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Disease, pollinator, and resource limitation influences on the reproductive biology and growing season of *Arisaema triphyllum*, Jack-in-the-pulpit

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DISEASE, POLLINATOR, AND RESOURCE LIMITATION INFLUENCES ON THE REPRODUCTIVE BIOLOGY AND GROWING SEASON OF ARISAEMA TRIPHYLLUM, JACK-IN-THE-PULPIT

By

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ABSTRACT

DISEASE, POLLINATOR, AND RESOURCE LIMITATION INFLUENCES ON THE REPRODUCTIVE BIOLOGY AND GROWING SEASON OF ARISAEMA TRIPHYLLUM, JACK-IN-THE-PULPIT

By

Jessica L. Cook

Arisaema triphyllum, Jack-in-the-pulpit, is a common spring ephemeral in eastern forests of North America. Female reproductive success in *A. triphyllum* has been shown to be limited by the amount of available pollen and resources. Disease has also been shown to limit female reproductive success in *A. triphyllum*; populations frequently contain plants infected with the systemic rust *Uromyces ari-triphylli*. Previous research demonstrated that disease reduces seed set in infected females, but results came from a single population over a single growing season. This study investigated the relative importance of pollen limitation, resource limitation, and disease in determining female reproductive success as well as the effects of disease on the emergence and growing season of vegetative plants at four populations in Michigan over two growing seasons. The results of the two year study indicated that although pollen limitation and disease both limit female reproductive success, disease has a much greater effect. Disease limited flowering period length, seed set frequency, and seed weight. The amount of available resources, i.e. the proximate environment, did not appear to affect female reproductive success. Infected vegetative individuals emerged earlier than healthy plants and had a shorter growing season. Plants growing in more alkaline soils had a longer growing season than plants growing in more acidic soils.

"I am still learning....." Michelangelo

This thesis is dedicated to loved ones and those still seeking the "rainbow connection" (Thanks Kermit)

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Chapter 1. Effects of pollen limitation, resource limitation, and disease on female reproductive success

Effective sexual reproduction in angiosperms requires the production of floral structures such as pollen, pollinator attractants and/or rewards, and ovules. These structures impose a resource cost; according to the principle of allocation, individual plants have a finite amount of resources to use for survival, i.e. growth and maintenance, as well as reproduction (Kay 1987; Fitter & Setters 1988; Nault & Gagnon 1988; Lovett Doust 1987; Delph & Meagher 1995; Worley & Harder 1996; Barbour et. al. 1999). These resources are allocated based on life history patterns that have evolved in response to natural selection, which acts to optimize survival and reproduction. Even after plants evolve a successful strategy of resource allocation for sexual reproduction and produce a flower or inflorescence, they must overcome additional challenges to produce fruit and seed, i.e. the ultimate goals of sexual reproduction. These limitations on fruit and seed production, hereafter referred to as female reproductive success, include pollen limitation and resource limitation.

Numerous studies have focused on systems where pollen limitation and/or resource limitation is present and identified the effects of each on female reproductive success. Female reproductive success is often limited by inadequate pollen deposition due to variability in pollinator activity and/or efficiency (Schemske et al. 1978; Rust 1980; Schemske & Horvitz 1984; Montalvo & Ackerman 1986; Campbell 1987; Young 1988; Zimmerman & Aide 1989; Andersson 1996; Waser et al. 1996; Herrera 2000). Resource availability has also been shown to limit female reproductive success (Went & Westergaard 1949; Cloudsley-Thompson & Chadwick 1964; Policansky 1981; Lovett Doust & Cavers 1982; Willson & Burley 1983; Bierzychudek 1984; Lubbers &

Christensen 1986; Lovett Doust et al. 1986; Ackerman & Montalvo 1990; Niesenbaum 1993).

A few studies have demonstrated that disease, in addition to pollen and resource limitations, can also affect female reproductive success. Disease can indirectly affect female reproductive success by altering pollinator behavior or reducing the amount of resources available for reproduction (Roy 1993 & 1996; Althizer et al. 1998) as well as directly by attacking reproductive structures of the host plant (Alexander 1987; Burdon 1987; Parker 1987; Clay 1986, 1987, & 1990). In this chapter I will examine pollen limitation, resource limitation, and disease in detail and discuss the specific ways that each can limit female reproductive success.

Pollen limitation

Many plants depend on pollinators for successful pollen transfer/deposition and flowers have evolved an incredibly wide range of shapes, sizes, colors and scents to influence pollinator behavior (Bell 1985; Haynes et al. 1991; Andersson 1996; Conner & Rush 1996; Menzel & Műller 1996; Raguso 2001; Pellmyr 2002). Most interactions between plants and their pollinators are mutualistic and typically include a floral reward, e.g. nectar or pollen, in exchange for pollen transfer. However, the plant is under selection to maximize its attractiveness to highly effective pollinators at the lowest cost. The two components of pollinator effectiveness are pollinator efficiency and quality of pollinator service. Pollinator efficiency simply refers to the number of pollen grains deposited on stigmas by a particular pollinator during a single visit, while the quality of pollinator service is determined by observing which insect visitors make contact with anthers and stigma (Bertin 1989).

Pollen limitation of female reproductive success appears to be common (Burd 1994). The fact that plants commonly invest a large proportion of a flower's biomass into pollinator attractants (e.g. scent) (Pleasants & Chaplin 1983; Southwick 1984) suggests that it is difficult for plants to acquire adequate pollinator service. In his recent review, Burd (1994) reported that pollen limitation was been documented for 159 of 258 (62%) plant species. He concluded that: 1) the magnitude of pollen limitation is often large, 2) percent fruit set and fruit output of hand-pollinated plants are significantly higher than that of unmanipulated controls, and 3) that most species experience variability in the level of pollen limitation within a season. He also reported that only a few species experienced a significant decline in fecundity as the result of receiving supplemental pollen, which suggests that negative effects from pollen overabundance are relatively uncommon. He suggests that pollen limitation may be an adaptive response of resource allocation strategies to a pollination environment that has large stochastic variation in pollen delivery and ovule fertilization (Burd 1994).

The majority of Burd's (1994) data measured pollen limitation by comparing the percentage fruit set of hand-pollinated plants with that of unmanipulated controls. Pollen limitation is typically assessed using artificial pollination. However, it can also be characterized by low stigmatic loads relative to ovule number (Snow 1986) or correlations of the number of pollinator visits with seed set (Real & Rathcke 1991).

Spring ephemerals are especially prone to pollen limitation since flowering occurs between the time when temperatures are warm enough for pollinator activity but prior to the canopy closure and pollinator abundance often fluctuates seasonally (Schemske et al. 1978). For example, only 33% of unmanipulated flowers of *Erythronium albidum* set

seed, compared with 78% of flowers outcrossed by hand (Schemske et al. 1978). Pollinators of *E. albidum* were active during brief, erratic periods when weather conditions allowed movement, and peak flowering rarely occurred during peak pollinator activity (Schemske et al. 1978). Studies conducted in many generalized pollination systems indicate that temporal variation in pollinator availability and activity reduces fruit and seed production. Zimmerman & Aide (1989) studied the generalized pollination system of the orchid *Aspasia pricipissa* and found that fruit production was limited by pollinator activity. Specifically, they observed that fruit set was over six times greater for hand-pollinated plants compared to unmanipulated controls. Campbell (1987) found that variation in fruit set among three populations of *Veronica cusickii* resulted primarily from differences in pollinator visitation. She observed an overall increase in fruit set for hand-pollinated plants, however there were differences in the degree of pollinator limitation among the three populations; in only one population was there was a significant difference in seed set between unmanipulated controls (44%) and hand-pollinated (91%) plants.

There are numerous examples of pollination systems where not all pollinators are equally effective (Schemske & Horvitz 1984; Montalvo & Ackerman 1986; Young 1988; Waser et al. 1996; Herrera 2000). Schemske & Horvitz (1984) found that floral visitors to the neotropical orchid *Calathea ovandensis* differed significantly in their ability to affect fruit set; this variation creates selection pressure on the plant to specialize on the most effective pollinator. Young (2002) compared the effectiveness of diurnal and nocturnal pollinators of *Silene latifolia* which opens its flowers in the evening but closes them by midmorning of the following day. She found that *S. latifolia* flowers exposed

only to nocturnal visitors, i.e. moths, produced more seed than flowers exposed only to diurnal visitors (i.e. bees, flies, and wasps). These results suggest that nocturnal flowering in *S. latifolia* is an adaptation for specialization on the more effective nocturnal pollinators.

According to Bosch & Waser (2001) plant density can indirectly affect female reproductive success either by changing the quantity and quality of pollination service or because density and reproduction are in response to the quality of the surrounding environment. Bosch & Waser (1999) found that seed set declined by one-third in sparse *Delphinium nuttallianum* and *Aconitum columbianum* populations relative to nearby dense populations. To determine whether reduced seed set was caused by reduced environmental quality or pollinator quality, Bosch & Waser (2001) manipulated the density of potted *D. nuttallianum* and *A. columbianum* plants and observed interspecific differences in how density affected reproductive success. For *A. columbianum*, visitation and seed set significantly increased with plant density, which is consistent with the interpretation that pollination quality is lower in sparse populations. In contrast, pollinator visitation and seed set for *D. nuttallianum* did not differ between dense and sparse arrays; this finding is consistent with the idea that environmental quality is lower in sparse populations (Bosch & Waser 2001). Rust (1980) found that the density of male plants influenced the likelihood of seed set in *Arisaema triphyllum* (L.) Schott (Araceae); increasing the density of males significantly increased the percentage of females setting seed but not seed production.

Resource Limitation

Successful pollination alone does not necessarily guarantee female reproductive success; as shown in the *D. nuttallianum* example, resource limitation can also influence seed production. Resource limitation is most apparent in extreme environments. Desert annuals must tolerate extremely dry conditions and time germination in response to sporadic precipitation; the length of time that moisture is available is very short and reproduction must occur quickly. Most desert annuals require approximately 60 mm of precipitation within a few hours of germination (Cloudsley-Thompson & Chadwick 1964). Among the fastest plants to complete its life cycle is *Boerrhavia repens* of the Sahara Desert. Plants of this species can go from seed to seed in as little as 10 days (Cloudsley-Thompson & Chadwick 1964).

Resource acquisition in spring ephemerals is restricted by light availability during the short interval between the first warm days of spring and full leaf expansion in the deciduous forest canopy. Water and nutrients are relatively abundant, but as the season progresses the amount of available light decreases (Salisbury 1916a&b; Morgan 1971; Taylor & Pearcy 1976; Muller 1978; Schemske et al. 1978). In Brownfield Woods near Urbana, Illinois, the amount of available light decreased from 50% of incident radiation in March to ~30% in mid-April to <10% in early May as leaf expansion occurred in the deciduous forest canopy (Schemske et al. 1978). Several studies have demonstrated that photosynthetic rates of spring ephemerals decline in the late spring as the canopy closes (Salisbury 1916; Anderson & Hubricht 1940; Sparling 1967; Risser & Cottam 1968; Heinrich 1976; Taylor & Pearcy 1976). Therefore, these plants have a

short period in which to acquire the energy from sunlight and nutrients necessary for growth and reproduction.

