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Cause and Impacts of the Early Season Collapse of Lilium grayi (Gray's lily), on Roan Mountain, TN/NC

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by

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ABSTRACT

Cause and Impacts of the Early Season Collapse of *Lilium grayi* (Gray's lily), on Roan Mountain, TN/NC

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A population of the rare Southern Appalachian endemic species *Lilium grayi*, (Gray's lily) Roan Mountain, TN/NC was monitored for 2 years to determine the cause and impact of an early season collapse. High concentrations of the *Lilium* spp. host-specific fungal phytopathogen, *Pseudocercosporella inconspicua* (G. Winter) U. Braun were associated with 19/20 symptomatic and 0/30 asymptomatic plants. Strength of the association between pathogen and disease and the replication of disease symptoms in 4/4 healthy hosts showed that *P. inconspicua* was the causal agent of the disease referred to as lily leaf spot. Disease had a severe impact on the population with 59% of mature and 98% of adolescent plants undergoing early senescence. Only 32% of mature plants produced capsules and they were frequently diseased. A recurring spatiotemporal pattern typical of an infectious disease suggested that the lily leaf spot disease is capable of causing sequential annual epidemics of unknown long-term consequences to the stability of the host population.

DEDICATION

This Thesis is Dedicated To:

The memory of my father, Robert Jackson Ingram Jr., I hope I have made you proud.

To my grandmother, Minette Cass Hatcher, whose unwavering love was the light by which I walked for so many years. Words cannot express how grateful I am.

To my grandfather, Jack Bell Hatcher, whose passion for horticulture helped me find the path to happiness. You may not know it but you are the example by which I follow.

To my mother, Elizabeth Sue Ingram, who taught me to strive for greatness. You taught me to never settle and most importantly, to never give up.

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"The mediocre teacher tells. The good teacher explains. The superior teacher demonstrates. The great teacher inspires." ― William Arthur Ward

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"The journey of a thousand miles begins with a single step." ― Lao Tzu
Thank you for helping me to take that first step.

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CHAPTER 1

INTRODUCTION

History of the Early Season Collapse of Lilium grayi *on Roan Mountain TN/NC*

Roan Mountain is a beautiful wilderness area that is host to an overwhelming diversity of habitats, flora, and fauna. These include the globally rare "Southern Appalachian grassy balds," a plant community found only a few mountaintops within the southeastern United States (Skinner 2002). Over the past 2 centuries the uniqueness of the habitat and the many disjunct and endemic species that occur within the grassy bald habitat on the Roan Mountain massif have attracted the attention of botanists and early ecologists. Examples of notable figures include Andre Michaux, Asa Gray, and D.M. Brown. Within the last 50 years a marked decline in the land area covered by the Southern Appalachian grassy balds has been noted (Brown 1949; Brown 1983; Weigl and Knowles 1995; Crawford and Kennedy 2009). The recent reduction in size of the grassy balds has been attributed to the encroachment of woody shrubs, especially Canada blackberry (*Rubus canadensis)* (Donaldson 2009). Observations that the grassy bald habitats on Roan Mountain are closing have spurred a renewed interest in understanding the ecology of the habitat and the many rare species it supports.

One of the more prominent plant species occurring in the grassy bald habitat is *Lilium grayi* S. Watson (Gray's lily). As a member of the family Liliaceae and the genus *Lilium*, *L. grayi* is considered a true lily. True lilies are perennial herbs of high aesthetic value and consequently are of great commercial significance. They are cultivated in many gardens and landscapes and the flowers are highly prized among florists (Skinner 2002). Gray's lily is a narrowly endemic species occurring only in high elevation sites, (1200-1990 m) from southwest

Virginia to east Tennessee and west North Carolina in the southern Appalachian Mountains of North America (Skinner 2002).

Gray's lily is a perennial plant that emerges from an unbranched rhizomatous bulb with associated scales. The leaves are arranged in 3 - 5 leaf whorls per plant with 3 - 12 leaves per whorl. The leaf blades have an acute apex and are elliptic to slightly lanceolate with leaf length from 1.9 - 5 times longer than the width. Inflorescences are racemose, with 1 - 16 nodding flowers that bloom from June-July (Smith 1998). The flowers are red-orange with purple to black spots on the inside of the petals, are unfragrant, and are primarily pollinated by ruby-throated hummingbirds (*Archilochus colubris* L. Trochilidae). The diagnostic characteristic of the species is tepals that are recurved for 2/3 - 9/10 of their length from the base. The anthers are magenta and the filaments are parallel to the style. Ovaries are triloculate and the mature fruit is a dehiscent capsule (Skinner 2002).

Molecular-based phylogenetic studies show *Lilium canadense* L. (Canada lily) as the sister taxa to *L. grayi* (Hayashi and Kawano 2000; Skinner 2002). In regions where *L. canadense* and *L. grayi* overlap, they frequently hybridize, producing the hybrid, *Lilium* x *pseudograyi* Grove. Although *L. grayi* can be found in other habitats such as forest seeps and boulder fields, the openness and high light conditions of the grassy bald habitat appear to promote the successful completion of its life cycle (Amoroso and Finnegan 2002; Coomans 2002). As the largest and most continuous of the extant Southern Appalachian grassy balds, Roan Mountain has been the focal point for the few studies conducted on the life cycle and ecology of *L. grayi* (Dunscombe 1994; Donaldson 2009; Powell 2011). The extent of suitable habitat on Roan Mountain for *L. grayi* and the diminutive size of previously reported populations elsewhere have

led to the conclusion that the population on Roan Mountain is likely the largest extant natural population of the species.

Gray's lily has a Global Heritage Rank of G3 and is considered a "Federal Species of Concern" in the United States (Donaldson 2009). In Tennessee, the status of the species is "Endangered", in Virginia it is ranked as "S-2" or very rare and imperiled, and in North Carolina its status is "Special Concern, Threatened" (USDA Plant Database 2013). There are only 112 known occurrences of *L. grayi*, with 27 of the reports attributable to historic accounts of non-extant localities (Coomans 2002). Recent work suggests that many of the extant populations of the species consisted of only 5-10 plants (Bates 2000). The state and federal listing of *L. grayi* is largely based on 7 major threats faced by the species in the wild: A restricted distribution and few remaining genetically pure populations; A high frequency of mammal browsing by deer (Murdock 1995; Bates 1998); Intrusion of *L. canadense* into the range of *L. grayi* and subsequent hybridization; Loss of habitat due to woody encroachment (Crawford and Kennedy 2009; Donaldson 2009); Reduced vigor and reproductive success; Over-collecting and poaching of the species due to its relative rarity and beauty; Early season collapse of the species due to an unknown disease (Coomans 2002).

The combination of the relative rarity and exceptional beauty of *L. grayi* has attracted the attention of photographers, hikers, naturalists, and amateur and professional botanists. This attention highlighted an early season collapse that was observed in populations of *L. grayi* on Roan Mountain and other sites (Bates 1999; Donaldson 2009; Powell 2011). While there were attempts to characterize and identify the cause of the early season collapse, these were unsuccessful in providing a definitive determination of the cause of disease (Bates 1995, 1998, 1998, 1999, 2000; Coomans 2002; Powell 2011).

The first investigation of the early season collapse of *L. grayi* was conducted by Bates and the North Carolina Plant Conservation Program. Their studies were presented in several reports that outlined the demography of *L. grayi*, the hypothesized cause of an early season collapse, the impact of disease on reproduction and recruitment, and the effect of canopy clearing on the occurrence and severity of disease (Bates 1995, 1998, 1998, 1999, 2000; Coomans 2002). Symptoms of the early season collapse were described as a yellowing or spotting of leaves and stems along with wilting of the leaves (Bates 1998; Coomans 2002). Moreover, there were severe reductions in within-season survivorship of juvenile and adult lilies. In 1997, rates of early season collapse were 100% for juveniles and 57% - 100% for adults on Bluff Mountain, Ashe County, NC and Sparta Bog, Allegheny County, NC (Bates 1998). In 1999, rates of early season collapse in 2 populations ranged from 39% - 100% for juveniles, and 95% of adults (Bates 1999).

The high rates of early season collapse were attributed to a fungal disease that drastically reduced host survivorship. Additionally, the high rate of early season collapse of juveniles was used as evidence of the reduction in recruitment of non-reproductively mature *L. grayi* into the reproductively mature lily population. A severe decrease in fecundity of adult plants was also noted with rates of capsule production per plant ranging from 0 - 52%. The low capsule production was attributed to fungal infection that caused an early season collapse of host plants before reproduction and, to a lesser degree, herbivory by deer (Coomans 2002). Increased rates of early season collapse were hypothesized to be associated with high soil moisture and canopy cover. However, Bates (2000) conclusions were reached without associating the early season collapse of lily plants with a diagnostic list of symptoms. Instead, methods suggest an assumption that all early season collapse of lily plants was caused by the fungal disease. Without

providing a means of differentiation between the many abiotic and biotic causes of early senescence, rates of early season collapse are subject to inflation. However, the absence of a symptom-pathogen association does not negate the observation that survivorship, fecundity, and recruitment of *L. grayi* was being apparently depressed from expectations (Coomans 2002).

Prior studies had difficulty in determining the causal organism of the early season collapse of *L. grayi*. The high prevalence of species of 3 common phytopathogenic genera on diseased host tissue was the basis for an association of the collapse of *L. grayi* with *Alternaria* species, *Botrytis* species, and *Colletotrichum* species. *Colletotrichum* sp. was concluded to be the causal organism of disease based on its prevalence among host disease samples (Bates 1998). However, there were several problems with the proposed determination of causality. First, hypothesized fungal pathogens were not identified to species. Consequently, it was not possible to compare the fungal species occurrence with known host range. Second, symptoms of the early season collapse did not match symptoms expected for infection by *Colletotrichum* sp. Last, diseased samples used for diagnosis were reported as dead lily stems and capsules (Bates 1998). For the last reason alone, it is not surprising that *Alternaria* sp., *Botrytis* sp., and *Colletotrichum* sp. were found to be highly prevalent because of the more aggressive growth of secondary pathogens on dead tissue. Because secondary fungal pathogens frequently out-compete primary fungal pathogens, an accurate diagnosis of disease is usually only possible from living tissues.

Nearly a decade after the initial studies, another preliminary investigation was conducted whose primary goal was to understand the demography of *L. grayi* with a secondary goal of identifying a candidate primary pathogen or abiotic cause of the early season collapse (Powell 2011). Powell (2011) further refuted claims of earlier studies by finding a lack of association between *Colletotrichum* sp. with symptoms of the early season collapse among the pathogen data

based on determination from several diseased samples sent to the North Carolina Plant Disease and Insect Clinic at North Carolina State University. Rather all diseased samples contained the phytopathogen, *Pseudocercosporella inconspicua* (G. Winter) U. Braun.

Braun (1995) cited the fungal phytopathogen *Pseudocercosporella inconspicua*, Phylum Ascomycota, Class Dothideomycete, as host-specific to the genus *Lilium*. It was first described as the causal organism of a leaf spot disease of *Lilium* when found on *L. martagon* in Switzerland in 1883. Since then, 8 of the 11 *Lilium* sp. hosts of *P. inconspicua* have been in Europe and Asia. There is one host species, *L. speciosum,* on which the disease has been reported in Asia, Europe, and North America (Manitoba, Canada). Past reports in Europe have implicated *P. inconspicua* as the cause of an early season decline of *Lilium* sp. cultivated in flower farms in Kiev, Ukraine (Zerova 1940). The disease was reported to have caused significant economic loss in nurseries as whole sections of nurseries were killed before or during flowering. There was also a report of a 1920 epidemic outbreak of *P. inconspicua* in cultivated *L. maximowjczii* in some districts of Hokkaido, Japan (Makoto 1925). The 3 other hosts of *P. inconspicua* have been *Lilium* species in North America, with 2 of the 3 host species reported from the United States.

All previous geographic reports from the United States have been restricted to sites east of the Mississippi River and in the more northerly states of Wisconsin, New York, and Connecticut. Of the 2 known hosts of *P. inconspicua* in the United States, *L. canadense* (Canada lily) is considered the sister taxon of *L. grayi.* Although *L. canadense* has a distribution that extends from the northern to the southern Appalachians, there have been no reports of *P. inconspicua* south of Connecticut. Since *L. canadense* is generally found below 1400 m in elevation, the climate at lower latitudes may not be suitable for *P. inconspicua* (Skinner 2002). However, the *L. grayi* population on Roan Mountain occurs at the highest elevation of its range,

where it is found at 1700 and 1900 m (Skinner 2002). At these elevations the climate is analogous to that of Nova Scotia.

Powell (2011) noted significant spatial patterns of health in the *L. grayi* population which are characteristic of an infectious disease. Different groupings or clusters of lily plants were identified where health was respectively better or poorer than that of the remainder of the population. Areas of each cluster type expanded in size over the course of the season. The spatial clustering and progressively decreasing health suggested the early season collapse was the result of an infectious process and/or localized environmental effects on health.

While previous studies have been conducted to identify an association of symptoms of the early season collapse and an abiotic or biotic precursor, the lack of sufficient evidence has precluded a formal determination of the causal agent of disease. However, the most noteworthy conclusions from preliminary studies include; identification of a candidate primary fungal phytopathogen, *Pseudocercosporella inconspicua*; illustration of spatial patterns of health that suggest an infectious process; documentation of a marked reduction in lily fecundity and recruitment; evidence of reduced survivorship in *L. grayi* populations on Roan Mountain and at other sites.

Within the last 2 decades there has been an increase in conservation and management activities within the grassy balds on Roan Mountain. Management has been conducted by agencies that include the United States Forest Service (USFS), Southern Appalachian Highlands Conservancy (SAHC), and the Appalachian Trail Conservancy (Donaldson 2009). However, management activities within the grassy balds often proceeded in the absence of investigations of the impacts on rare and endemic plant species (J. Donaldson and F. Levy pers. comm. 2011). Obviously, the long-term persistence of the early season collapse on Roan Mountain indicates

that the disease of *L. grayi* is not an isolated event but is rather a re-occurring pattern of unknown consequence to the stability and viability of the lily population. Without an understanding of the cause and characteristics of the early season collapse, the current management practices are unlikely to succeed in the conservation of *L. grayi*. Rather, uninformed management may exacerbate the disease and cause the already rare *L. grayi* to be become either increasingly rare or extirpated from Roan Mountain.

As management interventions continue and inevitably increase in frequency and intensity in the future, the need for empirical evidence on the ecology and impacts of disease increases. Information on the ecology and impact of disease could be used to formulate management approaches to conserving both the grassy bald habitat and *L. grayi*. Although *L. grayi* is not widely available commercially and therefore has little economic market value, it is a close relative of the more widely cultivated *L. canadense* and *L. superbum*. Therefore investigation of the early season collapse of *L. grayi* confers a 2-fold benefit. In addition to conserving the natural population of the species, information gained on the early season collapse of *L. grayi* may prove beneficial to future endeavors to control and/or manage the cause of the early season collapse in economically important lily species. Furthermore, *P. inconspicua* has been considered a significant plant pathogen in Europe and Asia with few reports from North America and even fewer in the United States. In the past, *P. inconspicua* infection has been listed as a destructive foreign disease of concern that had not yet been established in the United States (Hunt 1946). This listing of *P. inconspicua* was motivated in part by a European report of the pathogen as the cause of a severely destructive disease responsible for significant economic loss in the Ukraine (Zerova 1940). Confirmation of *P. inconspicua* outside of its previously known range may be sign that it is an emerging disease within the United States. If the pathogen is not

native to North America and is a new introduction, history has taught us that foreign pathogens introduced into natural populations have can have disastrous effects on native species. Two outstanding examples of this are the chestnut blight (*Cryptonectria parasitica* (Murrill) Barr.) and Dutch elm disease (*Ophiostoma ulmi* Buisman). In both cases the introduction of an exotic pathogen into the United States decimated native host populations.

Determination of the Causes of Plant Disease

The traditional method of providing proof of pathogenicity and the causal association between a pathogen and its disease symptoms has been through completion of Koch's Postulates (Koch 1893). Koch's Postulates are composed of a multistep process whereby a pathogen must be isolated from symptomatic host tissue and grown in pure culture, the pure culture must cause similar disease symptoms when used to inoculate a healthy host, and the pathogen must be re-isolated from the diseased experimental host and grown in a pure culture. For over a century this method was held as the basis for the study of pathogenic microorganisms of humans, animals, and plants and the standard for proving pathogenicity of a disease-causing organism within a specific host or hosts. Several pathogens causing many of the most important plant diseases of the past 200 years, including the American chestnut blight (*Cryptonectria parasitica*) and Dutch elm disease (*Ophiostoma ulmi)*, have been determined using Koch's Postulates.

Despite the prevalent use of Koch's Postulates, it is not the sole approach to evidencing a causal relationship of disease. Many consider a strict implementation of the method as exclusionary to the determination of many otherwise reportable disease-causing organisms (Rivers 1937; Evans 1976). Exclusionary aspects of Koch's Postulates are related to 3 complications frequently encountered investigating causal organisms of previously unreported disease. The 3 complications as related to fungal phytopathogens are: the isolation of the

candidate pathogen from diseased host tissue to yield a pure culture, the subsequent *in vitro* sporulation of the pathogen, and replication of disease symptoms within a healthy host following inoculation from a pure culture.

Isolation of a candidate fungal pathogen in pure culture is necessary to establish a causal relationship of disease with a pathogen and to provide inoculum needed for the replication of disease symptoms. Isolating a fungal pathogen from diseased host tissue provides support for a causal association by providing evidence for the internal presence of a pathogen within a host. If an organism were to be the cause of a disease, it would be expected to occur internally within its host. While not all internal fungal organisms are pathogens (i.e. mycorrhizal and endophytic fungi), all fungi that cause pathogenesis occur internally. However, in many cases the isolation and/or pure culture of a candidate pathogen is unattainable due to either difficulty in purification or inability to replicate *in situ* conditions necessary for growth (Laney et al. 2013). Difficulty in purification of primary pathogens is usually the result of numerous other pathogenic and non-pathogenic organisms which may co-occur within diseased host plant tissue. Numerous plant pathogens often coexist within a host because of colonization by secondary fungal pathogens. Once a host's defenses have been weakened by a primary pathogen, a multitude of secondary pathogens and saprophytic organism can invade diseased host tissue. Secondary pathogens often have more aggressive growth rates on artificial media than primary pathogens and as a result of competition, they can lead to difficulty in obtaining pure cultures of slow growing primary pathogens.

Pathogen substrate-specific requirements can also complicate attempts to isolate pathogens. For example, viruses are largely unable to live outside of their plant host or vector. Because of the obligate nature of viruses, it is often impossible to obtain and propagate a

previously unknown viral pathogen in culture. If one adhered to the strict implementation of Koch's Postulates, the failure to grow the viral causal organism in pure culture would prevent the establishment of a causal relationship of disease for numerous currently recognized pathogenic plant viruses (Louie et al. 2000; Laney et al. 2013). Examples of difficult to isolate pathogens are numerous but are most often associated with obligate parasites.

In virology determinations of causal organisms often use alternatives to Koch's Postulates (Hamilton et al. 1981; Louie et al. 2000; Laney et al. 2013). The cause of many viral diseases, such as the recently reported viruses Rose rosette virus (RRV) and Maize necrotic streak virus (MNSZ), would not have been possible through a strict implementation of Koch's Postulates (Louie et al. 2000; Laney et al. 2013). Recognizing the shortcomings of Koch's postulates has led researchers in virology to "work within the spirit of Koch's Postulates" (Hamilton et al. 1981). Through this interpretation of Koch's Postulates, 3 major factors are considered sufficient to prove an association: First, disease symptoms must be reproducible by exposure to the isolated disease agent. Second, the known host range must be considered and compared for congruity with occurrence of the disease agent. Third, an assessment of the symptomatology associated with the candidate pathogen and the similarity to symptoms occurring on previously reported hosts must be considered (Hamilton et al. 1981).

Additional standards for assessing a causal relationship between disease symptoms and a disease agent are widely used in epidemiology. One of the best examples is Hill's Criteria of Causation (Hill 1965). These criteria consist of 9 points that combined are considered sufficient to infer causation of disease by a pathogen or other disease agent. The points are:

1. A temporal relationship. Exposure to the disease agent must precede the exhibition of disease symptoms.
2. Strength of association. There must be a statistically supported association between the occurrence of signs of the pathogen and the presence of disease symptom. The stronger the association the more likely the pathogen is the cause of the disease.
3. Dose-response relationship. Higher levels of a disease agent increase the likelihood of the occurrence of a disease.
4. Consistency of the association. If a relationship is causal, the association of the pathogen and symptoms should be replicable using different methods.
5. Biological plausibility. In the case of pathogens, this requires consideration of previously known hosts and the presence of conditions favorable to the development of the disease.
6. Consideration of alternate hypotheses. Other possible explanations for the disease must be considered and eliminated.
7. Experiment. Ability to reduce disease through experimental application of preventive measures.
8. Specificity. The disease agent consistently produces the same symptoms within its host. This is a description of a one-to-one relationship whereby one disease agent produces one disease.
9. Coherence of the causal association. Requires consideration of all of the evidence to support the causal relationship in relation to current knowledge of the other like diseases.

To accommodate for the difficulties that arose within the current study's attempt to determine a causal relationship of disease by fulfilling Koch's Postulates, a combination of methods from virology and epidemiology were employed to determine the cause of the early season collapse of *L. grayi*.

The traditional means of identification of fungal species has been through the diagnosis of species-specific morphological characteristics (Denoyes and Baudry 1995; Kusayama and Tanina 2008). Diagnostic morphological characteristics include a combination of vegetative growth traits and reproductive structures. If a pure culture of a fungal pathogen is obtainable, observation of diagnostic vegetative growth traits is not difficult. The main complication encountered in the morphological diagnosis of fungi is the difficulty of obtaining reproductive structures. While some fungi will readily exhibit reproductive structures on media, the majority of fungi do not. For fungal pathogens that do not readily reproduce in culture, sporulation must be artificially induced. However, inducing sporulation of fungal pathogens is frequently difficult because of species-specific environmental and substrate requirements. For example, some groups of fungi such as the rusts, cercospora, and cercospora-like fungi have been reported to be notoriously difficult to culture and/or sporulate (Ekpo and Esuruoso 1978; Darvas and Kotze 1979). For some fungal species, entire studies have focused on identifying nutritional and environmental conditions necessary for optimal growth and sporulation (Ward and Friend 1979; Beckman and Payne 1983).

For many fungal pathogens of economically important crops, such as *Aspergillus flavus*, a prevalent mycotoxin-producing corn pathogen, there is a large body of literature on conditions necessary for growth and sporulation (Rai et al. 1978). However, comprehensive information is often lacking for host-specific fungal phytopathogens of plant genera of lesser economic value.

This lack of information is especially noteworthy for diseases of natural, non-agronomic plant populations. When confronted with the absence of supporting literature on pathogen culture and sporulation, the task of completing Koch's Postulates can range from onerous to near impossible.

The difficult and time consuming process of morphological identification of pathogens are reasons why diagnosis via molecular analysis has become increasingly widely used since the advent of polymerase chain reaction (PCR) in the 1980s. Diagnostic PCR fingerprinting systems of fungal phytopathogens can provide a more reliable and much less time time-consuming alternative to the morphological diagnoses of vegetative and reproductive structures. However, because of the effort and resources required for the optimization of molecular identification protocols, species-specific sequences are often only available for pathogens linked to plant diseases that cause significant economic losses and/or fungal species used in phylogenetic studies.

As the majority of fungal species are not associated with economically important plants, there is a noticeable absence of the species-specific DNA primers and sequences necessary for the identification of many fungal phytopathogens of native, non-economically important plants. Such is the case for pathogens of *L. grayi.* The unavailability of molecular diagnostics means that any study seeking to identify an unstudied fungal phytopathogen would bear the burden of creating a system and verifying its efficacy. Creating a diagnostic system is often quite difficult because of the high levels of intraspecific genetic diversity in natural populations. Because of the difficulties involved with molecular identification, morphological identification is currently still used in the diagnosis of candidate fungal pathogens with some frequency (Denoyes and Baudry 1995; Kasuyama and Tanina 2008). Because there were no molecular diagnostics for *P. inconspicua*, and the current study had a limited time frame, molecular identification was not

included. Instead, identification of the candidate pathogen was accomplished through diagnosis of vegetative and reproductive morphology.

Fungal Disease in Natural Populations

Disease within natural populations is thought to exist within a dynamic equilibrium between the host, pathogen, and environment but typically occurs at low, sometimes undetectable levels until some change occurs in any of the components of the disease triangle (Dinoor and Eshed 1984). The 3 components of the disease triangle are; (i) susceptibility of the host to the disease agent or pathogen caused by either a reduced capacity or complete inability to resist the disease, (ii) a pathogen capable of pathogenesis within a host plant, (iii) suitable environmental conditions to allow for infection and development of disease symptoms (Agrios 2005). To understand the dynamics of a disease within a natural population and be able to determine the likelihood and frequency of annual or periodic epidemics, one must first understand how each of the components of the disease triangle interacts with the other 2.

Burdon (1993) characterized the dynamic equilibrium of a disease system as a "demographic cycle of pathogens" and organized the cycle into 4 sequential phases: "the re-establishment, endemic, epidemic, and crash phases." Each of these 4 stages is critical to understanding the development of annual and periodic outbreaks of disease and also for inferring the possible outcomes to both the host and pathogen once the host is unable to sustain high levels of disease. The re-establishment phase is characterized by almost imperceptible levels of disease and is either a period of re-introduction or localized proliferation. This is followed by a period of endemism characterized by low levels of disease within the host population. The endemic phase is where most pathogen-host systems or pathosystems exist within a stable equilibrium and spend the largest proportion of time within the demographic cycle. The pathosystem exists

within this stable equilibrium until there is either an increase in host susceptibility, an increase in the pathogen's virulence, and/or a change in environmental conditions that is favorable to disease dispersal and/or development. Once one of these changes occurs, there is a shift to the third phase, an epidemic outbreak of disease.

Disease and disease outbreaks within natural plant populations are commonly considered to be a natural component of the ecology of an ecosystem but investigations report them to be fairly rare occurrences (Burdon 1993). Epidemics are characterized by initially low incidence of disease that increases exponentially either within and/or between years and occurs within a specific population in a defined geographic area. Disease that spreads over largely undefined geographic areas and ever expanding within and between seasons are often considered pandemics. Pandemics are often the result of local epidemics that spread from one or several local focal points of disease until the disease has been dispersed across one or many continents. Epidemics on the other hand are restricted by either the occurrence of specific environmental conditions necessary for the development of disease, host specificity and/or limited host geographic distribution (Agrios 2005). Epidemic outbreaks of disease are also largely characterized by significant foci of disease that spread and sometimes coalesce within or between seasons but do not spread outside of the environmental conditions found within a defined geographic area and host population (Burdon and Jarosz 1989).

Foci of disease are present because they represent locations of primary sources of inoculum and/or areas from which high levels of disease inoculum is dispersed. Disease foci usually create spatio-temporal patterns of disease incidence and severity. Spatial patterns of disease distribution are typically characterized by disease gradients with host plants nearer the center of the foci experiencing higher rates of disease incidence and severity and host plants

further away experiencing reduced levels. Initially low levels of disease characterize temporal patterns of disease during its establishment and is followed by a period of marked increase in incidence and severity of disease (Coulston and Ritters 2003; Maanen and Xu 2003).

The final phase of the demographic cycle of pathogens is the crash that is characterized by a decline in host and pathogen populations. At this point the survival of the pathogen is dependent on its ability to persist within either a reduced number or complete absence of hosts. During this period the pathogen can either face extinction or recycle into the re-establishment phase by employing 3 modes of survival. These modes of survival are specific to the type of pathogen and mode of infection employed by the host. Some pathogens are able to overwinter on alternate hosts until the re-emergence of its primary host (Agrios 2005). Others are able to persist systemically within the host plant until re-emergence. More often fungal pathogens are able to produce vegetative resting spores called sclerotia and/or overwinter saprophytically within the soil or on host tissue until favorable conditions and sufficient numbers of host are again present. Many of the mildews and molds are known to overwinter saprophytically on host tissue. Examples of pathogens overwintering on host tissue include *Pseudocercosporella herpitrichoides*, the cause of late eye-spot on wheat and *P. inconspicua* (Makoto 1925; Agrios 2005).

Factors Affecting Disease Outbreak in Natural Plant Populations

As all epidemics are not caused by the same type of organisms, all disease epidemics do not share the same patterns and dynamics. Several intrinsic traits of both the host and the pathogen and extrinsic traits of their shared environment ultimately determine the characteristics of each epidemic. The intrinsic traits of the host include: survivorship of the host, morphological and physiological risk factors for disease, fecundity, and phenology (Gilbert 2002). The intrinsic

traits of the pathogen include: virulence, dispersal pattern, phenology, and the ability of the pathogen to overwinter and/or persist in the habitat in either a reduced number or temporary absence of hosts (Xu and Ridout 1998). The extrinsic factors of the environment include favorable conditions for the development of disease, conditions necessary for reproduction of the pathogen, and/or abiotic conditions that either reduce or increase plant susceptibility. Environmental factors are often much harder to ascertain than intrinsic factors of the host or pathogen because of the dynamic way in which even local weather conditions change.

Though the factors to consider are many, the characteristics used to evaluate the level of significance and severity of epidemics in natural populations mainly focus on the ability of disease to inhibit perpetuation of the host species. These characteristics include the incidence of the disease within a season and ability of the pathogen to persist between seasons, the severity of disease, the effects of disease on host survivorship and/or senescence, effect of the disease on the fecundity of the host, and the capacity of the disease to inhibit the recruitment of new host plants within the host population (Dinoor and Eshed 1984; Jarosz and Burdon 1989; Maanen and Xu 2003).

Aside from host resistance and pathogen virulence, the largest determining factor of incidence and severity of disease is the number of rounds of disease propagule production that can be completed by the pathogen within a season (Gilbert 2002). The characteristics of the pathogen's disease cycle under favorable conditions and the rate of inoculum production are major components contributing to disease incidence and they are the determining factors of the rate of spread (Segarra et al. 2001). This is why an epidemiological study must first identify mechanisms of pathogen propagation through time.

A pathogen's disease cycle is categorized as either monocyclic or polycyclic. There are many monocyclic diseases but they are a minority when compared to the number of polycyclic diseases. An example of a monocylic disease is *Exobasidium splendidum* on *Vaccinium vitis-idaea*. The pathogen was reported to infect its host once within a season and then overwinter on plant debris (Pehkonen and Tovanen 2008). A pathogen that is monocyclic would be allowed only a single opportunity within a season for the pathogen to proliferate and infect its host. Additionally, the number of disease propagules that can be produced are largely dependent on the number of host plants infected from the previous season and for disease to reach epidemic proportions it typically is necessary to have a prolific pathogen that is capable of overwintering in or on a living plant host or plant debris for many years. Conversely, polycyclic diseases are capable of many rounds of successive reproduction within a single season. An example of a polycyclic disease is *Pseudocercopsorella herpotrichoides* on *Triticum* spp. (wheats). Previous reports of the epidemiology of this disease noted it as a polycyclic disease capable of many successive rounds of reproduction within a single season (Fitt et al. 1988). Thus, pathogens, such as *P. herpotrichoides* or *P. inconspicua*, are capable of exponential increases of disease propagules within a single season, with the potential to reach epidemic proportions on an annual basis (Burdon 1993).

