(\tau)$ to simulate the pitfall sampling of the chambers for each temperature treatment. To simulate fruiting, we randomly selected the

dehiscence date for each plant with a chamber from the previously determined logistic distribution,

$$date \sim Logistic\left(T_1 - \tau_0, 1/b_1\right)$$

for each temperature treatment. Finally, we simulated the dispersal of a fixed number of seeds (40) over 24 hours once a week for 5 weeks starting June 1 (day 152) within each chamber for each temperature treatment.

3.5.3 Comparison of the experimental response and models to gradual warming

The integration of press warming experiments with models of climate change still offers many challenges (Classen and Langley 2005; Rustad 2008). For models of species interactions, we must determine how to incorporate responses to short-term press warming on the interactions into models of the long-term dynamics of the interaction and its effects on the population density and dynamics for each species. Here we consider two possible methods to incorporate the response of ant and plant phenology to our simulated press warming experiment into models of myrmecochore population dynamics.

'Fixed' parameter model analysis

The seed dispersal rates for *A. rudis* have been measured numerous times, across numerous geographical regions and environmental gradients (reviewed in Ness et al. 2009). Therefore, a model that depends on the proportion of seeds dispersed as a function of temperature is a logical application of the response of myrmecochory to a short-term press warming

experiment. We determined the proportion of seeds dispersed over the five weeks as the sum of the number of seeds dispersed divided by the total number of seeds made available over the five weeks. We used the estimated fraction of dispersal based on our simulated experiment to parameterize *b*, the fixed proportion of seeds dispersed each year, to calculate the population density at equilibrium (Eq. 3) for each increase in warming. We call this the 'fixed model' analysis.

'Pressed' warming model analysis

The model we developed in Section 3.3 compliments the FLP press warming experiment, which measures all of the climate sensitive parameters we consider in our model. Therefore, we fit our model parameters based on data from our simulated press warming experiment to predict the myrmecochore population density at equilibrium at different levels of warming. We analyzed the simulated experimental results as follows. First, we used simple linear regression to determine the advance in the fruit dehiscence dates as the slope of the linear relationship between our average fruit dehiscence dates per chamber each year and the chamber temperature increase. Then, we fit our model, $P(\tau)$, for ant colony density using non-linear least squares regression and determined estimates of a, b_2, and T_2 based on the simulated sampled ant densities for each chamber across all years. The data collected in the FLP, allows us to quantify the parameters a, b_2, T_2 and T_1 based on the simulated experimental results to run our full model of plant population dynamics and acquire the new population density at equilibrium under pressed warming. We call this the 'press model' analysis.

'Gradual' warming model

We must compare the fixed and press models to the 'natural' dynamics of the myrmecochore population dynamics under climate change to determine the accuracy of our models based on data from the press warming experiment. In order to simulate natural warming dynamics, we used our full model of plant population dynamics to observe the effects of gradual warming over 100 years on the plant population density. Each year, we increased temperature by 1/100 of our goal temperature increase (1.5-5.5˚C) and determined the population density at the end of 100 years. We use the determined population density and stage-structure at equilibrium from our baseline case as the initial plant population density in the simulation. We call this the 'gradual model' analysis.

Model comparison

We simulated the above experiment and model analysis 100 times with R Statistical Software (R Development Core Team 2009) to provide a measure of the variability in our model predictions based on the measured experimental results. We compared the three different model outputs, fixed, press and gradual, with an ANOVA with model type as a factor. We grouped each comparison by temperature increase in order to compare the outcome of each model under different levels of warming. We used Tukey HSD multiple comparisons procedure to compare each model output grouped by temperature.

3.6 Results II: Press warming experiments and model analysis

We see that the fixed and press models differ significantly from the gradual model, but that the difference is conditional on the level of warming (Fig. 3.7). At low temperature increases, the difference between the gradual and press models is not statistically significant for temperature increase of 2˚C, and the difference is statistically, but not practically, significant for temperature increases of 1.5˚C. Practically, we see that the press model tracks the gradual model up to 2.5˚C at which point it veers from the gradual model and exhibits a steeper decline. The fixed model presents a steady decline in plant population density as temperature increases and is significantly lower than the press and gradual models at moderate temperature increases. In general, though, neither model based on the press warming experiment accurately projects the population density under warming in comparison to the result expected from more natural gradual warming, especially at high levels of warming.

3.7 Discussion

Climate change is affecting many ecosystems on Earth, but understanding the dynamics and consequences of those changes has proven very difficult. The literature on climate change and species interactions has tended to emphasize the potential problems associated with the decoupling of the phenologies (e.g., Visser et al. 2006; Memmott et al. 2007). We found that, in our system, because the fruiting times of myrmecochores and activity times of ants are relatively broad, that phenological decoupling should not occur or effect myrmecochore

population dynamics. However, in our model *A. rudis* abundance plays a major role in the persistence of the plant population. The results of our model analysis underscore the importance of understanding species' life histories in considering the population dynamics of mutualist partners and in particular, understanding the phenologies of species and the factors that influence those phenologies.