Seed production of the ephemeral *Thalictrum thalictroides* was limited by light availability late in the season (Lubbers & Christensen 1986). This study found that artificially shading plants caused a significant reduction in the percentage seed set and also decreased the probability of flowering again the following year (Lubbers & Christensen 1986). Niesenbaum (1993) found that fruit set in the understory shrub *Lindera benzoin* was limited by available light. Specifically, he observed that fruit set was significantly greater for branches in the sun versus in the shade, and low light levels limited flower production in the following growing season (Niesenbaum 1993).

These studies demonstrate that the availability of proximate resources, as determined by the current environment, can limit female reproductive success. However, the amount of stored resources can also exert an influence on female reproductive success (Policansky 1981; Lovett Doust et al. 1986; Ackerman & Montalvo 1990). Ackerman & Montalvo (1990) found that although hand-pollination of the pollen-limited orchid *Epidendrum ciliare* increased fruit production, the total seed crop mass per fruit decreased as fruit set increased, suggesting a finite amount of resources that must be partitioned among all pollinated ovules and developing fruits.

Over the course of its lifetime, the spring ephemeral *Arisaema triphyllum* accumulates energy resources in its corm and sexual expression is strongly correlated with corm size; plants from large corms are female while smaller corms produce male or vegetative plants (Policansky 1981; Lovett Doust & Cavers 1982; Bierzychudek 1982 & 1984; Lovett Doust et al. 1986). Manipulations of variables such as soil nutrients,

removal of corm tissue, and soil moisture have been shown to alter the sexual expression of this species (Pickett 1915; Schaffner 1922; Heslop-Harrison 1957). Female reproductive success in *A. triphyllum* has been shown to be limited by the amount of stored resources in the corm (Policansky 1981; Lovett Doust et al. 1986). Studies found that females with larger corms produced more flowers and had higher rates of successful fruit and seed production (Policansky 1981; Lovett Doust et al. 1986).

The studies cited above indicate that availability of both proximate and stored resources can limit female reproductive success. These limitations are most pronounced in extreme environments but also exert a significant influence on the female reproductive success of plants found in environments where the amount of available resources declines over the course of the growing season, e.g. spring ephemerals. Resource limitation can have a significant effect on female reproductive success in spring ephemerals such as *T. thalictroides*, *L. benzoin* and *A. triphyllum* because they have a short period of time to acquire the energy from sunlight necessary for growth and seed production.

Disease

Most studies on female reproductive success have concentrated on pollen limitation and resource availability, however disease can also affect reproduction both directly and indirectly. Direct effects include the elimination or alteration of reproductive structures on the plant, while indirect effects can take the form of interference in pollinator attraction or a reduction in the resources available for seed production.

Pathogens indirectly affect reproduction by altering the attractiveness of the host plant to potential pollinators. The rust fungus *Puccinia monoica* produces pseudoflowers on *Arabis* spp. that resemble true flowers in color, shape, size, nectar production, and

scent (Roy 1993). These pseudoflowers attract insects that facilitate the rust's reproduction in a way that is analogous to pollination; although insects visited the pseudoflowers equally as often as true flowers, the duration of visits was much longer on pseudoflowers (Roy 1993). Roy (1996) also found that this pathogen also affected female reproductive success of *Anemone patens*, which often co-occurs with the *Arabis holboelli* host. Pollen loss may occur as insects move between flowers and pseudoflowers, reducing the likelihood of effective pollination and subsequent seed production in *A. patens* (Roy 1996). She also observed that the presence of fungal spermatia on flower stigmas reduced female reproductive success; there was a significant decrease in seed set when spermatia and pollen were applied at the same time relative to the hand-pollinated controls (Roy 1996). Althizer et al. (1998) found that *Silene latifolia* plants infected with the anther smut fungus *Mycobotrium violaceum* often flower earlier in the season than healthy plants; a high proportion of disease may encourage insects to visit infected plants and facilitate disease spread early in the season. This study also discovered that bee pollinators of *Silene latifolia* showed a strong preference for healthy over infected flowers but bees with prior exposure to diseased flowers discriminated less against them than bees exposed only to healthy flowers (Althizer et al. 1998).

Pathogens that infect the roots, stems, and leaves can indirectly affect plant reproductive success by acting as a drain on energy, altering the allocation of resources within infected plants and subsequently affecting the quantity and quality of seeds produced (Burdon 1987). Foliar pathogens have been shown to cause substantial changes in the carbon allocation patterns among competing energy sinks (Livne and Daly 1966). This study found that the amount of newly fixed carbon being transported from the first

leaves of *Phaseolus vulgaris* was considerably less if the leaves were infected with the bean rust *Uromyces appendiculatus*; diseased leaves accumulated carbon that was fixed at a different location within the plant, thus acting as a net energy drain (Livne and Daly 1966). Diseased plants can also experience significant declines in the rate of photosynthesis as well as changes in assimilate partitioning and nitrogen metabolism (Burdon 1987). In some instances, alterations in the assimilate distribution pattern as a result of infection have taken the form of changes in the dry weight of roots, shoots, leaves, and reproductive structures.

Pathogens can reduce plant fitness by attacking reproductive structures in the host plant. For example, *M. violaceum* sterilizes infected *S. latifolia* plants by altering flower structure. In males, the pathogen causes the plant to replace anthers with spore sacs filled with teliospores (Baker 1947). Infected females produce male-like flowers containing spore sacs. Fungal endophytes can also directly alter host reproduction in a variety of ways. Plants infected by the *Balansieae* endophytes have substantially lower reproductive success than their healthy counterparts; infected plants do not produce viable inflorescences and are limited to vegetative reproduction (Clay 1986). However, other endophytes can actually increase the reproductive success of the host. Clay (1987 & 1990) found that grasses infected by the *Acremonium* endophytes produced more inflorescences and seeds than uninfected plants. He also observed that seeds from infected plants germinated more rapidly and the resulting seedlings grew faster than those produced by healthy plants (Clay 1987).

As is evident from the above studies, the presence of disease can indirectly affect reproductive success by altering the attractiveness of the host plant to potential

pollinators or by reducing the amount of resources available for seed production. Disease can also directly affect reproductive success of the host by changing or eliminating reproduction and subsequently altering host fitness. However, in some instances infection can benefit the host.

The Arisaema triphyllum-Uromyces ari-triphylli system

My study examines pollen limitation and resource limitation in *Arisaema triphyllum* (L.) Schott (Araceae). I also examine the influence of a systemic rust pathogen *Uromyces ari-triphylli* (Schw.) Seeler (Basidiomycetes) on female reproductive success and the growing season of vegetative plants.

Arisaema triphyllum, Jack-in-the-pulpit, is a native spring ephemeral whose distribution extends from the East Coast westward to the Great Plains states (Gleason & Cronquist 1991). These herbaceous perennials develop from a corm and possess one or two tripartite leaves that unfold in late April or early May. Individual plants can be vegetative, male, or female, but the sex of individual plants is not fixed; at the end of each growing season the plant makes a developmental "decision" about which sex to be the following year (Bierzychudek 1984). Larger plants tend to be female, medium sized plants are usually male, and small plants are vegetative. Heslop-Harrison (1957) demonstrated the strong causal relationship between corm size and sex; removing portions of the corm of female plants caused them to flower as males the following season. Bierzychudek (1984) found that sex determination was based on more than the current size of a plant; according to her model, current plant size, previous year's size, and previous year's sex were more reliable for predicting the current sex of individuals than a model containing only current plant size. Pickett (1915) also observed that

females switched to males as a result of decreased soil moisture. Schaffner (1922) found that the addition of soil nutrients, i.e. manure, enabled plants flowering as males to flower as females the following season. However this effect is not always evident; Bierzychudek (1984) found that supplementing plants with nutrient fertilizer did not increase the likelihood that a plant would switch sex.

Males and females produce a single inflorescence with numerous flowers. Inflorescences are open for approximately 3-4 weeks; in central Michigan flowering begins in late April and is completed by the end of June. *Arisaema triphyllum* has a kettle trap morphology that uses chemical attractants to deceive its thrip and fungus gnat pollinators (Rust 1980; Vogel & Martens 2000). In the male spathe, waxy papillae force insect pollinators down the inflorescence, causing them to wade through the pollen accumulated at the bottom of the inflorescence. Insects can escape the inflorescence through a small exit hole formed by a gap in the spathe edges (Rust 1980). The female spathe appears deceptively similar, however it lacks an exit hole. After depositing pollen on the stigmas during their efforts to escape, the pollinators become trapped and eventually die, hence this interaction is antagonistic because the plant provides no reward and ultimately kills its pollinators.

Jack-in-the-pulpit is self-incompatible and successful cross-pollination is necessary for females to mature berries, which contain between 1-8 seeds (Lovett Doust et al. 1986; Jessica Cook unpublished observation). Female reproductive success in *A. triphyllum* is often pollen-limited; Bierzychudek (1981) found that hand-pollinated *A. triphyllum* produced over an order of magnitude more seeds than naturally pollinated controls. Rust (1980) found that increasing the density of males did not increase seed

production per female but did significantly increase the percentage of females setting seed.

The quantity of stored resources in the underground corm can also limit female reproductive success in *A. triphyllum*. As previously noted, females with larger corms produce more flowers, fruits, and seed (Policansky 1981; Lovett Doust et al. 1986). However, resource limitation may not always be evident. Bierzychudek (1982) did not find a significant correlation between plant size and seed number or between plant size and seed weight. Additionally, the amount of resources stored in the corm can affect female reproductive success in the gender labile *A. triphyllum* by influencing the sexual state of individuals. The resource cost of maturing seed can lead to reductions in corm size, which increases the likelihood that an individual plant will flower as a male the following season (Bierzychudek 1984).

The systemic rust, *Uromyces ari-triphylli*, is common within populations of Jack-in-the-pulpits (Pady 1939). The rust was resident in nearly all *A. triphyllum* populations surveyed in southern Michigan (EE Mason, AM Jarosz & K Kampf, unpublished observation). This pathogen invades the leaves, inflorescence, and corm of *A. triphyllum*; many populations contain infected plants. Once a plant becomes infected, it remains diseased for the remainder of its life. The rust is transmitted to asexually produced cormlets but not to seed or pollen (Parker 1987). Although the mechanism of horizontal transmission is unknown, adult healthy plants do become infected. The disease overwinters in the corm and produces bright yellow aeciospores on the leaves and inflorescence (Pady 1939). Infections decrease leaf size and cause leaves to senesce prematurely, but flowering frequency appears to be equal for healthy and infected

individuals (Parker 1987). It is my hypothesis that the disease also causes the inflorescence to senesce prematurely as well, resulting in a shorter flowering period, which may affect opportunities for pollinator visitation. Parker (1987) found that infected females produced one-quarter of the number of seeds produced by healthy females. In addition, the proportion of healthy females setting seed was much greater than the proportion of diseased females setting seed. Parker (1987) proposed that these differences could be attributed to either reduced pollinator visitation or resource limitation in diseased plants.