Disease Assessment: Factors Affecting the Severity of Disease in Plant Hosts

Evaluating the severity of a disease on a host plant is of the utmost importance in understanding the impact of a disease on the host population. Disease severity is, "the proportion of area or amount of plant tissue that is diseased" (Agrios 2005). Essentially, a disease severity index provides a standardized measure of the point prevalence of disease symptoms on or within an individual host. However, disease severity typically does not consider the identity of affected

host plant structures (i.e. foliar, reproductive, etc.) or the effect of disease on infected host tissues (i.e. necrosis, stunting, etc.) Alternatively, the severity of a disease may be evaluated by a description of disease severity, the scope of affected host plant structures, and the acuteness of disease symptoms. By considering the proportion, scope, and acuteness of disease, the severity of a disease provides a means of describing the total impact of disease on the life cycle of the host.

Though plant pathogens characteristically infect certain structures such as roots and foliage, often a disease affects more than just the infected tissues. For this reason, an assessment of the scope of a disease must consider the effect of disease symptoms on all plant structures over the course of the disease. For example, *P. herpitrichoides* causes leaf spots on wheat but over the course of the disease, the pathogen is capable of affecting all plant structures by causing early senescence and limiting reproduction and crop yield.

Acuteness of disease is measured by the ability of a pathogen to inhibit host metabolic processes such as cellular respiration and photosynthesis and/or reproduction. Inhibition of a host's life cycle can be categorized as systemic (physically or chemically inhibiting the acquisition of nutrients such as nitrogen and phosphorus and resources such as water and gas exchange) or nonsystemic (affecting specific structures responsible for certain physiological processes such as photosynthesis or reproduction). Systemic and nonsystemic diseases can also be classified as having direct or indirect effects on their hosts. Direct or indirect effects are determined by the mode by which a pathogen impact's its host's life cycle. For example, disease that causes plant death by inhibiting transpiration through blockage of stomata would be considered an indirect effect, while disease that causes necrosis on foliar structures would be considered a direct effect.

The effects of most systemic diseases are indirect and act by reducing a host's ability to acquire resources. Many systemic diseases are roots diseases. For example *Rhizoctonia solani*, a systemic disease, is the cause of crown rot of soybean. This disease inhibits the movement of water and nutrients by degrading root tissue and causing root cell death through both mycotoxin production and mechanical means (Agrios 2005). Other systemic diseases, such as those caused by *Verticillium dahliae* (causal organism of wilt diseases within numerous crop species) (Bhat and Subbarao 1999), are capable of inhibiting the flow of photosynthates and macro- and micronutrients by blocking phloem structures within plants. Other fungal diseases reduce water uptake and transpiration by blocking xylem and/or stomatal pores (Agrios 2005). Fungal pathogens that block stomatal pores also have the additional effect of reducing the gas exchange necessary for cellular respiration and photosynthesis (Agrios 2005). However, some systemic fungal diseases, such as *Monilinia* sp. on blueberries, directly reduce host fitness by infecting reproductive structures. These diseases are generally less common and have a minimal effect on survival of the host due to their restricted effect on host fruit.

Non-systemic pathogens can interfere with host life cycles by directly inhibiting a host's capacity to assimilate nutrients and resources. Direct inhibition of photosynthesis can occur through disruption of photosynthetic pathways, chemical inhibition, and/or by reducing the number of photosynthetic organs or area through stunting, dwarfism, and/or necrosis. This is accomplished through the production of chemicals that inhibit particular pathways, through the reduction or over expression of certain genes, and also by causing cell death of photosynthetic structures. As a primarily foliar disease, the latter is the mechanism by which *P. inconspicua* causes the lily leaf spot disease (Agrios 2005).

Factors Affecting the Impact of Disease within Natural Populations

A natural plant population suffering from a highly acute disease, occurring with a high incidence rate, and resulting in frequent plant death does not always bring about a reduction in the viability of the host population. Instead, the impact of disease on reproduction and recruitment are 2 of the most important determinants of the ability of the host population to persist in the presence of a disease epidemic.

A major factor affecting host plant fecundity is the ability of a disease to infect and directly and/or indirectly cause severe symptoms on reproductive structures and/or cause seed pathogenicity. When a pathogen infects reproductive structures, it can effectively result in: decreased number of fruit, decreased number of seeds, and reduced seed viability (Agrios 2005). Phytopathogens can indirectly reduce the number of fruit produced by stressing a host plant and causing it to divert resources for survival (Stephenson 1981). Plant disease can also directly affect reproductively associated structures. For example, necrosis of pedicels by disease can result in the loss of flowers and fruit (Batta 2001). Disease can also cause pre-fertilization ovary loss (Cook 1930). Additionally, disease symptoms can be associated with the fruit themselves and cause immature fruit drop or necrotic fruit. An example of a fruit infection is Mummy berry disease on *Vaccinium* spp. (blueberry spp.) caused by *Monilinia vaccinii-corymbosi* (Cox and Scherm 2001).

Though disease effects on host fecundity can be variable, disease symptoms on maturing fruit can result in reduced seed maturation and/or reduced seed viability (Jarosz and Davelos 1995). Negative effects on seed viability can occur in 2 major ways: indirectly through necrosis of tissues responsible for providing nutrients to the developing seeds or by causing seed pathogenicity through diffusion of mycotoxins and/or infection. Disease can indirectly cause

reduced seed viability via nutrient deficiency by causing senescence of nutrient shuttling tissue (i.e. pedicels). Disease can also directly cause reduced seed viability by infecting seed.

A pathogen causing early senescence or infecting host reproductive structures is not always sufficient to cause a significant reduction in host fecundity. Instead, a significant reduction in host reproduction occurs when there are marked increases in the pathogen population before or during host reproduction. When life events occur in this order, mortality of above-ground structures can occur before reproduction is possible. Previous reports of *P. inconspicua* on *Lilium* spp. indicate that increased severity of disease often precedes host reproduction. Zerova (1940) noted that infection by *P. inconspicua* occurred at the beginning of the host vegetative period and continued until autumn. The rapid proliferation of *P. inconspicua* can be attributed to its polycyclic mode of reproduction. As with many leaf spotting diseases, the severity of a disease is largely dependent on the accumulation of secondary infections resulting from many successive rounds of postprimary reproduction. As more disease inoculum is produced exponentially, disease lesions on a plant become numerous within a short period of time. Because each round of pathogen reproduction requires time to complete, the longer the time available, the more severe the impact on host reproduction. If infection starts at the beginning of the growing season, *P. inconspicua* would have considerable time to increase exponentially before lily plants can reproduce. Thus, the polycyclic mode of pathogen reproduction and the host-pathogen phenology suggest that *P. inconspicua* is capable of severely reducing the fecundity of *L. grayi.*

Disease can further limit population increase by impacting recruitment of new individuals to reproductive maturity. In extreme cases, this can result in the elimination of sexual reproduction. Recruitment occurs when non-reproductively mature plants reach an age and/or

developmental stage necessary for reproduction and/or clonal propagation. An example of a disease that results in the elimination of sexual reproduction is the chestnut blight (causal organism, *Cryptonectria parasitica*) on *Castanea dentata,* (American chestnut). American chestnut trees reproduce at a minimum of 4 years of age. It is at approximately this age that the blight begins to girdle the chestnut trees, followed by stem death before reproduction. Because of the phenology of both the host and the pathogen, native populations of the American chestnut have been reduced to short-lived stump-derived sprouts.

Statement of Purpose

The purpose of this study is to investigate the cause and impact of an early season collapse of *L. grayi* on Roan Mountain TC/NC through studies of the disease-symptom association, host and pathogen phenology, spatial patterns, risk factors, and inoculation trials. The questions addressed were:

- Is the candidate primary phytopathogen *P. inconspicua* the causal organism of the early season collapse of *L. grayi*?
- What are the impacts of disease on host survivorship, reproduction, and recruitment?
- What is the extent and distribution of disease among the host population?
- Does the early season collapse of *L. grayi* on Roan Mountain constitute an epidemic?

CHAPTER 2

MATERIALS & METHODS

Symptomatology of the Early Season Collapse of Lilium grayi

Before disease symptoms of the lily leaf spotting disease could be associated with *P. inconspicua* it was crucial to compile a list of characteristic symptoms so that the disease could be reliably and efficiently distinguished from environmental stresses and/or other diseases. Therefore, the characteristic symptoms essential for a visual diagnosis using key morphological signs and *in situ* macroscopic observations were identified.

The lily leaf spot disease begins at one or many individual infection sites and initially presents as single or multiple amphigenous pale green lesion(s) of irregular margin occurring on one or many leaves. These pale green lesions are associated with a slight depression of the leaf epidermis. As the disease progresses, lesions become circular to elliptic and first turn yellow but rather quickly transition to a light tan color (Figure 1A). Tan necrotic lesions will often contain a white to grayish powdery substance, which is the conidial mass of *P. inconspicua* (Figure 1B). In most cases these lesions have no distinct margin. Instead, lesions are associated with a fading of the tannish coloration to green towards the edge of the lesion (Figure 1B). When a distinct margin is present, it is usually light brown. After all leaf tissue has senesced from a diseased leaf, that leaf still retains the tan color initially associated with the necrotic lesions. The spread of the disease within a plant is largely due to secondary inocolum that is produced almost continuously throughout the season within necrotic lesions. It is not the effect of any one tan lesion on a plant that causes early senescence but rather the accumulated effects of numerous different separate infections.

In the early stages of disease necrotic lesions within a leaf coalesce causing a general yellowing on the host leaf as photosynthetic activity declines (Figure 1C; 6A,C). Middle (Figure 2A) to late (Figure 2B) stages are characterized by the early senescence of multiple leaves that become dry and brittle (Figure 2A; 2B). The final stages of the disease are characterized by the death of all above-ground structures including any undeveloped reproductive structures, i.e., seed capsules (Figure 2C).

Symptoms observed in the field were grouped into five important diagnostic characteristics that were used for the visual assessment and identification of the lily leaf spotting disease.

1. Tan amphigenous necrotic lesions (Figure 1A)
2. Margin of necrotic lesions fading from tan to green (Figure 1B)
3. A white to gray powder within necrotic lesions (Figure 1B)
4. Coalescence of lesions to cause leaf necrosis (Figure 2A)
3. Retention of the tan coloration of the lily leaf after senescence (Figure 6C)

Characteristic tan lesions and conidia were also found on flowers (Figure 3; 4A; 6A), stems, pedicels (Figure 3; 4B; 4C), and seed capsules (Figure 4B; 5A; 5B; 5C). Extent of disease symptoms on maturing seed capsules ranged from small lesions with no conidial production to large and/or numerous lesions that often produced copious amounts of conidia (Figure 4B; 5A; 5B; 5C; 6B). Additionally, on heavily diseased plants there were seed capsules that aborted because of disease lesions and there were seed capsules that had fallen because diseased pedicels senesced (Figure 6B; 6C).

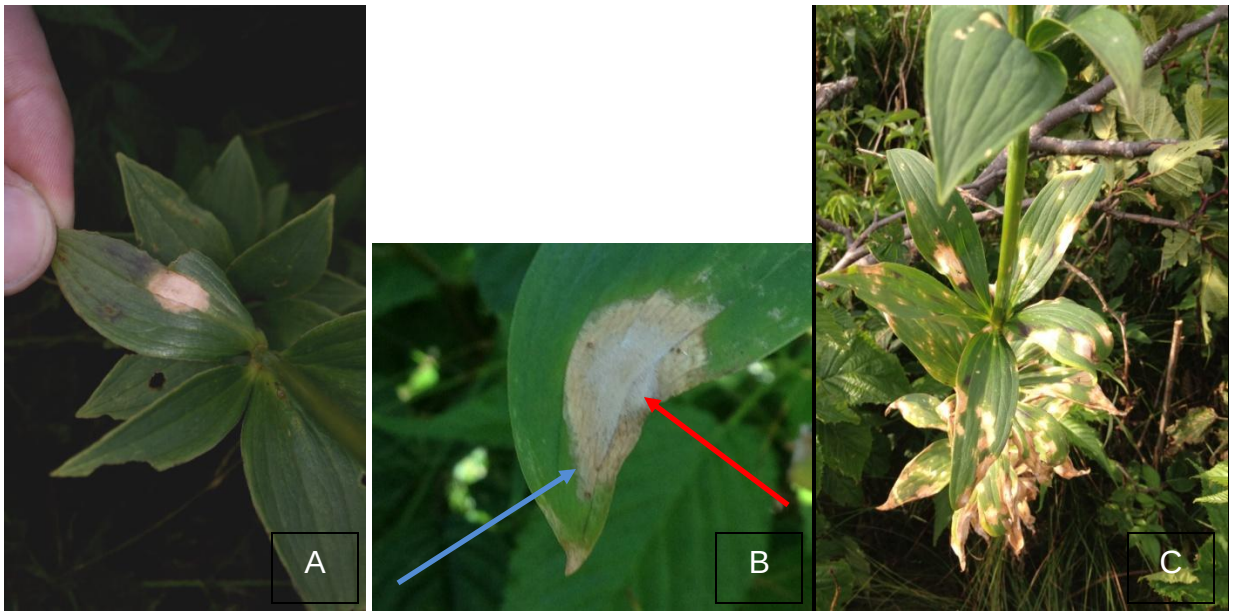


Figure 1. Photographs of *L. grayi* Plants Exhibiting Symptoms of the Lily Leaf Spot Disease. (A) Tan necrotic leaf spot on a lily leaf indicating the initial site of infection by the lily leaf spot disease. (B) Close up of an intact lily leaf with a tan lesion exhibiting no distinct margin (blue arrow) and with conidia of *P. inconspicua* appearing as a whitish powder (red arrow). (C) Lily plant exhibiting middle stages of decline and covered with numerous tan lesions.

Figure 2. Photographs of *L. grayi* Plants Exhibiting the Middle to Final Stages of the Lily Leaf Spot Disease. Middle (A) to late (B) stages of decline are characterized by 50-100% of leaves having experienced early senescence. In the final stage of decline (C) all leaves have senesced and chlorosis/necrosis of all other above ground structures is present.

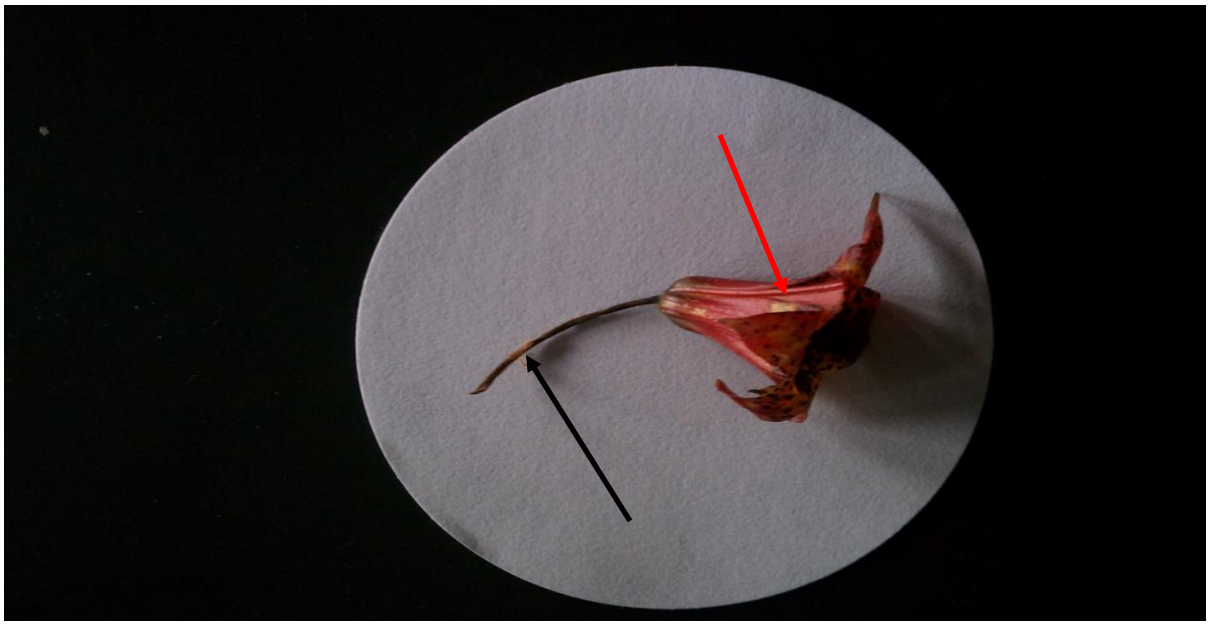


Figure 3. Photograph of a *L. grayi* Flower Exhibiting Symptoms of the Lily Leaf Spot Disease. Characteristic tan lesions are present on the tepals (red arrow) and on the pedicel (black arrow).

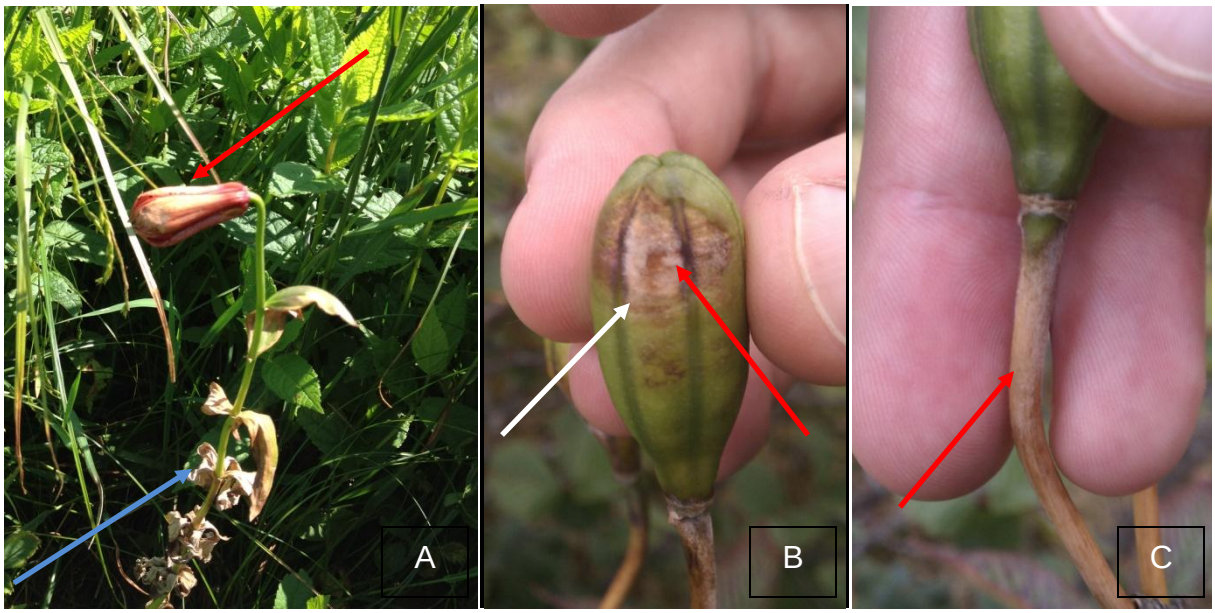


Figure 4. Photographs of *L. grayi* Plants Exhibiting Symptoms of the Lily Leaf Spot Disease on Reproductive Structures. (A) A heavily diseased *L. grayi* plant with characteristically dead tannish leaves (blue arrow) and tan necrotic lesions causing deformation of the flower (red arrow.) (B) Characteristic tan necrotic lesion (white arrow) on a maturing *L. grayi* seed capsule. The whitish substance within the tannish necrotic lesion and on the pedicel is a mass of conidia of *P. inconspicua* (red arrow.) (C) Tissue death at the base of a maturing *L. grayi* seed capsule and the attached pedicel as a result of the lily leaf spotting disease. The whitish substance on the pedicel is a mass conidia of *P. inconspicua,* (red arrow.)

Figure 5. Photographs of Maturing *L. grayi* Seed Capsules Exhibiting Symptoms of the Lily Leaf Spot Disease. (A) Necrotic lesion formed at base of lily seed capsule. (B) Tan lesion and whitish powder on a maturing seed capsule. (C) Seed capsule with lesions covering more than 50% of the capsule.

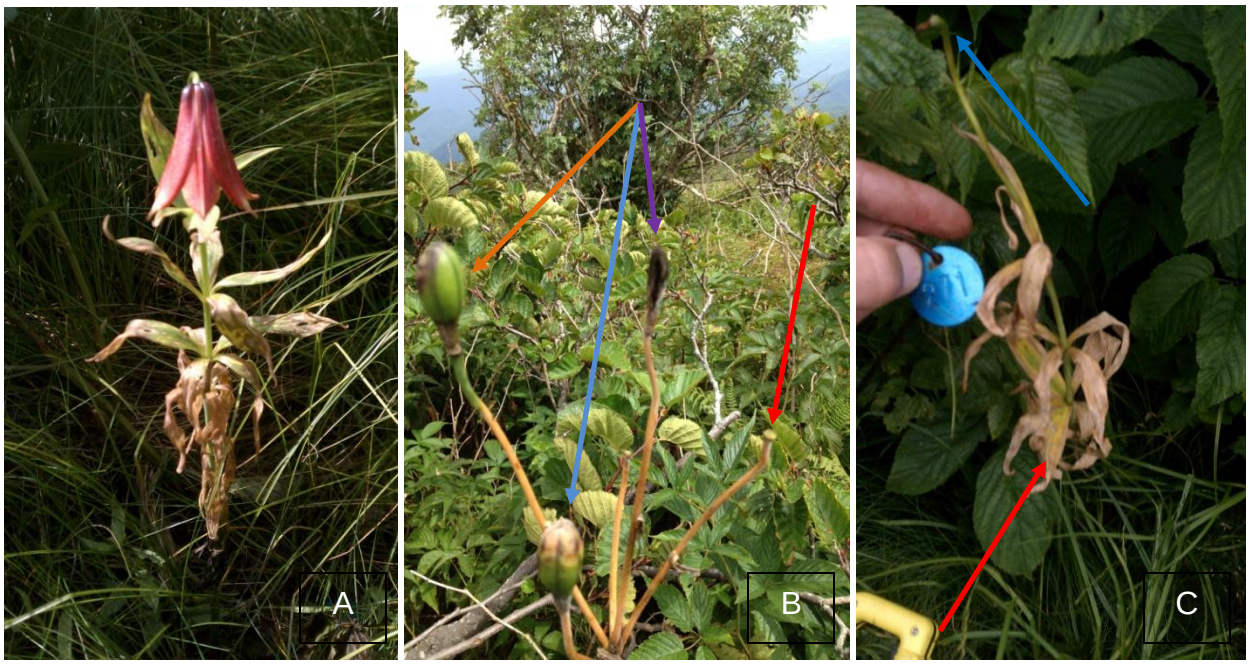


Figure 6. Photographs of the Effects of the Lily Leaf Spot Disease on Reproductively Mature *L. grayi* Plants. (A) In addition to weakening flowering plants,(B) heavily diseased lily plants were noted as having aborted seed capsules (red arrow) and also intact seed capsules exhibiting various levels of disease ranging from slightly diseased (orange arrow), to significantly diseased (light blue arrow), and dead (purple arrow.) (C) An aborted seed capsule (blue arrow) as a result of severe infection by the lily leaf spot disease along with chlorosis (red arrow).

Diagnosis of the Candidate Pathogen, Pseudocercosporella inconspicua

Identification and diagnosis of *P. inconspicua* was made using a Nikon Eclipse 80*i* compound microscope and NIS Elements Digital Imaging Software to view and measure morphological characteristics of conidia. Morphological traits of the conidia of *P. inconspicua* described by Braun (1988) were used to diagnose the pathogen. Measurements of 20 conidia on each slide were taken for: apex to base length, width at the widest point, and width at both the apex and midpoint. The number of cells within conidia and the presence or absence of a truncated base was also recorded. The genus *Pseudocercosporella* (Deighton) U. Braun is comprised of 81 species of necrotrophic phytopathogenic fungi known to occur on members of at least 41 plant families all over the world. Species of the genus are host-specific with host species restricted to single plant families (Braun 1995). The most common symptom of infection is leaf spotting.

The microscopic morphology of *Pseudocercosporella* species is characterized by internal/primary and less often, external/secondary mycelia. Hyphae are hyaline, septate, branched, and smooth. Stromata can be lacking to well-developed with hyaline to slightly pigmented coloration and are often imbedded within the leaf epidermis. Conidiophores are morphologically different than the vegetative hyphae with cells either septate or non-septate and forming singly to fasciculately. On conidiophores, hyaline conidia are formed singly, less often in simple or branched chains, are from fusiform to filiform in shape, and are more than twice septate (i.e. two or more septa within a conidium). The walls of conidia are thin with an unthickened hilum. Qualitative and quantitative diagnostic conidial characteristics are: hyaline; smooth; formed individually on conidiophores that arise from the stromata; fusiform; straight to

slightly falcate; 30-110 μm in length; 2-6 μm in width; 1-7 septate; more narrow towards the apex; tip blunt to subacute; base narrowed, unthickened, and truncated or rounded (Braun 1995).

Cultivation of the Host, Lilium grayi

Lilium grayi plants were cultivated to provide healthy hosts for inoculation trials in attempts to complete Koch's Postulates. Cultivated plants were kept disease free in a growth chamber until the time of their use. Three *L. grayi* bulbs were collected from the field. Each bulb had approximately 10 - 30 scales that were removed to be grown as separate individuals. The bulbs and the scales were collected in late November 2012, a date that provided an initial cold treatment in the field. At each collection site, up to 3 inches of soil around the stem and below the bulb were taken to ensure that the entire bulb was intact. In the laboratory, bulbs and scales were separated and placed into plastic bags containing moistened soil collected from the field. The plastic bags were left partially open to allow for respiration and then placed in the crisper tray of a (4° C) refrigerator for one month. At the end of the month, bulbs and scales were removed from the refrigerator and planted in soil from the field in separate pots. Pots were placed in a growth chamber on a 12 hr light, 16° C/ 12 hr dark, 12° C, regimen.

Culture of the Pathogen, Mycosphaerella martagonis, *(Anamorph:* Pseudocercosporella inconspicua*)*

To obtain a pure culture of *P. inconspicua* 2 cultures of *Mycosphaerella martagonis* CBS 284.49, were obtained from the Centraalbureau voor Schimmelcultures (CBS-KNAW) Fungal Biodiversity Centre in Utrecht, Netherlands. One of the cultures was left intact and the second was subcultured onto 10 petri dishes containing oatmeal agar (OA) with streptomycin (30 mg/L) prepared to the manufacturer's specifications. One of these was placed in the refrigerator and was subcultured as needed. The other 9 subcultures were grown in ambient conditions to observe

the vegetative and reproductive structures. Subcultures obtained from CBS 284.49, showed optimal growth between 20 - 24 °C. Mycelial characteristics observed in these cultures were: hyaline hyphae, internal growth, minimal to absent in aerial secondary growth, and sparse branching. Sporulation was achieved on petri dishes with thick OA media and cultured for around 8 to 12 weeks or until the periphery of the agar began to dry out. The location of sporulation within the petri dish was the point at which growing mycelia met the margin of the dried agar.

Observations of growth characteristics of the CBS culture aided in the identification of field isolates by providing a standard culture for comparison.

Isolation of the Fungal Pathogen, Pseudocercosporella inconspicua*, from Diseased Host Tissue*

Pure cultures of *P. inconspicua* isolated from diseased host tissue were needed to establish a causal relationship between the pathogen and the symptoms of the lily leaf spot disease (Agrios 2005). During 2011 and 2012 several sets of visually symptomatic diseased leaf material were collected from the field and used to isolate *P. inconspicua* in pure culture. Field isolates were placed in 50 mL centrifuge tubes to prevent contamination and desiccation. Diseased leaves were processed in the laboratory by excising living green tissue (1 - 2 cm^2) close to disease lesions. Excised leaf sections were then surface sterilized before transfer to media.

Surface sterilization of leaf sections was accomplished by placing leaf sections in 250 ml beakers containing a solution of 5% ethanol, stirring continuously for 30 seconds. Underneath a sterile laminar flow hood, leaf sections were removed with sterile forceps and placed in a 250 ml beaker containing 10% bleach solution for 5 minutes, stirring continuously. After 5 minutes, leaf sections were placed in 250 ml beakers containing sterile deionized water for 30 seconds to wash the bleach solution. After the wash, single leaf sections were placed on potato dextrose agar

(PDA) media, prepared to the manufacturer's specifications and containing streptomycin (30 mg/L). Each petri dish was placed in ambient conditions and monitored for fungal growth (Agrios 2005). Oatmeal agar, water agar, water with host tissue, half and quarter concetrations of PDA, and Mycosel were also used as culture media.

Petri dishes were observed for fungal hyphae emerging from the cultured leaf sections daily for the first week and twice weekly for subsequent weeks. As hyphae emerged subcultures were prepared by excising agar sections containing hyphae of interest and transferring those sections onto petri dishes containing PDA. Cultures of field isolates could and often did have hyphae with different growth characteristics. The different hyphal growth characteristics were used to isolate different fungal species and resulted in several subcultures for each field isolate. After subcultures were prepared, petri plates containing the original field isolates were placed in ambient conditions for further observation of hyphal growth. Field isolate subcultures were left in ambient conditions for up to a month to allow for further growth. In these, fungal growth patterns were compared to those of *P. inconspicua* as described in Braun (1995). Field isolate subcultures were also compared to samples from the CBS culture. Plates that showed signs of excessive aerial growth or of any coloration outside of white were destroyed because this type of growth was inconsistent with characteristics of *P. inconspicua*. Cultures that fit the diagnostic growth characteristics were placed in a growth chamber to initiate sporulation to allow for a positive identification. The conditions used to initiate sporulation were 12 hrs UV light and 12 hrs dark at 24° C.

Viral Testing of Diseased Lilium grayi

Chlorosis, necrotic lesions, and purpling were characteristic viral symptoms observed among lilies of the study population (Figure 7). Of these symptoms, chlorosis and necrotic lesions have also been observed in association with the lily leaf spotting disease (Powell 2011). To provide evidence that the symptoms observed in association with the lily leaf spotting disease were not due to a viral pathogen, several lily leaf samples were tested for common lily viruses. Leaf samples were collected in July 2011, from 8 *L. grayi* specimens located within the demography transect. These leaf specimens were sent to a plant disease diagnostic company (AgDia Elkhart, IN) for viral testing. All samples were collected during the same outing and were placed in separate labeled bags. Samples were refrigerated overnight and mailed the next morning.

Of the 8 samples submitted for viral testing, three exhibited characteristic symptoms of the leaf spot disease (necrotic lesions, chlorosis, and a powdery substance within lesions), 3 exhibited disease/stress symptoms not characteristically associated with the leaf spotting disease (purpling, browning, and mottling), and 2 were collected from healthy plants exhibiting no signs of stress or disease. The diagnostic viral panel consisted of 7 viruses known to infect members of the genus *Lilium*. The viruses tested for were: Cucumber mosaic virus (CMV), Impatiens necrotic spot virus (INSV), Lily symptomless virus (LSV), Potyvirus group (POTY), Tobacco ringspot virus (TRSV), Tomato aspermy virus (TAV), and Tomato spotted wilt virus (TSWV).