In addition to observational studies of the responses of species to climate change, experiments are an important component of climate change research. However, experiments are useful only in as much as we understand the context of the temporal and spatial scale at which climate changes will actually occur (Rustad 2008). The results of our simulation study suggest that with modest levels of warming, the population dynamics of myrmecochores based on press experiments are similar to those that we may observe with long-term gradual climate changes. At higher temperatures, the results of the models based on press experiments and natural climate change diverge. Further, because our models do not incorporate plasticity and local adaptation explicitly, actual differences may be greater than those we observe here. Therefore, further model development and analysis is necessary to determine how best to integrate models and experiments to predict future myrmecochore population dynamics.

3.7.1 Complex effects of climate change

Though we do not intend our model, in its current form, to be a predictive model about climate change, we may still draw parallels between our model development and the use of

models that do project future population dynamics under climate change. In a projection of population dynamics with climate change, it is impossible to take into consideration every nuanced response to warming. In our model, for example, we do not incorporate the potential decrease in germination rate due to warming, though we examine its possible effects with our model analysis. We also do not consider explicitly the possible adaptations that may buffer against the negative effects of climate change. For example, an increase in vegetative reproduction may allow the myrmecochore population to persist in the absence of its keystone disperser (Pudlo et al. 1980) at higher densities than our model would predict. However, we can explore the effect of such adaptations through the analysis of the response of the population density at equilibrium to varied levels of dispersal and vegetative reproduction (see Section 3.4.1).

We must also be aware of our assumption that fruiting phenology will continue to advance linearly. More likely, there exists some natural threshold for how early fruiting can occur in a year (see Fig. 1 Sparks et al. 2000), especially in the southern edges of a species' range. Once the fruiting date reaches that threshold, it is possible that other, non-climate, factors (e.g. photoperiod) determine the timing of the fruiting season (Walther 2010). In addition, simply observing a shift in the temporal overlap of interacting species may not be sufficient to determine if the interaction breaks down (e.g. Memmott et al. 2007). Instead, the abundance of the two interacting species may play an important role in the persistence of the mutualism (Hegland et al. 2009), as did the density of *A. rudis* in our model. The importance of *A. rudis* density to the persistence of myrmecochores under climate change is in part due

to our focus on only *A. rudis* as the keystone disperser of myrmecochores in our model. Other ant species in our system can disperse seeds; though, at least under current conditions, they do so less frequently and effectively than *A. rudis* (Ness et al. 2009). Therefore, while we recognize the limitations of our model of the effects of climate change on population dynamics and species interactions, we also recognize the necessity of simplicity in order to construct useful models of complex dynamics.

3.7.2 Suggestions for climate change studies of species interactions

Though a useful tool, we emphasize the importance of context in the interpretation of the outcomes of press warming experiments. Large-scale experiments like the Future Life Project offer an opportunity to examine a large number of variables under warming at different geographic extremes. Without press experiments, it would be difficult to evaluate cause-and-effect relationships or validate and inform models (Rustad 2008). In contrast, press warming experiments do not provide information on the long-term response of species under gradual climate change, including possible adaptations or rapid evolution. In addition, predictions and extrapolations based on the short-term data of a short-term response to climate change may not tract the natural response of the system (results herein; Brooker et al. 2007; Rustad 2008). The issue remains how we might interpret the measured responses to press warming experiments in the context of gradual climate change and model predictions of the response of population dynamics to climate change. The answer may lay partly in the use of the two other evaluations of the response to climate change: long-term (historical) observation and climate gradient studies.

The integration of long-term observational studies and climate gradients studies with short-term experiments provides the necessary information to model both the transient and enduring effects of climate change on species interactions (Rustad 2008; Klabwijk et al. 2009) and will provide more accurate predictions of future population dynamics (Visser et al. 2006). In addition, the use of climate gradient studies provides observations in time and space of the variability of species interactions (Rustad 2008). We know that phenotypic plasticity may allow species to adapt to changes in partner timing in species interaction (Visser and Both 2005; Visser et al. 2006), or even compensate for the diminished abundance of a partner (e.g., increased vegetative reproduction; Pudlo et al. 1980). Therefore, the advantage of the natural climate gradients that exist may offer a method to validate and inform model predictions of future population dynamics and the persistence of species interactions.

3.7.3 Future research

We based the current formulation of our model mainly on historical observation of phenology and expert opinion. Over the next three years, the Future Life Project will provide more data on the short-term dynamics of ant and myrmecochore populations under warming scenarios. In addition, we hope to continue to collect data on myrmecochore phenology, myrmecochore population dynamics and ant population dynamics along both latitudinal and elevational climate gradients (e.g., Zelikova et al. 2008; Appendix III). The synthesis of each study will allow us to further develop our model such that we can predict future plant

population density under more detailed models of climate change (e.g., IPCC models), and not based simply on the projected temperature increase above current conditions at 2100.

The number of press warming experiments in the literature continues to grow (reviewed in Rustad 2008), but it seems as though models to compliment such experiments are still scarce (Classen and Langley 2005; Rustad 2008). Therefore, we recognize an increasing need for the collaboration of ecologists and mathematicians so that informed predictions of the effects of climate change can aide in management and policy decisions. In addition, we see that it is not simply a matter of integrating models and experiments, but also long-term observation and climate gradient studies in order to understand the effects of climate change on species interactions and to project the effects of climate change on future population dynamics. We hope our study underlines the importance of understanding the intricacies of species interactions and the caution necessary to interpret transient dynamics in the context of long-term projections of gradual processes such as climate change.