This study investigated the effects of pollen limitation, resource limitation, and disease on the female reproductive success and growing season of *A. triphyllum*. While other work has investigated these factors singly or in pairs, this is the first study to investigate all three simultaneously. My goal was to investigate the relative importance of each factor within *A. triphyllum* populations in Michigan. Specifically, in this study I have:

1. Conducted a hand-pollination experiment and compared the seed set frequency of hand-pollinated versus naturally pollinated females to determine the effects of pollen limitation;
2. Measured four environmental variables (light availability, soil pH, soil moisture, & soil temperature) near each plant to determine the effects of resource limitation on emergence date, growing season length, & flowering period length; and
3. Compared the flowering period length, inflorescence size, seed set frequency, seed number, seed weight, and pollen viability of healthy and infected plants to determine the effects of disease.

Since the disease has the potential to alter many stages of the host life cycle, I also investigated the effects of disease on the growing season of vegetative plants. This last study was initiated to compare the relative emergence and growing season length of healthy and diseased individuals.

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Chapter 2. Female reproductive success in *Arisaema triphyllum*: effects of pollen limitation, resource limitation and disease

Introduction

Life history patterns are shaped by limited energy or nutrient resources that force trade-offs in the amount of resources that can be devoted to growth, maintenance and reproduction (Freeman & Herron 2004). The amount of resources a plant devotes to reproduction should be geared towards optimizing trade-offs under the current environmental conditions in order to maximize fitness. The production of reproductive structures, e.g. ovules, is the first step toward achieving female reproductive success but other obstacles must be overcome to successfully produce seed. Pollen limitation, resource limitation, and disease can reduce female reproductive success.

Variation in pollinator activity and/or efficiency can lead to inadequate pollen deposition on the stigmas of flowers (Schemske et al. 1978; Rust 1980; Bierzychudek 1981; Waser 1983; Schemske & Horvitz 1984; Montalvo & Ackerman 1986; Campbell 1987; Young 1988 & 2002; Zimmerman & Aide 1989; Andersson 1996; Waser et al. 1996; Herrera 2000). As a consequence, the number of ovules fertilized may be smaller than the maximum number a plant can mature into seed with the amount of energy and nutrient resources available. Schemske et al. (1978) observed that fluctuations in pollinator abundance limited female reproductive success in several spring ephemerals; hand-pollination increased seed production relative to unmanipulated controls. Two studies have indicated that female reproductive success in the spring ephemeral *Arisaema triphyllum* is pollen-limited (Rust 1980; Bierzychudek 1981). Rust (1980) observed that seed production of *A. triphyllum* females significantly declined with increasing distance from potential pollen donors; females within one meter of a male plant had significantly

higher seed production than females more distant from males. Bierzychudek (1981) observed a striking difference in female reproductive success between hand-pollinated and naturally pollinated plants; plants given supplemental pollen produced over an order of magnitude more seeds than plants relying solely on insect pollinators.

Resource limitation can also influence female reproductive success, particularly for plants in extreme environments or those growing in areas where the amount of available resources, e.g. light or water, declines over the course of the growing season. Desert annuals have evolved life history strategies to cope with extremely arid conditions such as germinating in response to infrequent precipitation (Went & Westergaard 1949; Cloudsley-Thompson & Chadwick 1964). The effect of resource limitation on female reproductive success is particularly apparent in spring ephemerals whose flowering and growth are restricted by the changing pattern of light incidence on the forest floor during the interval between the first warm days of spring and full leaf expansion of the deciduous forest canopy.

At the onset of the growing season, light, water, and nutrients are relatively abundant but light levels decline dramatically as the season progresses (Salisbury 1916a&b; Morgan 1971; Taylor & Pearcy 1976; Muller 1978; Schemske et al. 1978). Schemske et al. (1978) observed that the amount of available light decreased from 50% of incident radiation in March to ~30% in mid-April to <10% in early May as canopy expansion occurred in Brownfield Woods near Urbana, Illinois.

Several studies have demonstrated that a decrease in light levels can reduce seed production in spring ephemerals. Lubbers & Christensen (1986) found that low levels of light late in the growing season limited seed production of *Thalictrum thalictroides* and

that artificial shading resulted in a significant reduction in percentage seed set. Fruit set on individual branches of the understory shrub *Lindera benzoin* was affected by light availability; branches in the sun had greater fruit set than those in the shade (Niesenbaum 1993). In addition to proximate resources, the amount of stored resources can affect female reproductive success; several studies have demonstrated that among female *A. triphyllum*, plants with larger corms had higher rates of seed production and matured a greater number of seeds compared to plants with smaller corms (Policansky 1981; Lovett Doust et al. 1986).

Although numerous investigations have examined how pollen limitation and/or resource limitations affects female reproductive success, few have been conducted in systems where disease is also a potential limitation. Most plant-pathogen interactions are detrimental to the host plant (Pady 1939; Baker 1947; Alexander 1987; Burdon 1987; Parker 1987; Clay 1986, 1987, & 1990; Roy 1993; Althizer et.al. 1998) and disease can limit female reproductive success by reducing or eliminating the reproduction of the host plant (Pady 1939; Alexander 1987; Burdon 1987; Parker 1987; Clay 1990). *Mycobotrium violaceum*, a systemic fungal pathogen of *Silene latifolia*, sterilizes female flowers by aborting the pistils and converting it to a male-like flower containing fungal spore sacs instead of anthers. Infection by *Balansieae* endophytes reduces female reproductive success of the host; diseased individuals are unable to produce viable inflorescences or seeds and are limited to vegetative reproduction (Clay 1986). Parker (1987) was the first to investigate the effects of the systemic rust pathogen *Uromyces ari-triphylli* (Schw.) Seeler (Basidiomycetes) on female reproductive success of *Arisaema triphyllum*. The proportion of diseased females setting seed was significantly lower than the proportion of

healthy females setting seed and infected females produced significantly fewer seeds than healthy females. However, Parker's (1987) conclusions were based on the performance of plants in a single population over a single growing season.

Although the majority of plant-pathogen interactions are negative, there are a few instances when the presence of a systemic pathogen can actually increase the female reproductive success of the host (Clay 1987 & 1990). Grasses infected by *Acremonium* endophytes produce more seeds than uninfected plants, and have increased rates of seed germination and seedling growth (Clay 1987).

The spring ephemeral *Arisaema triphyllum* (L.) Schott (Araceae) is particularly amenable for investigating limitations on female reproduction, measured here as seed set. Previous studies have found that seed set is affected by pollen limitation (Rust 1980; Bierzychudek 1981), resource limitation (Policansky 1981; Lovett Doust et al. 1986) and disease caused by *Uromyces ari-triphylli* infections (Parker 1987). However, no study has investigated these three factors simultaneously. The goal of this study was to determine the relative importance of pollen limitation, resource limitation and disease on female reproductive success in *A. triphyllum* populations in Michigan. Pollen limitation was assessed by comparing seed set in unmanipulated and hand-pollinated females. Resource limitation was evaluated by examining correlations between seed set and environmental factors: light, soil pH, soil, moisture, & soil temperature. The importance of disease was investigated by comparing seed set of healthy and diseased females.

Materials & Methods

Experiments were conducted in April-September of 2002 & 2003 at two populations, located in the Lott and Hudson woodlots in central, lower peninsula

Michigan. Both sites are part of Michigan State University's natural areas holdings and are located within 5 miles campus (http://www.cpp.msu.edu/nat_area/). Incidence (i.e. percentage of infected plants within a population) of *U. ari-triphylli* infection was approximately 50% at each site.

Each year approximately 150 flowering plants were selected randomly in each population. Flowering phenology of each inflorescence was estimated by checking plants every second day to determine the "start" and "stop" dates for flowering. Flowering "start" criterion was the complete expansion of the two halves of the spathe with no visible overlap around the spadix. Flowering was considered to "stop" when the inflorescence itself had senesced or the senescence of the peduncle occurred, causing the flower to topple over. Inflorescence height for each plant was measured as the distance indicated in Figure 1. Disease status was assessed by checking the spathe and leaves for the presence of any of the multiple spore types associated with *U. ari-triphylli* infection.

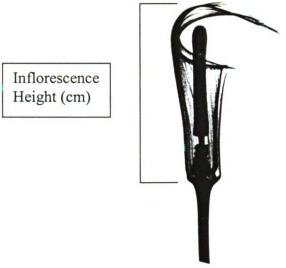

Figure 1. Inflorescence height measurement. Photo courtesy of Albert G. Richards.

The influence of resources on flowering phenology and seed set was evaluated by measuring localized environmental variables (i.e., light, soil pH, soil moisture and soil temperature) at approximately 50 plants in each population. Measurements were made at the beginning, middle, and end of the flowering season since the environment can fluctuate over time, e.g., light levels decrease as leaves in the forest canopy expand. In 2002, light was measured as light intensity using an Extech Model 401025 Foot Candle/ Lux Meter placed approximately one inch above the plant leaf. All light measurements were made on overcast days between 12-4 pm. In 2003, light was measured as percent canopy cover using a spherical densitometer placed approximately one inch above the plant leaf. Soil pH and moisture were measured at a depth of three inches using a Kelway Soil Tester HB-2. Soil temperature was measured at a depth of 2.5 inches using a Tele-tru thermometer in 2003 only.

Fruits were collected in September of both years and the number of seeds produced by each inflorescence was counted. Seeds were defleshed prior to counting and weighing. Individual seed weights were recorded in 2002, while only the total weight of all seed from a single inflorescence was recorded in 2003. Individual seed weights were initially measured because of concern for differences in seed weight distributions between healthy and diseased plants, however, upon graphical examination of the 2002 data it was determined that the distributions were approximately the same.

Analysis of variance (ANOVA) using Type III tests were carried out using PROC MIXED (SAS Institute 1997) to determine the significance of environmental factors on flowering phenology and seed set. Least square means were reported to correct for the influence of other main effects in the unbalanced design. Additionally, for inflorescence

height we also used the Protected Fisher's LSD (least squares difference) to do pair-wise mean comparisons.

Hand-pollination experiment

In the observational work described above, any potential influence of disease and environmental factors on seed set is confounded with reduced seed set due to limited pollinator service. A hand-pollination experiment was carried out in both years to determine whether seed set was pollen-limited. Pollen from ~60 healthy males and ~60 diseased males was used to pollinate 116 healthy females and 97 diseased females using a 2 X 2 factorial design. Control plants (N=166) received no supplemental pollen and included females designated specifically for this experiment as well as females marked for the flowering phenology experiment. In 2002 all females came from Hudson and Lott populations, but the number of diseased females at Hudson was low (N=14). To ensure an adequate sample size in 2003, diseased females from a third site, Webber, were added. These females were used in the analyses for the hand-pollination experiment but were excluded from the seed production analyses for replication purposes, i.e. so that the seed data from 2002 (Lott & Hudson females only) and 2003 could be combined for analyses. Pollen was applied with a camel hair brush when the flowers in the female spathe were observed to be open. The brush was swabbed around one or more male spathes before being wiped on the flowers in the female spathe. Plants of a single genet are incompatible, and it is likely that clumps of plants are the clonal progeny of a single individual (Bierzychudek 1984). In order to reduce the risk of incompatibility, pollen donors were selected randomly from a distance of at least 5m away from the recipient

female. Females were monitored for seed set and the same seed measurements used in the flowering period experiment were applied.