Figure 7. Photograph of a *L. grayi* Plant Included in the Viral Testing that Exhibited the "Purpling" Symptom.

Measuring Background Levels of Pseudocercosporella inconspicua *Conidia:*

A Conidial Baseline Study

A host-specific fungal pathogen is expected to produce the highest concentrations of asexual propagules on its host. Because *P. inconspicua* is known to be host-specific to *Lilium* species, the concentrations of *P. inconspicua* conidia are expected to be the highest on diseased *L. grayi* and much lower to completely absent on nonhost neighboring plants. To compare the conidial load on host and nonhost plants, *L. grayi* and neighboring plants of other species were non-destructively sampled and concentrations of *P. inconspicua* conidia were quantified. Included in the conidial counts were *Rubus canadensis* L., *Rumex acetosella* L., *Angelica triquinata* Michx., *Alnus viridis* (Chaix) DC spp. *crispa* (Aiton) Turrill (Table 1).

Before sampling, all lily plants were given a visual diagnosis of disease presence/absence and severity. Each plant was sampled by pressing a 2 cm^2 piece of translucent tape to the epidermis of a single leaf. The tape was then removed, applied to a cover slip, and placed on a

microscope slide. All slides were placed in a cooler at approximately 4° C until they were viewed.

Slides were viewed under a compound microscope at 100x and 400x magnification for the presence and concentration of the diagnostic conidia of *P. inconspicua*. Concentrations of conidia were categorized as: 0 = "absent", 1 - 10 = "very low – low", ≥11 = "medium – high". Comparisons were conducted using the Freeman-Halton extension of the Fisher's exact test of a 3 x 3 contigency table with rows corresponding to plant species/lily disease status and columns representing the categories of *P. inconspicua* conidial concentrations (Freeman and Halton 1951).

Table 1. Identity of Species and Number Plants Sampled (N) within the Conidial Baseline Study

Species	Disease Status	N
Lilium grayi S. Watson, Gray's lily	Healthy	10
Lilium grayi S. Watson, Gray's lily	Diseased	10
Rubus canadensis L., Smooth blackberry	N/A	13
Angelica triquinata Michx., Filmy angelica	N/A	2
Alnus viridis (Chaix) DC ssp. *crispa* (Aiton) Turrill, Green alder	N/A	4
Rumex acetosella L., Sheep's sorrel	N/A	1

Association of the Pathogen, Pseudocercosporella inconspicua*, with the Symptoms of the Early Season Collapse of* Lilium grayi

To examine the pathogen-host relationship and to test for an association between disease symptoms and signs of disease, (i.e. conidia of *P. inconspicua*), plants were visually diagnosed and then sampled for conidia. Sampling of plants and preparation of slides was conducted using the, "scotch tape method" (A. Windham, pers. comm., 2012.) The scotch tape method is a means

by which conidia were obtained by pressing transparent tape to the epidermis of a single leaf and then gently removing the tape, taking care not to remove any of the leaf in the process. Tape sections were then pressed to a coverslip, placed on a microscope slide, and sealed using clear fingernail polish to prevent desiccation and inhibit germination. To safeguard against degradation, slides were brought into the laboratory and placed into cold storage until viewed. For each sample plant, 3 leaf epidermis samples were taken from the top of each leaf, the bottom of the same leaf, and the top and bottom of a different leaf. Leaves sampled on plants diagnosed as nonsymptomatic for the leaf spot disease were chosen at random. Leaf samples from symptomatic plants were taken from within tannish necrotic lesions.

Slides were manually scanned for the presence of conidia using a Nikon Eclipse 80*i* compound microscope. NIS Elements Digital Imaging Software was used to measure morphological characteristics of conidia at 200x and 400x magnification. Measurements of 20 conidia on each slide were taken for apex to base length, width at the widest point, and width at both the apex and base. The number of cells within conidia and the presence or absence of a truncated base was also recorded. When conidia were present, conidial concentration were estimated on a scale that ranged from absent or 0, very low (1 - 50), low (51 - 99), medium (100 - 499), high (500 - 999), and very high ($\geq$1000). All slides were photographed for documentation and future review.

Fisher's exact test of a 2 x 2 contingency table was used to test for an association between the visual diagnosis of disease and the presence of *P. inconspicua*. This approach tested the validity and reliability of the visual diagnosis and subsequently provided support for the use of the disease severity scale used in *Investigation of the Impacts of the Early Season Collapse on*

Non-reproductively Mature Lilies (p. 54) and *Investigation of the Impacts of the Early Season Collapse on Reproductively Mature Lilies* (p. 56).

Reproducing the Symptoms of the Early Season Collapse: Inoculation of Healthy Hosts within the Field

The prerequisite for any infectious disease is the ability of a specific pathogen to infect and cause pathogenesis within its host. Without this ability, it is highly unlikely that a given organism is the cause of a specific disease. To determine the infectivity of *P. inconspicua* on *L. grayi*, inoculation trials of healthy plants were conducted under *in situ* conditions. If the symptoms of the lily leaf spot on *L. grayi* were the result of infection by the candidate primary pathogen *P. inconspicua*, inoculation of healthy lily plants should result in the replication of disease symptoms. However, if no causal relationship exists between *P. inconspicua* and the symptoms of the lily leaf spot disease then inoculated host plants should exhibit no symptoms of the disease.

Between August 10 and September 6 2011, 4 healthy plants located outside of the demography transect were inoculated with conidia of *P. inconspicua*. All of these plants had an absence of disease symptoms and were located in areas where no disease symptoms had been observed on neighboring lily plants. Additionally, inoculation trials were conducted late in the season. This period was chosen on the assumption that by this time in the season most infections would have progressed beyond the latency period and resulted in display of disease symptoms.

Lily leaves that exhibited characteristic symptoms of lily leaf spot disease and supported abundant conidia were collected in the field (Figure 8). Portions of these leaves exhibiting tan necrotic lesions were used to inoculate four healthy/asymptomatic plants. On each test plant, leaves were inoculated by pressing inocula tissue onto a thumbnail abrasion on the upper surface

of the test leaf. Inocula were applied to 2 different locations within the same leaf. The first application location was on the abrasion made during treatment. The second application was on an undamaged section of the leaf. Another leaf served as a control and was abraded but received no inoculum. The health status, disease symptoms, and spread of disease symptoms on study plants were recorded every 2 weeks for 6 weeks.

Figure 8. Photograph of a Diseased *L. grayi* Leaf Used as Inocula within the Field Inoculation Trials. The whitish substances (black arrows) in necrotic lesions are masses of conidia of *P. inconspicua*.

Investigation of the Impacts of the Early Season Collapse on Non-reproductively Mature Lilies

Foliar diseases, such as *P. inconspicua,* can have differential effects on plant hosts depending upon the developmental stage at which infection occurs (Ashton & Macauley 1972). Distinguishing impacts at different plant developmental stages can uncover trends that may prove important to understanding the epidemiology. To determine the impact of the lily leaf spot disease on the health and survivorship of non-reproductively mature lilies, disease severity and mortality of seedling and juvenile lilies were monitored in 7 separate 1 m^2 plots. In 2011, three plots were located in areas subject to browsing by goats the previous year. In 2012, new plots were chosen that were distributed along the length of the demography transect outside of experimentally browsed areas. Because of the patchy distribution of *L. grayi,* plot locations were not randomly chosen but were located in areas where at least 10 or more seedlings and/or juveniles were present. Some plots had as few as 2 seedlings or as many as 27. Similarly, abundance of juveniles varied. Plants were noted as either juvenile (leaf whorl(s) but no flowers)

or seedling (single strap leaf only.) Plant height or length of strap leaf, number of whorls, leaves per whorl, and disease severity were recorded every 2 weeks over an approximately 14-week period. Disease severity was quantified using a 6 point scale: 0, 1, 1.5, 2, 2.5, 3, with 0 indicating a dead plant, 1 indicating that over 90% of the plant was diseased and/or dying, 1.5 indicating that 50% - 90% of the plant was diseased and/or dead, 2.0 indicating that 25% - 50% of the plant was diseased or dying, 2.5 indicating that 25% or less of the plant was diseased and/or dead, and 3 indicating excellent health. The basis of this scale is detailed under *Symptomatology of the Early Season Collapse of* Lilium grayi (p. 40). For seedlings, the disease severity scale had limitations caused by an inability to consistently distinguish between symptoms caused by environmental stresses such as leaf burn and/or shading and those caused by disease. To overcome this shortcoming, during the 2012 season samples of characteristically symptomatic leaves were taken from plots, placed on slides, and diagnosed as having a presence or absence of *P. inconspicua*. The methods of sampling and diagnosis of disease are the same as those described under *Diagnosis of the Candidate Pathogen,* Pseudocercosporella inconspicua (p. 45).

To determine if there was a differential effect of disease on different developmental stages of lilies, survivorship was calculated and compared to that of reproductively mature lilies.

To compare the increase in disease severity between plots and between developmental stage within and between years, change in plot mean disease severity was tested for significant differences. Because initial sampling dates differed between study years (June 11 in 2011 and May 22 in 2012) comparison by date was not used for analysis. Instead, rates of change in disease severity within plots were used to calculate slopes using linear regression as implemented by the REG procedure in SAS v.9.3 (SAS Institute 2011). Slopes of disease

severity were then compared using ANOVA, as implemented by the regression procedure in SAS v.9.3.

To test for interactions between disease severity and other variables (year, plot identity, sampling date, and developmental stage) repeated measures ANOVA was conducted as implemented by the GLM procedure in SAS v. 9.3.

Investigation of the Impacts of the Early Season Collapse on Reproductively Mature Lilies

Impacts and distribution of the early season collapse on the life cycle of reproductively mature *L. grayi* were investigated by monitoring the location, distribution, and severity of disease within two sequential growing seasons. Monitoring was conducted within a line transect following the Appalachian Trail and extending to part of the Grassy Ridge Trail. Reproductive plants were given unique coordinates based on their location. Unique coordinates allowed location-specific data to be collected throughout the growing season and relocation of plants in subsequent seasons.

The transect was located within the grassy balds atop Roan Mountain starting at the elevation sign on top of Jane Bald (36.105982 N, 82.093620 W) and extending east approximately 300 meters before the memorial rock on top of Grassy Ridge (36.103659 N, 82.081861 W) (Figure 9). An attempt was made to include all mature plants occurring approximately 2 to 9 meters on either side of the edge of the trails. Sampling occurred every 2 weeks for approximately 14 weeks over the growing season, extending from May to September.

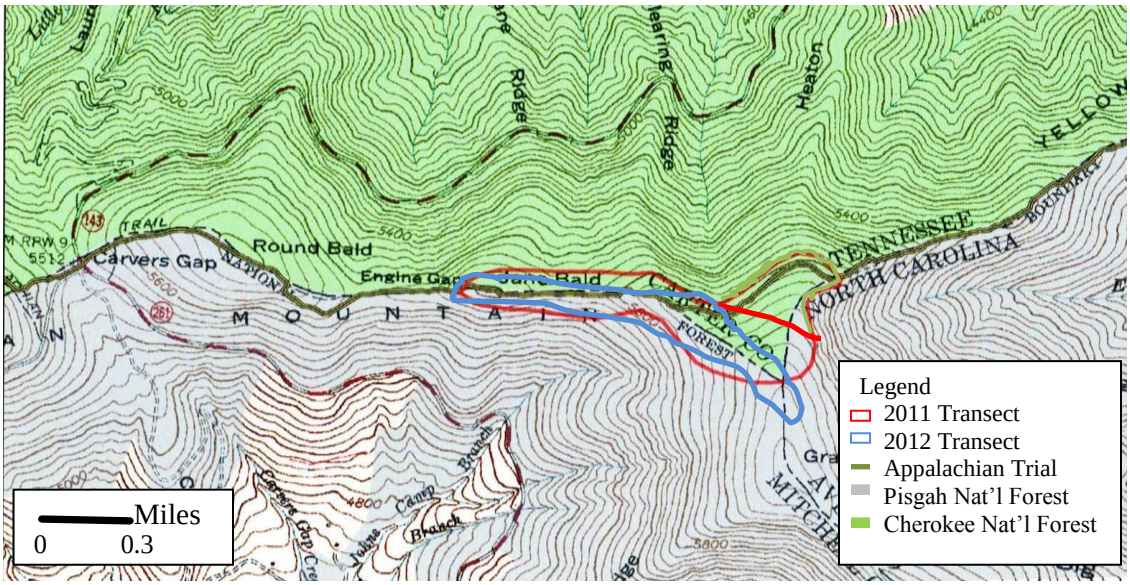


Figure 9. Topographic Map of the Study Area within the Grassy Balds of the Roan Mountain Massif in Roan Mountain,TN/NC. (USGS 2012)

At the onset of the project in 2010 (Powell 2011) and each subsequent year of the study, the transect was systematically searched for tagged and previously untagged reproductive lily plants. Previously untagged plants were given a unique identification number and included in the multi-year study. Inclusion of new plants each year resulted in an increased number of plants every year of the study with 100 plants in 2011 and 120 plants in 2012. The increased number of individuals in the study was likely not a result of an increase in population numbers but rather a product of multiple years of intensive surveying of the same area combined with failure of some plants to appear above ground on an annual basis (C. Ulrey, pers. comm. 2010).

The following indicators of vegetative and reproductive health and vigor were measured: height (cm), number of flowers/fruit, number of leaf whorls, number of leaves per whorl, plant health status, number of infected whorls, indications of physical damage from browsing/herbivory (mammal or insect), and reproductive damage (disease on pedicels, flowers, or fruit). The disease severity scale was based on the disease symptoms detailed within *Symptomatology of the Early Season Collapse of* Lilium grayi (p. 40). Leaf whorls exhibiting

disease symptoms were recorded as infected if there were disease symptoms on any of the leaves contained within a whorl. Browsing was recorded as insect- or mammal-mediated and was inferred by the nature of the mechanical damage present.

To describe the extent and severity of disease, host survivorship (disease specific mortality and all other causes), incidence and location of disease, percentage of plants that lived to reproduce, and percentage of plants with disease on reproductive structures were tabulated. Incidence rates were calculated by excluding plants from the at-risk population that were browsed and had not exhibited disease symptoms. Plants that had shown symptoms of disease before being browsed were included in the number of disease cases. This was done both for disease incidence rates and rates of reproductive damage by disease. When assessing host survivorship, disease-specific mortality and mortality from all causes were calculated. Disease-specific mortality excluded plants that had a disease severity scale value of $\geq$1.5 and included all plants with a disease severity scale value $\leq$1.0. Mortality from all causes included all plants with a disease severity scale value $\leq$1.0.

A primary study goal was to determine whether the disease phenomenon on Roan Mountain was the result of an infectious process caused by a pathogenic organism and/or the result of environmental factors. Because disease is caused by susceptible hosts coming into direct contact with inoculum of a pathogen, disease incidence and severity is expected to be higher on plants near initial sources of inoculum than those further away (Jarosz and Burdon 1989). The spatial dependence of disease distribution results in 2 characteristics for an infectious process: spatial clustering of disease incidence and a disease gradient (Burdon 1984). To determine if the lily leaf spot disease on Roan Mountain is the result of an infectious process, the location of

disease incidence and interplant distances were tested for spatial clustering and a gradient of disease.

To determine whether there were clusters of disease severity, cluster analysis was conducted using the scan statistic and implemented using SaTScan software (Kulldorff 1997). The scan statistic is the maximum number of diseased or healthy individuals in a specified area. Significance of the scan statistic indicates a cluster and is assessed with 1000 Monte Carlo simulations. A purely spatial analysis was chosen that used an ordinal probability model and scanned for both high and low values. The shape of the scanning window was a circle with a maximum cluster size not exceeding 50% of the population**.** Level of significance was set to $P \leq 0.05$. Analyses were conducted for each of the 9 sampling events in 2011 and each of the 7 sampling events in 2012 for the variables disease severity and number of diseased whorls. By identifying foci for these 2 variables, it was possible to track the change in the area encompassed by clusters. To visualize the location and extent of clusters, 2D scatter plots were produced using the software, Spatial Analysis in Macroecology (SAM v. 4.0) and manually drawing circles to encompass plants in a cluster. Circles of red (disease cluster) or green (healthy clusters) and yellow (cluster of high numbers of diseased whorls) or light green (cluster of low numbers of diseased whorls) were used to indicate significant clusters. To determine whether there were clusters based on plant vigor, cluster analysis was also performed on plant height, number of whorls, number of leaves per whorl, and number of fruit. The expectation was that disease severity and number of diseased whorls would follow similar patterns of clustering due to both representing indicators of the lily leaf spot disease. The measures of plant vigor were expected to show clustering patterns similar to that of health and/or number of diseased whorls only if a trait was associated with the disease.

Identification of environmental or morphological risk factors linked to the incidence and severity of the lily leaf spot disease was accomplished through correlation analysis and a multivariate analysis of risk factors using logistic regression (Levy et al. 2009). To test for associations between disease severity, plant vigor metrics, browsing, plant location, and disease on reproductive structures, correlation analysis was conducted using the CORR procedure in SAS. Multivariate analysis was conducted using logistic regression as implemented by the LOGISTIC procedure in SAS. Logistic regression was conducted with disease severity as both a binary and an ordinal response variable. In the binary analyses disease scale values of 0.0 - 2.0 were considered diseased and 2.5 and 3.0 were considered healthy. To account for the effects of natural senescence on host plants, the disease scale value 2.0 was considered healthy for the last 2 sampling dates in 2011 and 2012. The ordinal response variable followed the disease scale. To exclude irrelevant variables from the logistic regression model, inclusion of a predictor variable was initially determined by including and removing variables to obtain the lowest possible Model Fit Statistics. Additionally, predictor variables were subsequently included or excluded from the model by both forward and backward stepwise selection as implemented by the STEPWISE SELECTION option in the PROC LOGISTIC procedure in SAS v.9.3 with an inclusion and removal criteria of 0.35. In the forward stepwise selection a predictor was included in the model and then excluded or not based on significance from a univariate analysis. In the backwards selection all variables started in the model and were subsequently removed or retained based on model significance. The variables tested for inclusion were: height, proportion of yellow whorls for the same sampling event, number of whorls, number of leaves per whorl, number of mature fruit at end of season, x-coordinate, y-coordinate, and heavily browsed.

To further test the hypothesis of an infectious process, a test for a gradient of disease severity was used. For this spatial autocorrelation was used to test for similarity of disease severity among host plants at various distances. Spatial autocorrelation was conducted on disease severity, number of disease whorls, and measures of plant vigor using Moran's I as implemented by SAM v. 4.0 (Real and McElhany 1996). Plants were grouped into 6 equal distance classes of approximately 300 m. Moran's I was calculated for each of the distance classes within each sampling event. Results were represented on a correlogram generated within SAM v.4.0.

Spatial autocorrelation analysis was conducted with the expectation that if disease was the result of an infectious process, then a disease gradient would be present with plants in closer proximity having more similar disease severity than those at further distances (Jarosz and Burdon 1989). Additionally, if disease reached epidemic proportions, disease gradients would be expected to disappear as the severity of disease spread to most plants. However, if disease were not the result of an infectious process but rather was the result of abiotic causes, disease severity would not be expected to either be randomly distributed or to form a steep gradient that remained stable throughout the season.

Investigation of the Impact of Pseudocercosporella inconspicua *on the Seed Viability of* Lilium grayi

The effect of plant disease on seed viability can be quite variable (Burdon 1993). Effects can range from complete inviability to no effects. To determine the effect of the lily leaf spot disease on the viability of *L. grayi* seed, a germination and seed viability study was conducted.

Due to complications in germinating *L. grayi* seed, 3 separate germination studies were attempted using different protocols. The germination study conducted in 2011 used seed collected in 2010 by Powell (2011). Six seed capsules from 6 different maternal plants were

weighed intact and then both the empty capsule and total contained seed were counted and weighed separately. Seed counts were also recorded. Using sterile techniques, 60 seeds from each plant were divided into 4 subsets of 15 seeds. Each subset of seeds was wrapped in cheesecloth to prevent seed loss or separation and placed far enough apart to not touch within sealable plastic bags containing 600 mL of milled peat moss moistened with 400 mL of boiled deionized water. Each bag had an unsealed corner for air exchange. For a period of 5 weeks germination bags were placed in ambient conditions. After this period, they were moved to a germination chamber set 4° C for 10 weeks. At the end of the 10 weeks the bags were again placed in ambient conditions.

The germination attempt conducted in 2012 utilized seed collected in 2011. Twenty mature seed capsules from 20 different plants were collected in mid September, 2011. Fifteen of the seed capsules were collected from diseased maternal plants and 5 seed capsules were collected from maternal plants exhibiting no symptoms of disease. Morphometric data on the seed and capsules were collected in the same manner as the first germination study. Seeds were removed from seed capsules and placed in petri dishes containing 4 pieces of filter paper moistened with a solution of fungicide (2% Captan / 98% dH2O). Care was taken not to leave standing water. Petri dishes were placed at ambient conditions in a plastic bag for 5 weeks. After 5 weeks bags were removed and placed in a germination chamber at 4° C for 8 weeks. Once germination occurred, seedlings were counted and removed over the course of 4 weeks.

The third germination trial was conducted in 2013. In 2012, 30 seed capsules from plants of differing health status were collected from the field and brought into the laboratory for germination. Of the 30 capsules 12 had signs and symptoms of the leaf spotting disease on them while the other 18 capsules exhibited no signs or symptoms of disease. Seed capsules were

placed at room temperature in the laboratory to promote drying and prevent rotting. Once seed capsules had dried, they were placed in individual plastic tubes at $\leq$0 °C until use. Weight was measured for intact capsule, capsule only, and seed only. Seed count, coloration, and level of deformity were also recorded. Seeds were handled wearing gloves and using a metal scoop. Seeds were placed in 50 mL tubes with approximately 40 mL of milled peat moss moistened with 30 mL of sterile water. Excess water was removed by loosely placing the cap on and inverting the tube. Each tube was then placed in ambient conditions for 5 weeks and then moved to a germination chamber at 4 °C for 8 weeks. At the end of the 8 weeks tubes were placed in ambient conditions to await germination. Once germination started, seedlings were counted and removed over the course of 4 weeks.

The purpose of the seed viability study was to determine the relative viability of seed originating from moderately to severely diseased capsules through comparison of the weight, size, and number of seeds from healthy and diseased capsules. To test for differences in the morphological characteristics of seed from disease capsules, comparisons of capsule weight, capsule length, seed weight, seed length, and seed count from diseased and healthy capsules were conducted using a student t-test as implemented by the TTEST procedure in SAS.

If the lily leaf spot disease on lily seed capsules were to negatively impact the viability of seed, disease symptoms would be expected to be associated with characteristics such as reduced seed weight and count. Associations were assessed using the Pearson correlation coefficient as implemented by CORR procedure in SAS (PROC CORR).

CHAPTER 3

RESULTS

Diagnosis of the Candidate Pathogen, Pseudocercosporella inconspicua

The morphological characteristics of conidia obtained from within diseased host lesions conformed to those of *P. inconspicua* (Braun 1995). Conidia were erumpent and occurred singly on conidiophores attached to stromata embedded in leaf epidermis. Conidia appeared septate, hyaline, and fusiform with blunt or rounded tips and rounded to truncated bases (Appendix J; K). Conidia were also attenuated from the mid-point to the apex (Appendix I). Mean conidial length was 83.70 μm with a standard deviation of 15.08 μm and a range of 42.43 – 123.52 μm (Appendix G). Mean conidial width was 4.96 μm with a standard deviation of 1.03 μm and a range of 2.27 – 6.75 μm (Appendix H).

Isolation of the Fungal Pathogen, Pseudocercosporella inconspicua*, from Diseased Host Tissue*

Eight attempts were made to isolate *P. inconspicua* from symptomatically characteristic diseased leaf tissue of *L. grayi*. Several isolates matching the morphology of CBS cultures (CBS 284.49) were obtained but due to an inability to induce sporulation none were positively identified as *P. inconspicua*. Approximately 90% of attempts to isolate from diseased tissue nearest necrotic lesions resulted in several fungal types within a single petri dish. Only by excising leaf sections of green tissue in proximity to, but not touching the margin of necrotic lesions, were cultures containing fungi with a single set of morphologically constant vegetative characteristics obtainable. PDA media made to the manufacturer's specifications with streptomycin (30 mg/L) proved to be the most appropriate media for isolation purposes because mycelia were more easily observed growing on this media type due its clarity. This made identification of key vegetative characteristics much easier as compared to oatmeal agar (OA).

Viral Testing of Lilium grayi

All 8 samples tested negative for all 7 of the viruses they were tested for, which were the following: Cucumber mosaic virus (CMV), Impatiens necrotic spot virus (INSV), Lily symptomless virus (LSV), Potyvirus group (POTY), Tobacco ringspot virus (TRSV), Tomato aspermy virus (TAV), and Tomato spotted wilt virus (TSWV).

Measuring Background Levels of Pseudocercosporella inconspicua *Conidia: A Conidial Baseline Study*

There was either an absence or very low levels of *P. inconspicua* conidia on plant species other than *L. grayi*. Additionally, all but 2 diseased *L. grayi* had *P. inconspicua* conidia in medium to high concentrations while all healthy *L. grayi* had an absence of conidia. The association between high concentrations of *P. inconspicua* conidia and diseased *L. grayi* was significant, P<0.0001 (Table 2).

Table 2. Contingency (3 x 3) Table Comparing Concentrations of *P. inconspicua* Conidia Present on Samples on *L. grayi* and Other Species Using the Freeman-Halton Extension of the Fisher's Exact Test. "N/A" = not applicable.

Concentration of *P. inconspicua* conidia					
		Absent	Very low- Low	Medium- High	
Species	Disease Status				Column Total
L. grayi	Healthy	10	0	0	10
L. grayi	Diseased	1	1	8	10
Other species	N/A	15	5	0	20
Row Total		26	6	8	40
	Fisher's exact test: P = <0.0001				

Association of the Pathogen, Pseudocercosporella inconspicua, *with the Symptoms of the Early Season Collapse of* Lilium grayi

Diagnosis of the lily leaf spot disease by visual inspection (i.e. host symptoms) is highly predictive of diagnostic signs (i.e. conidia) of *P. inconspicua*. Slides prepared in the field indicated high concentrations of *P. inconspicua* were consistently and significantly associated with tan necrotic lesions occurring on leaves of *L. grayi* plants suffering from the lily leaf spot disease (Figure 10; Table 3). In all but one instance, when a plant was diagnosed as "diseased" there were high concentrations of *P. inconspicua* conidia within tan lesions. Conversely, when a plant was diagnosed as "healthy" there was an absence of *P. inconspicua* conidia.

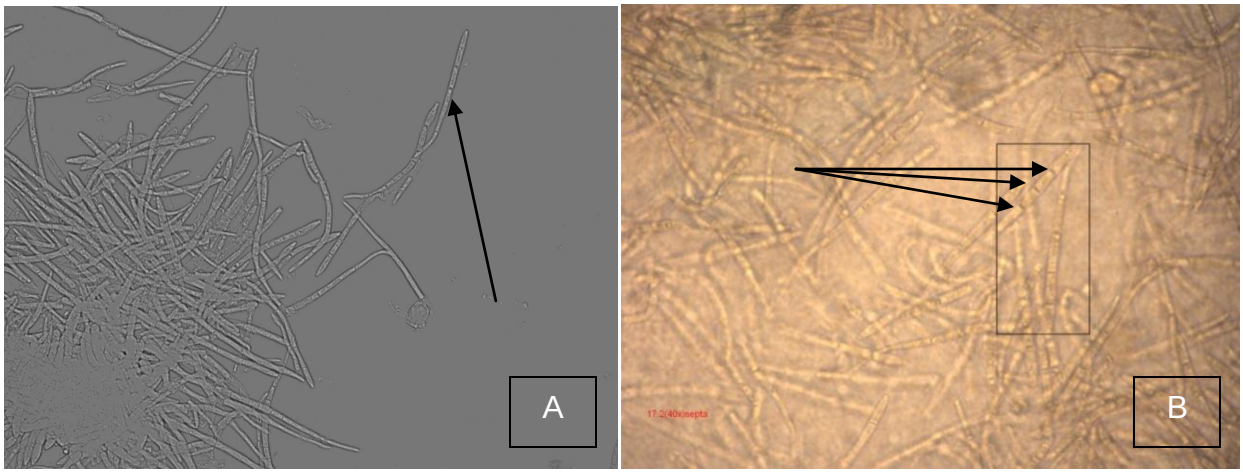


Figure 10. Photographs of Diagnostic Conidia of *P. inconspicua* from Field Samples of Diseased *L. grayi.* (A) Slide obtained from a characteristically symptomatic plant within the demography transect in 2011. The numerous tubular structures are the conidia and the single fusiform structure (black arrow) is a conidium, 200 x magnification. (B) Slide obtained from a characteristically symptomatic plant within the demography transect in 2011. Several hyaline conidia (black box) of *P. inconspicua*. Diagnostic septation (black arrows) within conidium, 400 x magnification.

Table 3. Contingency (2 x 2) Table Comparing Visual Diagnosis of Disease and the Presence of *P. inconspicua* Conidia on *L. grayi* Using Fisher's Exact Test

Visual Diagnosis			
	Healthy	Diseased	
Pathogen			Column Total
Absent	31	1	32
Present	0	19	19
Row Total	31	20	51
$\chi^2 = 42.96$; df = 3; P <0.0001			

Figure 11. Photographs of Field Samples Being Collected for Pathogen-Symptom Association. (A) Microscope slides used for lily leaf spot disease diagnosis. (B) Plant visually diagnosed as positive for lily leaf spotting disease. Leaf lesions (black arrows) that were sampled as part of the disease symptom association analysis.

Reproducing the Symptoms of the Early Season Collapse: Inoculation of Hosts within the Field

The symptoms of the lily leaf spot disease were transmittable through contact with host tissue that had tested positive for high concentrations of *P. inconspicua.* Once disease symptoms were exhibited, disease spread rapidly to other leaves and structures on study plants. Of 5 plants inoculated in the field, 4 showed disease symptoms within 2 weeks (Figure 12B; 13; 14). One plant was excluded due to defoliation by mammal browsing.

With the exception of a defoliated plant, all but 1 of the inoculated plants died as a result of the rapid proliferation of disease symptoms. In total 3 of the 4 plants experienced an early season collapse of above-ground structures (Figure 12A). The initial development of disease symptoms occurred between the first and second observation, 2 days to 1 week after inoculation (Figure 13; 14). At a week after inoculation control leaves exhibited no signs or symptoms of disease. By the last 2 sample events at 1 month after inoculation, disease symptoms had developed on control leaves (Figure 14). The appearance of disease symptoms was presumably caused by spread of the disease. The 1 unbrowsed plant not experiencing early season collapse by the end of the trial had symptoms of the lily leaf spot disease but they were isolated to the inoculated experimental leaf (Figure 12B).