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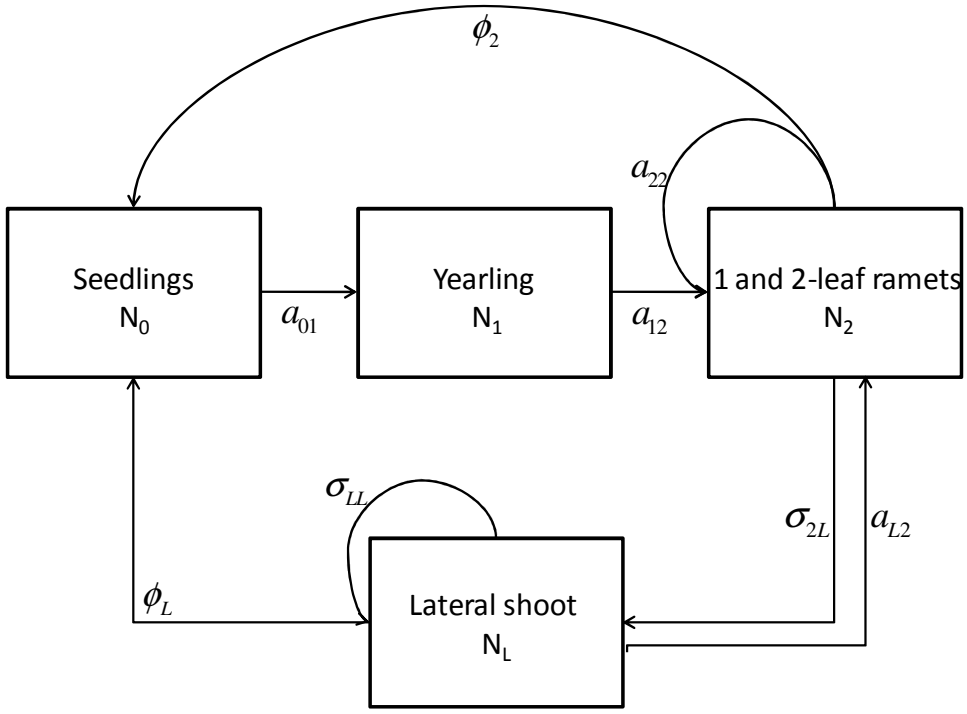

Figure 3.1

Figure 3.1: The life cycle of *Asarum canadense* (similar to Fig. 1; Damman and Cain (1998)) at the ramet level from year-to-year. The parameters a_{ij} represent the transition rates between stage *i* and stage *j*, σ_{iL} represent the production of lateral shoots per individual in stage *i*, and ϕ_i is the fecundity, or production of seedlings per individual in stage *i*.

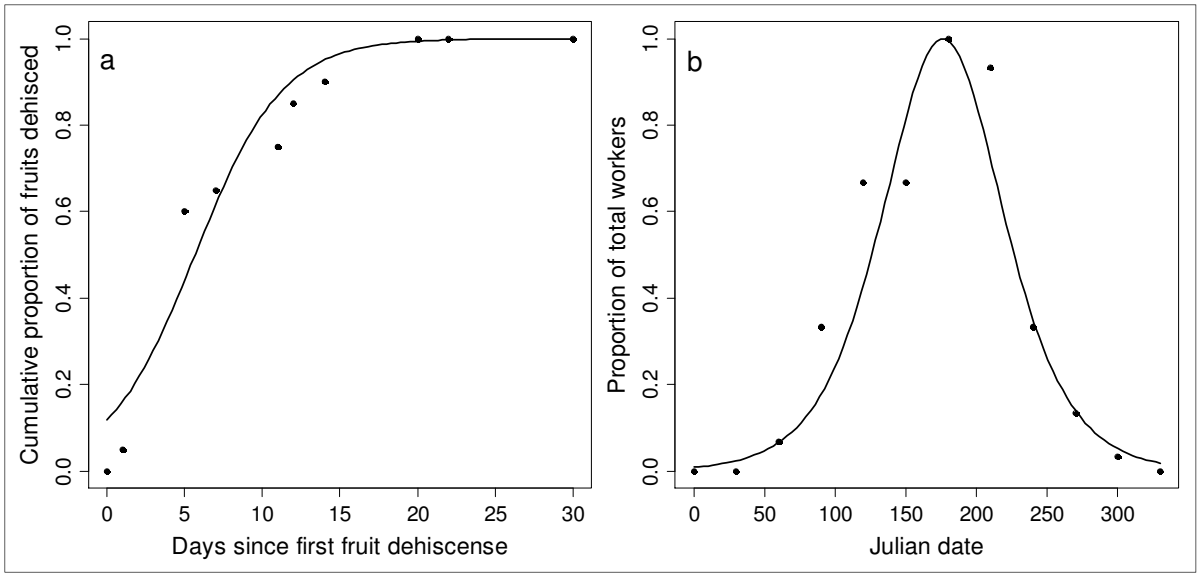

Figure 3.2

Figure 3.2: The non-linear least squares regression fit for a) the model of fruit dehiscence of *A. canadense* based on data from Smith et al. (1989) and b) the model of ant foraging phenology for *A. rudis* base on data from Dunn et al. (2007).