Analyses of the data were performed using PROC GENMOD of SAS to do logistic regressions. Main effects (population, disease status, year, and sex) were tested using Likelihood Ratio tests; significance of *a priori* contrasts were assessed by Chi-Square tests. Contrasts were used to compare 1) seed set frequency of hand-pollinated vs. unmanipulated control females and healthy vs. diseased females and 2) probability of setting seed for healthy and diseased females from a healthy vs. diseased pollen donor. Analysis of variance (ANOVA) using Type III tests were carried out using PROC MIXED (SAS Institute 1997) to determine the significance of disease on seed production. Least square means were reported to correct for the influence of other main effects in unbalanced design. PROC GLM (SAS Institute 1997) was used to determine if pollen loading from hand pollination had a significant effect on seed number.

Fungicide trial

It is possible that resource limited plants are more susceptible to infection. If true, then the effects of resource limitation and infection would be confounded. Curing plants of disease would allow an independent assessment of the roles of both disease and resource availability. Just prior to the 2003 field season, 500mL of the fungicide Tilt® (propiconazole) at a concentration of 14g/L was applied to the soil surrounding the corms of 45 females that were infected in 2002. My intent was to eliminate the effects of disease and pollen limitation (by including treated females in the hand pollination experiment), thus allowing me to isolate the effects of resource limitation on female reproductive success. Upon successful treatment, I intended to compare the seed set frequency and

seed production of treated females with that of diseased females and females who were healthy their entire lives. Unfortunately, only a few of the treated plants emerged; none produced an inflorescence and all had malformed leaves.

Results

Healthy plants had a higher probability of being male than diseased plants (X^2 = 21.94; $p < 0.01$), with 66% of healthy flowering plants being male and only 48% of diseased plants flowering as male.

Inflorescence Height

Inflorescence height differed between the sexes and between healthy and diseased females (Table 2.1). However, significant interactions between sex and disease status and between population and year occurred (Table 2.2). Both healthy and diseased females were significantly larger than males (Table 2.1). However, females were more affected by disease, since inflorescence height for diseased females was significantly smaller (9%) than healthy females. In contrast, inflorescence height for healthy and diseased males did not differ statistically although diseased male inflorescences were, on average, 4% smaller than their healthy counterparts. Inflorescences were smaller in 2002 compared to 2003 (Table 2.3). In 2002, mean inflorescence height at Hudson was significantly smaller than at Lott (Table 2.3). Inflorescence size was larger at both sites in 2003, and the inflorescences at Lott and Hudson did not differ.

Table 2.1. Least square mean inflorescence height (cm) and standard error values for healthy & diseased male and female *A. triphyllum*.

	Healthy		Diseased		Overall	
Sex	Number	Mean	Number	Mean	Number	Mean
Male	384	5.95±0.043 a	101	5.73±0.083 a	485	5.84±0.046
Female	196	7.14±0.059 b	110	6.50±0.095 c	306	6.82±0.057
Overall	580	6.55±0.037	211	6.11±0.065		

* means followed by the same letter do not differ significantly

Table 2.2. Analysis of variance for the effects of population, sex, disease status and year on inflorescence height.

Main Effect	df	Mean Square	F Value	Pr > F
Population	1	1.50	2.30	0.1295
Sex	1	124.11	190.38	<0.0001
Pop*Sex	1	0.68	1.05	0.3059
Disease status	1	21.98	33.72	< 0.0001
Pop*Disease	1	0.04	0.07	0.7998
Sex*Disease	1	5.66	8.68	0.0033
Year	1	10.16	15.58	<0.0001
Pop*Year	1	2.56	3.93	0.0478
Sex*Year	1	1.56	2.39	0.1228
Disease*Year	1	0.35	0.54	0.4626
Error	779	.68		

Table 2.3. Least square mean inflorescence height (cm) and standard error values for *A. triphyllum* at Hudson and Lott populations in 2002 & 2003.

	2002		2003	
Population	Number	Size	Number	Size
Hudson	163	6.08±0.082 a	210	6.47±0.081 c
Lott	234	6.32±0.057 b	183	6.46±0.060 c

* means followed by the same letter do not differ significantly

Flowering period length

Flowering period was affected by year, sex, disease status, and population with significant sex by year and population by disease interactions (Table 2.4). The interaction between year and sex was due to males in 2003 flowering for a shorter period than flowers from males in 2002 or females (Table 2.5). The flowering period for females did not differ between years, and was similar to the flowering period for males in 2002.

Table 2.4. Analysis of variance for the effects of population, sex, disease status and year on flowering period length of healthy & diseased *A. triphyllum* at Hudson and Lott populations in 2002 & 2003.

Main Effect	df	Mean Square	F Value	Pr > F
Population	1	407.48	15.96	< 0.0001
Year	1	364.33	14.27	0.0002
Pop*Year	1	53.61	2.10	0.1478
Sex	1	287.99	11.28	0.0008
Sex*Year	1	700.07	27.42	< 0.0001
Pop*Sex	1	53.87	2.11	0.1470
Disease status	1	3640.78	142.60	< 0.0001
Disease*Year	1	62.81	2.46	0.1170
Pop*Disease	1	247.40	9.69	0.0019
Sex*Disease	1	0.51	0.02	0.8920
Error	778	25.53		

Table 2.5. Least square mean flowering period length (days) and standard error values for male and female *A. triphyllum* in 2002 & 2003.

	2002		2003		Overall	
Sex	Number	Mean	Number	Mean	Number	Mean
Male	201	25.4±0.408 a	182	21.8±0.377 b	383	23.6±0.291
Female	95	24.9±0.471 a	102	25.3±0.473 a	197	25.1±0.355
Overall	296	25.1±0.319	282	23.5±0.317		

* means followed by the same letter do not differ significantly

Healthy plants had significantly longer flowering periods than diseased plants, and the flowering period of healthy plants at each site were similar (Table 2.6). The disease by population interaction was due to a differential response of disease at the two sites. Flowering period of diseased plants at the Hudson site was 26% shorter than healthy plants. In contrast, diseased plants at Lott were less affected by infection; the reduction in flowering period was only 15%.

Table 2.6. Least square mean flowering period length (days) and standard error values for healthy and diseased A. triphyllum at Hudson and Lott populations in 2002 & 2003.

	Healthy		Diseased		Overall	
Pop.	Number	Mean	Number	Mean	Number	Mean
Hudson	320	26.9±0.324 a	53	19.9±0.720 b	373	23.4±0.402 a
Lott	260	27.3±0.324 a	157	23.3±0.411 c	417	25.3±0.261 b
Overall	790	27.1±0.229	210	21.6±0.410		

* means followed by the same letter do not differ significantly

There was no evidence that proximate resources affected flowering period, since no single or combination of environmental factors (light availability, soil moisture, soil pH or soil temperature) had a significant effect on flowering period length in either year and or population (Appendix A). The means for environmental variables in the 2002 and 2003 flowering seasons are in Appendix B.

Seed Set Frequency

Rust infection had a significant affect on a female's ability to set seed (X^2= 64.80 and $p<0.0001$, df =1); an infected female was five times less likely to set seed than a

healthy female (Table 2.7). Pollen limitation also affected the probability of a female setting seed. For healthy females, hand-pollination increased the probability of seed set by 22% (Table 2.7; X^2=12.40 and p=0.0004, df =1). The disease status of the male used to donate pollen did not influence the probability of seed set in hand-pollinated healthy females. When the pollen donor was a healthy male the probability of a healthy female setting seed was 79% (n=58), which was similar to seed set when the pollen donor was a diseased male (81%, n = 58; X^2 = 0.05 and p = 0.816, df =2). Hand-pollination of diseased females had little effect, increasing seed set probability by a non-significant 3% (X^2=0.311 and p>0.25, df =1). Thus, disease undercuts a female's ability to set seed to the extent that pollen limitation is no longer a consequence.

In contrast to the significant effects of rust infection and pollen limitation, there were no consistent relationships between seed set and environmental variables. In 2002, there was no evidence that any environmental factor (light availability, soil moisture, soil pH or soil temperature) affected seed set. In 2003, seed set increased with higher soil pH and higher soil moisture (Table 2.8).

Table 2.7. Seed set frequency of unmanipulated controls and hand-pollinated healthy & diseased *A. triphyllum* females from Hudson, Lott, and Webber.

Female recipient	Naturally pollinated	Hand pollinated
Healthy	0.58 (N=102)	0.80 (N=116)
Diseased	0.11 (N=64)	0.14 (N=97)

Table 2.8. Logistic regression to test the effects of environmental variables (light, soil pH, soil moisture, soil temperature) on seed set frequency in 2003.

Main Effect	df	Chi Square	Pr > Chi Square
Population	1	0.48	0.4868
Disease status	1	5.69	0.0171
% Cover	1	0.05	0.8284
Soil pH	1	6.98	0.0082
Soil moisture	1	4.49	0.0341
Soil temperature	1	1.04	0.3084

Seed production

For all females setting seed, regardless of whether or not they were hand-pollinated or unmanipulated, the number of seed produced was not influenced by year, population or disease status (Table 2.9). Pollen loading, however, had a significant effect on seed production; hand-pollination increased the number of seeds produced relative to their unmanipulated counterparts (Table 2.10). The sample sizes differ between Table 2.7 and Table 2.10 because for 11 females (of the 173 total females setting seed) the seed could not be collected. Average weight of seed from healthy females was 19% heavier than seed from diseased females (Table 2.11), indicating that diseased females are constrained in their ability to provision seed. For healthy females only, seeds produced by control plants were significantly heavier than those produced by hand-pollinated females; it is possible that the control plants were able to allocate more resources into each seed because they produced fewer seeds than hand-pollinated plants (Tables 2.10 & 2.11). Seed weight was also influenced by a significant interaction between population and year (Table 2.12). Seed weights at Lott did not differ between 2002 and 2003 (Table 2.13). At Hudson, seed weight in 2003 was greater than seed weights in 2002. Seed weights at Hudson were generally higher than seed weights at Lott.

Table 2.9. Analysis of variance for effects of population, disease status and year on the number of seeds produced by healthy & diseased *A. triphyllum* setting seed at Hudson and Lott populations in 2002 & 2003.

Main Effect	df	Mean Square	F Value	Pr > F
Population	1	1084.18	0.79	0.3745
Disease status	1	27.45	0.02	0.8873
Year	1	2538.90	1.85	0.1762
Pop*Disease	1	3677.98	2.68	0.1034
Pop* Year	1	1619.41	1.18	0.2783
Disease*Year	1	45.01	0.34	0.5620
Error	151	1372.38		

Table 2.10. Least square mean number of seeds produced by hand-pollinated and unmanipulated healthy and diseased *A. triphyllum* females setting seed in Hudson and Lott populations in 2002 &2003. Numbers represent an average for those females that set at least one seed on their inflorescence.