Of the 5 inoculated plants, 2 were located in areas within the transect (approx. x=1100 m and x=1600 m) where disease clusters were absent and disease had not been observed on neighboring plants. Inoculated plants located within these areas suffered a similar precipitous decline as plants located in areas where disease was observed on other plants within 10 meters. At the end of the season all inoculated plants had high concentrations of *P. inconspicua* conidia.

Figure 12. Photographs of *L. grayi* Plants Used in Field Inoculation Trial on September 6, 2011. (A) *Lilium grayi* after experiencing early-season collapse. Note the characteristic tan leaves (white arrows) that are present after the plant dies. (B) The only study plant that lived until the last sampling date. Note that the lily leaf spot disease tan lesion was restricted to the experimental inoculated leaf (white arrow.)

Figure 13. Photographs of the Control and Experimental Leaves on a *L. grayi* Plant Used Within the Field Inoculation Trial on August 12, 2011. (A) Control leaf with no disease symptoms. (B) Inoculated experimental leaf exhibiting the first signs of a developing lesion (white arrow) characteristic of the lily leaf spot disease.

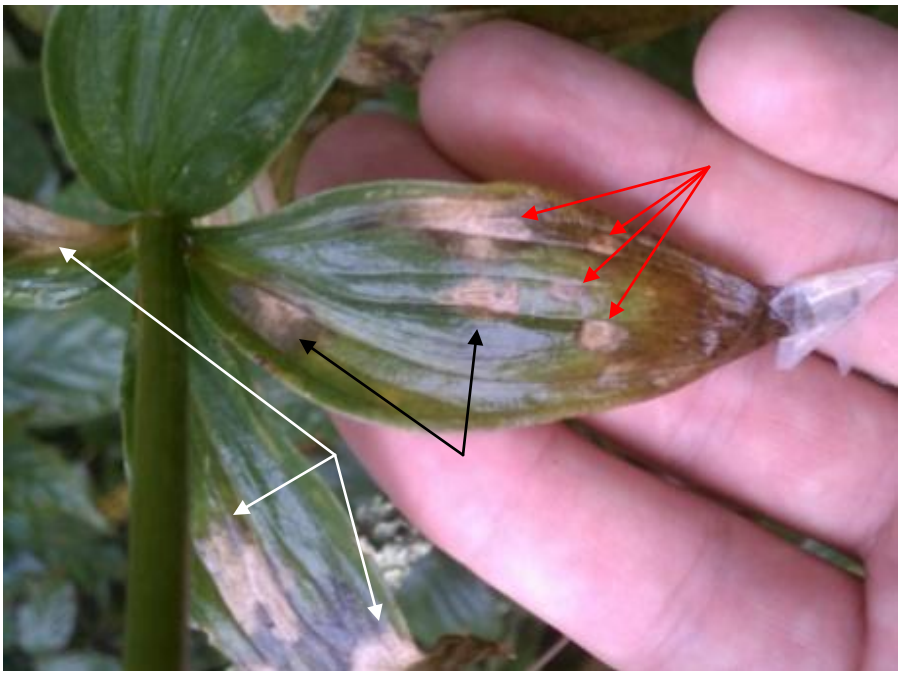


Figure 14. Photograph of the Experimental Leaf on a *L. grayi* Plant Used Within the Field Inoculation Trial on August 20, 2011. Characteristic tan necrotic lesions are present on the 2 inoculation sites (black arrows) and on 4 other locations within the experimental leaf (red arrows). Tan necrotic lesions had also spread to neighboring leaves within the same leaf whorl (white arrows).

Investigation of the Impacts of the Early Season Collapse on Nonreproductively Mature Lilies

Impact on Seedling Recruitment and Juvenile Survival

Within-season survivorship of nonreproductively mature lilies was very low with mortality rates of 100% in 2011 and 99.4% in 2012 (Table 4). While not all mortality could be attributed to disease, all plots had plants exhibiting symptoms of the leaf spotting disease. In 2011 and 2012, similar patterns of disease severity were observed, whereby the mean disease severity of adolescent lilies increased rapidly until all seedlings and almost all juveniles had senesced by mid-season (Figure 16). However, early senescence of seedlings and juveniles occurred a month earlier in 2012 than in 2011 (Figure 16).

Figure 15. Photograph of a Plot of *L. grayi* Seedlings Necrotic from the Lily Leaf Spot Disease on July 7, 2011. Seedlings exhibited a general blackening and tan necrotic lesions (white arrows) characteristic of the lily leaf spot disease.

Table 4. Mortality Rates for Death from All Causes for Seedling and Juvenile Lilies in 2011 and 2012.

Developmental Stage	**Percent Mortality from all Causes (Number of Plants) by Year**	
	2011	2012
Seedling	100.0% (83)	100.0% (77)
Juvenile	100.0% (36)	98.8% (43)
All non-reproductive lilies	100.0% (119)	99.4% (120)

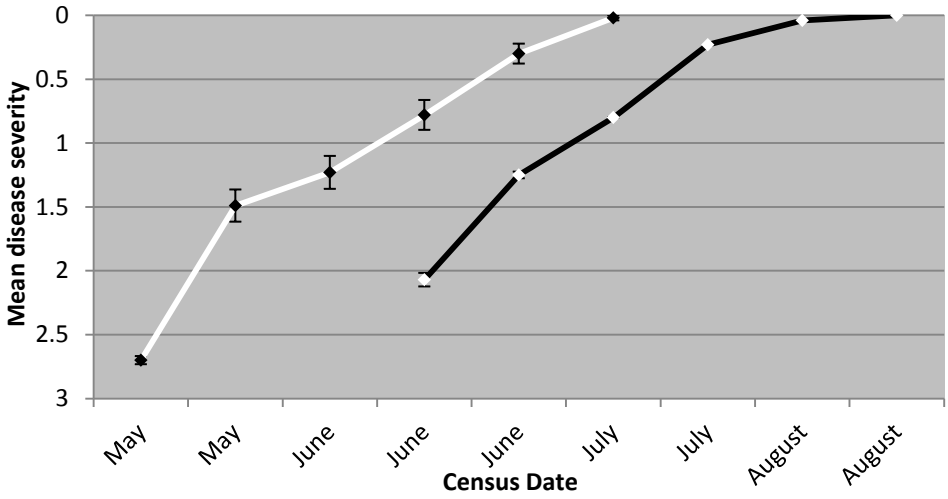


Figure 16. Bimonthly Change in Mean Disease Severity of Nonreproductively Mature Lilies in 2011 and 2012. Mean disease severity was calculated from the pooled seedling and juvenile data from all plots within a year. Error bars represent SEM.
Year line color code: 2011 = black; 2012 = white.

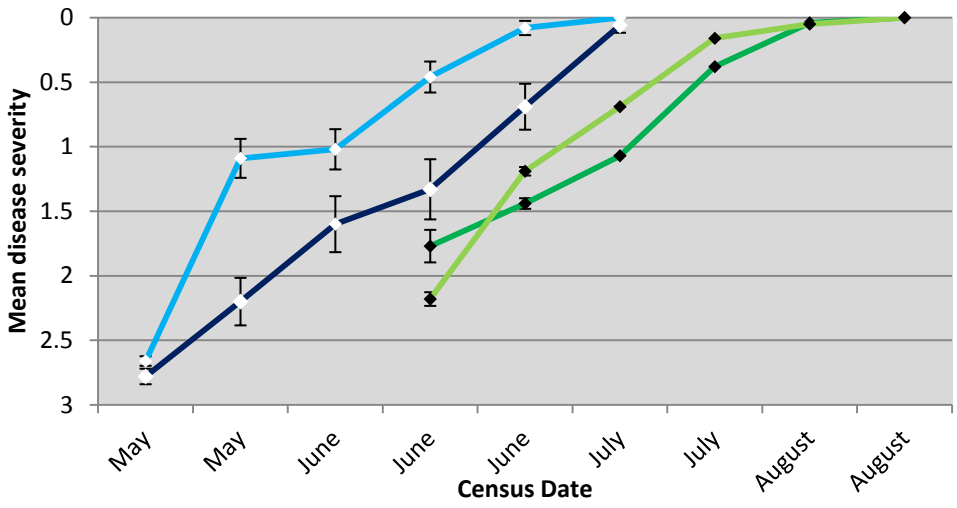


Figure 17. Bimonthly Change in Mean Disease Severity of Seedlings and Juveniles in 2011 and 2012. Mean disease severity was calculated from pooled data of all seedlings within all plots and the pooled data of all juveniles within all plots for each study year. Error bars represent the SEM. ("β" = rate of change), Developmental Stage and Year color code:
Seedlings 2011 (β = - 0.424) = light green; Juveniles 2011 (β = - 0.393) = dark green;
Seedlings 2012 (β = - 0.483) = light blue; Juveniles 2012 (β = - 0.526) = dark blue.

Because sampling events occurred at different times in 2011 and 2012, comparisons of disease severity among sampling events between years was not possible. Instead, comparison of disease progression was accomplished by using change in mean disease severity of seedlings and juveniles within a growing season to calculate slopes. These slopes represent the rates of increase in disease severity. Juvenile lilies experienced a significantly more rapid rate of increase in disease severity in 2012 as compared to 2011 (Table 6). Rates of change in mean disease severity were not significantly different for either all non-reproductive lilies between years (Table 5), seedlings between years (Table 7), and seedlings and juveniles within years similar (Table 8; 9).

Table 5. Results of One-way ANOVA Comparing Rates of Change (β coefficients of linear regression of disease severity over time) in Disease Severity of Nonreproductive Lilies in 2011 and 2012

Source	Rate of Change in Disease Severity (β)	Adj R- square	T	P
Non-reproductive lilies, 2011	-0.368	0.843		
Non-reproductive lilies, 2012	-0.501	0.869		
Difference in slopes between years	-0.132	0.853	-1.20	0.265

Table 6. Results of One-way ANOVA Comparing Rates of Change (β coefficients of linear regression of disease severity over time) in Disease Severity of Juvenile Lilies in 2011 and 2012

Study year	Rate of Change in Disease Severity (β)	Adj R- square	T	P
Juveniles, 2011	-0.336	0.928		
Juveniles, 2012	-0.513	0.926		
Difference in slopes between years	-0.176	0.926	-2.30	0.050

Table 7. Results of One-way ANOVA Comparing Rates of Change (β coefficients of linear regression of disease severity over time) in Disease Severity of Seedling Lilies in 2011 and 2012

Study year	Rate of Change in Disease Severity (β)	Adj R-Square	T	P
Seedlings, 2011	-0.400	0.734		
Seedlings, 2012	-0.487	0.790		
Difference in slopes between years	-0.087	0.747	-0.580	0.579

Table 8. Results of One-way ANOVA Comparing Rates of Change (β coefficients of linear regression of disease severity over time) in Disease Severity of Seedlings and Juvenile Lilies in 2011

Adolescent Lily Developmental Stage	Rate of Change in Disease Severity (β)	Adj R-Square	T	P
Seedling	-0.400	0.734		
Juvenile	-0.336	0.928		
Difference in slopes between developmental stages	-0.064	0.786	-0.570	0.585

Table 9. Results of One-way ANOVA Comparing Rates of Change (β coefficients of linear regression of disease severity over time) in Disease Severity of Seedling and Juvenile Lilies in 2012

Adolescent Lily Developmental Stage	Rate of Change in Disease Severity (β)	Adj R-Square	T	P
Seedling	-0.487	0.790		
Juvenile	-0.513	0.926		
Difference in slopes between stages	-0.026	0.846	-0.20	0.846

Despite a similar pattern in the rate of change in mean disease severity, the effect of disease on seedling and juvenile lilies on Roan Mountain was not uniform. Repeated measures ANOVA showed a main effect of the study year on disease severity and significant interactions between plots within years, between developmental stages within years, and between years within developmental stage (Table 10). The main effect of the study year indicates that disease severity was different between the 2 years. This difference of disease severity in study years suggests that disease was more severe in one year as compared to the other. The interactions suggest that disease severity of seedlings and juveniles was dependent upon plot, study year, and developmental stage. Because plots were located in different locations along the length of the demography transect, significant interactions suggests that there was an environmental component to the occurrence and severity of disease. Additionally, because of different sampling dates and plot locations in 2012, interactions resulting from the comparison of between-year plot effects (i.e. between plots, between plots in different years, between different developmental stages within plots in different years) on mean disease severity were not readily interpretable and were therefore not included.

Table 10. Results of Repeated Measures ANOVA Testing the Effects of Developmental Stage, Plot Identity, and Study Year on Mean Disease Severity. "Developmental Stage" = seedling or juvenile. **

Source	df	MS	F	P
Between Study Years	1	36.58	2.37	<.0001
Between Plots (within Study Year)	12	15.43	17.34	<.0001
Error	198	0.89		

Source	df	MS	F	P
Between Study Years	1	36.58	8.18	<.0001
Between Developmental Stages	1	7.77	0.42	0.0035
Between Study Years (Within Developmental Stage)	2	18.34	20.61	<.0001
Between Developmental Stages (within Study Year)	1	4.47	8.18	<.0001
Between Plots*Between Developmental Stages(within Study Year)	24	10.40	0.43	<.0001
Error	198	0.89		

** Study plots, different between years.

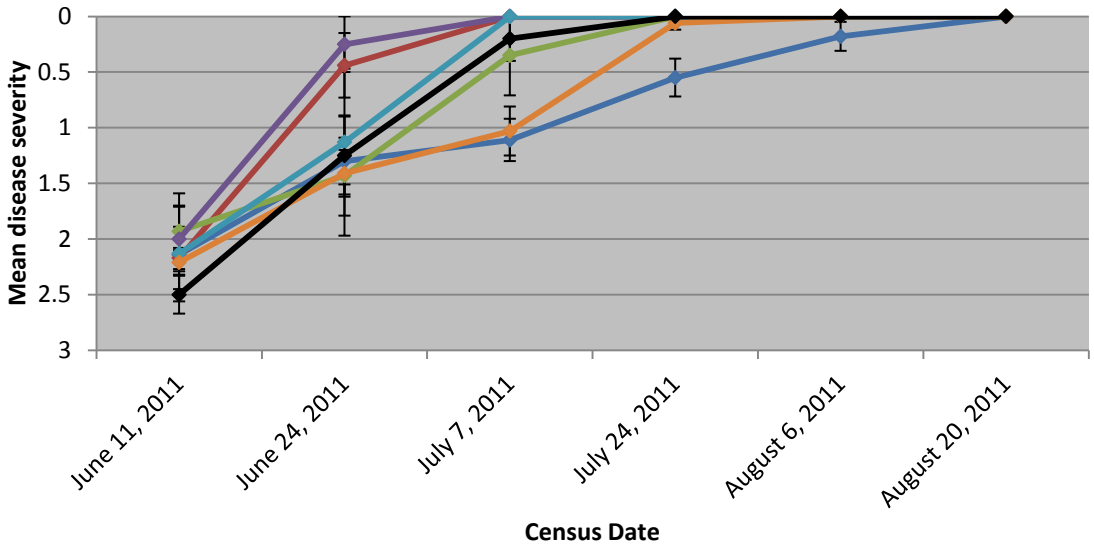


Figure 18. Bimonthly Change in Mean Disease Severity of Seedlings Within Each Nonreproductively Mature Lily Plot in 2011.
Plot color code: Plot 1 = dark blue; Plot 2 = red; Plot 3 = green; Plot 4 = purple; Plot 5 = light blue; Plot 6 = orange; Plot 7 = black.

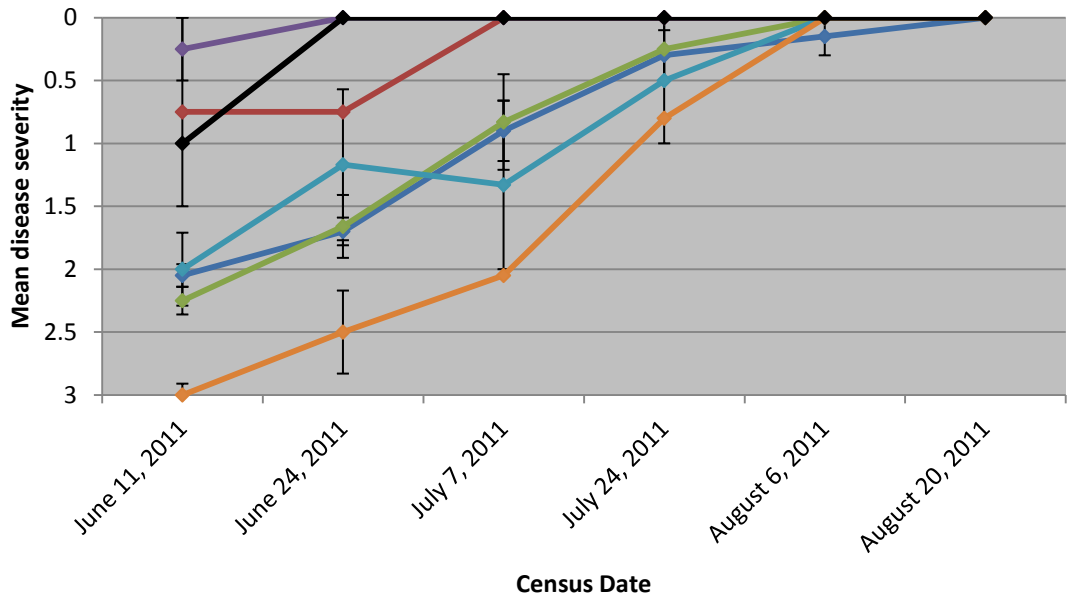


Figure 19. Bimonthly Change in Mean Disease Severity of Juveniles Within Each Nonreproductively Mature Lily Plots in 2011

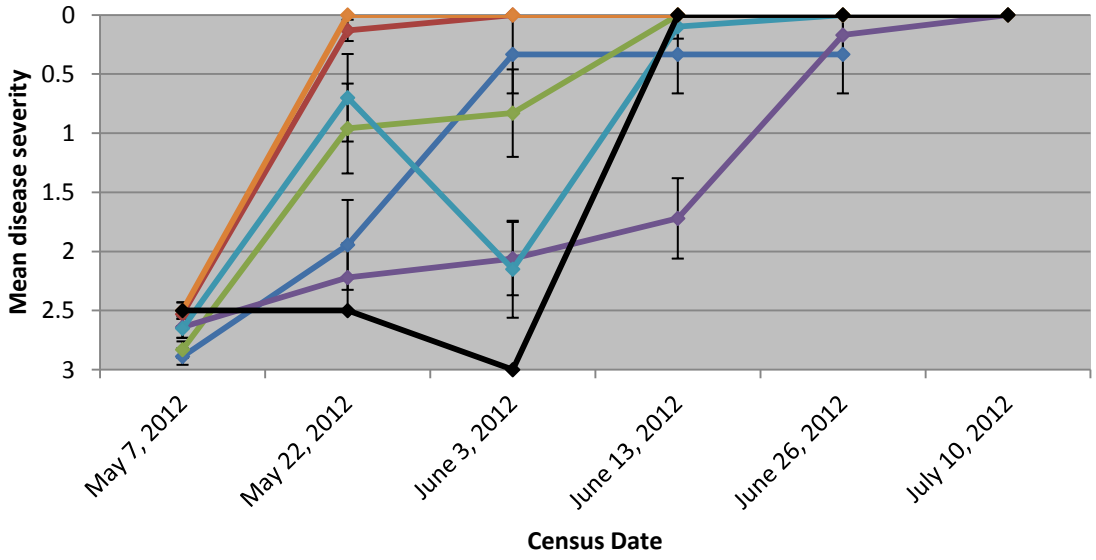


Figure 20. Bimonthly Change in Mean Disease Severity of Seedlings Within Each Nonreproductively Mature Lily Plots in 2012

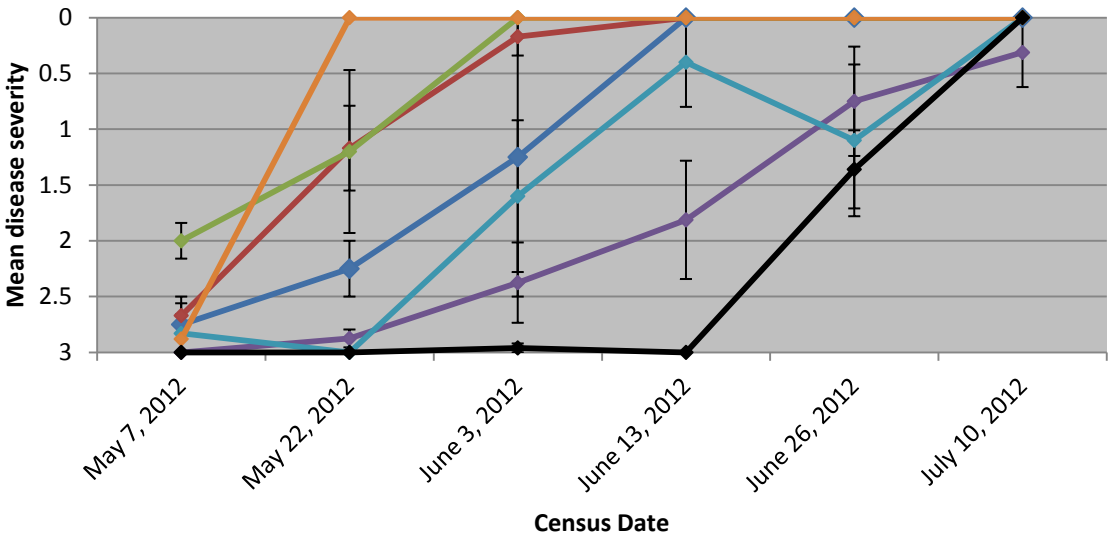


Figure 21. Bimonthly Change in Mean Disease Severity of Juveniles Within Each Nonreproductively Mature Lily Plot in 2012

Table 11. Number of Seedlings and Juveniles within Individual Nonreproductively Mature Lily Plots in 2011 and 2012

	2011			2012		
Plot	Number of Seedlings	Number of Juveniles	Total	Number of Seedlings	Number of Juveniles	Total
1	22	10	32	9	2	11
2	9	2	11	15	6	21
3	7	6	13	12	5	17
4	4	4	8	18	8	26
5	4	3	7	10	5	15
6	27	10	37	10	4	14
7	10	1	11	2	14	16
Total	n = 83	n = 36	n = 119	n = 76	n = 44	n = 120

Investigation of the Impacts of the Early Season Collapse on Reproductively Mature Lilies

Impacts of Lily Leaf Spot Disease on Reproduction

By the end of the season in 2011 and 2012 rates of disease prevalence among reproductively mature lilies were >90% (Table 12). Disease curves of disease incidence over time suggest marked but linear increases from late May until early July in 2011 and 2012 (Figure 22) while disease curves of the proportion of disease incidence were sigmoid and suggested

periods of exponential increase within the same period of time (Figure 23). The reason for these different trends is because additional plants were added to the study from June until July in both years because of the high number of plants lost to mammal browsing.

Disease impacts on host survivorship were considered within and between seasons. Within season host survivorship was greatly reduced with 70% of plants in 2011 and 59% of plants experiencing disease-specific early collapse of above-ground structures. The within-season impact of disease was exacerbated by a high frequency of mammal browsing. The combined effects of browsing and disease resulted in <90% early season mortality in 2011 and 2012 (Table 12). The impact of disease on host survivorship of plants heavily diseased in 2011 was also quite severe with only 24% of moderately to heavily diseased plants reemerging the following year. However, only 29 of the 94 plants from the 2011 season reemerged in 2012. Additionally, of the 29 plants from 2011 that re-emerged in 2012 over half of them were moderately to heavily diseased the previous year (Table 12).

The impact of the lily leaf spot disease on the life cycle of *L. grayi* was most apparent in effects on reproductive success (Table 12). Because of high rates of early season collapse before or during seed maturation, many flowering plants either failed to produce or produced fewer seed capsules than the number of flowers present on the plant (Table 12). The reduction in mature seed capsules was further exacerbated by high levels of mammal browsing. Additionally, a high percentage of the plants that lived to produce seed exhibited necrotic lesions on maturing seed capsules (Table 12; Figure 5).

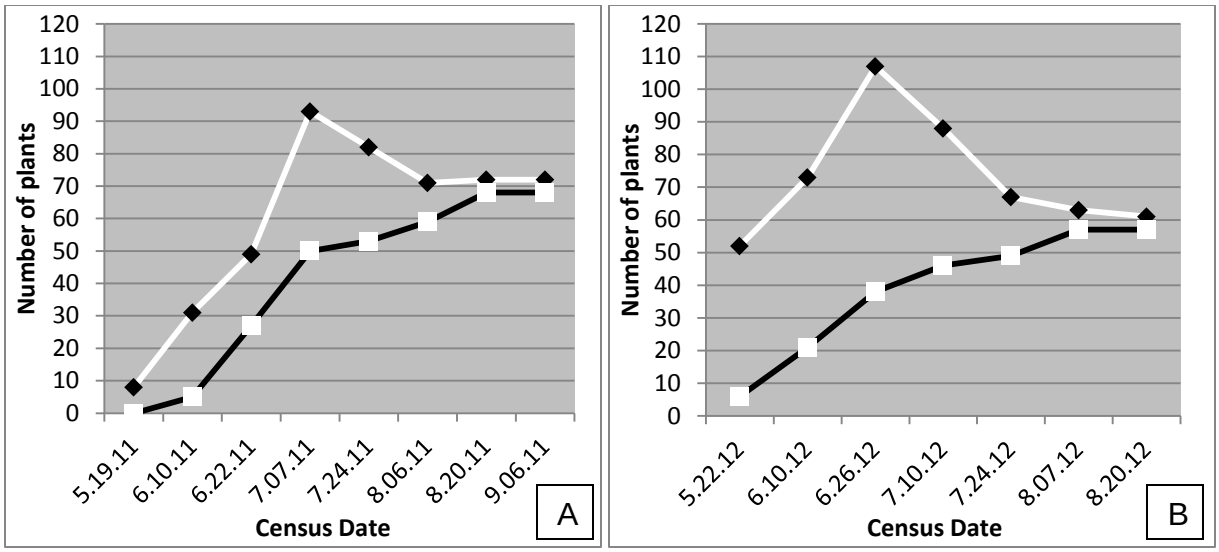


Figure 22. Disease-Progress-Curves of the Number of Cases of the Lily Leaf Spot Disease Among Reproductively Mature Lily Plants in 2011 (A) and 2012 (B). The total number of plants was included for each census date. Number of plants line color code:
Cases of the lily leaf spot disease = black; Total number of lily plants = white.

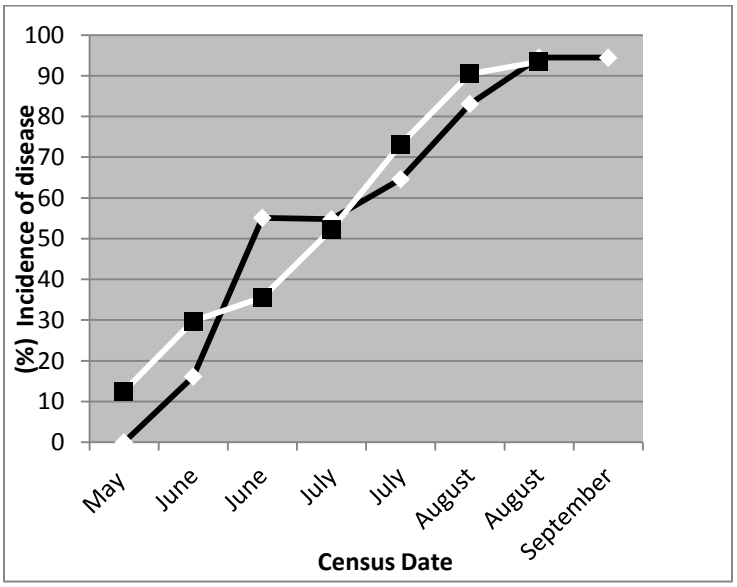


Figure 23. Disease-Progress-Curve of the Proportion of Incidence of the Lily Leaf Spot Disease Among Reproductively Mature Lily Plants in 2011 and 2012.
Study year line color code: 2011 = black; 2012 = white.

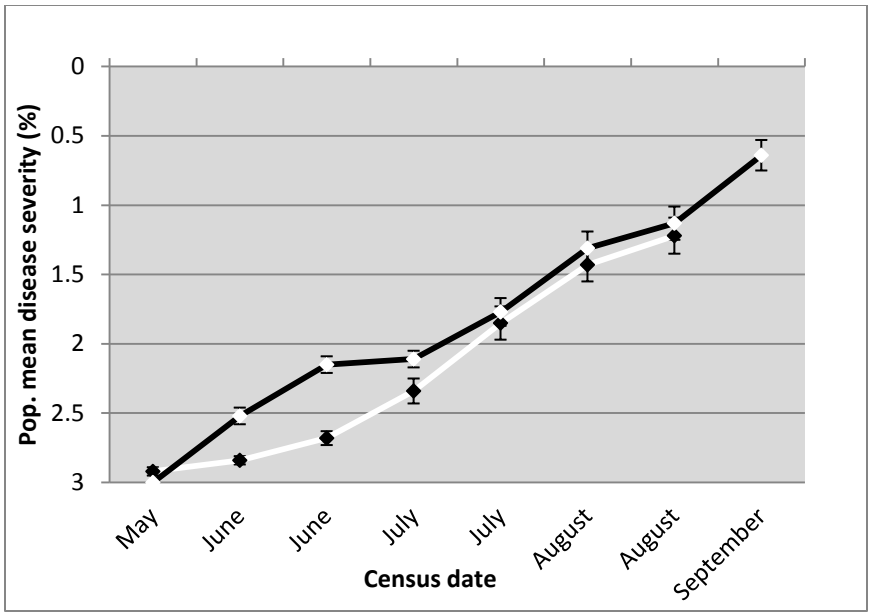


Figure 24. Disease-Progress-Curve of the Population Mean Disease Severity of Reproductively Mature Lily Plants in 2011 and 2012.
Study year line color code: 2011 = black; 2012 = white.

Table 12. Rates of Host Browsing, Survivorship, Mortality, Disease Prevalence, Reproductive Damage from Disease (lesion on capsule or pedicel), and Reproductive Success of Reproductively Mature Lilies in 2011 and 2012

	2012	2011
	Proportion (%)	Proportion (%)
Plants browsed	83/120 (69%)	47/94 (50%)
Disease-specific mortality (browsed excluded)	22/37 (59%)	33/47 (70%)
Prevalence at end of season (browsed excluded)	34/37 (92%)	44/47 (94%)
Mortality from all causes	93/120 (78%)	76/94 (81%)
Prevalence of reproductive damage from disease (browsed excluded)	25/37 (68%)	39/47 (83%)
Plants producing seed capsules	27/120 (25%)	30/94 (32%)
Capsule producing plants with disease on reproductive structures	17/27 (63%)	25/30 (83%)
Number of mature seed capsules/ Total Number of Flowers (browsed excluded)	37/60 (62%)	33/94 (35%)
Number of mature seed capsules/ Total Number of Flowers	37/144 (26%)	33/166 (20%)
Re-emergence of 2011 Plants	29/94 (31%)	
Re-emergence of Moderately to Heavily Diseased Plants	17/71 (24%)	

Risk Factors for Disease

None of the morphological characteristics of lilies measured by this study were identified as risk factors of the lily leaf spot disease and there was no correlation between any of the lily morphological characteristics and disease severity (Table 13). The only variable consistently associated with disease severity in all logistic regression models and therefore identified as a risk factor, was the location (x-coordinate) of a plant along the length of the transect (Table 13). Point estimates of the effect of the x-coordinate on disease severity were positive and were of a similar magnitude for all dates. Positive point estimates indicated that plants nearer the distal end of the transect were less diseased than plants nearer the start of the transect. The multivariate analysis was conducted on all sample events from both seasons. The fourth and fifth sampling events were chosen for illustration purposes because disease was most common at these times.