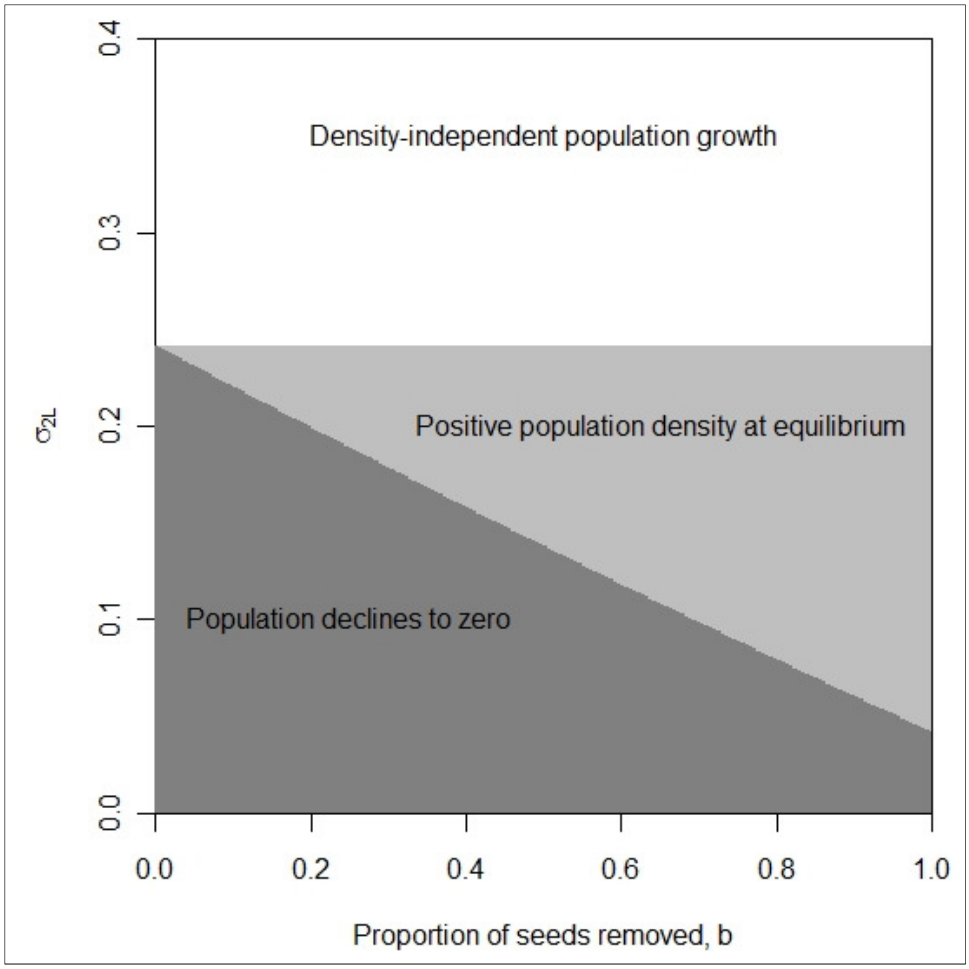

Figure 3.3

Figure 3.3: A comparison of the effects of the proportion of seeds removed to the level of vegetative reproduction for mature adults, σ_{2L}. Notice that as vegetative reproduction increases for mature adults, density-dependence no longer affects the population and the population density inflates as it would for a density-independent population, regardless of the level of seed dispersal.