Disease Status	Unmanipulated	Hand-pollinated
Healthy	33.09 a	47.21 b
	N=57	N=87
Diseased	15.00 c	37.73 ab
	N=7	N=11

* means followed by the same letter do not differ significantly

Table 2.11. Least square mean seed weight (g) for hand-pollinated & unmanipulated healthy & diseased *A. triphyllum* females setting seed in Hudson & Lott populations.

Disease Status	Unmanipulated	Hand-pollinated
Healthy	0.0497 a	0.0447 b
	N=57	N=87
Diseased	0.0325 c	0.0336 c
	N=7	N=11

* means followed by the same letter do not differ significantly

Table 2.12. Analysis of variance for effects of population, disease status and year on the average weight of seeds produced by healthy & diseased *A. triphyllum* at Hudson and Lott populations in 2002 & 2003.

Main Effect	df	Mean Square	F Value	Pr > F
Population	1	0.0002	2.33	0.1288
Disease	1	0.0004	4.21	0.0419
Year	1	0.0003	2.69	0.1031
Pop*Disease	1	0.0000	0.55	0.4602
Pop* Year	1	0.0006	5.85	0.0168
Disease*Year	1	0.0002	2.09	0.1507
Error	151	0.0001		

Table 2.13. Least square mean seed weight and standard error for *A. triphyllum* females setting seed in Hudson & Lott populations in 2002 & 2003.

	2002		2003	
Population	Number	Seed weight (g)	Number	Seed weight (g)
Hudson	41	0.0397 ± 0.004 b	43	0.0504 ± 0.004 c
Lott	44	0.0383 ± 0.003 a	28	0.0390 ± 0.003 ab

*means followed by the same letter do not differ significantly

Discussion

Female reproductive success in *Arisaema triphyllum* was influenced predominantly by disease and pollen limitation. Proximal resources available to the plant (e.g. light and moisture) had almost no influence on seed production. *Uromyces ari-triphylli* infection had numerous adverse effects on flowering and seed production. Diseased plants flowered for a shorter period and had smaller inflorescences than their healthy counterparts. Females infected by *U. ari-triphylli* were less likely to set seed, and produced lighter seeds. *Uromyces ari-triphylli* infections are not benign and would be expected to significantly reduce the fitness of diseased plants. Thus, my work suggests that the *Arisaema triphyllum-Uromyces ari-triphylli* interaction is typical of many natural

plant-pathogen systems where infections decrease host fitness (Burdon 1987; Jarosz & Davelos 1995).

My work corroborates the results of Parker (1987) who found that diseased plants produced only one-quarter of the number of seeds produced by healthy plants (means: 2.6 and 12.3 seeds, respectively). It was not clear from Parker's work whether the reduction in seed number was due to fewer diseased females setting seed or to a smaller number of seeds being produced by all diseased females. My work indicates that the reduction in seed number can be attributed to a much lower proportion of diseased females setting any seed. Diseased females that set seed produced nearly the same number of seeds as their healthy counterparts, but the average seed weight for diseased plants was significantly lower than for healthy females. This pattern of reduced seed weight suggests that disease reduces a plant's ability to provision seed.

Parker (1987) also reported that diseased plants senesced earlier than healthy plants. As reported in the next chapter, I found a similar trend whereby the growing season of infected plants was reduced by an average of 2 weeks. My work on flowering phenology indicates that rust infections also reduce significantly the flowering period of both male and female plants. Parker's work investigated a single *A. triphyllum* population over one growing season, hence it was difficult to interpret the generality of his findings. My study incorporated two additional populations over two growing seasons; it indicates that Parker's observations are consistent across space and time.

Prior to my study, it was not known whether the lower proportion of diseased females setting seed was due to reduced pollinator visitation or physiological effects of disease that reduce a female's ability to mature seed. Pathogen infection may alter

pollinator visitation in a number of ways. Infection could interfere with the production of olfactory attractants used by *A. triphyllum* to lure pollinators (Vogel & Martens 2000). Hence the attractiveness of diseased inflorescences may be reduced relative to the inflorescences of healthy plants. Diseased plants flower for a shorter length of time than healthy plants, which reduces opportunities for pollinator visitation. However, the results of my hand-pollination experiment suggest that the major effect of *U. ari-triphylli* infection is to inhibit the maturation of seed and the reduced ability of infected females to attract pollinators is of only minor importance to female reproductive success. A scant eleven percent of diseased females set seed naturally; when supplemental pollen was provided, the proportion of diseased females setting seed only increased to 14%, a full 44% below the proportion of healthy females setting seed naturally.

The hand-pollination experiment also demonstrated that female reproductive success is indeed limited by the availability of pollen, which is similar to the findings of earlier work by Bierzychudek (1981) and Rust (1980). Bierzychudek (1981) observed that hand-pollinated females produced over an order of magnitude more seeds than unmanipulated females (mean = 43.2, 1.0 respectively). My data do not display such striking trends but I found that pollen loading had a significant effect on seed production; hand-pollinated females produced on average 42.5 seeds, while unmanipulated control females produced on average 24.0 seeds. Rust (1980) found that *A. triphyllum* females within one meter of a male plant produced significantly more seeds than females more distant from males (mean = 33.5, 7.8 respectively). He also observed that pollinator movement was random within the one meter distance and that increased density of male plants significantly increased the percentage of females setting seed but not the number

of seeds produced; he proposed that increased male density may decrease pollen movement (Rust 1980). I did not take density into consideration in this study nor did I directly study pollinator movement, but Rust's (1980) findings support the idea that pollen limitation plays a role in limiting the female reproductive success of *A. triphyllum*. Given the dynamics of this plant-pollinator interaction, chronic pollen limitation is to be expected; the reward for insects successfully transferring pollen is death (Rust 1980). Therefore, pollen transfer is a chance event that occurs only when *A. triphyllum* manages to dupe insects into visiting multiple inflorescences.

Healthy females in the hand-pollination experiment exhibited significant differences in average seed weight; unmanipulated controls produced heavier seed than hand-pollinated plants. My findings suggest a trade-off between the number of seed that can be produced and the provisioning of individual seed. Control plants, which produced fewer seeds, are likely able to allocate a larger proportion of resources into each seed, resulting in a higher average seed weight. Hand-pollinated plants produced more seeds, but had to allocate available resources among a larger number of progeny, which likely resulted in a lower average seed weight. Seed weight was also influenced by population and year, however, and this suggests that differences in the net amount of resources available to individual plants resulting from variation across time and space could alter the proportion of resources that individual plants allocate to seed production. Long-term studies, possibly with a common garden experiment, are needed to address this issue further.

I did not find that localized environmental conditions had a significant effect on reproduction in *A. triphyllum*. The only significant correlations were between seed set

frequency and soil pH and soil moisture. This contrasts with earlier reports that resource limitations do have strong effects on reproductive success (Lovett Doust & Cavers 1982; Lovett Doust et al. 1986). The difference in findings may be due to the methods by which the availability of resources was measured. My work concentrated on the effects of the current physical environment (i.e., light, soil moisture, soil temperature and soil pH) while earlier studies measured stored resources in the corm. The corm may effectively ameliorate environmental fluctuations by accumulating nutrients in storage tissues during environmentally favorable years and releasing nutrients when conditions are less favorable. Several studies have noted that corms and plants can shrink or increase in size over time (Lovett Doust & Cavers 1982; Bierzychudek 1984; Lovett Doust et al. 1986). The ability of the corm to buffer the influence of the current environment may explain the limited number of environmental correlations detected in the current study.

Female reproductive success in *A. triphyllum* is strongly influenced by pollen limitation and disease, and may also be influenced by the microclimate. Hence it is apparent that several factors must converge for a female to successfully set seed. This study reinforces earlier work by Parker (1987) that *U. ari-triphylli* infections have a strong adverse affect on female reproductive success in *A. triphyllum*, and on the flowering phenology of both male and female plants. My work demonstrates that disease is just as important as pollen limitation in determining seed set in *A. triphyllum*. The influence of disease has largely been ignored in examinations of potential limitations on female reproductive success and the general influence of disease on female reproductive success remains to be determined. The effect of pathogens is obvious when infections

result in sterilization of flowers (Baker 1947; Alexander 1987; Althizer et al. 1998) or inhibition of flowering (Clay 1986 & 1987). The potential for foliar pathogens to have adverse effects on seed set has been demonstrated under greenhouse conditions by Jarosz et al. (1989), however few studies have demonstrated the effects of infection under field conditions (Roy & Bierzychudek 1993), and other work suggests that foliar pathogens have little influence on seed set (Jarosz & Burdon 1992). This work demonstrates that the influence of pathogens is variable across systems, since *U. ari-triphylli* infections adversely impact seed set in *A. triphyllum* under field conditions. Disease incidence within *A. triphyllum* populations must be taken into account when assessing the population level consequences of reduced female reproductive success due to disease; in populations with few diseased plants the adverse effects of *U. ari-triphylli* infections may be minimal. As disease incidence increases, population growth may become increasingly compromised.

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Chapter 3. Effects of disease and resource limitation on the growing season of vegetative *Arisaema triphyllum*.

Introduction

The timing of events, e.g. emergence and senescence, over the course of the growing season can be critical to survival and reproduction (Rathcke & Lacey 1985) and plants experience trade-offs for early vs. late emergence. Previous studies indicate that plants emerging early in the growing season have a longer period to attain resources for growth and reproduction, which leads to larger, more competitive plants with higher reproductive success than plants emerging late in the season (Baskin & Baskin 1972; Marks & Prince 1981). The trade-off for early emergence is a higher mortality due to less favorable environmental conditions (e.g., inadequate light levels, cold temperatures) early in the growing season (Baskin & Baskin 1972; Marks & Prince 1981). Plants emerging late in the growing season experience lower mortality rates and more favorable environmental conditions but have less time to acquire resources (Baskin & Baskin 1972; Marks & Prince 1981). Early emerging *Leavenworthia stylosa* had higher mortality than individuals emerging late in the season because of below average temperatures, but early plants produced more fruits and seed per plant due to the longer growing season (Baskin & Baskin 1972). Similarly, Marks & Prince (1981) observed that *Lactuca serriola* seedlings emerging in the winter months produced more seeds than individuals that emerged in the spring or summer because of the duration of the rosette stage, however the increased reproductive success of the winter seedlings was offset slightly by their higher mortality rate.