Morphological characteristics, such as plant height, leaves per whorl, and number of whorls, were positively correlated with each other both within and between years (Appendix A). Positive correlations between morphological characteristics suggest that within a year, plants that are taller have more whorls with more leaves per whorls. Between years, the pattern of correlation suggests consistency in phenotypic characteristics from season to season. Height and number of mature seed capsules produced within a season were also positively correlated. Positive correlations of height and number of seed capsules suggest that taller plants were more likely to successfully complete their reproductive cycle and reproduce. With the exception of number of leaf whorls on a plant, no morphological characteristics were correlated with location, i.e., x-coordinate and/or y-coordinate (Appendix A).The lack of correlations between the x- and y-coordinates of a plant and all morphological characteristics except number of whorls on a plant, suggest that plant location is not associated with plant vigor. Negative correlations

between number of leaf whorls on a plant and the x-coordinate of a plant suggested that plants closer to the distal end of the transect had fewer whorls.

There were significant correlations between the indicators of disease (disease severity scale, number of diseased whorls on a plant, and reproductive damage by disease) (Appendix B). It is important to remember that a decrease in the disease severity value is reflective of an increase in disease severity. The number of diseased whorls on a plant was negatively correlated with the disease severity scale within a year. The consistent correlation with disease severity indicated that the number of diseased whorls on a plant served as a good proxy for disease severity by showing that plants having more diseased whorls had increased disease severity within a year. The number of diseased whorls on a plant in 2012 was positively correlated with reproductive damage due to disease within the same year. Positive correlations between the number of diseased whorls on a plant and reproductive damage by disease suggest that disease symptoms on lily reproductive structures are associated with the inoculum load of a plant. The number of diseased whorls in 2011 was positively correlated with reproductive damage in 2012. Plants with reproductive damage from disease were negatively correlated with disease severity scale value within 2012 (Appendix B). This pattern of correlation suggests that increased severity of disease was associated with infection of reproductive structures.

With the exception of reproductive damage by disease, indicators of disease were consistently correlated with the location of a plant on the x-axis of the transect. The x-coordinate was negatively correlated with the number of diseased whorls and positively correlated with disease severity. This pattern suggests a nonrandom distribution of disease with plants located at the distal end of the transect experiencing reduced disease severity compared to plants near the beginning of the transect.

Table 13. Risk Factors for Disease using Stepwise Ordinal Logistic Regression

July 10	2012 Ordinal Response Model					July 24					
Parameter	df	Estimate	S.E.	Wald	Pr >	Parameter	df	Estimate	S.E.	Wald	Pr >
Intercept 3	1	-1.9714	0.460	18.36	<.0001	Intercept 3	1	-5.194	1.388	14.00	0.0002
Intercept 2.5	1	0.2835	0.381	0.55	0.4569	Intercept 2.5	1	-1.821	1.200	2.30	0.1292
Intercept 2	1	1.0482	0.417	6.31	0.0120	Intercept 2	1	-1.173	1.187	0.97	0.3231
Intercept 1.5	1	1.4746	0.458	10.33	0.0013	Intercept 1.5	1	-0.887	1.184	0.56	0.4536
Intercept 1	1	1.8667	0.513	13.21	0.0003	Intercept 1	1	-0.138	1.187	0.01	0.9069
x	1	1.1530	0.001	9.16	**0.0025**	x	1	1.205	0.001	9.70	**0.0018**
y	1	-0.0911	0.064	2.02	0.1552	HT12	1	0.038	0.018	4.73	0.0295
N=74						N=61					
July 7	2011 Ordinal Response Model					July 24					
Parameter	df	Estimate	S.E.	Wald	Pr >	Parameter	df	Estimate	S.E.	Wald	Pr >
Intercept 3	1	-2.3991	1.392	2.96	0.0849	Intercept 3	1	-5.0261	0.987	25.94	<.0001
Intercept 2.5	1	-0.3095	1.359	0.05	0.8198	Intercept 2.5	1	-2.5541	0.854	8.95	0.0028
Intercept 2	1	1.6850	1.375	1.50	0.2203	Intercept 2	1	-1.1820	0.810	2.13	0.1444
Intercept 1.5	1	2.5601	1.405	3.32	0.0683	Intercept 1.5	1	-0.7097	0.805	0.77	0.3781
Intercept 1	1	3.4448	1.471	5.48	0.0192	Intercept 1	1	-0.1285	0.810	0.02	0.8739
x	1	1.0880	0.001	3.68	**0.0550**	x	1	1.2330	0.001	17.90	**<.0001**
WH11	1	-0.2847	0.190	2.23	0.1345	HT11	1	0.0118	0.009	1.59	0.2061
LeavesW11		0.2366	0.142	2.78	0.0949						
N=91						N=81					

Spatial Analyses of Disease

Disease on mature lilies was not randomly distributed in the population. Instead, 2 major patterns of disease distribution were noted: First, there was a disease gradient; Second, there were spatial clusters of high and low disease severity.

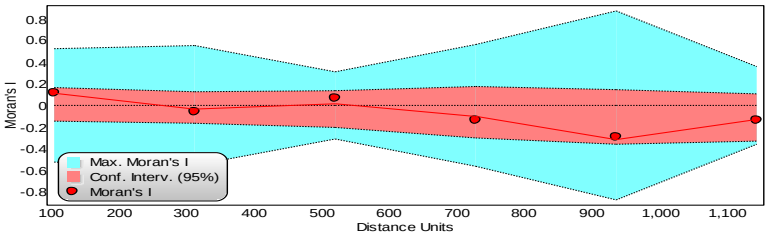
Spatial autocorrelation conducted on disease severity indicated a disease gradient within reproductively mature lilies. The disease gradient was manifested as a trend in which plants in close proximity to each other, i.e. 100 meters or less, showed positive autocorrelation for disease severity while plants at more distant intervals had a negative autocorrelation (Table 14; Table 16). At middle distance classes there was no evidence of autocorrelation with disease severity. However, there were exceptions to this general pattern in both years.

Early in the 2011 season evidence of a disease gradient was largely absent but from the middle until the end of the season there was positive autocorrelation of disease severity for plants within the 100 m distance class and negative autocorrelation for plants within the 300 m distance class (Table 14). The similarity of disease severity of plants within 100 m and dissimilarity of the disease severity of plants within 300 m provided evidence of a gradient of disease that persisted throughout the second half of the season in 2011. In 2012 a gradient of disease severity was present at the beginning of the season and dissipated as the season progressed. The dissipation of the gradient was likely due to an increased prevalence and severity of disease during the second half of the growing season in 2012 (Table 16).

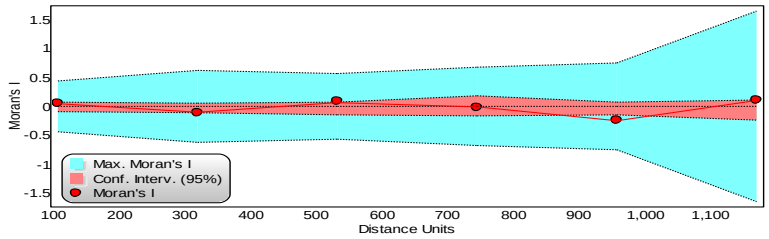
There were no significant autocorrelations for any morphological or reproductive traits except the number of diseased whorls on a plant. With few exceptions, the distance classes that were significantly autocorrelated with the number of diseased whorls on a plant were the same as those significantly autocorrelated with disease severity for the entire season in 2011 and from the beginning of the season until the fifth sampling date in 2012 (Table 15; 17). Spatial structure of diseased whorls was likely lost by midseason in 2012 due to the increased prevalence of the disease in that year.

Table 14. Correlograms Showing Results of Spatial Autocorrelation Analysis of the Disease Severity of Reproductively Mature Lilies in 2011. Census Dates 2 – 8.

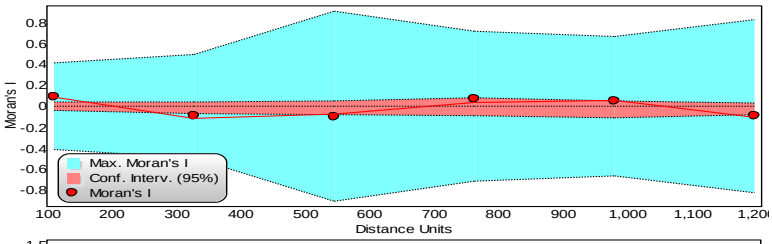
Census Date June 10, 2011				
Class	**Dist.**	**Moran's**	**P**	**I max**
1	103.95	0.118	0.156	0.524
2	311.85	-0.054	0.407	0.557
3	519.75	0.068	0.422	0.308
4	727.65	-0.140	0.161	0.560
5	935.55	-0.289	0.050	0.876
6	1143.45	-0.133	0.111	0.359
Expected Moran's I = -0.033				



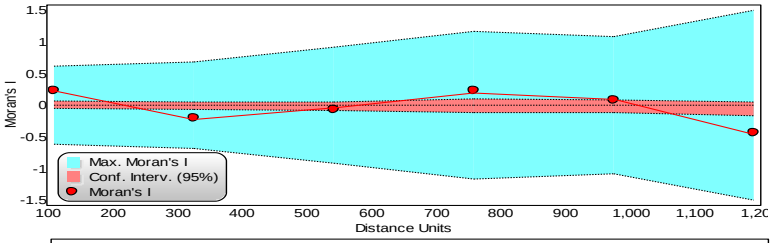
Census Date June 22, 2011				
Class	**Dist.**	**Moran's**	**P**	**I max**
1	106.40	0.061	0.276	0.444
2	319.22	-0.105	0.035	0.631
3	532.04	0.095	0.080	0.569
4	744.86	-0.019	0.819	0.668
5	957.68	-0.232	0.005	0.742
6	1170.50	0.109	0.136	1.646
Expected Moran's I = -0.021				



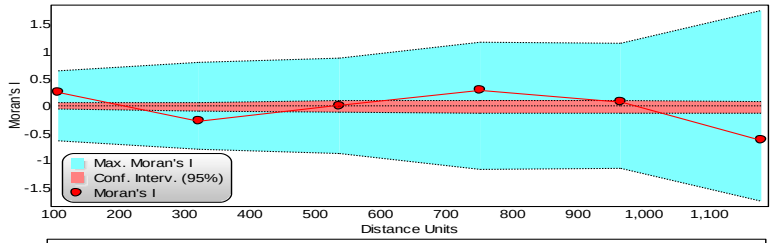
Census Date July 7, 2011				
Class	**Dist.**	**Moran's**	**P**	**I max**
1	108.85	0.092	0.005	0.412
2	326.57	-0.089	0.015	0.497
3	544.29	-0.104	0.010	0.908
4	762.01	0.070	0.095	0.719
5	979.73	0.048	0.166	0.668
6	1197.45	-0.093	0.020	0.829
Expected Moran's I = -0.011				



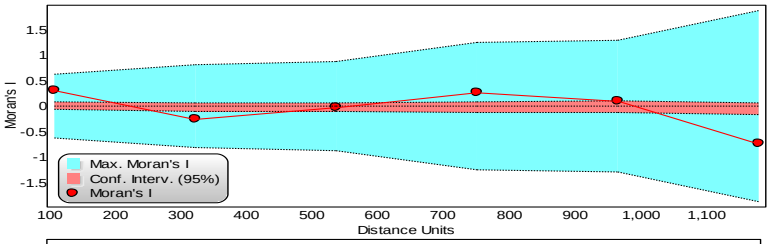
Census Date July 24, 2011				
Class	**Dist.**	**Moran's**	**P**	**I max**
1	108.27	0.237	0.005	0.613
2	324.83	-0.199	0.005	0.681
3	541.38	-0.071	0.080	0.922
4	757.93	0.232	0.005	1.178
5	974.48	0.085	0.085	1.093
6	1191.04	-0.440	0.005	1.504
Expected Moran's I = -0.012				



Census Date August 6, 2011				
Class	**Dist.**	**Moran's**	**P**	**I max**
1	107.30	0.258	0.005	0.640
2	321.91	-0.267	0.005	0.798
3	536.52	0.003	0.925	0.879
4	751.14	0.295	0.005	1.177
5	965.75	0.080	0.106	1.161
6	1180.36	-0.632	0.005	1.759
Expected Moran's I = -0.014				



Census Date August 20, 2011				
Class	**Dist.**	**Moran's**	**P**	**I max**
1	107.30	0.305	0.005	0.631
2	321.91	-0.255	0.005	0.812
3	536.52	-0.015	0.704	0.885
4	751.14	0.271	0.005	1.261
5	965.75	0.114	0.050	1.287
6	1180.36	-0.726	0.005	1.878
Expected Moran's I = -0.014				



Census Date September 6, 2011				
Class	**Dist.**	**Moran's**	**P**	**I max**
1	107.30	0.264	0.005	0.656
2	321.91	-0.152	0.015	0.719
3	536.52	-0.040	0.327	0.749
4	751.14	0.166	0.015	1.198
5	965.75	0.037	0.347	1.351
6	1180.36	-0.625	0.005	2.114
Expected Moran's I = -0.014				

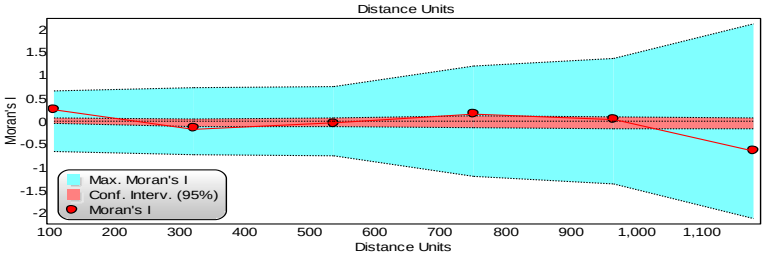


Table 15. Correlograms Showing Results of Spatial Autocorrelation Analysis of the Number of Yellow or Diseased Whorls of Reproductively Mature Lilies in 2011. Data outputs and correlograms of Moran's I are shown for sample dates 4-8.

Census Date July 7, 2011				
Class	**Dist.**	**Moran's**	**P**	**I max**
1	108.85	0.070	0.015	0.366
2	326.57	-0.039	0.216	0.458
3	544.29	-0.140	0.010	0.821
4	762.01	-0.044	0.317	0.726
5	979.73	0.042	0.251	0.548
6	1197.45	-0.004	0.764	0.695
Expected Moran's I = -0.011				
Census Date July 24, 2011				
Class	**Dist.**	**Moran's**	**P**	**I max**
1	108.27	0.231	0.005	0.602
2	324.83	-0.089	0.015	0.561
3	541.38	0.135	0.010	0.81
4	757.93	-0.081	0.111	0.838
5	974.48	-0.054	0.166	1.050
6	1191.04	-0.509	0.005	1.339
Expected Moran's I = -0.012				
Census Date August 6, 2011				
Class	**Dist.**	**Moran's**	**P**	**I max**
1	107.30	0.319	0.005	0.606
2	321.91	-0.298	0.005	0.859
3	536.52	0.010	0.809	0.910
4	751.14	0.321	0.005	1.246
5	965.75	0.045	0.286	1.262
6	1180.36	-0.715	0.005	1.847
Expected Moran's I = -0.014				
Census Date August 20, 2011				
Class	**Dist.**	**Moran's**	**P**	**I max**
1	107.30	0.301	0.005	0.555
2	321.91	-0.148	0.010	0.732
3	536.52	-0.055	0.216	0.867
4	751.14	0.290	0.005	1.265
5	965.75	-0.006	0.894	1.354
6	1180.36	-0.762	0.005	1.964
Expected Moran's I = -0.014				
Census Date September 6, 2011				
Class	**Dist.**	**Moran's**	**P**	**I max**
1	107.30	0.299	0.005	0.656
2	321.91	-0.148	0.010	0.719
3	536.52	-0.054	0.141	0.749
4	751.14	0.294	0.005	1.198
5	965.75	-0.001	0.995	1.351
6	1180.36	-0.773	0.005	2.114
Expected Moran's I = -0.014				

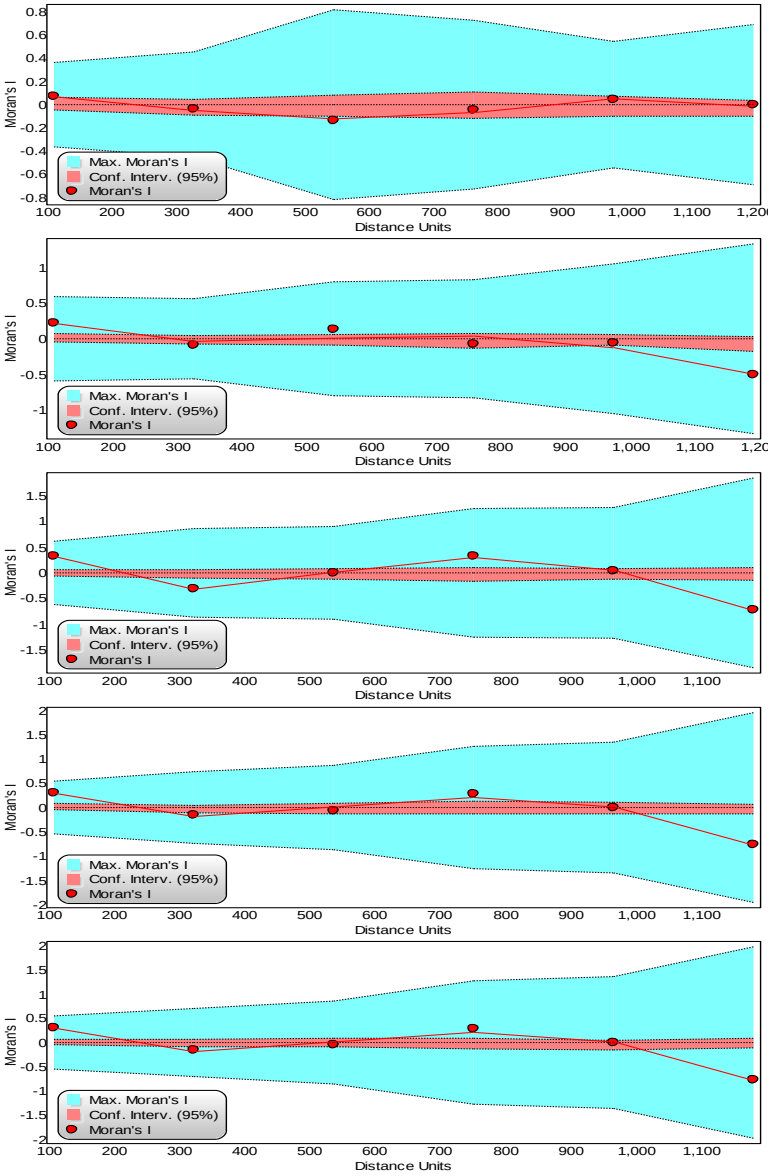


Table 16. Correlograms Showing Results of Spatial Autocorrelation Analysis of the Disease Severity of Reproductively Mature Lilies in 2012. Census Dates 1 – 7.

Census Date May 22, 2012				
Class	**Dist.**	**Moran's**	**P**	**I max**
1	109.19	0.320	0.005	0.867
2	327.58	-0.043	0.402	0.548
3	545.96	-0.070	0.161	0.418
4	764.35	-0.074	0.352	0.650
5	982.73	-0.198	0.01	0.752
6	1201.12	-0.414	0.005	0.876
Expected Moran's I = -0.022				

Census Date June 10, 2012				
Class	**Dist.**	**Moran's**	**P**	**I max**
1	109.19	0.274	0.005	0.691
2	327.58	0.151	0.005	0.490
3	545.96	-0.062	0.181	0.543
4	764.35	-0.069	0.201	0.721
5	982.73	-0.324	0.005	0.834
6	1201.12	-0.392	0.005	1.037
Expected Moran's I = -0.015				

Census Date June 26, 2012				
Class	**Dist.**	**Moran's**	**P**	**I max**
1	110.30	0.077	0.015	0.467
2	330.90	0.066	0.060	0.377
3	551.50	-0.107	0.020	0.725
4	772.10	-0.124	0.025	0.843
5	992.70	-0.044	0.246	0.638
6	1213.30	-0.070	0.030	0.698
Expected Moran's I = -0.011				

Census Date July 10, 2011				
Class	**Dist.**	**Moran's**	**P**	**I max**
1	110.30	0.119	0.005	0.510
2	330.90	0.120	0.010	0.403
3	551.50	-0.078	0.050	0.584
4	772.10	-0.138	0.035	0.832
5	992.70	-0.039	0.302	0.641
6	1213.30	-0.233	0.005	0.823
Expected Moran's I = -0.014				

Census Date July 24, 2012				
Class	**Dist.**	**Moran's**	**P**	**I max**
1	110.30	0.102	0.025	0.625
2	330.90	0.124	0.025	0.530
3	551.50	-0.018	0.623	0.391
4	772.10	-0.295	0.005	0.790
5	992.70	-0.109	0.065	0.760
6	1213.30	-0.238	0.030	0.977
Expected Moran's I = -0.017				

Census Date August 7, 2011				
Class	**Dist.**	**Moran's**	**P**	**I max**
1	110.30	0.033	0.538	0.482
2	330.90	0.148	0.020	0.444
3	551.50	-0.048	0.251	0.491
4	772.10	-0.429	0.005	0.800
5	992.70	-0.031	0.477	0.791
6	1213.30	-0.118	0.055	0.954
Expected Moran's I = -0.018				

Census Date August 20, 2011				
Class	**Dist.**	**Moran's**	**P**	**I max**
1	110.30	-0.007	0.894	0.369
2	330.90	0.066	0.131	0.458
3	551.50	0.008	0.829	0.375
4	772.10	-0.273	0.005	0.800
5	992.70	0.005	0.960	0.799
6	1213.30	-0.103	0.085	0.805
Expected Moran's I = -0.019				

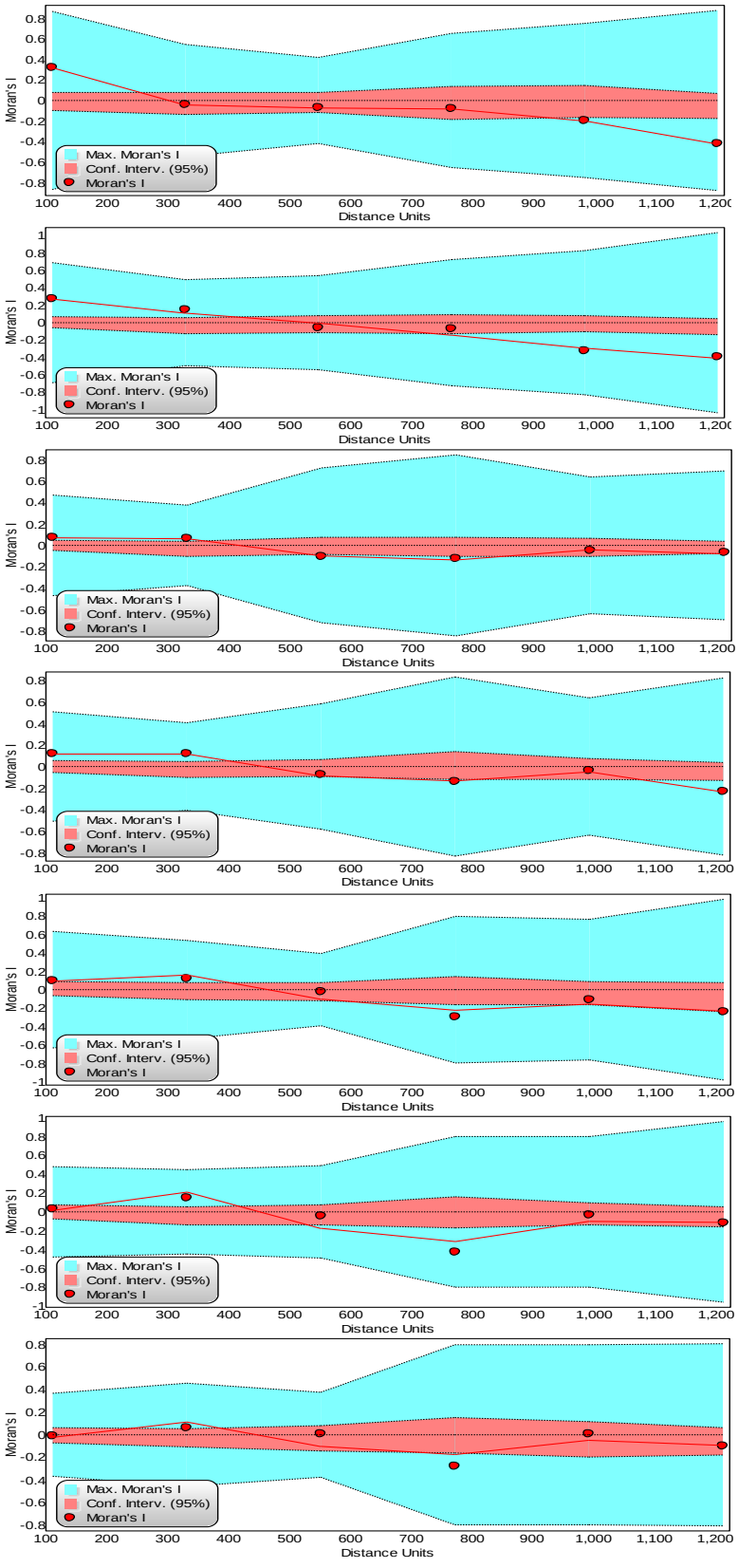


Table 17. Correlograms Showing Results of Spatial Autocorrelation Analysis of the Number of Yellow or Diseased Whorls of Reproductively Mature Lilies in 2012. Census Dates 1 – 7.

Census Date May 22, 2012				
Class	**Dist.**	**Moran's**	**P**	**I max**
1	109.19	0.174	0.005	0.819
2	327.58	-0.032	0.588	0.452
3	545.96	-0.046	0.397	0.352
4	764.35	-0.047	0.583	0.551
5	982.73	-0.119	0.070	0.652
6	1201.12	-0.264	0.010	0.767
Expected Moran's I = -0.022				

Census Date June 10, 2012				
Class	**Dist.**	**Moran's**	**P**	**I max**
1	109.19	0.287	0.005	0.679
2	327.58	0.134	0.010	0.487
3	545.96	-0.057	0.176	0.521
4	764.35	-0.034	0.513	0.711
5	982.73	-0.312	0.005	0.823
6	1201.12	-0.440	0.005	1.038
Expected Moran's I = -0.015				

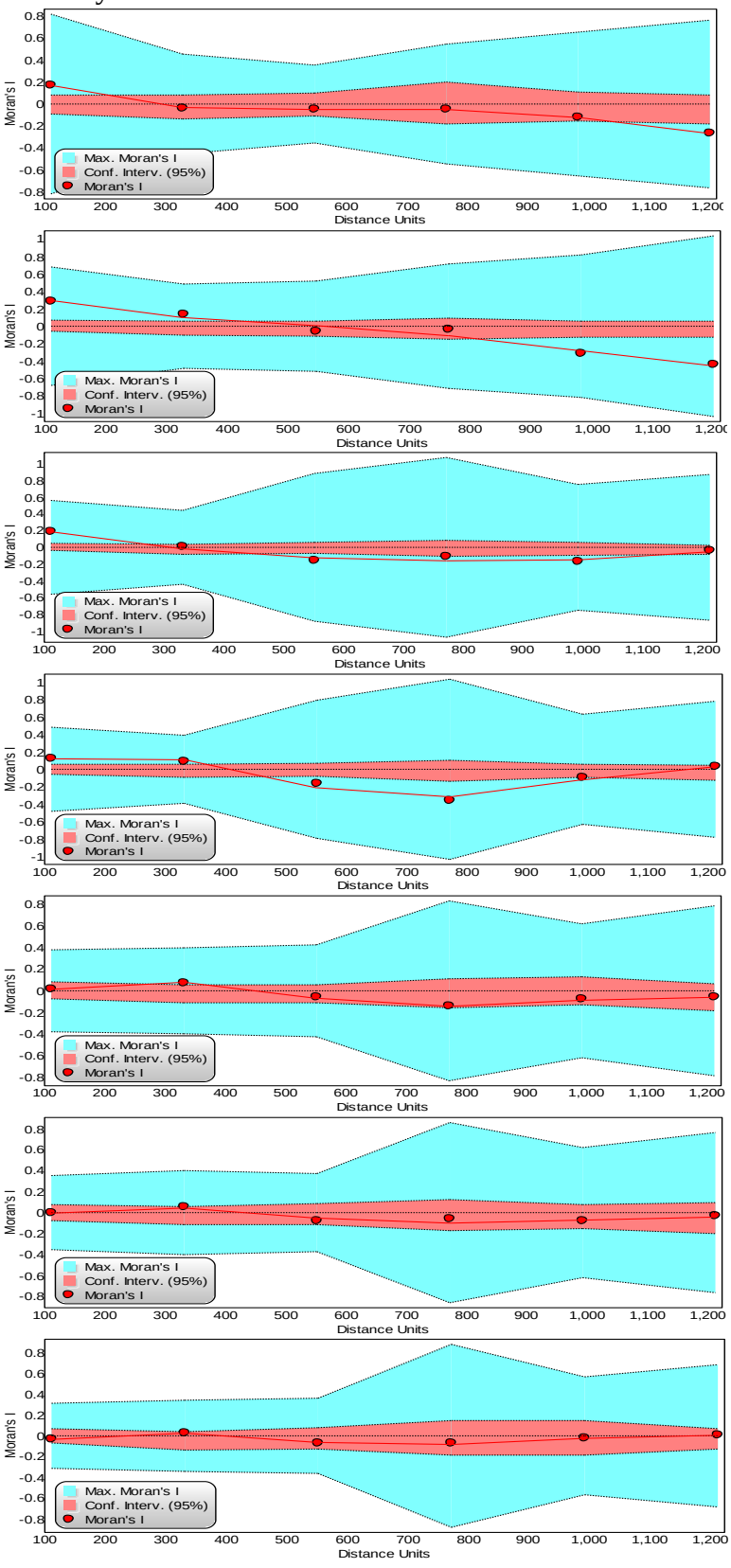
Census Date June 26, 2012				
Class	**Dist.**	**Moran's**	**P**	**I max**
1	110.30	0.187	0.005	0.564
2	330.90	0.014	0.467	0.443
3	551.50	-0.160	0.010	0.886
4	772.10	-0.111	0.030	1.075
5	992.70	-0.162	0.005	0.753
6	1213.30	-0.041	0.141	0.871
Expected Moran's I = -0.011				