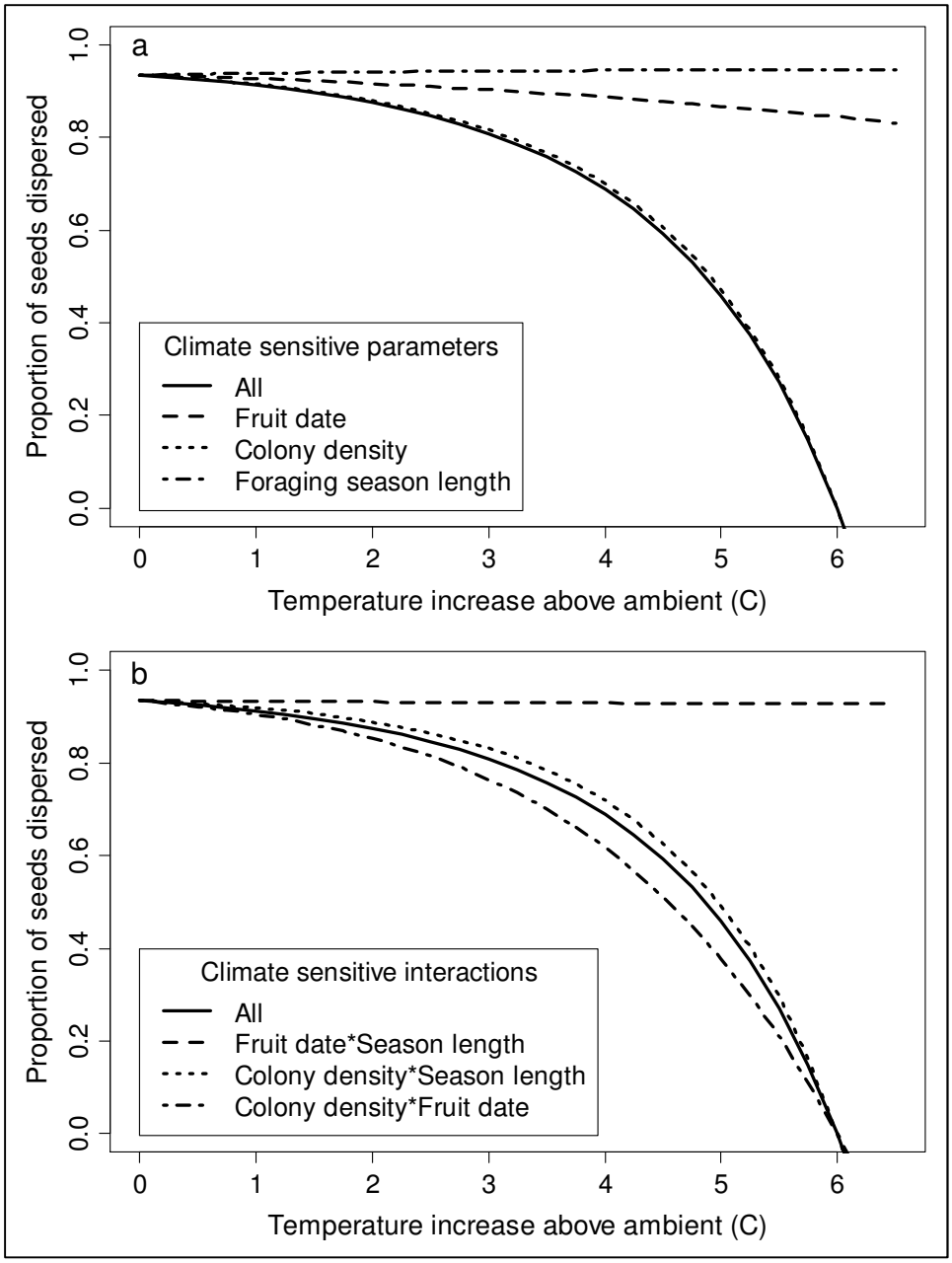

Figure 3.4

Figure 3.4: The influence of each climate-sensitive parameter on the proportion of seeds dispersed by ants over a year as we vary a) just the individual parameters, and b) the interactions of the parameters with temperature. 'All' represents all three parameters as they varied with each temperature increase above the average ambient temperature.