The timing of leaf senescence at the end of the growing season is a strictly controlled physiological process that can be induced by both abiotic (e.g., drought, nutrient availability) and biotic (e.g., pathogens, herbivory) environmental stresses as well as endogenous factors such as hormone levels and aging (Munne-Bosch & Alegre 2004). These two types of cues interact in the initiation and progression of leaf senescence; environmental stresses can speed up senescence by influencing reproductive development and hormone levels while endogenous factors can enhance the plant's ability to carry out leaf senescence under stress (Munne-Bosch & Alegre 2004). Our study examined potential environmental stresses rather than endogenous factors. In order to cope with environmental stresses, plants have evolved mechanisms that allow them to initiate leaf senescence in order to reallocate nutrients to reproductive organs or eliminate water consumption by older, less productive leaves; this ability to regulate leaf senescence is an adaptation that enables plants to complete their life cycle in times of stress (Gan & Amasino 1997). Although previous findings indicate that plants in many ecosystems induce leaf senescence in response to abiotic environmental stresses such as limited water and nutrients (especially Nitrogen) (de Castri 1981), biotic stresses such as pathogens could also lead to premature leaf senescence.

Previous research has demonstrated that pathogens do alter host life history (Alexander 1987; Clay 1986 & 1987; Parker 1987). For example, the anther smut *Mycobotrium violaceum* alters the life history of *Silene latifolia* to maximize pathogen dispersal by causing infected females to produce anther-like structures containing teliospores instead of pistillate flowers (Alexander 1987). Clay (1986 & 1987) found that

choke pathogens inhibit reproduction by replacing the inflorescence of infected plants with fungal reproductive structures.

In Chapter two, I demonstrated that systemic rust infection by *Uromyces ari-triphylli* adversely affected reproduction in *Arisaema triphyllum* by shortening the flowering period of infected plants, and reducing both seed set frequency and seed weight. Parker's (1987) study suggested that infections should also reduce the growing period of plants by causing early senescence. My goal in this study was to determine the extent to which disease reduces the growing season length by causing early senescence and whether it also had an effect on plant emergence. I compared the emergence and growing season length of healthy and diseased vegetative *A. triphyllum* over two growing seasons. I also attempted to determine whether potential environmental stresses (i.e. resource limitation) such as light, soil pH, soil moisture, and soil temperature had an effect on emergence and growing season length.

Materials & Methods

Experiments were conducted in April-September of 2002 & 2003 at four populations in central, lower peninsula Michigan. Three of the sites (Lott, Hudson, & Elsesser woodlots) are part of Michigan State University's natural areas holdings and are located within 5 miles campus (http://www.cpp.msu.edu/nat_area/). The fourth site, Webber, is located on private property approximately 20 miles northwest of campus and permission was obtained from the land owners prior to initiation of the study. Lott & Hudson populations were highly diseased because the incidence of *U. ari-triphylli* infection was approximately 50%. The other two populations, Webber & Elsesser, had a disease incidence of less than 10%. A series of $1m^2$ rectangular plots (0.25m X 4m) were

set up at various locations in each population to obtain an adequate sampling of microclimates within each site. Since plants within a clump are likely to be clones, long rectangular plots were used to ensure that multiple plant genotypes were monitored within each plot. All plants emerging within each plot were monitored for date of emergence, date of senescence, and disease status. In 2002 date of emergence was determined using weekly leaf length measurements and when the measurements were within 1-2 mm of the previous week's measurement the leaf was considered fully expanded. Growth in *A. triphyllum* is determinant (Bierzychudek 1984), and I expected leaf expansion to stop within a few weeks of the start of the growing season. However, leaves kept expanding well into the growing season. Therefore, emergence date criterion in 2003 was changed to the date when the shoot first emerged from the soil. Date of senescence was determined by weekly monitoring of plants and the senescence criterion was the total lack of chlorophyll in the leaf. Plants were considered to be diseased if any of the multiple spore types of *U. ari-triphylli* were present. Approximately 175 plants were sampled in each low disease population and 350 plants were sampled in each high disease population. A larger sample size was used in the high disease populations so approximately 175 healthy and 175 diseased plants were monitored at each site.

To determine whether environmental conditions influenced plant emergence and growing season length, light, soil pH, soil moisture and soil temperature (2003 only) measurements were taken in the middle and at the ends of each plot. Environmental measurements were made at the beginning, middle, and end of the growing season because of potential fluctuations in these factors over the course of the growing season e.g. overstory canopy closure. In 2002 light intensity was measured using an Extech

Model 401025 Foot Candle/ Lux Meter placed approximately one foot above the soil. All light measurements were made on overcast days between 12-4 pm. In 2003 light was measured as percent canopy cover using a spherical densiometer (Forestry Suppliers) placed approximately one foot above the soil. Soil pH and soil moisture were measured at a depth of three inches using a Kelway Soil Tester. In 2003, soil temperature was measured at a depth of 2.5 inches using a Tele-tru thermometer.

PROC MIXED in SAS was used to conduct analyses of variance (ANOVAs) and Type III tests to determine the significance of the effects of population and disease in the models for growing season length and emergence date (SAS Institute 1997). A preliminary analysis to determine whether environmental variables were highly correlated with each other was carried out using PROC CORR. Environmental variables early in the season were not highly correlated (i.e., > 0.85) with each other (Appendix 3), so I decided to exclude interaction terms from the emergence model. Only early environmental measures were used to evaluate correlations with plant emergence, since no plant emerged after the mid season measures were taken. With regard to growing season length, measurements made early, mid and late season were also not highly correlated; the average correlation between measures was 0.210; the highest correlation was 0.79 between soil temperature (early) and soil temperature (mid) (Appendix C). Therefore, I decided to leave all early, mid and late season measurements in the growing season length model but exclude the interaction terms. PROC GENMOD in SAS was used to conduct logistic regressions and Type III tests were utilized to determine the effects of environmental variables (light, soil pH, soil moisture, soil temperature) on emergence and growing season length.

Least square means were used because of unbalanced sample sizes. Since emergence criteria differed between 2002 and 2003, emergence and growing season data for each year were analyzed separately.

Results

Plant emergence

Plant emergence was influenced by disease status and population site in both 2002 and 2003 (Table 3.1). Somewhat surprisingly, average emergence date for diseased plants was significantly earlier than healthy plants in both years (Table 3.2). The influence of population was more variable across years (Table 3.3). Plants at Elsesser were among the latest to emerge in 2002 and 2003, while plants at Hudson were in the earliest group both years. The relative emergence of plants at Webber and Lott changed between years. Plants at Webber had the second earliest emergence in 2002 and the earliest emergence in 2003. The Lott population had the most significant change across years. Plants at this site were the latest to emerge in 2002, but grouped with earlier emergence in 2003.

Table 3.1. Analysis of variance of Julian emergence dates for the effects of population & disease for vegetative *A. triphyllum* at all four populations in a)2002 & b)2003.

a) Analysis of variance of Julian emergence dates in 2002.

Main Effect	df	Mean Square	F Value	Pr > F
Population	3	3638	37.59	< 0.0001
Disease status	1	5633	58.20	< 0.0001
Pop*Disease	3	134	1.39	0.1291
Error	1188	96.79		

b) Analysis of variance of Julian emergence dates in 2003.

Main Effect	df	Mean Square	F Value	Pr > F
Population	3	756.89	9.69	< 0.0001
Disease status	1	564.73	7.23	0.0073
Pop*Disease	3	101.54	1.30	0.2718
Error	1048	78.11		

Table 3.2. Least square mean Julian emergence dates for healthy and diseased *A. triphyllum* in 2002 and 2003.

Year	Population	Julian emergence date*
2002		
	Healthy	153.65±0.36
	Diseased	148.05±0.64
2003		
	Healthy	124.95±0.33
	Diseased	122.94±0.67

*means and standard errors. Healthy and diseased values within a year are significantly different.

Table 3.3. Least square mean Julian emergence dates for *A. triphyllum* in Hudson, Webber, Elsesser, and Lott populations in 2002 and 2003.

Year	Population	Julian emergence date*
2002		
	Hudson	146.17±0.56 a
	Webber	150.24±0.95 b
	Elsesser	152.79±0.80 c
	Lott	154.19±0.55 c
2003		
	Webber	121.90±0.85 d
	Hudson	122.74±0.63 d
	Lott	123.31±0.57 d
	Elsesser	127.84±0.88 e

*means and standard errors. Values followed by the same letter do not differ significantly

Despite differences in environmental measures within and among populations, none of the environmental factors (light availability, soil moisture, soil pH or soil temperature) early in the season were found to have a significant effect on plant emergence in 2002 (Table 3.4). However in 2003 percent cover and soil pH early in the season had significant effects on emergence (Table 3.5). The amount of cover early in the year influenced plant emergence with individuals in more open microhabitats emerging later than individuals in closed areas. Soil pH also affected plant emergence; plants in more alkaline soils tended to emerge earlier than individuals in more acidic

soils. Mean values for the environmental variables in 2002 & 2003 can be found in Appendix D.

Table 3.4. Analysis of variance for the effects of population, disease, and environmental variables early in the season on plant emergence in 2002.

Main Effect	df	Mean Square	F Value	Pr > F
Population	3	756.89	18.42	< 0.0001
Disease status	1	564.73	57.67	< 0.0001
Pop*Disease	3	101.54	1.73	0.1599
Light intensity	1	15.47	0.16	0.6887
Soil pH	1	312.28	3.23	0.0726
Soil moisture	1	125.68	1.30	0.2546
Error	1185	96.68		

Table 3.5. Analysis of variance for the effects of population, disease, and environmental variables early in the season on plant emergence in 2003.

Main Effect	df	Mean Square	F Value	Pr > F
Population	3	629.76	8.23	< 0.0001
Disease status	1	574.66	7.51	0.0063
Pop*Disease	3	107.13	1.40	0.2402
Light intensity	1	615.98	8.05	0.0046
Soil pH	1	471.36	6.16	0.0133
Soil moisture	1	101.01	1.32	0.2508
Soil temp.	1	30.61	0.40	0.5262
Error	1042	76.52		

Growing season length

In 2002, growing season length was influenced by an interaction between disease status & population (Table 3.6). Healthy plants always had longer growing seasons than their diseased counterparts (Table 3.7). On average, the growing season for healthy plants was more than two weeks longer than the growing season of diseased plants (mean: healthy = 56.63 days; diseased = 41.28 days). The degree to which disease affected growing season length varied among populations. At Elsesser, Hudson, and Lott the growing season for diseased plants was approximately three-fourths that of healthy

plants. The effect of disease was more severe at Webber where the growing season of diseased plants was only 63% of the growing season for healthy plants. The effect of population was also evident in the healthy plants. At Elsesser, healthy plants had the longest growing season, which was more than three weeks longer than the shortest growing season at Lott. For diseased plants, the range across populations for the diseased plants was 16.75 days. Growing season lengths were similar for diseased plants at the Hudson and Lott populations. In contrast, the growing season for healthy plants at Hudson was significantly longer than that for healthy plants at Lott.

Table 3.6. Analysis of variance of growing season length for the effects of population and disease on healthy & diseased vegetative *A. triphyllum* at all four populations in 2002.

Main Effect	df	Mean Square	F Value	Pr > F
Population	3	141173	30.46	< 0.0001
Disease status	1	50958	109.51	< 0.0001
Pop*Disease	3	1316	2.83	0.0375
Error	1188	465.33		

Table 3.7. Least square mean growing season length for healthy & diseased vegetative *A. triphyllum* at all four populations in 2002.