Census Date July 10, 2011				
Class	**Dist.**	**Moran's**	**P**	**I max**
1	110.30	0.127	0.005	0.477
2	330.90	0.095	0.020	0.388
3	551.50	-0.165	0.005	0.792
4	772.10	-0.352	0.005	1.031
5	992.70	-0.095	0.025	0.628
6	1213.30	0.031	0.296	0.777
Expected Moran's I = -0.014				

Census Date July 24, 2012				
Class	**Dist.**	**Moran's**	**P**	**I max**
1	110.30	0.019	0.764	0.374
2	330.90	0.076	0.095	0.396
3	551.50	-0.058	0.166	0.427
4	772.10	-0.142	0.060	0.828
5	992.70	-0.078	0.171	0.615
6	1213.30	-0.059	0.206	0.784
Expected Moran's I = -0.017				

Census Date August 7, 2011				
Class	**Dist.**	**Moran's**	**P**	**I max**
1	110.30	-0.002	0.995	0.354
2	330.90	0.060	0.156	0.403
3	551.50	-0.077	0.146	0.378
4	772.10	-0.062	0.402	0.863
5	992.70	-0.079	0.106	0.627
6	1213.30	-0.032	0.362	0.765
Expected Moran's I = -0.018				

Census Date August 20, 2011				
Class	**Dist.**	**Moran's**	**P**	**I max**
1	110.30	-0.029	0.608	0.316
2	330.90	0.029	0.432	0.346
3	551.50	-0.065	0.166	0.358
4	772.10	-0.069	0.392	0.879
5	992.70	-0.020	0.698	0.565
6	1213.30	0.013	0.608	0.688
Expected Moran's I = -0.019				



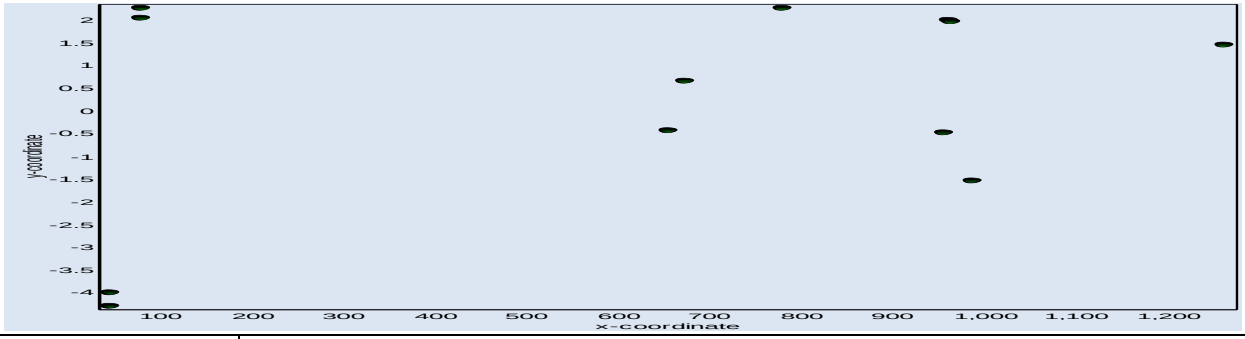
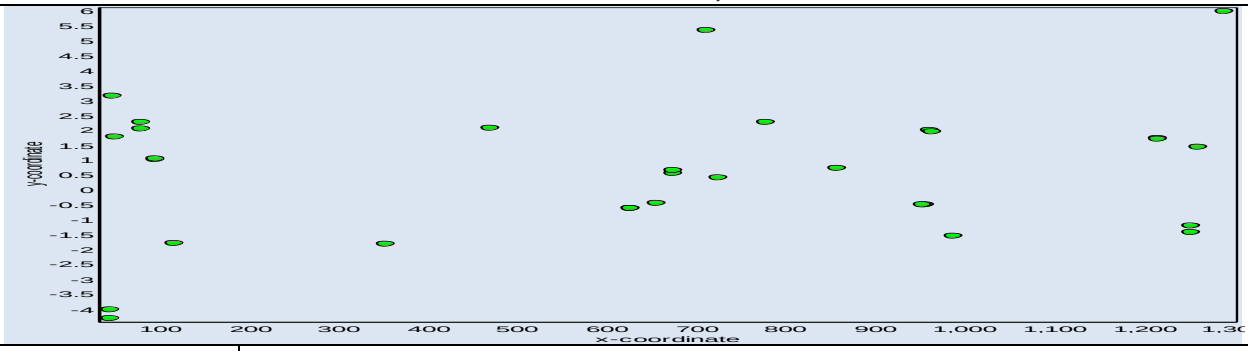
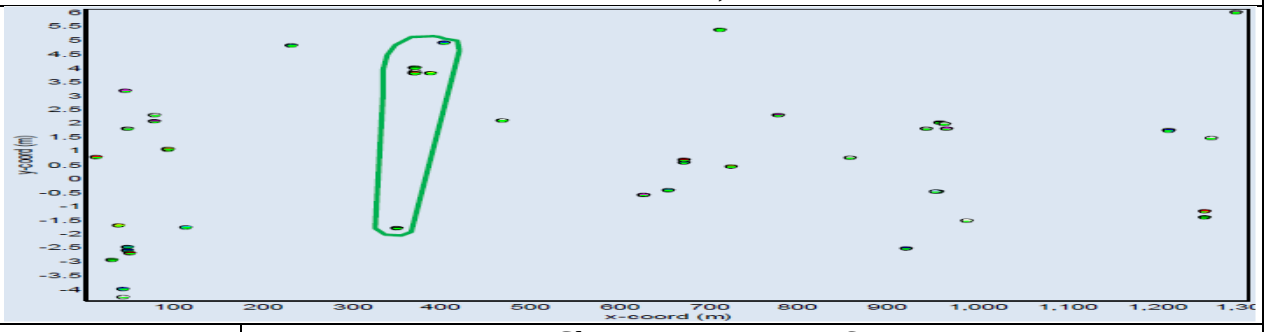
Disease clusters were detected from the middle until the end of the season in 2011 and from the beginning until the middle of the season in 2012 (Table 18; 19). Maximum disease cluster size was reached on July 24 in 2011 and June 26 in 2012. In census dates following the cluster maximum, disease clusters had a tendency to decrease in physical extent and in number of plants (Table 18; 19). The only exception was the cluster of diseased plants whose location was centered at transect point 14.7 m (Table 18). This disease cluster nearly doubled in number of plants from 18 to 29 and more than doubled in size from a radius of 68.8 m to 224.9 m from the seventh to the eighth census date in 2011(Table 18).

In both years, a large health cluster was located at approximately 1000-1300 m (Table 18; 19). There was only 1 other health cluster in 2011 that appeared on the third census date, was centered at transect point 351.8 m, and had disappeared by the following census date. This short lived health cluster was likely the result of the poor health of neighboring plants in disease clusters that appeared during the fourth census date in 2011 (Table 18). Clusters of healthy plants appeared earlier in the season than disease clusters in 2011 and at the same time in 2012. In both years, health clusters experienced similar temporal patterns in which clusters increased in size until a maximum for area occupied and number of plants was reached. After the maximum, these clusters drastically decreased in size and in numbers of plants until the season ended. The only exception was the cluster of healthy plants centered at transect point 1255.5 m, that declined from 29 to 16 plants and then increased to 30 within the second – fourth census dates of 2012, (Table 19). Once a cluster of diseased plants was detected it did not vary in location for sequential sampling events within that season but rather followed a general pattern of growing in number of plants and magnitude of size until a maximum was reached.

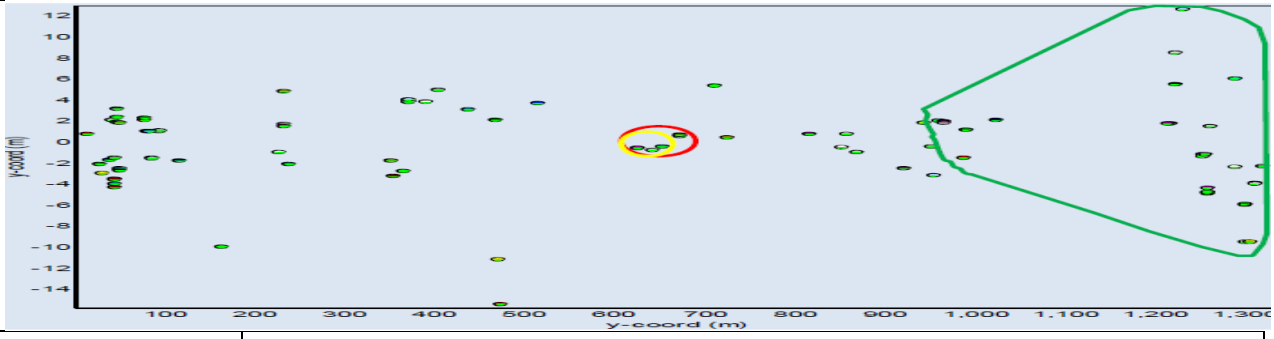
Clusters of high and low numbers of yellow or diseased whorls were present from the middle to the end of the season in 2011 and from the beginning until the middle of the season in 2012. A cluster of high numbers of yellow or diseased whorls was coincident with a disease cluster centered at transect point 14.7 m in 2011 and 78.8 m in 2012 (Table 18; 19). The clusters of high number of yellow or diseased whorls appeared at the same time as the disease cluster in 2011 but appeared one sampling date earlier than the diseased cluster in 2012 (Table 18; 19). The clusters of low numbers of yellow or diseased whorls were coincident with the health cluster centered at 1255.5 m in 2011 and 2012.

None of the morphological or reproductive traits of lily plants occurred in clusters. The lack of clustering suggests 2 points. First, because neither morphological nor reproductive traits were in groupings associated with disease or health clusters, it is unlikely that any of these traits are risk factors for disease. If a trait was a risk factor it would be expected to coincide with clusters of diseased plants. Second, because neither morphological nor reproductive traits were in groupings, differences in physical environment were unlikely to be solely responsible for differences in plant vigor and/or disease severity. If differences in physical environment were to impact the location of disease clusters and/or plant vigor then traits would be expected to occur in groupings.

Table 18. Scatterplots Illustrating the Results of the 2011 Cluster Analysis of Disease Severity and Number of Diseased Whorls

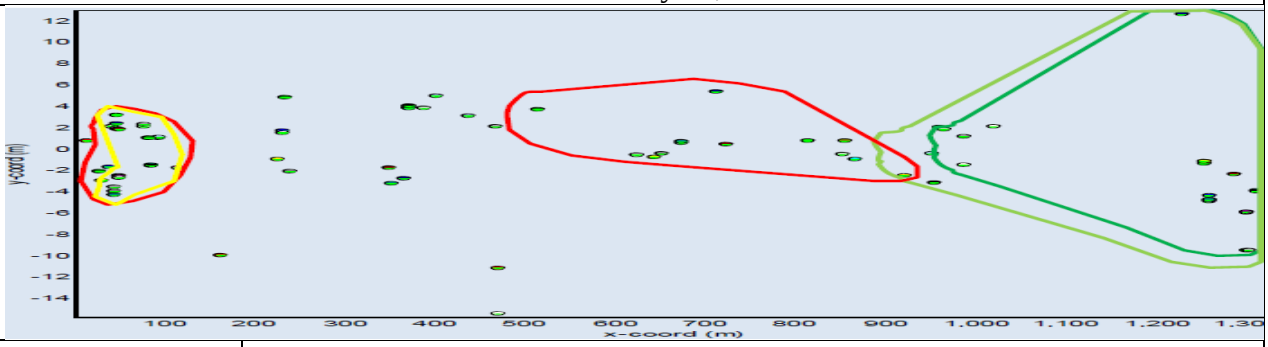
Census Date May 19, 2011				
				
Population Mean Disease Severity (N = 12)	Cluster Mean Disease Severity			
	Diseased	Cluster Center (x-coordinate)	Healthy	Cluster Center (x-coordinate)
3.0	N/A	N/A	N/A	N/A
Census Date June 10, 2011				
				
Population Mean Disease Severity (N = 31)	Cluster Mean Disease Severity			
	Diseased	Cluster Center (x-coordinate)	Healthy	Cluster Center (x-coordinate)
2.52	N/A	N/A	N/A	N/A
Census Date June 22, 2011				
				
Population Mean Disease Severity (N = 49)	Cluster Mean Disease Severity			
	Diseased	Cluster Center (x-coordinate)	Healthy (N = 9)	Cluster Center (x-coordinate)
2.15	N/A	N/A	2.50	351.83

Census Date July 07, 2011



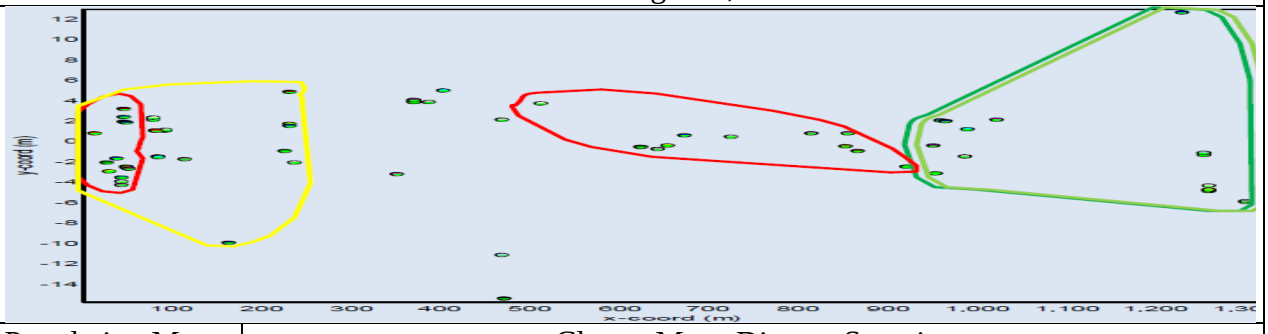
Population Mean Disease Severity (N = 93)	Cluster Mean Disease Severity			
	Diseased (N = 6)	Cluster Center (x-coordinate)	Healthy (N = 29)	Cluster Center (x-coordinate)
2.12	1.00	644.23	2.45	1255.50

Census Date July 24, 2011



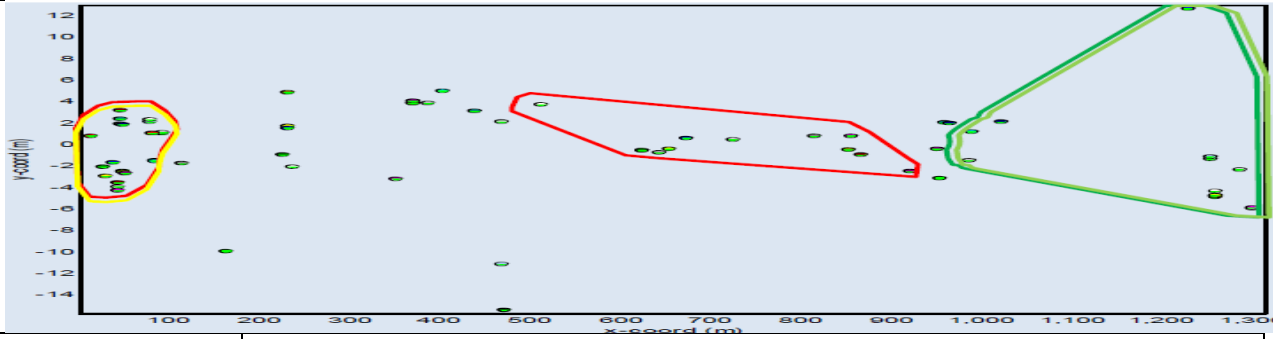
Population Mean Disease Severity (N = 83)	Cluster Mean Disease Severity			
	Diseased	Cluster Center (x-coordinate)	Healthy (N = 21)	Cluster Center (x-coordinate)
1.77	(N = 23); 1.50	85.30	2.55	1255.50
	(N = 14); 0.71	712.60		

Census Date August 6, 2011



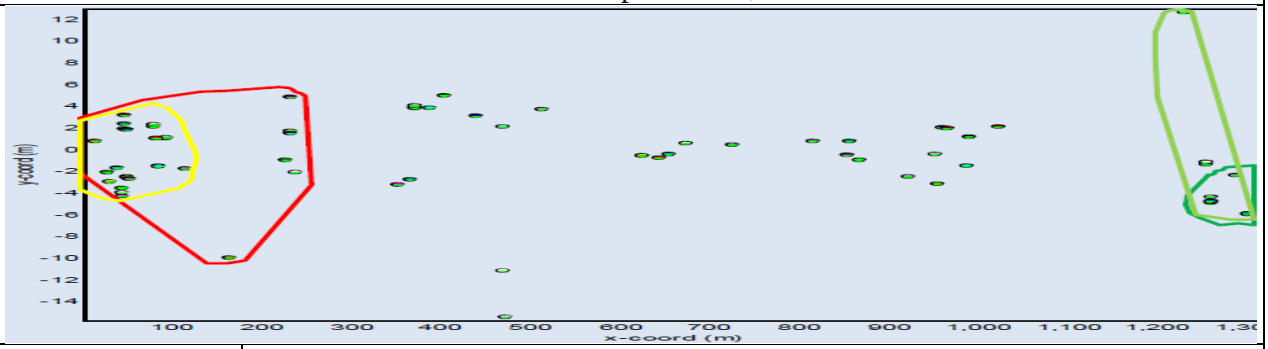
Population Mean Disease Severity (N = 71)	Cluster Mean Disease Severity			
	Diseased	Cluster Center (x-coordinate)	Healthy (N = 16)	Cluster Center (x-coordinate)
1.31	(N = 18); 0.69	14.66	2.34	1255.50
	(N = 12); 0.42	725.46		

Census Date August 20, 2011



Population Mean Disease Severity (N = 73)	Cluster Mean Disease Severity			
	Diseased	Cluster Center (x-coordinate)	Healthy (N = 15)	Cluster Center (x-coordinate)
1.13	(N = 18); 0.33	14.66	2.27	1255.50
	(N = 12); 0.21	725.46		

Census Date September 6, 2011

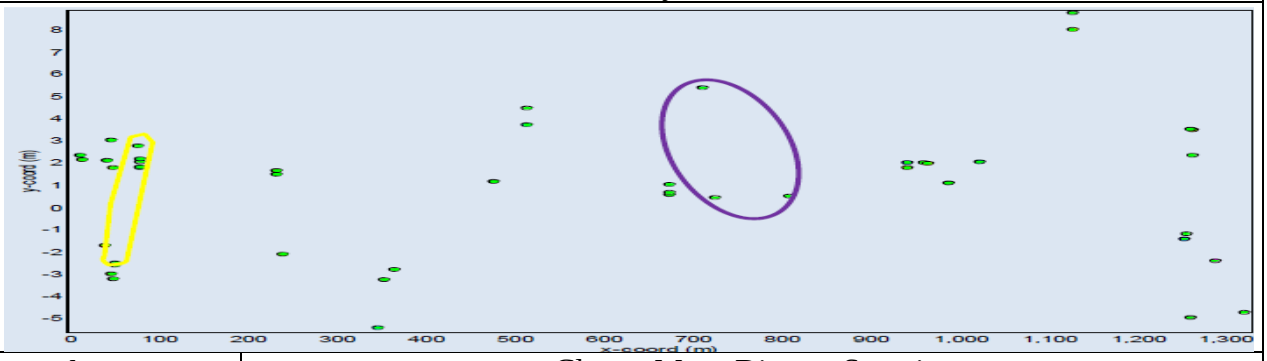


Population Mean Disease Severity (N = 74)	Cluster Mean Disease Severity			
	Diseased (N = 29)	Cluster Center (x-coordinate)	Healthy (N = 9)	Cluster Center (x-coordinate)
0.61	0.10	14.66	2.00	1289.24

*Each dot represents a plant. Dots enclosed by circles represent; “Red” = disease cluster; “Dark Green” = health cluster; “Yellow” = cluster of high numbers of diseased whorls; “Light green” = cluster of low numbers of diseased whorls; “Purple” = number of whorls. N = the number of plants in the respective cluster.

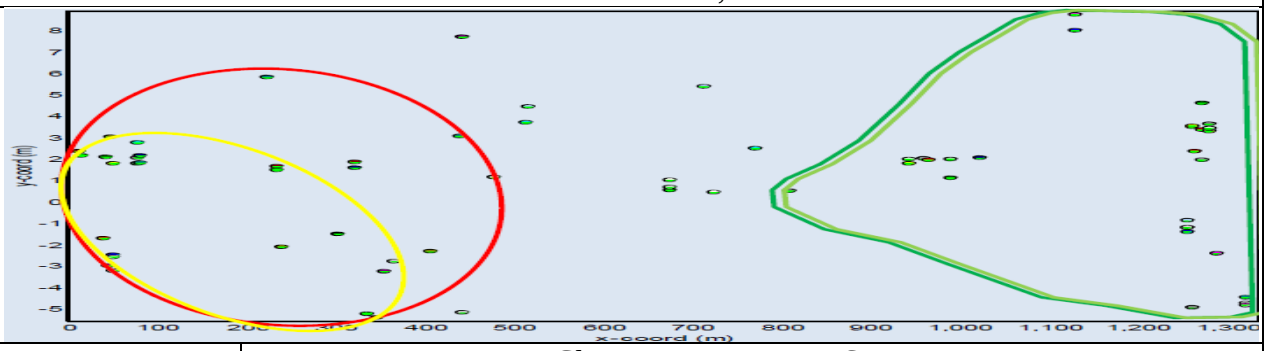
Table 19. Scatterplots Illustrating the Results of the 2012 Cluster Analysis of Disease Severity and Number of Diseased Whorls

Census Date May 22, 2012



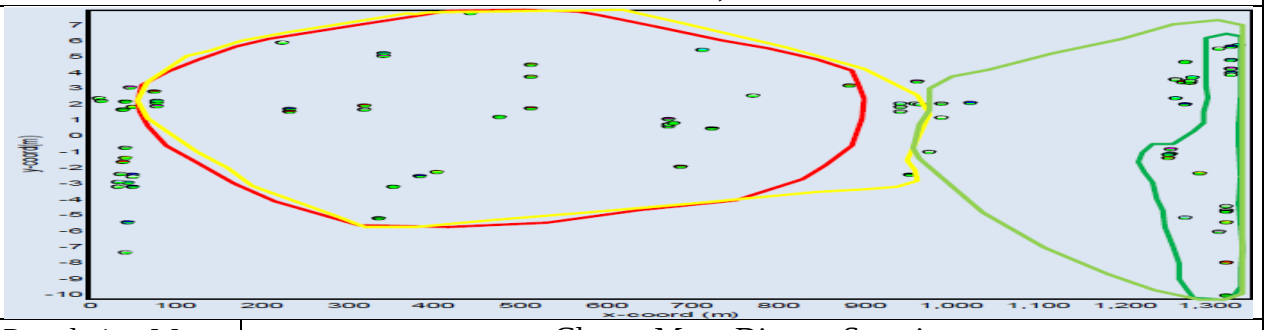
Population Mean Disease Severity (N= 52)	Cluster Mean Disease Severity			
	Diseased	Cluster Center (x-coordinate)	Healthy	Cluster Center (x-coordinate)
2.92	N/A	N/A	N/A	N/A

Census Date June 10, 2012



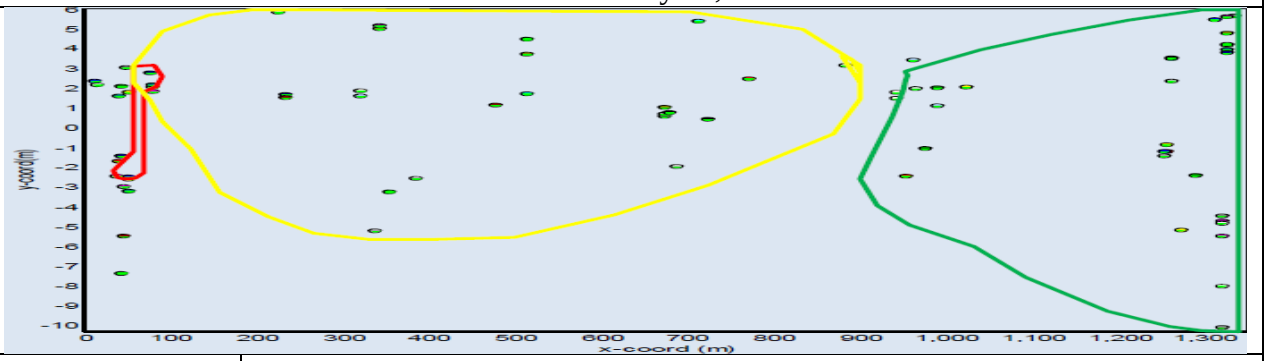
Population Mean Disease Severity (N = 73)	Cluster Mean Disease Severity			
	Diseased (N = 26)	Cluster Center (x-coordinate)	Healthy (N = 29)	Cluster Center (x-coordinate)
2.84	2.63	233.41	2.98	1255.50

Census Date June 26, 2012



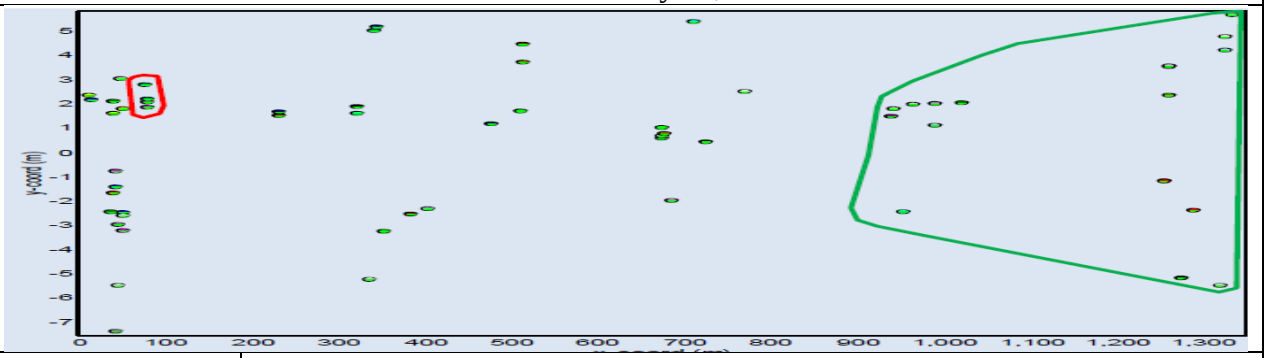
Population Mean Disease Severity (N= 107)	Cluster Mean Disease Severity			
	Diseased (N = 29)	Cluster Center (x-coordinate)	Healthy (N = 16)	Cluster Center (x-coordinate)
2.68	2.32	478.80	3.00	1322.19

Census Date July 10, 2012



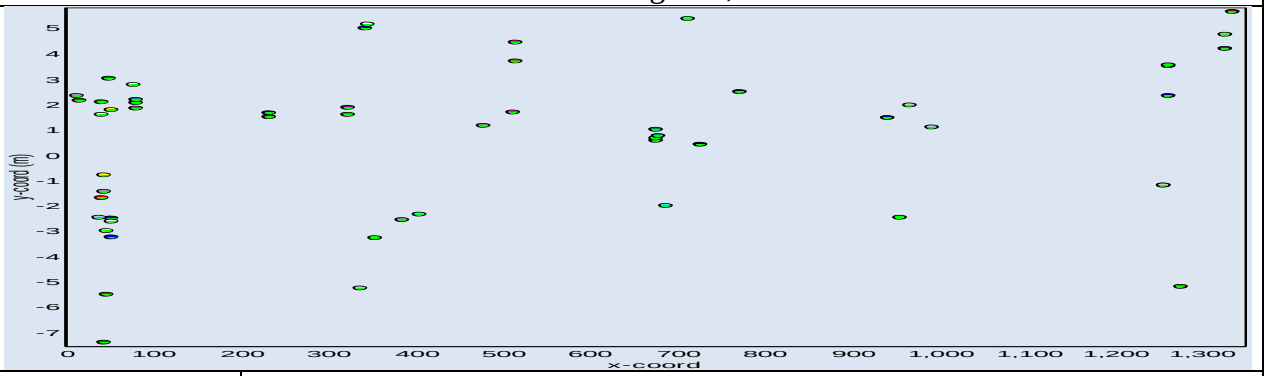
Population Mean Disease Severity (N= 88)	Cluster Mean Disease Severity			
	Diseased (N = 4)	Cluster Center (x-coordinate)	Healthy (N = 30)	Cluster Center (x-coordinate)
2.35	0.00	78.80	2.70	1255.50

Census Date July 24, 2012

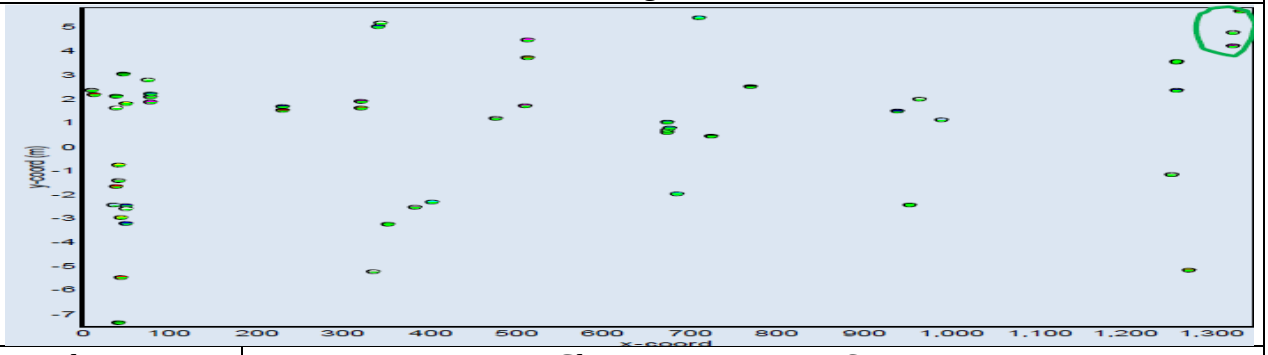


Population Mean Disease Severity (N= 67)	Cluster Mean Disease Severity			
	Diseased (N = 4)	Cluster Center (x-coordinate)	Healthy (N = 18)	Cluster Center (x-coordinate)
1.86	0.00	78.80	2.38	1256.78

Census Date August 7, 2012



Population Mean Disease Severity (N= 63)	Cluster Mean Disease Severity			
	Diseased	Cluster Center (x-coordinate)	Healthy	Cluster Center (x-coordinate)
1.44	N/A	N/A	N/A	N/A

Census Date August 20, 2012				
				
Population Mean Disease Severity (N= 61)	Cluster Mean Disease Severity			
	Diseased	Cluster Center (x-coordinate)	Healthy	Cluster Center (x-coordinate)
1.22	N/A	N/A	1.83	1335.49

*Each dot represents a plant. Dots enclosed by circles represent; "Red" = disease cluster; "Dark Green" = health cluster; "Yellow" = cluster of high numbers of diseased whorls; "Light green" = cluster of low numbers of diseased whorls; "Purple" = number of whorls. N = the number of plants in the respective cluster.