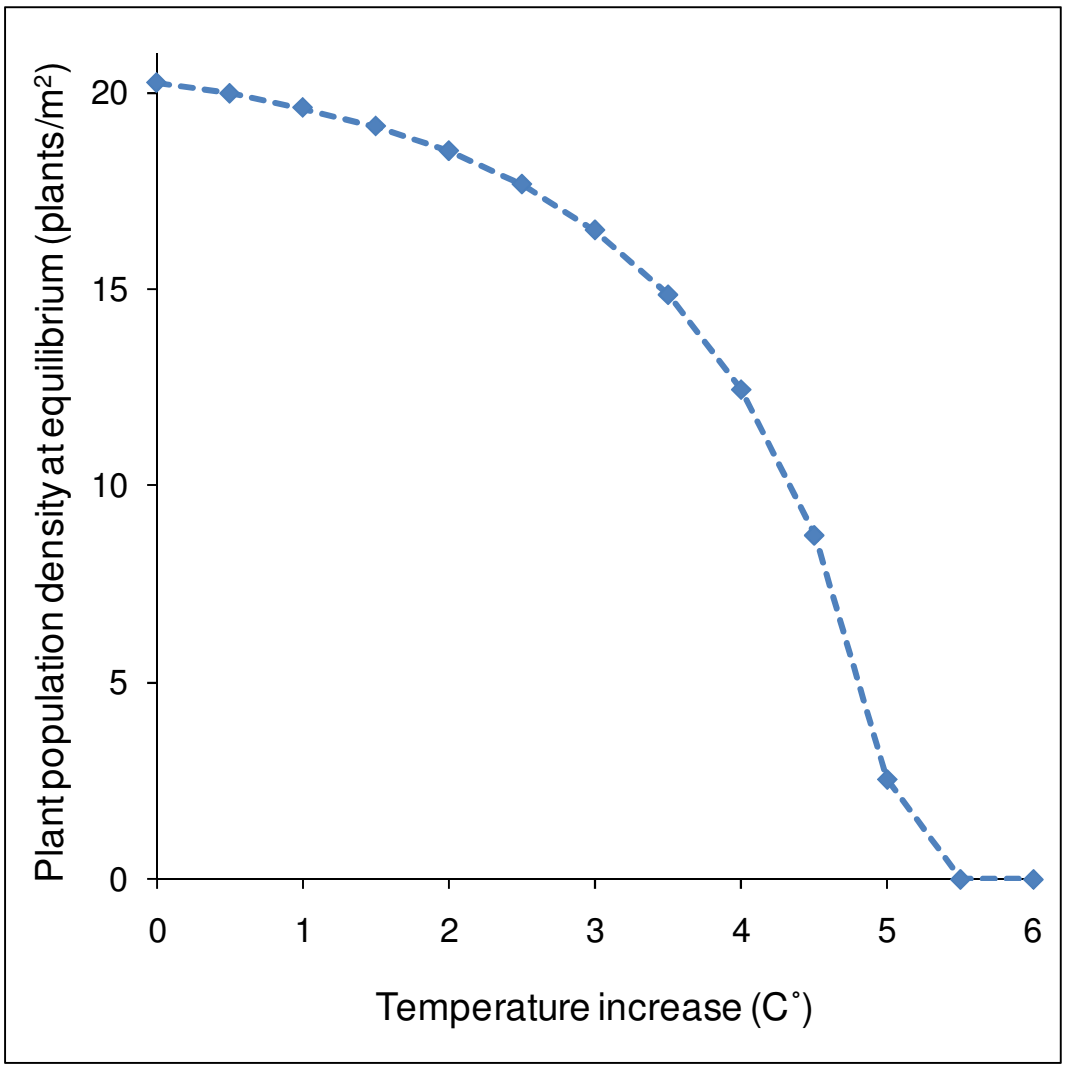

Figure 3.5

Figure 3.5: The plant population density at equilibrium under each warming scenario (increase above the average ambient temperature), with the peak fruit dehiscence date (T_1), the ant foraging season length (b_2), and the colony density (a) all varied with temperature as described in Section 3.3.4.

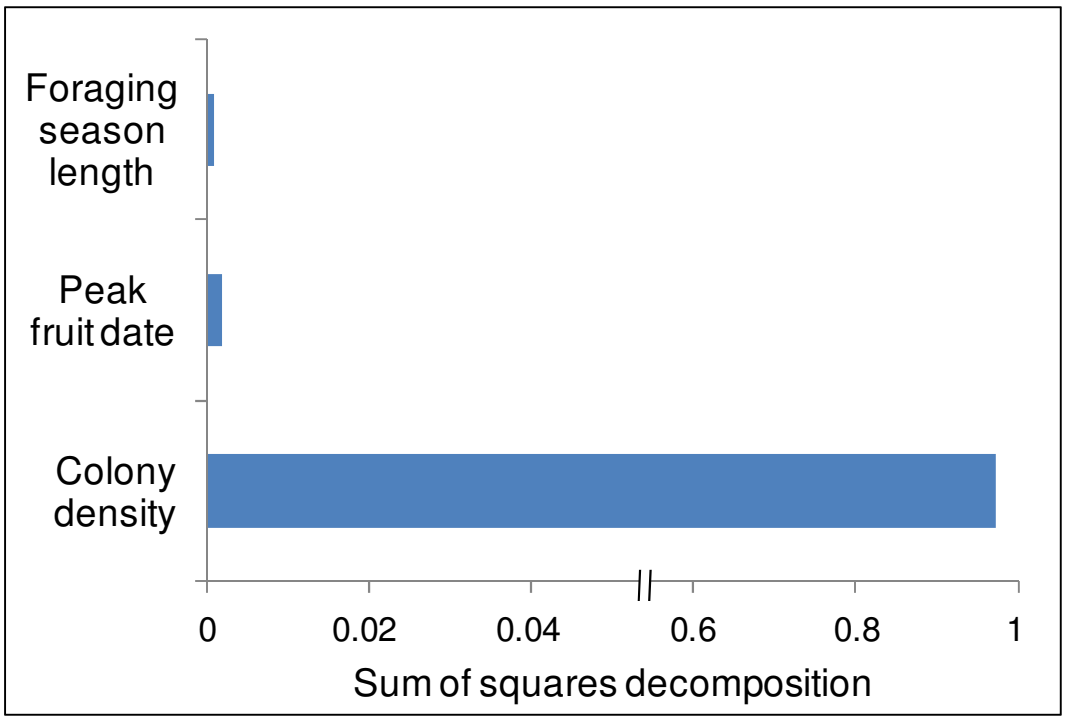

Figure 3.6

Figure 3.6: Sum of squares decomposition when we consider the main effects of the three climate sensitive parameters - the peak fruit dehiscence date (T_1), the ant foraging season length (b_2), and the colony density (a) - each at different levels not dependent on temperature, on the total plant population density at equilibrium.