Population	Growing season length (days)				
	Number	Healthy[a]	Number	Diseased[a]	Proportion Diseased/Healthy
Elsesser	107	71.40±2.08	59	54.08±2.81	0.757
Webber	203	64.16±1.51	31	40.19±3.87	0.626
Hudson	327	52.85±1.19	103	38.66±2.02	0.732
Lott	252	49.20±1.36	114	37.33±2.12	0.759

[a]means and standard errors. Healthy and diseased values within a population are significantly different.

In 2003, both disease status and population had a significant effect on growing season length but there was no interaction (Table 3.8). The average growing season for plants, regardless of disease status, differed among the populations (Table 3.9). As in

2002, plants at Elsesser had the longest growing season, which was three weeks longer than the shortest growing season at Webber (Table 3.9). The relationship between Hudson and Lott populations with respect to growing season length did not remain constant between years (Tables 3.7 & 3.9). In 2002, plants at Hudson had a longer growing season than plants at Lott but in 2003 the opposite was true. Diseased plants had a mean growing season that was significantly shorter (15 days) than their healthy counterparts (Table 3.10).

Table 3.8. Analysis of variance of growing season length for the effects of population and disease on healthy & diseased vegetative *A. triphyllum* at all four populations in 2003.

Main Effect	df	Mean Square	F Value	Pr > F
Population	3	896.48	20.99	< 0.0001
Disease status	1	30487	71.28	< 0.0001
Pop*Disease	3	85.54	0.20	0.8955
Error	1048	427.71		

Table 3.9. Least square mean growing season length and standard errors for vegetative *A. triphyllum* at all four populations in 2003.

Population	Number	Growing season length (days)
Elsesser	135	93.13±2.07 a
Lott	310	79.10±1.34 b
Hudson	401	76.00±1.47 b c
Webber	210	72.06±1.98 c

*means followed by the same letter do not differ significantly

Table 3.10. Least square mean growing season length and standard errors for healthy & diseased vegetative *A. triphyllum* in 2003.

Disease Status	Number	Growing season length (days)
Healthy	853	87.44±0.78
Diseased	203	72.71±1.56

Soil pH was the only environmental variable to have a consistent, significant effect on growing period length in both years (Tables 3.11 & 3.12); plants growing in

more alkaline soils had a longer growing season than plants growing in more acidic soils. However, the portion of the growing season in which pH exerted a significant effect varied between years; in 2002 the effect was more pronounced in the beginning and middle of the growing season (Table 3.11) but in 2003 the effect was predominantly found in the beginning and end of the growing season (Table 3.12). In 2003, soil moisture levels in the middle and end of the growing season also had a significant effect on growing period length; plants growing in wetter soils had a longer growing period length than plants growing in drier soils (Table 3.12).

Table 3.11. Analysis of variance of environmental data at all four populations for early(1), middle (2), and end(3) of the growing season in 2002.

Main Effect	df	Mean Square	F Value	Pr > F
Population	3	7036.23	15.26	< 0.0001
Disease status	1	50083.6	108.62	< 0.0001
Pop*Disease	3	1152.72	2.50	0.0584
Light 1	1	0.46	0.001	0.9512
Soil pH 1	1	3591.89	7.79	0.0054
Soil moisture 1	1	239.77	0.52	0.4730
Light 2	1	442.65	0.96	0.3282
Soil pH 2	1	1895.08	4.11	0.0427
Soil moisture 2	1	428.81	0.93	0.3340
Light 3	1	1240.33	2.69	0.1014
Soil pH 3	1	1166.56	2.53	0.1119
Soil moisture 3	1	534.86	1.16	0.2807
Error	1168	461.09		

Table 3.12. Analysis of variance of environmental data at all four populations for early(1), middle (2), and end(3) of the growing season in 2003.

Main Effect	df	Mean Square	F Value	Pr > F
Population	3	2269.89	5.57	0.0009
Disease status	1	31786.56	78.00	< 0.0001
Pop*Disease	3	232.29	0.57	0.6327
% cover 1	1	268.96	0.66	0.4166
Soil pH 1	1	6422.51	15.76	< 0.0001
Soil moisture 1	1	12.22	0.03	0.8728
Soil temp. 1	1	497.17	1.22	0.2705
% cover 2	1	419.74	1.03	0.3094
Soil pH 2	1	16.30	0.04	0.8448
Soil moisture 2	1	2436.97	5.98	0.0146
Soil temp. 2	1	73.35	0.18	0.6695
% cover 3	1	20.38	0.05	0.8266
Soil pH 3	1	2436.97	5.98	0.0147
Soil moisture 3	1	3068.63	7.53	0.0062
Soil temp. 3	1	175.23	0.43	0.5111
Error	1034	407.52		

Discussion

Infections by the systemic fungal pathogen, *Uromyces ari-triphylli*, altered the life history of vegetative *Arisaema triphyllum* by shortening the growing season and promoting early emergence. My findings support those of Parker (1987) who noted that flowering *A. triphyllum* senesced earlier than healthy flowering individuals. Early emergence does ameliorate the effects of early senescence to some degree, but the growing season of the average diseased plant remained only three-quarters that of comparable healthy plants. The combined effects of a shorter growing season and the nutritional demands of the pathogen probably contribute to the temporal trend where diseased *A. triphyllum* decrease in size across years (EE Mason, personal communication). Overall, resource limitation, as determined by the proximate

environment, does not appear to have a strong influence on emergence and growing season length of *A. triphyllum*.

My results present the intriguing possibility that emergence is altered in diseased *A. triphyllum* for the benefit of the pathogen. Early emergence by diseased plants may increase the probability of contagious spread for *U. ari-triphylli*. Lesions on infected plants do not erupt and disperse spores until the leaf begins to unfurl. This means that inoculum for infectious spread of the pathogen is not available until two or more weeks after the infected individual emerges from the ground. If the pathogen can shift emergence so that infected plants emerge earlier than healthy individuals, then spread to succulent new material on healthy plants may be maximized.

Alteration of host life history by pathogens is known for several host-parasite systems. Life history of *Silene latifolia* infected by the anther smut, *Mycobotrium violaceum*, is altered in a manner that promotes pathogen spread. Infected females produce anther-like structures containing pathogen teliospores instead of pistillate flowers. Similarly, the anthers of males produce pathogen teliospores instead of pollen. Infection also influences flowering behavior. Healthy males produce more flowers than healthy females, but female flowers remain on the plants for a longer period of time (Alexander 1987). Infected plants produce the male number of flowers and flowers are retained on the plant nearly as long as healthy female (Alexander 1987). Thus, the pathogen alters the plant to maximize its dispersal. Choke pathogens get their name because they inhibit reproduction of the plant. The inflorescence of infected plants is replaced by reproductive structures of the pathogen which promote pathogen spread during the host's flowering period (Clay 1986 & 1987). Thus, it seems possible that the

early emergence exhibited by infected *A. triphyllum* is an adaptation by the pathogen to maximize its infectious spread within a population.

My findings relating to the effect of disease on the growing period corroborate earlier work by Parker (1987); he observed that diseased *A. triphyllum* have reduced leaf longevity, which likely contributed to the shorter growing period length of diseased plants in my study. A shortened growing season could have pronounced effects on resource acquisition in *A. triphyllum*; individuals have only a brief period of time to utilize available resources for survival and to accumulate photosynthetic reserves in the corm. Accumulation of these corm resources is essential for vegetative *A. triphyllum* to make the transition to flowering as a male or female; previous studies have demonstrated that sexual expression *A. triphyllum* is correlated with overall plant size which is directly related to the amount of corm resources (Heslop-Harrison 1957; Policansky 1981; Bierzychudek 1982 & 1984; Lovett Doust & Cavers 1982; Lovett Doust et al. 1986; Parker 1987). The disease probably acts as a drain on a plant's energy and nutrient resources, which could further reduce the resources available to produce cormlets or an inflorescence. In consequence, disease could alter population composition by altering the proportion of vegetative, male, and female plants. Ultimately, disease may decrease the persistence of *A. triphyllum* populations in the understory, which could affect the structure of the forest community.

Environmental variables (light, soil pH, soil moisture, and soil temperature) had no consistent effect on emergence or growing period length suggesting that proximate resources are not important influences on these aspects of the life history. Past studies have reported that the amount of stored resources in the corm of *A. triphyllum* can affect

the timing of transition from vegetative to flowering as well as female reproductive success (Lovett Doust & Cavers 1982; Bierzychudek 1984; Lovett Doust et al. 1986). These stored resources may compensate for reduced nutrient accumulation when environmental conditions are less favorable. Essentially, the corm may function to "average" the effects of the proximate environment and buffer a plant's response to fluctuations in light incidence, soil pH, soil moisture, and soil temperature from one growing season to the next. Soil pH was the only environmental variable that consistently influenced plant emergence and growing period length. Previous findings by Giesler et al. (1998) indicate that there is a strong relationship between soil characteristics (e.g. soil pH & nitrogen content) and plant productivity. Although my study did not measure productivity, it is possible that the differences in emergence and growing period length of plants growing in different soil pH conditions could indirectly influence productivity. I observed that plants growing in more alkaline soils emerged earlier and had a longer growing period than plants growing in acidic soil, which could be associated with an increase in productivity. This relationship between early emergence/longer growing period and productivity is purely speculative, however, and further investigation is needed.

Although light and percent cover did not exert a significant influence on growth period, I speculate that the significant effect of population could be attributed to differences in light levels among the four sites. I observed that plants at Elsesser population had the longest growth period in 2003, which could relate to the fact it consistently had a lower percent canopy cover than any of the other four populations over the growing season. Similarly, plants at Webber population had the shortest growth

period in 2003, which might be due to the fact it consistently had a higher percent canopy cover than any of the other four populations over the growing season. These statements are highly speculative, however, and additional research is needed.

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Chapter 4. Conclusions

The results of my study indicate that the *Arisaema triphyllum-Uromyces ari-triphylli* interaction is typical of many plant-pathogen interactions; infection reduces the reproductive success of the host (Alexander 1987; Burdon 1987; Parker 1987; Jarosz et al. 1989; Jarosz & Burdon 1992; Roy 1993; Jarosz & Davelos 1995). Disease sabotages the efforts of *A. triphyllum* to maximize female reproduction on two different levels: 1) limiting the time an inflorescence is receptive to pollen, and 2) limiting the flow of resources to pollinated ovules and developing fruits. My findings demonstrate the importance of pathogens in shaping plant communities by reducing individual fitness, which also has implications at the population level (e.g. reduced competitive ability).

Pollen limitation was also an important limitation on female reproductive success in *A. triphyllum*; my results corroborate the findings of Rust (1980) and Bierzychudek (1981). For healthy females, hand-pollinated plants were more likely to set seed than unmanipulated controls and produced more seeds, but the seeds were smaller than those of the controls. This suggests a trade-off between seed number and the amount of resources allocated to each seed, and could have implications for seedling survival in *A. triphyllum*, but additional studies are needed.