Investigation of the Impact of Pseudocercosporella inconspicua *on Seed Viability of* Lilium grayi

Lily seeds originating from diseased capsules were smaller in size, fewer in number, discolored, deformed, and often had signs of fungal growth on the seed coat (Appendix M).

Morphological characteristics of capsules and seed were significantly different from those of healthy capsules (Table 20; 21; 22). Capsule weight, seed weight, and seed count were negatively correlated with presence of disease on seed capsules (Table 23). The associations between reduced seed weight and lower seed count compared to seeds from healthy capsules suggest that disease is capable of reducing seed vigor and production.

The effect of disease on seed germination was indecisive because of complications of contamination by fungi that burned emerging cotyledons and effectively rendered all seed inviable.

Table 20. Results of the Student t-Test Comparing Number of Seeds (seed count per capsule) within Diseased and Healthy Capsules

	DF	N	Mean	Std Dev	Min-Max	F	T	P
Healthy (Capsule)		14	200.9	24.2	142- 226			
Diseased (Capsule)		13	162.3	35.1	104- 237			
Pooled	25		38.6	29.9			3.35	0.0025
Satterthwaite	21		38.6				3.31	0.0033
Folded F	12					2.11		0.1975

Table 21. Results of the Student t-Test Comparing Capsule Weight (g) of Diseased and Healthy Capsules

	DF	N	Mean	Std Dev	Min-Max	F	T	P
Healthy (Capsule)		14	0.25	0.07	0.15- 0.36			
Diseased (Capsule)		13	0.18	0.07	0.09- 0.32			
Pooled	25		0.07	0.07			2.75	0.0109
Satterthwaite	25		0.07				2.75	0.0110
Folded F	12					1.05		0.9260

Table 22. Results of the Student t-Test Comparing Seed Weight (g) of Diseased and Healthy Capsules

	DF	N	Mean	Std Dev	Min-Max	F	T	P
Healthy (Capsule)		14	0.77	0.15	0.49- 0.07			
Diseased (Capsule)		13	0.31	0.26	0.07-0.85			
Pooled	25		0.46	0.21			5.71	<.0001
Satterthwaite	19		0.46				5.61	<.0001
Folded F	12					2.86		0.0715

Table 23. Results of the Correlation Analysis of the Capsule Weight, Seed Weight, and Seed Count of Diseased and Healthy Capsules. Upper value is the Pearson correlation coefficient, lower value is the P value associated with each correlation coefficient.

Trait (N=27)	Seed Weight	Seed Count	Diseased
Capsule Weight	0.7739 <.0001	0.7512 <.0001	-0.4819 0.0109
Seed Weight	1.0000	0.8253 <.0001	-0.7526 <.0001
Seed Count		1.0000	-0.5571 0.0025

CHAPTER 4

DISCUSSION

Determination of the Causal Organism of the Early Season Collapse of Lilium grayi

Through the combination of a large body of evidence supporting a causal relationship, the consistent replication of disease symptoms in healthy hosts, and the diagnosis of pathogen reproductive structures on host tissue postinoculation, the "spirit of Koch's Postulates" has been sufficiently fulfilled. However, due to the inability to acquire a pure culture of *P. inconspicua* from diseased lily tissue the fulfillment of Koch's Postulates in its strict sense was ultimately prevented.

Attempts to fulfill Koch's Postulates led to several complications. These included: difficulty in cultivation of the host, *L. grayi,* difficulty obtaining pure culture of *P. inconspicua* from host tissue, and a lack of published reports on species-specific DNA primers and growth conditions promoting sporulation of *P. inconspicua*. The slow growth rate and persistence of secondary pathogens from within diseased host tissue resulted in numerous failed attempts at isolating a pure culture of *P. inconspicua* despite use of several different types of media at differing concentrations. Secondary pathogens invariably outgrew *P. inconspicua*. Similarly, attempts to obtain a pure culture by isolating *P. inconspicua* through the use of serial dilutions of conidia present within foliar disease lesions were unsuccessful. Again, these trials failed due to competitive exclusion of *P. inconspicua* by more aggressive secondary pathogens. Examples of these secondary pathogens include *Alternaria* sp., *Fusarium* sp., and *Botrytis* sp.

At the onset of the current investigation, the candidate pathogen based on Powell (2011) was considered the most likely causal organism for several reasons. First, host tissue samples used for diagnosis were living and exceptional care was taken to ensure their integrity prior to

analysis. Second, the candidate pathogen, *P. inconspicua*, was present in all diseased samples and absent in all non-diseased samples. Third, the symptoms caused by *P. inconspicua* on its known hosts matched those of the early season collapse. Finally, *P. inconspicua* is host-specific to *Lilium* spp.

The current investigation has supported the hypothesis that *P. inconspicua* is the causal organism of disease. Initial diagnosis of conidia found in high concentrations within disease lesions on lily host tissue were identified as *P. inconspicua* G. Winter (U. Braun), a phytopathogen host-specific to *Lilium* species, on the basis of diagnostic morphological characteristics of the asexual conidia. Further evidence indicated that disease symptoms on *L. grayi* were strongly associated with high concentrations of the diagnostic conidia (Table 2; 3). Additionally, an absence or low concentration of *P. inconspicua* conidia was observed on non-diseased *L. grayi* and other non-host species. This indicates that conidia on diseased *L. grayi* were not a result of high background levels within the environment but were instead most likely due to the sporulation of the pathogen on its host. More evidence of a causal association was provided by the replication of disease symptoms in field inoculation trials. Field inoculation trials evidenced the high level of consistency and specificity of the causal relationship between *P. inconspicua* and the early season collapse by inducing disease and replicating disease symptoms in all healthy hosts inoculated in the field. Additionally, as the inoculation of healthy hosts was conducted late in the season, i.e. late July, exhibition of disease symptoms as the result of latent infections could largely be dismissed.

Earlier reports that attributed the causation of early season collapse to *Colletotrichum* sp. with associated *Alternaria* sp. and *Botrytis* sp. were not supported (Bates 1997). Those conclusions had been reached in the absence of a rigorous disease association study based on

diagnoses from dead lily tissue and included only a single *L. grayi* sample from the Roan Mountain population. Furthermore, species in the genera *Alternaria* and *Botrytis* are most often opportunistic or secondary pathogens that are predominantly associated with older senescing tissues and/or stressed plants (Agrios 2005). These secondary pathogens suggest that the samples were likely degraded and not appropriate for diagnosis of the primary fungal pathogen. Additionally, while foliar disease phytopathogens occur within each of the 3 fungal genera, the disease symptoms of these pathogens on other *Lilium* hosts only superficially matched those of the early season collapse of *L. grayi*. The consideration of known lily pathogens within *Botrytis* sp. and *Colletotrichum* sp. as alternate hypotheses for the cause of the early season collapse and the reasoning for their exclusion is discussed in the following section. Members of the genus *Alternaria* are not discussed because current literature suggests that they are not considered primary pathogens of diseases of *Lilium* sp. (Kameneskey and Okubo 2003, p. 103).

There are currently 2 species of *Botrytis* sp. that are known to cause significant disease in *Lilium* sp. in commercial cultivation (Kameneskey and Okubo 2003, p. 103). The first, *B. cinerea*, is most often associated with floral structures where it leads to flower blight or gray mold in lily species such as *L. longiflorum* Thunb. The second, *Botrytis elliptica*, is associated with foliar structures and causes Botrytis Blight on numerous species of *Lilium* (Hou and Chen 2003; Feng et al. 2007). The symptoms associated with both *Botrytis* species start as oval to elliptical-shaped, reddish-brown to tan leaf spots with purple margins. As lesions coalesce a general blighting occurs that can cause early senescence of the host (Horst 2008, p. 159). One of the most characteristic disease symptoms of *Botrytis* on species of *Lilium* is concentric rings within lesions that give the appearance of a bulls-eye. While the symptoms of *B. cinerea* do not exclude it as a possible pathogen, experimental data have shown a slower rate of development of

symptoms on lily leaves, as compared to *B. elliptica* (Hou and Chen 2003). Due to the slow rate of development of disease symptoms on foliar structures, *B. cinerea* is not a likely candidate primary pathogen of the early season collapse of lilies on Roan Mountain. Conversely, *B. elliptica* could be considered a candidate primary pathogen. Exhibition of tan necrotic lesions on foliar structures that result in whole leaf death closely matches the symptoms associated with the early season collapse of lilies (Bates 1997). Additionally, Botrytis Blight is reported to occur more frequently and with greater severity in cool, wet environments, similar to the mountaintop environment on Roan Mountain (Feng et al. 2007). Thus, *B. elliptica* could be considered a likely candidate pathogen causing the early season collapse of *L. grayi*. However, the symptoms of the early season collapse do not include lesions forming bulls-eye patterns nor the diagnostic conidia of *B. elliptica*. In the absence of the distinct symptoms of disease with a pathogen of *Botrytis*, the argument that *Botrytis* spp. are associated with the early season collapse must be considered weak.

Colletotrichum sp. was also a reasonable hypothetical candidate for the causal organism of disease, as at least 2 species of the genus are pathogens associated with 2 prevalent diseases of *Lilium* species. The first species *C. lilii,* has been reported as the causal organism of Black Scale of bulbs of several members of *Lilium* (Plakidas 1944). Because the lily disease of *L. grayi* on Roan Mountain was primarily a leaf spot disease, *C. lilii* as a scale rot disease is unlikely to be responsible for the early season collapse. The second species, *C. liliacearum,* has been reported as the causal organism of an anthracnose on members of *Lilium* (Feng et al. 2007). Although *C. liliacearum* has been reported as a major disease of *Lilium* species in cultivation, the symptoms of the early season collapse of *L. grayi* did not match those of an anthracnose. Additionally, this study found no evidence that symptoms of the early season collapse were associated with *C.*

liliacearum. The differences between observed disease symptoms and those of a *Colletrotrichum* pathogen and the absence of *in situ* reproductive structures for the later on host lilies suggest that *Colletotrichum* sp. were also unlikely to be the cause of the early season collapse.

Viruses were also investigated as the possible causal organisms of disease. Although common viral symptoms such as necrotic lesions, chlorosis, and reduced yield were associated with the early season collapse, no evidence supported a virus as the cause of the early season collapse. Instead, there was an absence of common lily viral diseases within the lily population. Additionally, there were no other characteristic symptoms of viral infection such as mosaicism, stunting, dwarfing, galls, or tumors found within the sample lily population.

One of the shortcomings of this study was a failure to investigate the potential role of bacterial pathogens in the early season collapse. Because Koch's postulates were not fulfilled in their strict sense, the possibility that a bacterial pathogen may form a disease complex with *P. inconspicua* cannot be conclusively excluded. However, there was evidence to suggest that no bacterial phytopathogen was present. First, diagnostics from prior studies suggested a fungal pathogen as the cause of disease. These diagnoses were based on host tissue analyzed by the North Carolina Plant Disease and Insect Clinic. These analyses failed to identify bacterial pathogens in any of the samples from either the Bates (1998) or the Powell (2011) studies. Second, the symptoms associated with the lily disease did not conform to those of a bacterial disease (Bates 1998; Powell 2011). While these factors are not sufficient to exclude a bacterial phytopathogen, they strongly suggest that it is unlikely.

The *Lilium* sp. host-specific phytopathogen *Pseudocercosporella inconspicua* was found to be the most likely cause of the early season collapse of *Lilium grayi.* Evidence for the causal relationship included: morphological diagnosis of the candidate pathogen to species on diseased

host tissue; the establishment of a temporal relationship of disease through the consistent replication of specific disease symptoms within healthy hosts (Figure 12; 13; 14); a very strong association between symptoms of the disease and high concentrations of diagnostic conidia (Table 3); biological plausibility based on known host specificity and the occurrence of *P. inconspicua* on closely related species of *Lilium* (including the sister taxa to *L. grayi*), consensus of symptomatology as reported for other species of *Lilium*, and a similarity of the climate of the study site to the climates associated with the geographic range of the pathogen; the consideration and exclusion of alternate possible causes of disease.

Epidemiology of the Lily Leaf Spot Disease

A risk assessment analyses indicated that neither incidence nor severity of the disease was affected by any one or combination of risk factors associated with the morphology of the host plant. Regardless of the analysis (i.e. correlation, backward/forward or ordinal/binary logistic regression), the only variable to have a consistent and significant association with disease incidence or severity was the position of a plant on the x-axis of the transect (Appendix A; Table 13). Plants at the western end (Jane Bald) were more likely to be severely diseased than plants at the eastern end (Grassy Ridge Bald). This relationship was likely due to the clustering pattern of disease in which a health cluster was located at the eastern end and disease clusters were located toward the west. This pattern was observed in both years. While the spatial pattern is characteristic of a disease epidemic, further spatial analyses of disease provided additional support for the epidemic determination.

In 2011 and 2012 marked increases in proportion disease incidence from late May until early July resulted in sigmoid disease-progress curves (Figure 23) that conform to the expectation for an epidemic caused by a polycyclic disease (Burdon 1993; Agrios 2005).

Regardless of time within the season or type of host organ from which conidial samples were taken, characteristic necrotic lesions contained *P. inconspicua* conidia. Observations of conidia throughout the growing season strongly suggest that *P. inconspicua* is capable of a polycyclic mode of reproduction. This conclusion is further supported by the polycyclic nature of closely related pathogens (i.e., *Pseudocercosporella herpotrichoides*, *Mycosphaerella fijinensis*, *Pseudocercosporella capsellae*).

Spatial analyses identified 2 patterns of the lily leaf spot disease also associated with disease epidemics. First, there were spatial foci or clusters of plants where disease severity was significantly higher or lower than that of the population mean (Table 18; 19). At the beginning of the season in 2012, a cluster of plants with a higher than expected number of diseased whorls was observed at the western end of the transect. This location was coincident with the same location of the large disease cluster from both years (Table 19). Coincidence of early disease symptoms with a subsequent disease cluster suggests that this location is a significant source of primary inoculum and it probably serves as a major area from which the annual epidemic initially spreads. As the growing seasons progressed, a health cluster remained relatively constant in size while the disease clusters expanded in size and increased in disease severity until near the end of the growing season. Clusters disappeared or were smaller late in the season because of a high level of disease had spread throughout the population (Table 12; Figure 23). Further, the areal extent of disease clusters increased between seasons. In 2011 there was a large disease cluster and a secondary disease cluster. As the season progressed, these disease clusters grew in area but remained separated in space. In 2012 there was only one disease cluster that grew to extend beyond the area covered by both disease clusters of the previous year i.e., the prior disease clusters had coalesced.

Zadoks and Van Den Bosch (1994) recognized the pattern of disease clusters expanding in area within a growing season as a characteristic of a "1st order epidemic." Additionally, the pattern of cluster expansion between seasons suggests the lily leaf spot disease developed faster and spread further in 2012 than in 2011. This conclusion is further supported by plants within the 2012 disease cluster having reached a mean disease severity value of <1.0 by July 10, whereas disease clusters in 2011 did not reach a mean disease severity value of <1.0 until 2 – 4 weeks later (Table 18; 19). The increased rates of disease spread and development from 2011 to 2012 suggest 4 interpretations. First, the lily leaf spot disease may be increasing in intensity as a result of a change in host-pathogen susceptibility. If the increase of disease in 2012 was the result of a change in host-pathogen susceptibility then it would be expected to result in the introgression of disease into regions of health clusters. Instead, the main health cluster remained stable in location and size between years. Second, the lily leaf spot disease may be increasing in intensity as a result of changes in annual host population sizes and densities. A density-dependent relationship of disease has been noted as a characteristic of many plant diseases (Burdon and Chivlers 1982). As host density increases, disease intensity increases. Previous investigations of the demography of *L. grayi* have suggested that population sizes may naturally fluctuate as a result of sporadic emergence patterns (Ulrey 2009, pers comm.) If the increase of disease intensity were the result of an increase in host population size, then an increased density of plants within disease clusters would be expected. However, because the current investigation of disease did not include all developmental stages in the spatial analyes, accurate estimates of the density were not possible. Third, environmental conditions in 2012 were more favorable for the development of disease as compared to 2011. Environmental conditions are dynamic and are rarely constant from year to year. Because of the relationship between the environment and disease, annual fluctuations in

weather can drastically change the course of disease (Burdon et al. 1989). For example, in the absence of favorable conditions, a disease may remain in the endemic phase of the "demographic cycle of pathogens" for one or several sequential seasons, while favorable conditions can result in a rapid shift from endemism to epidemic within a single season (Burdon 1993). If the increase of disease intensity were the result of more favorable conditions in 2012, the spatial patterns similar to those observed would have been expected (i.e., earlier appearance of disease clusters and faster expansion).

As *P. inconspicua* is a polycyclic disease, it requires many successive rounds of inoculum production to reach epidemic levels. Successive rounds of production requires sufficient time for several cycles of favorable and unfavorable conditions (i.e., wet/dry or warm/cool). As time determines the amount of secondary inoculum that can be produced, favorable conditions earlier in a growing season can exponentially increase the amount of inoculum produced. Early favorable conditions can result in a greater intensity of disease as compared to later (Maanen and Xu 2003).

If the increase in disease intensity were the result of an increase of favorable conditions earlier in the 2012 compared to 2011, spatial clusters in 2012 would be expected to appear earlier in the season, expand faster throughout the season, and decrease in size earlier than in 2011. The spatio-temporal patterns of disease clustering in 2012 conformed to these expectations. Four, the lily leaf spot disease may be increasing in intensity as a result of the accumulation of inoculum from sequential annual epidemics. A previous report has suggested that *P. inconspicua* is capable of overwintering on dead lily host tissue (Makota 1925). If correct, under favorable conditions *P. inconspicua* could accumulate in the environment at higher levels with each subsequent year. Through the accumulation of inoculum between years, higher numbers of plants are expected to

be infected early in the season by the primary inoculum. This would result in increasing disease intensity each subsequent growing season until the disease cycle was broken and/or until the host population experiences a crash (Burdon 1993). If the increase in disease intensity were the result of the accumulation of inoculum between years, then increased disease incidence earlier in the season and more rapid expansion and reduction of disease clusters would be expected. In 2012 the number of cases of disease early in the season were 4 times higher and the proportion of disease incidence was 2 times greater than in 2011 (Figure 22; 23). Additionally, disease clusters appeared earlier in the season, increased more rapidly in size, and decreased more rapidly compared to 2011. These factors strongly suggest that the increase in disease severity in 2012 compared to 2011 was the result of a combination of favorable environment and the accumulation of inoculum.

In 2011 and 2012 a disease cluster was located near the western end of the transect and a health cluster was located near the eastern end of the transect. The consistency in location of clusters from year to year suggests 2 alternative explanations. First, the location of clusters is environmentally determined and reflects favorable or unfavorable environmental conditions. Second, disease susceptibility may be genetically determined, in which case health clusters represent groupings of resistant plants (Burdon et al. 1989). The evidence from field inoculation trials supports the environmental hypothesis because experimental host plants located at the eastern end of the study transect (i.e. near a health cluster) contracted disease in a similar manner to experimental host plants near the middle of the transect. If plants within the eastern end of the transect differed in intrinsic resistance they would have been expected to either not become infected or to have reduced severity of disease. Instead, the 2 experimentally inoculated plants located in the region of excellent health experienced the early season collapse. Although this

conclusion is based on a small sample size (n = 4), disease induction following inoculation was clear.

A disease gradient represents the second spatio-temporal pattern of disease epidemics. A disease gradient was evident among mature lily plants in 2011 and 2012 as shown by spatial autocorrelation. Pairings of plants in close proximity (100 m) were similar in disease severity and plants at the further distance class (300 m) plants were significantly dissimilar (Figure 14). As the 2011 season progressed, there was a reduction in the similarity and dissimilarity in these distance classes. At the beginning of the 2012 season pairings of plants within 100 m of each other were significantly similar in disease severity and plants within 980 m of each other were significantly dissimilar (Figure 16). By the second sample date in 2012 the minimum distance for plants to be similar in their disease severity status had shifted to 300 m while the minimum distance for dissimilarity remained the same (980 m). By the third sample date in 2012 there was a reduction in significance of similarity and dissimilarity (Figure 16). There was also a shift in the minimum distance necessary for plants to be dissimilar in disease severity. By the end of the season in 2012 significance of similarity within the 100 m distance class had disappeared and spatial autocorrelation become reflective of the clustering structure of disease within the population.

The similarity of plants at the closest distance class and dissimilarity of plants at the next further distance class early in the season in 2011 and 2012 suggests a gradient in disease severity. The gradient of disease may be caused by an infectious process with local dispersal (Fitt et al. 1987; Burdon et al. 1989). Additionally, the reduction in significance of similarity of proximal clusters as seasons progressed conforms to the patterns associated with an epidemic. As disease becomes more prevalent and disease severity becomes more homogenous, the gradient

can be expected to dissipate (Xu and Ridout 1998). The disease gradients suggested that the disease was more intense in 2012 than in 2011 because the minimum distance for similarity was larger in 2012 (300 m) than in 2011 (100 m). This shift suggests the disease became more widespread in 2012. The reduction in significance that occurred earlier in 2012 than in 2011, indicated that disease had developed faster and spread further in 2012 compared to the 2011.

Impact of Disease on Host Survivorship

Disease symptoms were noted on all above-ground plant structures of moderately to heavily diseased lily plants (Figure 1; 3; 4; 5; 6) but were most prevalent and severe on foliar structures and maturing seed capsules. Additionally, these 2 tissues were the only locations with conidia of *P. inconspicua* intact on conidiophores imbedded in the epidermis (Figure 1; 4). Intact conidia on conidiophores indicate that leaves and maturing capsules are sites of secondary inoculum production, while stems and pedicels are not.

Host survivorship was considered within-season and between-seasons. Because *L. grayi* is a perennial plant that annually dies back to an underground bulb, reemergence of the lily plant was the only nondestructive approach to quantifying between-season survivorship. With only 24% of the plants that were moderately to heavily diseased in 2011 re-emerging in 2012, the disease appears to be severely reducing host survivorship. However, previous reports of host phenology suggest that an accurate assessment of between-season survivorship may be complicated because mature plants may not reemerge on an annual basis (C. Ulrey 2009, pers. comm.). An example of a perennial plant species with a variable emergence pattern, is *Prasophyllum correctum* D.L. Jones (Coates et al. 2006.) In the current study only 31% of plants in 2011 reemerged in 2012 (Table 12). Additionally, if the lily leaf spot disease were responsible for the reduction in host reemergence then a lower number of previously diseased plants would

be expected to reemerge as compared to nondiseased plants. However, of the 31% of plants that re-emerged in 2012 over half of them had been moderately to heavily diseased the previous year. This suggests that disease severity in the prior year was not the sole or principal determinant of reemergence within the current study. Future investigations of *P. inconspicua* should attempt to determine the impact of disease on between-season survivorship of *L. grayi* through a thorough investigation of survival of bulbs of diseased plants, *in situ* and under laboratory conditions.

The impact of disease on within-season host survivorship was easier to ascertain. In 2011 and 2012 a high percentage of plants became infected by *P. inconspicua*, and of those infected a large proportion experienced early season decline of above-ground structures (Table 12). The association of high rates of disease incidence with high percentages of within-season mortality of above-ground structures suggests that the lily leaf spot disease on *L. grayi* is a disease capable of reducing the within-season host population.

Impact of Disease on Host Reproduction

The lily leaf spot disease greatly reduced the fecundity of *L. grayi* through a combination of indirect and direct effects. Disease indirectly reduced reproductive output by causing an early season collapse of high proportions of plants before seed maturation. Senescence of plants before seed maturation can be attributed to the phenologies of *L. grayi* and *P. inconspicua*. Because disease first appears at host emergence, nearly the entire season is available for development of disease symptoms and numerous rounds of reproduction by *P. inconspicua*. As a consequence of the polycyclic mode of reproduction employed by *P. inconspicua*, an exponential increase of disease incidence occurred before and during seed set (Figure 22; 23). Additionally, exponential increases in population mean disease severity were observed during seed capsule maturation (Figure 24).

The temporal coincidence of epidemic levels of disease and host reproduction resulted in a high percentage of plants undergoing early season collapse before reproduction could be completed (Table 12). Failure of host persistence through the reproductive cycle had the obvious effect of reducing the amount of mature seed capsules with a reduction in host fecundity. There was no evidence to suggest that *P. inconspicua* indirectly reduced reproductive output by retarding development or causing plant stress. Some diseases reduce fecundity by inhibiting bud formation during disease stress (Dinoor and Eshed 1984). However, because *L. grayi* emerges each year with predeveloped reproductive structures, *P. inconspicua* likely had no developmental effect on the formation of buds. Though not investigated, disease may be capable of reducing bud formation in the following year. In addition to abiotic stresses, disease has been shown to impact the formation of reproductive structures in a host plant in a sequential season (Primack and Hall 1990.)

Disease directly reduced reproductive output by infecting reproductive structures. Disease lesions were observed on pedicels, flowers, and seed capsules. Lesions on pedicels often resulted in necrotic loss of reproductive structures (Figure 3; 4) while lesions on seed capsules often resulted in capsule abortion or reduced seed count and/or viability (Figure 4; 5; 6). In addition to reduced seed counts, disease lesions on seed capsules were associated with reduced capsule and seed weight (Table 20; 21; 22; 23). Furthermore, seed from entirely diseased seed capsules were blackened, deformed, and smaller than seed within nondiseased capsules. The reduced weight of seed and irregularity of seed appearance strongly suggest that the lily leaf spot disease reduces host fecundity by reducing seed viability. However, because a comprehensive germination study was not completed, no firm conclusions are appropriate in regard to the

impact of disease on seed viability. Future studies should include attempts to determine the impact of disease on seed viability through germination trials and common garden experiments.

Impact of Disease on Host Recruitment

High rates of early season collapse among adolescent lilies suggested that the lily leaf spot disease is capable of causing a drastic reduction in recruitment of *L. grayi* seedlings and juveniles. Similar rates of increase in disease were observed for seedlings between years, non-reproductively mature lilies combined between years, and for seedlings and juveniles within years (Table 5; 6; 7; 8; 9). The similarity of rates of increase in disease severity between years suggests that disease spread, severity, and progression of symptoms are undergoing only minor annual fluctuations among adolescent lilies. Furthermore, as different plots were chosen in 2012, the patterns and rates of change in disease severity are expected to be representative of the effect of the disease on the seedling and juvenile population and not simply estimates for specific plots.

There were, however, within season differences in mean disease severity among plots (Table 10). Because plots were placed at different locations along the length of the transect, the differences in disease severity among plots suggests either areas of increased or reduced host resistance and/or an environmental effect on disease.

P. inconspicua had a disproportionately destructive influence on the above-ground mortality of adolescent *L. grayi*. As leaf spot diseases effectively kill their host via necrosis of photosynthetic tissues (Burdon 1993), adolescent *L. grayi* with relatively few leaves decline more rapidly. Unfortunately, disease severity of individual nonreproductively plants was not tracked throughout the season but were instead tallied as groups in plots. To accurately compare rates of declines between adolescent and adult lilies, individual seedlings, juveniles, and reproductively mature lilies should be monitored using the same experimental design.

The exceptionally high rates of early season collapse of *L. grayi* seedlings and juveniles suggests 2 negative effects on host recruitment: a reduced number of plants are reaching reproductive maturity, and the abbreviated growing season for adolescent lilies may result in delayed maturity. However, it is important to note that the effect of the disease on below-ground structures was not investigated directly. Additionally, because individual non-reproductively mature *L. grayi* were not tracked each season, reemergence rates were not obtainable. Without understanding the effect of disease on the between-season survivorship of adolescents, the long-term impact of disease on recruitment remains largely unanswered. Future studies of *P. inconspicua* on *L. grayi* should include a multi-year demography study to track the annual emergence and developmental progress along with disease incidence and severity of individual non-reproductively mature lily plants. In either case, the growing season for non-reproductively mature seedlings is shortened as a result of the lily leaf spot disease. This is obviously a negative effect because of the limits it places on the period of above-ground activity.

Conclusions

Although the attempts to complete all the tenets of Koch's postulates were partly unsuccessful, a large body of evidence was amassed to demonstrate the causal association of symptoms of the lily leaf spot with the fungal pathogen, *P. inconspicua*. As *P. inconspicua* has never been definitively reported on *L. grayi*, this study extends the pathogen's host range. Moreover, because the previous reports of *P. inconspicua* within the United States have been restricted to northern states, this study also extends the geographic range of *P. inconspicua* to include Tennessee and North Carolina. Previous reports of disease epidemics caused by *P. inconspicua* in the Ukraine and Japan have indicated that the pathogen is capable of causing economic loss of lilies in cultivation. Accordingly, *P. inconspicua* has been considered a

destructive foreign pathogen not yet established within the United States. This new report of *P. inconspicua* causing a disease epidemic so far outside of its previous range may be an indication that it is an emerging infectious disease within the United States. Furthermore, because *P. inconspicua* has never been reported on lily hosts in the western United States it may pose a future threat to commercial lily cultivation within the many floral nurseries located within that region.

Spatio-temporal patterns associated with the lily leaf spot disease conformed to patterns expected of highly infectious polycyclic diseases annually cycling through the "demographic cycle of pathogens" with an outcome of sequential disease epidemics. Host within-season survivorship, fecundity, recruitment, and seed viability were greatly reduced as a result of infection by *P. inconspicua*. Long-term effects of disease on the host population were difficult to ascertain because of a lack of data on below-ground survival and prior reports of non-annual patterns of emergence for *L. grayi*. However, there was evidence that the lily leaf spot disease is capable of reducing host population size over time through a combination of reduced fecundity and delayed or arrested maturity of non-reproductive plants.

Studies of disease within natural populations indicate that annually recurring epidemics are capable of resulting in host population crashes (Burdon 1993). Because *L. grayi* is a rare plant of limited distribution, a marked reduction in population sizes may initiate a trend of population reduction leading to extinction. Long-term conservation of *L. grayi* will require consideration of the interacting-effects of disease, habitat loss, poaching, and mammal browsing.

REFERENCES

Agrios, G.N. 2005. Plant Pathology. 5th ed. London, UK: Elsevier Academic Press; p.

Amoroso JL, Finnegan JT. 2002. Natural Heritage Program List of the Rare Plant Species of North Carolina. North Carolina Natural Heritage Program.

Ashton DH, Macauley BJ. 1972. Winter leaf spot disease of seedlings of *Eucalyptus regnans* and its relation to forest litter. Transactions of the British Mycological Society 58(3): 377–386.

Bates M. 1995. Annual report on taxa in the national collection for North Carolina Botanical Garden, 1995. Raleigh (NC): North Carolina Plant Conservation Program, Dept. of Agriculture and Consumer Services (NC); Available by request from: U.S. Fish and Wildlife Service.

Bates M. 1998. North Carolina Plant Conservation Program *Lilium grayi* Annual Report, 1997. Raleigh (NC): North Carolina Plant Conservation Program, Dept. of Agriculture and Consumer Services (NC); Available by request from: U.S. Fish and Wildlife Service.