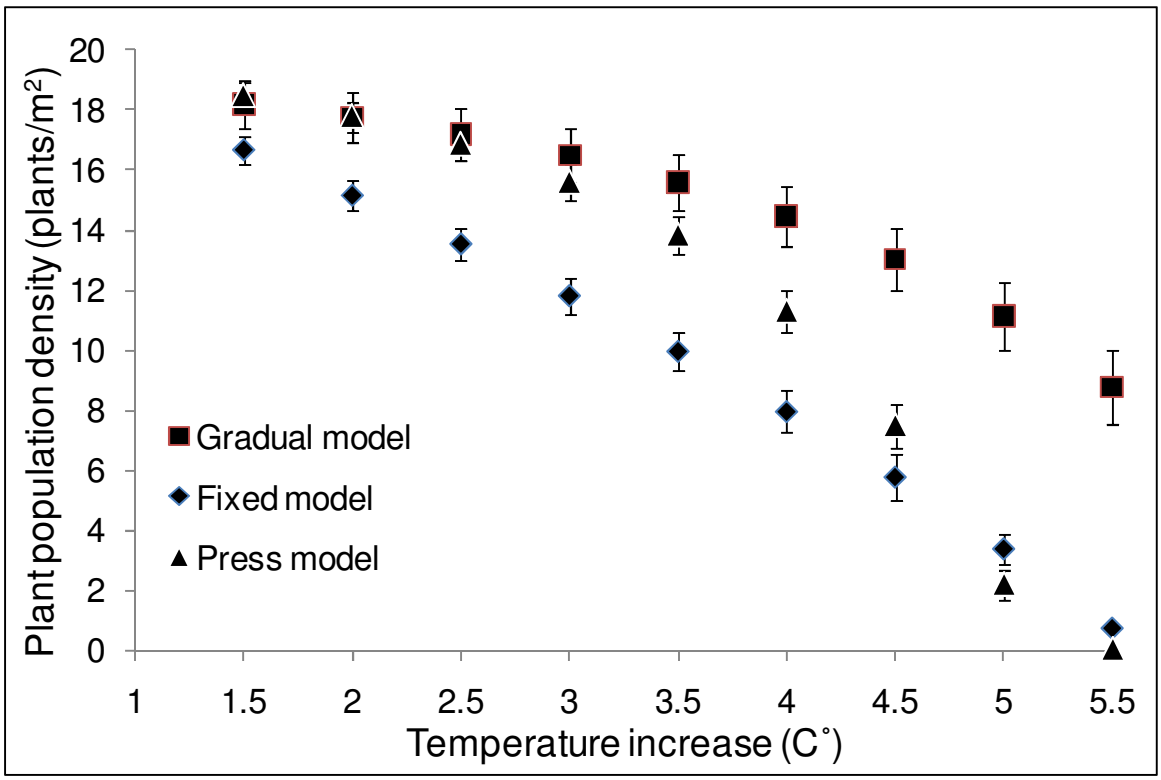

Figure 3.7

Figure 3.7: The results of the 100 simulation of the press warming experiment and the dynamics of the population density with gradual warming. We compared the projected plant popultion density after 100 years of gradual warming to the results of the fixed and press models based on the press warming experiment. The error bars on each point represent the standard deviation of the outcomes of each model for the 100 simulations.

APPENDICES

Appendix I

The Model

The model uses an integrodifference equation to combine the demographic matrix $\mathbf{B}$ for *H. arifolia* and the movement matrix $\mathbf{K}(x, y)$ for seed dispersal to find the population density for each stage at location x at time t+1:

$$\mathbf{n}(x, t+1) = \int_{-\infty}^{\infty} [\mathbf{K}(x, y) \circ \mathbf{B}]\mathbf{n}(y, t)dy, \qquad \text{(A.1)}$$

where ◦ stands for the Hadamard product (element by element multiplication) (Neubert and Caswell 2000).

Population Spread Rate

To calculate the speed of invasion, we must first make some assumptions. First, in addition to the requirement for the population projection matrix $\mathbf{B}$ to be positive and primitive, we must assume the dominant eigenvalue of $\mathbf{B}$ is greater than one (which is true for our data) to ensure the population will still grow when small. Second, we assumed that all dispersal kernels have a moment-generating function (mgf), $m(s)$, with shape parameter s, to ensure an upper bound exists for the rate of spread (i.e., spread rate cannot be infinite). Finally, we assume that if the population reaches a steady state, i.e. the stable-stage distribution, then the population has a traveling wave front of a fixed shape that moves at a constant rate, c. Based on these assumptions, Neubert and Caswell (2000, Appendix A) show that the upper bound on the invasion wave speed, c^*, is

$$c^* = \min_{s>0}\left[\frac{1}{s}\ln \rho(s)\right], \tag{A.2}$$

where $\rho(s)$ is the dominant eigenvalue of the matrix $\mathbf{B} \circ \mathbf{M}(s)$.

Dispersal Kernels

The dispersal kernels for the three dispersal scenarios are as follows.

Autochorous dispersal

The autochorous dispersal kernel for movement from the plant to a new location x_0 follows a half-normal distribution. We generated 1000 distances from a half-normal distribution based on the observation the plants do not have the ability to disperse seeds further than 2cm from the base. The mean is 0.53cm and the standard deviation is 0.40 for the half-normal. Since an explicit formula for the half-normal does not exist, we fit an empirical moment generating function (see below) to the generated data to determine $\mathrm{M}_0(s)$, the moment generating function for autochorous dispersal.

Primary Dispersal

The composite moment generating function for primary dispersal, from location x_0 to location x_1 is

$$\mathrm{M}_1(s) = \left[1 - p_1 + p_1 \mathrm{M}_1'(s)\right]\mathrm{M}_0(s). \tag{A.3}$$

where $\mathrm{M}_1'(s)$ is the mgf for the measured dispersal kernel for ant dispersal from the plant to the nest and each seed has a probability p_1 of being dispersed by an ant. We assume the two processes, plant dispersal and initial ant dispersal, are independent.

Secondary Dispersal

The composite mgf for secondary dispersal is

$$\mathrm{M}_2(s) = (1-p_1)\,\mathrm{M}_0(s) + (1-p_2)\,p_1\,\mathrm{M}_1'(s)\,\mathrm{M}_0(s) + p_2\,p_1\,\mathrm{M}_2'(s)\,\mathrm{M}_1'(s)\,\mathrm{M}_0(s). \qquad \text{(A.4)}$$

Where $\mathrm{M}_2'(s)$ is the mgf for the measured (re)dispersal kernel for redispersal from the nest and p_2 is the probability of redispersal from the nest.