In terms of resource limitation, the proximate environment does not appear to be as important as disease or the previous environment (as indicated by stored resources in the corm) in influencing the female reproductive success or growing season of *A. triphyllum*. Past studies have demonstrated that the amount of stored resources affects female reproductive success but these investigations utilized different methods to assess resource limitation, e.g. measuring corm size to determine the amount of stored resources

(Policansky 1981; Lovett Doust et al. 1986). The inability to correlate proximate environmental variables with flowering and seed set seems to indicate that the corm "averages" the environment, and buffers the plant's response to temporal variability in factors such as light incidence, soil moisture, soil temperature and soil pH. I would have liked to test the effects of resource limitation by directly measuring the amount of stored resources as indicated by corm diameter, i.e. replicating the approach of Lovett Doust et al. (1986), but there were practical and technical constraints. Additionally, if distinct environmental effects had been observed in the first year, I would have initiated more manipulative measures in the second growing season such as a shading experiment or artificial creation of light gaps. My study did suggest that soil pH had small but significant effects on seed set, emergence and growing period. The mechanism by which soil pH affects these important life history traits of *A. triphyllum* is unknown, but would be worth pursuing.

Our findings with respect to the effects of disease on seed set frequency corroborate Parker's (1987) results. This study however has not only expanded the assessment of the effects of disease by studying multiple populations over two growing seasons, but also enabled the in-depth examination of the numerous other ways that disease affects female reproductive success, e.g. flowering phenology. The numerous effects of disease on the life history of *A. triphyllum* all point to the reduced fitness of diseased individuals compared to their healthy counterparts. If a female plant becomes diseased and is unable to produce seed, reproductive success and genetic contribution to the next generation might be maintained by flowering as a male in subsequent years; we observed however that healthy plants had a higher probability of being male than

diseased plants. If disease did favor flowering as a male, the structure of *A. triphyllum* populations could shift to a more male biased ratio. For infected females able to maintain seed production, their progeny would be free of disease but have fewer stored resources both for establishment and sustenance, i.e. the length of time before the seedling would need to rely on photosynthesis for energy would be shortened.

My work also suggests that infection alters the pattern of emergence in *A. triphyllum*. Diseased plants emerge earlier in the season than healthy plants. This pattern may increase the rate of infectious spread of the pathogen by making spores of the pathogen available as the healthy plants begin their growing period. Further studies are needed to confirm this hypothesis. Disease status of individual plants could be manipulated with inoculation and disease curing experiments that would determine definitively if infection alters plant emergence. The clonal nature of *A. triphyllum* reproduction could be utilized in this work to examine the change in emergence as clones are infected.

For further study I would like to examine how the multitude of the above effects influence population structure and persistence in *A. triphyllum*. I do not think that this disease necessarily poses a threat to the existence of this species for several reasons. First, there are likely populations within the geographical range of *A. triphyllum* where the pathogen is absent. Second, the existence of resistant *A. triphyllum* genotypes within infected populations is a distinct (but uninvestigated) possibility. Finally, variation in disease severity among infected populations suggests that the effects of the pathogen on the life history of *A. triphyllum,* e.g. female reproductive success & competitive ability, pose more of a threat in highly diseased populations than in low disease populations.

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Appendices

Appendix A. Analysis of variance of environmental data at Hudson and Lott populations for the a) 2002 and b) 2003 flowering seasons.

a) Analysis of variance of environmental data at Hudson and Lott populations for the 2002 flowering season.

Main Effect	df	Mean Square	F Value	Pr > F
Population	1	87.63	3.16	0.0785
Disease	1	1005.49	36.26	<0.001
Light	1	79.58	2.87	0.0936
Soil pH	1	19.41	0.70	0.4065
Light*pH	1	80.42	2.90	0.0918
Soil moisture	1	16.36	0.59	0.4440
Light*moisture	1	71.82	2.59	0.1108
pH*moisture	1	17.75	0.64	0.4261
Light*pH*moisture	1	72.10	2.60	0.1100
Error	99	27.73		

b) Analysis of variance of environmental data at Hudson and Lott populations for the early(1), middle (2), and end(3) of the flowering season in 2003.

Main Effect	df	Mean Square	F Value	Pr > F
Population	1	61.37	3.33	0.0722
Sex	1	101.97	5.56	0.0211
Disease	1	163.59	8.92	0.0039
% Cover 1	1	0.18	0.01	0.9078
Soil pH 1	1	3.85	0.21	0.6484
Soil moisture 1	1	19.99	1.09	0.3011
Soil temp. 1	1	2.93	0.16	0.6879
% Cover 1*pH 1	1	1.65	0.09	0.7593
% Cover 1*moist.1	1	48.60	2.65	0.1081
% Cover 1*temp.1	1	0.18	0.01	0.9166
pH 1*moist.1	1	18.89	1.03	0.3136
pH 1*temp.1	1	1.47	0.08	0.7730
moist.1*temp.1	1	26.96	1.47	0.2298
% Cover 2	1	2.57	0.14	0.7077
Soil pH 2	1	0.73	0.04	0.8444
Soil moisture 2	1	33.56	1.83	0.1810
Soil temp. 2	1	36.13	1.97	0.1643
% Cover 2*pH 2	1	0.18	0.01	0.9177
% Cover 2*moist.2	1	0.18	0.11	0.7431
% Cover 2*temp.2	1	2.02	2.06	0.1559
pH 2*moist.2	1	50.80	2.77	0.1005
pH 2*temp.2	1	34.48	1.88	0.1750
moist.2*temp.2	1	41.99	2.29	0.1345
% Cover 3	1	3.48	0.19	0.6608
Soil pH 3	1	11.00	0.60	0.4411
Soil moisture 3	1	4.03	0.22	0.6398
Soil temp. 3	1	19.62	1.07	0.3039
% Cover 3*pH 3	1	4.95	0.27	0.6082
% Cover 3*moist.3	1	0.00	0.00	0.9593
% Cover 3*temp.3	1	16.14	0.88	0.3512
pH 3*moist.3	1	0.00	0.00	0.9564
pH 3*temp.3	1	15.22	0.83	0.3645
moist.3*temp.3	1	1.83	0.10	0.7562
Error	71	18.34		

Appendix B. Mean values of light availability, soil pH, soil moisture, and soil temperature for Hudson (HU) and Lott (LS) at the beginning, middle, and end of the 2002 and 2003 flowering seasons.

	Hudson	Lott
2002		
Early season measures:		
Light (Lux)	815.2	309.2
Soil pH	6.6	6.9
Soil Moisture (%)	67.6	64.5
Mid season measures:		
Light (Lux)	297.8	429.3
Soil pH	6.0	6.6
Soil Moisture (%)	72.0	78.3
Late season measures:		
Light (Lux)	1160.3	1370.5
Soil pH	6.8	7.0
Soil Moisture (%)	61.6	62.0
2003		
Early season measures:		
% Cover	12.0	12.1
Soil pH	6.8	6.9
Soil Moisture (%)	66.4	65.2
Soil Temperature (°C)	6.9	9.4
Mid season measures:		
% Cover	33.6	40.1
Soil pH	6.5	6.9
Soil Moisture (%)	71.1	69.5
Soil Temperature (°C)	9.2	12.0
Late season measures:		
% Cover	79.5	74.0
Soil pH	6.5	6.8
Soil Moisture (%)	72.5	74.1
Soil Temperature (°C)	14.0	15.0

Appendix C. Correlations among environmental variables in 2002 (above diagonal) and 2003 (below diagonal).

Var.	Cover/ Light early	pH early	Moist early	Temp early	Cover/ Light mid	pH mid	Moist mid	Temp mid	Cover/ Light late	Temp late	pH late	moist late
Cover/ Light early		-0.232	0.376 *		0.188	-0.353	-0.065		-0.141		0.197	0.376
pH early	-0.114		-0.427 *		0.061	0.609 *	0.124		0.438 *		-0.340 *	0.278 *
Moist early	-0.033	-0.328 *			0.181	-0.305 *	0.259 *		-0.100		0.268 *	0.100
Temp early	0.005	0.343 *	0.061									
Cover/ Light mid	-0.055	0.134	-0.053	0.005		-0.001	0.095		0.230		0.129	0.066
pH mid	0.028	0.462 *	-0.065	0.582 *	-0.028		-0.153		0.394 *		-0.037	0.117
Moist mid	-0.075	-0.113	0.413 *	-0.362 *	-0.036	-0.402 *			0.226		-0.105	0.441 *
Temp mid	0.119	0.280	0.109	0.794 *	0.105	0.544 *	-0.422 *					
Cover/ Light late	0.117	-0.127	0.024	-0.018	0.421 *	0.030	-0.175	0.011			-0.011	0.180
Temp late	-0.283	-0.032	0.236	-0.387 *	-0.042	-0.435	0.444 *	-0.223	-0.164			
pH late	0.109	0.416	-0.049	0.147	-0.028	0.104	-0.097	0.304 *	-0.212	0.433 *		-0.492 *
Moist late	-0.069	-0.044	0.267	0.188	-0.088	0.217	0.261 *	0.146	-0.054	-0.411 *	-0.564 *	

*indicates significance for Prob > |r| under H0: Rho=0 at 0.05 level

Appendix D. Mean values of light intensity, soil pH, soil moisture, and soil temperature (2003 only) for all four populations at the beginning, middle, and end of the a) 2002 and b) 2003 growing season.

a) mean environmental values in 2002.

	Hudson	Elsesser	Webber	Lott
2002				
Early season measures:				
Light (Lux)	904.7	532.1	446.0	456.2
Soil pH	6.5	6.5	7	7
Soil Moisture (%)	65.0	63.8	46.2	59.5
Mid season measures:				
Light (Lux)	272.0	137.1	106.2	342.7
Soil pH	6.0	6.1	6.3	6.5
Soil Moisture (%)	72.3	77.1	73.1	81.0
Late season measures:				
Light (Lux)	977.2	844.3	1048.1	1325.8
Soil pH	6.8	6.3	6.2	6.5
Soil Moisture (%)	69.9	68.1	80.5	81.3

b) mean environmental values in 2003.

	Hudson	Elsesser	Webber	Lott
2003				
Early season measures:				
% Cover	12.0	10.4	22.4	16.4
Soil pH	6.7	6.5	6.6	6.7
Soil Moisture (%)	68.6	54.4	62.5	62.5
Soil Temperature (°C)	8.9	6.7	7.8	11.5
Mid season measures:				
% Cover	81.6	75.9	80.2	81.8
Soil pH	6.6	6.5	6.6	6.8
Soil Moisture (%)	75.3	67.7	73.3	58.9
Soil Temperature (°C)	13.7	12.3	12.9	14.8
Late season measures:				
% Cover	82.4	78.1	88.7	84.3
Soil pH	6.9	6.5	6.7	6.7
Soil Moisture (%)	57.9	70.3	63.5	73.7
Soil Temperature (°C)	21.0	18.8	17.7	16.9