Empirical Moment Generating Function

In order to avoid assumptions about the distribution of the dispersal kernels $k_1'(x_1 - x_0)$ and $k_2'(x_2 - x_1)$, we will use a nonparametric estimator of the moment generating function for $\mathrm{M}_1'(s)$ and $\mathrm{M}_2'(s)$ (Clark et al. 2001). Our data are radial distances ($r = |x\text{-}y|$) from either a seed depot (or "plant") or an *A. rudis* nest. Given N radial distances, $r_1,...,r_N$, we assume the distances are independent, identically distributed random variables. Then we can estimate the moment generating function with the directional empirical moment generating function,

$$\mathrm{M}_{\mathrm{N}}^{\mathrm{E}}(s) = \frac{1}{N}\sum_{i=1}^{N} I_0(sr_i), \quad 0 \le s < \infty \qquad \text{(A.5)}$$

where I_0 is the modified Bessel function of the first kind and zeroth order (Neubert and Parker 2004; Lewis et al. 2006). We chose the directional moment generating function because it provides an unbiased estimate of wave speed (Lewis et al. 2006). We assume that dispersal is identical in all directions and thus the rate of movement is the same in all directions.

Appendix II

Demographic projection matrices for *Hexastylis arifolia* and *Asarum canadense*

Table A1.1 *Asarum canadense* average population projection matrix with population growth rate λ=1.062 (Cain and Damman 1997)

		Year t			
	Stage	**Seedling**	**Yearling**	**Lateral shoot**	**Mature**
Year t+1	**Seedling**	0.000	0.000	0.0875	0.390
	Yearling	0.500	0.000	0.000	0.000
	Lateral Shoot	0.000	0.000	0.015	0.155
	Mature	0.000	0.730	0.703	0.828

Table A1.2 *Hexastylis arifolia* average population projection matrix with population growth rate λ=1.017 (Giladi 2004)

		Year t						
	Stage	**Seedling**	**Sub-adult**	**Non-reproductive**	**Small**	**Medium**	**Large**	**Dormant**
Year t+1	**Seedling**	0.000	0.000	0.000	0.000	0.003	0.215	0.000
	Sub-adult	0.631	0.468	0.023	0.006	0.006	0.000	0.103
	Non-reproductive	0.124	0.345	0.541	0.087	0.017	0.020	0.289
	Small	0.000	0.026	0.243	0.503	0.113	0.025	0.402
	Medium	0.000	0.000	0.032	0.207	0.318	0.152	0.081
	Large	0.000	0.000	0.013	0.106	0.410	0.677	0.125
	Dormant	0.030	0.079	0.108	0.078	0.110	0.100	0.000

Appendix III

Flowering phenology along a latitudinal gradient

We acquired data from Project Budburst (http://www.budburst.ucar.edu/) for the first bloom date of *Sanguinaria canadensis* based on volunteer observations ranging from Tennessee to Minnesota. We determined an estimate of mean annual temperature based on the elevation and latitude of each observation. We used simple linear regression and found that for every degree increase in mean annual temperature, the bloom date of *S. canadensis* advances 2.4 days ($t = -6.98$, $p<0.0001$; Fig. A.1)

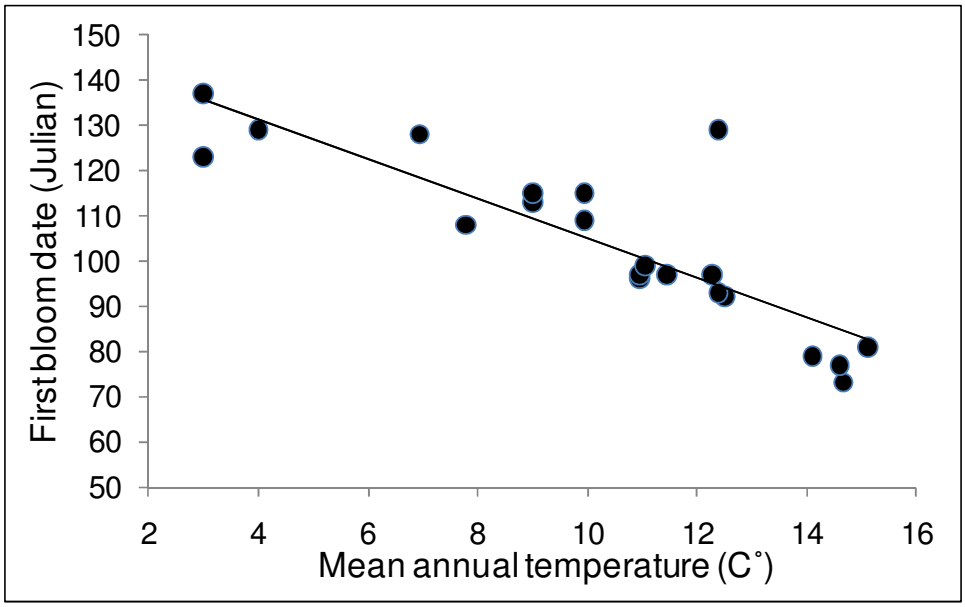

Figure A.1

Figure A.1: The first bloom dates as it corresponds to the mean annual temperature at the location of record (dots) along a latitudinal gradient and the best-fit line from the simple linear regression.