Bates M. 1998. North Carolina Plant Conservation Program *Lilium grayi* Interim Report, 1998. Raleigh (NC): North Carolina Plant Conservation Program, Dept. of Agriculture and Consumer Services (NC); Available by request from: U.S. Fish and Wildlife Service.

Bates M. 1999. North Carolina Plant Conservation Program Monitoring protocol for *Lilium grayi*, 1998. Raleigh (NC): North Carolina Plant Conservation Program, Dept. of Agriculture and Consumer Services (NC); Available by request from: U.S. Fish and Wildlife Service.

Bates M. 2000. North Carolina Plant Conservation Program *Lilium grayi* Annual Report, 2000. Raleigh, NC: North Carolina Department of Agriculture and Consumer Services.

Batta YA. 2001. Effect of fungicides and antagonistic microorganisms on the Black Fruit Spot disease on Persimmon. Dirasat 28(2, 3): 165-171.

Beckman PM, Payne GA. 1983. Cultural techniques and conditions influencing growth and sporulation of *Cercospora zeae-maydis* and lesion development in corn. Phytopathology 73(2): 286-289.

Bhat RG, Subbarao KV. 1999. Host range specificity in *Verticillium dahlia*. Phytopathology. 89(12): 1218-1225.

Brown DM. 1941. Vegetation of Roan Mountain: a phytosociological and successional study. Ecol Mono. 11: 61-97.

Braun U. 1988. Studies on *Ramularia* and allied genera (II). 47(3-4): 335-349.

Burdon JJ, Chivlers GA. 1982. Host density as a factor in plant disease ecology. Annu Rev Phytopathol. 20: 143-166.

Burdon JJ, Jarosz AM, Kirby GC. 1989. Pattern and patchiness in plant-pathogen interactions-causes and consequences. Annu Rev Ecol Syst. 20: 119-136.

Burdon JJ. 1993. The structure of pathogen populations in natural plant communities. Annu Rev Phytopathol. 31: 305-323.

Coates F, Lunt ID, Tremblay RL. 2006. Effects of disturbance on population dynamics of the threatened orchid *Prasophyllum correctum* D.L. Jones and implications for grassland management in south-eastern Australia. Biological Conservation 129(1): 59-69.

Coomans RJ. 2002. Effects of fungicide application and canopy clearing on growth and reproduction in Gray's lily (*Lilium grayi)*. Raleigh (NC): North Carolina Plant

Conservation Program, Dept. of Agriculture and Consumer Services (NC); Available by request from: U.S. Fish and Wildlife Service.

Coulston JW, Riitters KH. 2003. Geographic analysis of forest health indicators using spatial scan statistics. Environ Manage. 31(6): 764-773.

Cox KD, Scherm H. 2001. Gradients of primary and secondary infection by *Monilinia vaccinii-corymbosi* from point sources of ascospores and conidia. Plant Disease 85(9): 955-959.

Crawford CJ, Kennedy LM. 2009. Spatial and temporal patterns of tree encroachment into a Southern Appalachian Grass/Heath bald using tree rings. Natural Areas Journal 29(4): 367-375.

Darvas JM, Kotzé JM. 1979. Cercospora spot of avocados. South African Avocado Growers' Association Research Report. 3: 38-39.

Dinoor A, Eshed N. 1984. The role and importance of pathogens in natural plant communities. Annu Rev of Phytopathol. 22: 443-466.

Denoyes B, Baudry A. 1995. Species identification and pathogenicity study of French *Colletotrichum* strains isolated from strawberry using morphological and cultural characteristics. Phytopathology 85(1): 53-57.

Donaldson J. 2009. Baa-tany in the Balds. Appalachian Trail Journeys 5(4): 10-15.

Ekpo EJA, Esuruoso OF. 1978. Growth and sporulation of *Cercospora cruenta* and *Cercospora canescens* in culture. Can J Bot. 56(3): 229-233.

Evans AS. 1976. Causation and disease: The Henle-Koch Postulates revisited. Yale J Biol Med. 49(2): 175-195.

Feng C, Li Y, Na L, Gong Y. 2007. The survey and identification of *Lilium* spp.diseases in Yuxi City. Journal of Fujian College of Forestry 20(2): 162-164.

Fitt BDL, Gregory PH, Todd AD, McCartney HA, Macdonald OC. 1987. Spore dispersal and plant disease gradients; a comparison between two empirical models. J Phytopathol. 118(3): 227-242.

Freeman GH, Halton JH. 1951. Note on exact treatment of contingency, goodness of fit and other problems of significance. Biometrika 38: 141-149.

Gilbert GS. 2002. Evolutionary ecology of plant diseases in natural ecosystems. Annu Rev Phytopathol. 40: 13-43.

Hamilton RI, Edwardson JR, Francki RIB, Hsu HT, Hull R, Koenig R, Milne RG. 1981. Guidelines for the identification and characterization of plant viruses. J Gen Virol. 54(2): 223-241.

Hayashi K, Kawano S. 2000. Molecular systematics of *Lilium* and allied genera (Liliaceae): phylogenetic relationships among *Lilium* and related genera based on the *rbcL* and *matK* gene sequence data. Plant Species Biol. 15(1): 73-93.

Hill AH. 1965. The Environment and disease: Association or causation? Proc Roy Soc Med. 58(5): 295-300.

Hou PF, Chen CY. 2003. Early stages of infection of lily leaves by *Botrytis elliptica* and *B. cinerea*. Plant Pathol Bull. 12: 103-108.

Hunt NR. 1946. Destructive plant diseases not yet established in North America. Bot Rev. 12(10): 593-627.

Jarosz AM, Burdon JJ. 1991. Host-pathogen interactions in natural populations of *Linum marginale* and *Melampsora lini*: II. Local and Regional Variation in Patterns of Resistance and Racial Structure. Evolution 45(7): 1618-1627.

Jarosz AM, Davelos AL. 1995. Tansley Review No. 81. Effects of disease in wild plant populations and the evolution of pathogen aggressiveness. New Phytol. 129(3): 371-387.

Kamenetsky R, Okubo H, editors. 2012 Ornamental Geophytes: From Basic Science to Sustainable Production. Florida: CRC Press 103 p.

Kasuyama S, Tanina K. 2008. Leaf spot of cabbage *Brassica oleracea* L. (capitata group) and cauliflower *Brassica oleracea* L. (botrytis group) caused by *Pseudocercosporella capsellae*. Bulletin of the Agricultural Experiment Station, Okayama Prefectural General Agriculture Center 26: 55-60.

Kulldorff M. 1997. A spatial scan statistic. Commun Stat Theory Methods 26(6): 1481-1496.

Laney AG., Keller KE, Martin RR, Tzanetakis IE. 2011. A discovery 70 years in the making: characterization of the Rose rosette virus. J Gen Virol. 92(7): 1727-1732.

Levy F, Leman S, Sarubbi FA, Walker ES. 2009. Nosocomial transmission clusters and risk factors in *Moraxella*. Epidemiology & Infection 137: 581-590.

Louie R, Redinbaugh MG, Gordon DT, Abt JJ, Anderson RJ. 2000. Maize necrotic streak virus, a new maize virus with similarity to species of the Family Tombusviridae. Plant Disease 84(10): 1133-1139.

Mannen A van, Xu X-M. 2003. Modelling plant disease epidemics. Eur J Plant Pathol. 109(7): 669-682.

Makoto H. 1925. On a Cercosporellose of the cultivated lily, [in Japanese]. Annals of the Phytopathological Society of Japan 1(6): 20-30.

Pehkonen T, Tolvanen A. 2008. Relationships between *Vaccinium vitis-idaea*, frequency of its fungal pathogen *Exobasidium splendidum* and environment. Arct Antarct Alp Res. 40(3): 561-567.

Plakidas AG. 1944. Black scale: a disease of Easter lily bulbs. Phytopathology 34(6): 556-571.

Powell J. 2011. Impacts of goat browsing and disease on *Lilium grayi*, Gray's lily, on Roan Mountain [undergraduate thesis]. [Johnson City, (TN)]: East Tennessee State University.

Primack RB, Hall P. 1990. Costs of Reproduction in the Pink Lady's Slipper Orchid: A Four-Year Experimental Study. Am Natur. 136(5): 638-656.

Rai JN, Tewari JP, Sinha AK. 1967. Effect of environmental conditions on sclerotia and cleistothecia production in aspergillus. Mycopathologia et Mycologia Applicata 31(3-4): 209-224.

Real LA, McElhany P. 1996. Spatial pattern and process in plant-pathogen interactions. Ecol. 77(4): 1011-1025.

Rivers TM. 1937. Viruses and Koch's postulates. J Bacteriol. 33(1): 1-12.

SAS Institute Inc. 2011. SAS/STAT® 9.3 User's Guide. Cary, NC: SAS Institute Inc.

Segarra J, Jeger MJ, Van den Bosch F. 2001. Epidemic dynamics and patterns of plant diseases. Phytopathology 91(10): 1001-1010.

Skinner, M.W. 2002. *Lilium grayi*. In: Flora of North America Editorial Committee, eds. 1993+. Flora of North America North of Mexico. 16+ vols. New York and Oxford. 26: 197.

Smith, R.M. 1998. Wildflowers of the Southern Mountains. Knoxville, Tennessee: The University of Tennessee Press.

Stanley WM. 1935. Isolation of a crystalline protein assessing the properties of tobacco-mosaic virus. Science 81(2113): 644-645.

Stephenson AG. 1981. Flower and fruit abortion: proximate causes and ultimate consequences. Annu. Rev. Ecol. Syst. 12: 253-279.

USGS topographic map: the USGS store [Internet]. 2012. US Department of the Interior. [Updated 2012 May 7: Cited 2013 August 1]. Available from: http://store.usgs.gov.

Weigl PD, Knowles TW. 1995. Megaherbivores and Southern Appalachian Grass Balds. Growth and Change 26(3): 365–382.

Xu X.-M, Ridout MS. 1998. Effects of initial epidemic conditions, sporulation rate, and spore dispersal gradient on the spatio-temporal dynamics of plant disease epidemics. Phytopathology 88(10): 1000-1012.

Zadoks JC, Van Den Bosch F. 1994. On the spread of plant disease: A theory of foci. Annu Rev Phytopathol. 32: 503-521.

Zerova Mme MY. 1940. Diseases of Lilies in Kieff nurseries [in Russian]. Botanichnii Zhurna 1(1): 143-147.

APPENDICES

APPENDIX A

Results of the Correlation Analysis of Lily Morphological Traits, Capsule Production, Browsed Status, Reproductive Damage by Disease, and Plant Location. Correlation coefficients are presented. Significance ($p>0.05$) is denoted by red. Key to abbreviations; "x" = x-coordinate; "y" = y-coordinate; "Ht" = max. plant height; "Lv/wh" = max. number of leaves within a whorl; "Capsule" = number of mature capsules per plant; "Browse" = plant browsed by mammal; "RD" = disease lesions on pedicel, flower, or capsules; "Wh" = number of whorls per plant:

Variable	y	Ht '11	Ht '12	Lv/wh '11	Lv/wh '12	Capsule '11	Capsule '12	Browse '11	Browse '12	RD '11	RD '12	Wh '11	Wh '12
x	0.031	-0.067	-0.124	0.229	0.008	-0.102	-0.295	-0.149	0.398	0.098	-0.559	-0.232	-0.320
y		-0.066	-0.01	-0.221	0.134	-0.312	-0.078	0.057	-0.015	-0.382	-0.096	0.113	0.049
Ht 2011			0.736	0.476	0.516	0.535	0.038	-0.117	0.115	0.011	-0.005	0.496	0.613
Ht 2012				0.463	0.534	0.073	0.267	-0.14	-0.229	-0.006	0.122	0.540	0.489
Lv/wh '11					0.658	0.382	-0.019	-0.109	0.238	0.068	-0.238	0.207	0.345
Lv/wh '12						0.271	0.125	-0.241	0.101	-0.055	-0.002	0.387	0.544
Capsule 2011							-0.138	0.37	0.202	0.243	0.279	0.256	-0.027
Capsule 2012								0.098	0.573	0.018	0.515	0.236	0.421
Browse 2011									-0.244	-0.533	-0.005	-0.080	-0.048
Browse 2012										0.022	-0.502	-0.108	-0.313
RD 2011											0.068	-0.001	-0.128
RD 2012												0.145	0.248
Wh '11													0.545

APPENDIX B

Results of the Correlation Analysis of Disease Severity, Browsed Status, Reproductive Damage by Disease, Capsule Production, and Plant Location (x-coordinate). Correlation coefficients are presented for 2011 (’11) and 2012 (’12). Significance ($p < 0.05$) is denoted by red.

Variable	Browsed by Mammals '11	Browsed by Mammals '12	Disease on Repr. Structures '11	Disease on Repr. Structures '12	Capsules Produced '11	Capsules Produced '12	x-coordinate
Diseased Whorls (4) '11	0.052	0.104	-0.048	-0.128	-0.168	-0.008	-0.101
Diseased Whorls (5) '11	0.134	-0.176	-0.077	0.044	-0.187	0.035	-0.467
Diseased Whorls (6) '11	0.181	-0.383	-0.067	0.514	-0.163	0.108	-0.495
Diseased Whorls (7) '11	0.145	-0.367	-0.085	0.438	-0.169	0.212	-0.531
Diseased Whorls (8) '11	0.145	-0.367	-0.085	0.438	-0.169	0.212	-0.531
Diseased Whorls (1) '12	-0.167	0.021	-0.212	0.343	0.540	-0.069	-0.397
Diseased Whorls (2) '12	-0.234	-0.227	0.199	0.659	0.538	0.328	-0.529
Diseased Whorls (3) '12	-0.241	-0.212	0.169	0.438	0.372	0.206	-0.283
Diseased Whorls (4) '12	-0.261	-0.206	0.158	0.389	0.269	0.197	-0.233
Diseased Whorls (5) '12	-0.284	-0.224	0.258	0.437	0.250	0.209	-0.265
Diseased Whorls (6) '12	-0.074	-0.319	0.093	0.357	0.047	0.278	-0.254
Diseased Whorls (7) '12	-0.240	-0.281	0.241	0.292	0.167	0.245	-0.136
Disease Severity (2) '11	-0.037	0.000	0.141	0.000	0.212	0.775	0.380
Disease Severity (3) '11	0.239	0.000	-0.081	0.123	0.152	0.343	-0.158
Disease Severity (4) '11	-0.126	0.003	0.131	0.035	0.234	0.012	0.188
Disease Severity (5) '11	-0.281	0.209	0.215	-0.041	0.289	0.032	0.391
Disease Severity (6) '11	-0.273	0.159	0.223	0.223	0.319	0.091	0.471
Disease Severity (7) '11	0.145	0.261	0.178	-0.156	0.328	0.039	0.514
Disease Severity (8) '11	0.145	0.262	0.098	-0.164	0.349	0.049	0.502
Disease Severity (1) '12	-0.060	0.057	0.012	-0.462	-0.267	-0.008	0.499
Disease Severity (2) '12	0.209	0.172	-0.162	-0.633	-0.550	-0.297	0.516
Disease Severity (3) '12	0.229	0.120	0.121	-0.421	-0.532	-0.083	0.232
Disease Severity (4) '12	0.312	0.083	0.045	-0.449	-0.526	-0.017	0.354
Disease Severity (5) '12	0.305	-0.070	-0.083	-0.482	-0.483	0.071	0.397
Disease Severity (6) '12	0.331	0.005	-0.172	-0.373	-0.519	0.099	0.327
Disease Severity (7) '12	0.404	0.039	-0.404	-0.375	-0.524	0.079	0.274

APPENDIX C

Reproductively Mature Gray's Lily Demographic and Disease Severity Data Collected During 2011.
Key to variables: "ID" = Plant identification number; "x" = position on the x-axis of the study transect; "y" = position on the y-axis of the study transect; "Ht" = maximum plant height (cm); "Wh" = number of leaf whorls; "LW" = maximum number of leaves within a whorl; "DW" = number of diseased whorls; "D.S." = disease severity scale value (D.S. and DW data for each sampling event are located below the corresponding census date); "Br" = mammal browsed ("0" = no, "1" = yes); "Ca" = mature capsules per plant; "DR" = disease lesions on pedicels, flowers, or capsules ("0" = no, "1" = yes); N/A = data was not available.

	Plant Coordinate					Census Date																
						5.19.11		6.10	6.22	7.07.11		7.24.11		8.06.11		8.20.11		9.06.11				
ID	x	y	Ht	Wh	LW	DW	D.S.	D.S.	D.S.	DW	D.S.	DW	D.S.	DW	D.S.	DW	D.S.	DW	D.S.	Br	Ca	DR
499	0014.66	00.77	96	8	6				2.5	0	2.5	0	2.5	6	1.5	8	0.0	8	0.0	0	0	1
498	0028.91	-02.10	126	8	6					3	2.0	7	1.5	8	1.0	8	1.0	8	0.0	0	4	1
283	0032.30	-02.96	86	7	6				2.5	0	2.0	4	2.0	7	1.0	7	0.0	7	0.0	1	0	1
282	0039.71	-01.70	107	8	5				2.0	1	2.0	3	2.0	8	1.0	8	1.0	8	0.0	0	1	1
431	0041.45	02.10	64	7	5					2	2.0	0	2.0	7						1	0	1
432	0044.20	-01.53	60	7	7					1	2.0									1	0	0
433	0045.38	-03.53	74	6	5					0	2.0									1	0	1
261	0045.11	-04.00	44	7	5	0	3.0	1.5	1.0	7	0.0	7	0.0	7	0.0	7	0.0	7	0.0	0	0	1
204	0047.50	01.90	76	7	6					1	2.5	1	2.5	1	2.0	6	1.5	6	0.0	0	1	0
235	0044.20	-04.30	80	7	8	0	3.0	2.5	2.5	1	2.0	7	1.5	7	1.0	7	0.0	7	0.0	1	0	1
434	0048.66	02.35	87	7	5					3	1.5	7	0.0	7	0.0	7	0.0	7	0.0	1	0	0
435	0049.99	01.78	72	7	5			2.5	2.5	0	2.0	7	0.0	7	0.0	7	0.0	7	0.0	1	0	0
260	0047.80	03.15	111	9	6			2.5	2.5	5	1.5	9	0.0	9	0.0	9	0.0	9	0.0	1	0	0
285	0050.65	-02.50	67	7	5				2.0	0	2.0	7	0.0	7	0.0	7	0.0	7	0.0	1	0	0
286	0050.65	-02.60	47	6	5				2.0	0	2.0	6	0.0	6	0.0	6	0.0	6	0.0	1	0	0
287	0051.50	-02.70	49	6	4				2.0	0	2.0	1	2.0	4	1.5	6	1.5	6	0.0	0	0	1
436	0085.30	-01.52	93	10	6					1	2.5	2	2.5	1	2.5	1	2.0	1	2.0	0	5	0
437	0085.60	-01.57	94	8	8					0	2.0	1	2.0	1	2.0	1	2.0	1	2.0	0	3	1
275	0094.20	01.03	54	6	4			2.5	2.0	4	1.5	6	0.0	6	0.0	6	0.0	6	0.0	0	0	1
274	0094.21	01.05	67	6	5			2.0	2.0	1	2.0	4	1.5	6	0.0	6	0.0	6	0.0	0	1	0

	Plant Coordinate					Census Date																
						5.19.11		6.10	6.22	7.07.11		7.24.11		8.06.11		8.20.11		9.06.11				
ID	x	y	Ht	Wh	LW	DW	D.S.	D.S.	D.S.	DW	D.S.	DW	D.S.	DW	D.S.	DW	D.S.	DW	D.S.	Br	Ca	DR
213	0078.80	02.05	79	6	6	0	3.0	2.0	2.0	0	2.0	1	2.0	6	1.0	6	1.0	6	0.0	0	2	0
214	0079.00	02.27	65	5	5	0	3.0	2.0	2.0	0	2.0	4	1.5	5	0.0	5	0.0	5	0.0	0	0	0
438	0082.20	01.00	75	7	8					1	2.0	6	1.5	7	0.0	7	0.0	7	0.0	0	2	1
439	0083.20	01.00	58	6	4					1	2.0	6	1.0	6	0.0	6	0.0	6	0.0	0	0	1
300	0227.31	-01.01	83	7	6					0	2.5	0	2.5	1	2.0	1	2.0	1	0.0	0	0	1
440	0233.41	01.65	59	5	6					0	3.0	2	2.0	5	1.5	5	1.0	5	0.0	0	1	1
441	0233.36	01.50	63	6	6					0	2.5	2	2.0	6	1.5	6	1.0	6	0.0	0	1	1
442	0239.56	-02.10	73	7	5					4	1.5	7	1.0	7	0.0	7	0.0	7	0.0	0	0	1
218	0115.73	-01.79	53	6	6			3.0	2.0	1	2.0	6	0.0	6	0.0	6	0.0	6	0.0	0	0	1
444	0354.26	-03.25	78	6	7					1	2.0	2	2.0	2	2.0	2	2.0	2	2.0	1	0	0
445	0366.76	-02.80	85	7	8					1	2.0	1	2.0						1.0	0	1	0
238	0351.53	-01.80	79	6	6			3.0	2.5	0	2.0	0	2.0							0	2	0
297	0372.56	03.81	77	7	8				2.5	1	2.5	1	2.5	0	2.5	2	2.5	2	2.0	0	0	0
296	0372.56	03.99	73	7	6				2.5	1	2.5	1	2.5	0	2.0	1	2.0	1	2.0	1	0	0
295	0372.56	03.85	68	6	5				2.5	1	2.5	1	2.5	1	2.0	1	2.0	1	1.5	1	0	0
294	0372.56	03.79	67	6	5				2.5	1	2.5	1	2.5	1	2.0	2	2.0	2	1.0	1	0	0
293	0372.61	03.79	70	6	5				2.5	0	2.5	0	2.5	1	2.0	3	2.0	3	0.0	1	0	0
292	0372.71	03.99	64	6	5				2.5	1	2.5	1	2.5	1	2.0	4	2.0	4	0.0	1	0	0
291	0389.71	03.79	70	7	6				2.5	0	2.5	0	2.5	1	2.0	6	1.5	6	0.0	1	0	0
290	0403.91	04.91	84	7	5				2.5	1	2.0	3	2.0	3	2.0	6	1.5	6	1.0	0	3	1
447	0472.71	-15.45	65	7	6					0	3.0	0	3.0	0	2.5	0	2.5	0	2.0	1	0	0
222	0469.66	02.08	72	7	6			2.5	2.0	5	1.5	7	1.0	7	1.0	7	0.0	7	0.0	0	0	1
288	0471.33	-11.20	156	8	7					0	2.5	0	2.5	1	2.0	3	2.0	3	2.0	0	6	1
450	0514.87	03.70	55	5	7					0	2.5	5	1.5	5	0.0	5	0.0	5	0.0	1	0	0
451	0644.23	-00.76	103	6	8					5	1.0	6	1.0	6	1.0	6	1.0	6	0.0	0	0	1
272	0626.85	-00.60	116	8	7			2.5	2.0	5	1.5	8	1.0	8	1.0	8	0.0	8	0.0	0	0	1
271	0626.75	-00.60	74	5	6			2.5	2.0	5	1.0	5	0.0	5	0.0	5	0.0	5	0.0	0	0	1

	Plant Coordinate					Census Date																
						5.19.11		6.10	6.22	7.07.11		7.24.11		8.06.11		8.20.11		9.06.11				
ID	x	y	Ht	Wh	LW	DW	D.S.	D.S.	D.S.	DW	D.S.	DW	D.S.	DW	D.S.	DW	D.S.	DW	D.S.	Br	Ca	DR
230	0654.61	-00.43	50	6	6	0	3.0	2.5	1.5	6	0.0	6	0.0	6	0.0	6	0.0	6	0.0	1	0	0
231	0674.29	00.56	96	7	5			2.5	2.5	4	1.5	0	1.0	7	0.0	7	0.0	7	0.0	1	0	0
232	0674.37	00.67	80	4		0	3.0	2.5	2.0	0	1.5	4	1.0							1	0	0
278	0712.60	05.37	111	7				2.0	2.0	0	2.0	0	1.5							1	0	0
279	0725.46	00.43	112	9	6			3.0	2.5	0	2.5	0	2.0	6	1.5	8	1.5	8	1.0	0	1	1
244	0779.21	02.27	60	5	5	0	3.0	2.5	2.0											1	0	0
453	0816.81	00.75	59	6	6					6	1.0	6	0.0	6	0.0	6	0.0	6	0.0	1	0	0
246	0858.23	00.74	113	7	8			3.0	2.5	2	2.0	0	2.0	7	1.5	7	0.0	7	0.0	1	0	0
250	0957.47	-00.48	59	3	6	0	3.0	2.5	2.0											1	0	0
248	0961.02	02.01	74	6	6	0	3.0	2.5	2.5	1	2.5	1	2.0	2	2.0	2	2.0	2	0.0	1	0	0
247	0965.42	01.97	81	8	7	0	3.0	3.0	2.5	1	2.5	1	2.5	2	2.0	6	1.5	6	0.0	0	0	1
454	0869.21	-01.00	89	7	6					7	1.0	7	0.0	7	0.0	7	0.0	7	0.0	1	0	0
452	0956.82	-03.20	96	7	6					1	2.0	1	2.0	7	0.0	7	0.0	7	0.0	1	0	0
455	0853.78	-00.50	91	7	10					1	2.0	1	0.0	7	0.0	7	0.0	7	0.0	1	0	0
280	0953.82	-00.48	60	5	5			2.5	2.0	1	2.0	1	2.0	1	2.0	1	2.0	1	0.0	0	1	1
255	0988.80	-01.54	143	8	11		3.0	3.0	2.5	1	2.5	0	2.5	1	2.5	2	2.5	2	2.0	0	5	1
456	0990.32	01.11	104	7	8					1	2.5	1	2.5	0	2.5	1	2.5	1	2.0	0	1	1
253	0943.97	01.79	136	8	10				2.5	0	2.0									0	0	0
257	1255.50	-01.20	59	6	6			2.5	2.0	1	2.0	1	2.0	0	2.0	1	2.0	1	0.0	0	0	1
258	1255.50	-01.42	57	8	6			2.5	2.0	1	2.0	1	2.0	0	2.0	3	2.0	3	0.0	0	0	1
457	1256.78	-01.19	76	7	8					0	3.0	0	2.5	2	2.0	3	2.0	3	2.0	0	1	1
458	1260.50	-04.39	76	6	5					1	2.5	1	2.5	0	2.5	0	2.5	0	2.5	0	1	1
459	1261.02	-04.80	75	5	6					0	3.0	0	3.0	0	2.5	1	2.5	1	2.5	0	1	1
460	1261.14	-04.95	72	5	6					0	3.0	0	3.0	0	2.5	1	2.0	1	2.0	0	1	1
461	1261.14	-04.85	75	5	7					1	2.5	1	2.5	1	2.0	3	2.0	3	2.0	0	1	1
462	1261.20	-04.80	71	5	8					0	2.5	0	2.5	0	2.5	1	2.5	1	2.0	0	1	1
262	1217.26	01.75	49	8	6			2.5	2.0	1	2.0									1	0	1

	Plant Coordinate					Census Date																
						5.19.11		6.10	6.22	7.07.11		7.24.11		8.06.11		8.20.11		9.06.11				
ID	x	y	Ht	Wh	LW	DW	D.S.	D.S.	D.S.	DW	D.S.	DW	D.S.	DW	D.S.	DW	D.S.	DW	D.S.	Br	Ca	DR
263	1216.36	01.73	43	8	6			2.5	2.0	1	2.0									0	0	1
256	1263.49	01.46	103	7	8	0	3.0	3.0	2.5	0	2.5									0	0	0
264	1291.56	06.00	75	6	5			2.5	0.0	6	0.0									0	0	0
463	1223.36	05.50	65	5	5					0	2.5									0	0	0
464	1223.36	08.50	75	8	6					0	2.5									0	0	0
465	1231.38	12.60	67	6	5					0	3.0	0	3.0	0	3.0	1	2.0	1	2.0	0	0	0
466	1289.24	-02.40	58	5	8					0	3.0	0	3.0			0	2.5	0	2.5	0	1	1
467	1302.31	-09.50	62	5	6					0	2.5	0	2.5									
468	1302.31	-06.02	72	5	5					0	2.5	0	2.5	0	2.5	1	2.5	1	1.5	0	1	1
469	1302.31	-05.89	81	5	6					0	2.5	0	2.5	1	2.5	0	2.5	0	2.0	1	1	1
470	1306.97	-09.50	52	5	5					0	2.5	0	2.5							0	0	1
471	1313.97	-03.95	49	6	7					0	2.5	0	2.5							0	0	1
472	1313.97	-04.01	49	6	7					0	2.5	0	2.5							0	0	1
473	1320.97	-02.30	53	6	10					0	2.0									1		
289	0966.71	01.80	69	6	5				2.0	0	2.0	0	2.0							1		
476	0438.97	03.08	74	5	5					2	1.5	2	1.5			5	1.0	5	0.0	0	1	1
475	1023.49	02.04	119	7	7					0	3.0	0	3.0	1	2.5	2	2.0	2	1.0	0	1	1
277	0234.16	01.60	47	5	5				2.0	1	2.0	0	2.0	1	2.0	2	2.0	2	0.0	0	0	1
477	0045.40	-03.60	76	5	5					0	3.0	1	2.0	1	1.0	5	0.0	5	0.0	1	0	1
478	0164.11	-10.00	123	6	12					0	3.0	0	2.5	5	2.0	5	1.5	5	0.0	0	5	1
299	0234.16	04.80	83	7	6				2.0	1	2.0		1.0		0.0		0.0		0.0		0	

APPENDIX D

Reproductively Mature Gray's Lily Demographic and Disease Severity Data Collected During 2012.
Key to variables: "ID" = Plant identification number; "x" = position on the x-axis of the study transect; "y" = position on the y-axis of the study transect; "Ht" = maximum plant height (cm); "Wh" = number of leaf whorls; "LW" = maximum number of leaves within a whorl; DW = number of diseased whorls; "D.S." = disease severity scale value (D.S. and DW data for each sampling event are located below the corresponding census date); "Br" = mammal browsed ("0" = no, "1" = yes); "Ca" = mature capsules per plant; DR = disease lesions on pedicels, flowers, or capsules ("0" = no, "1" = yes); N/A = data was not available.

	Plant Coordinate					Census Date																
						5.22.12		6.10.12		6.26.12		7.10.12		7.24.12		8.07.12		8.20.12				
ID	x	y	Ht	Wh	LW	DW	D.S.	DW	D.S.	DW	D.S.	DW	D.S.	DW	D.S.	DW	D.S.	DW	D.S.	Br	Ca	DR
282	0039.71	-01.70	086	8	7	0	3.0	2	2.5	2	2.0	2	1.5	8	1.0	8	1.0	8	0.0	0	2	1
431	0041.45	02.10	046	6	5	0	2.5	1	2.5	1	3.0	1	2.5	2	2.5	2	2.0	2	0.0	0	1	1
435	0049.99	01.78	046	7	7	0	3.0	0	3.0	0	3.0	1	2.5	2	2.0	4	2.0	4	1.5	0	1	1
285	0050.65	-02.50	071	7	7	0	3.0	0	3.0	0	3.0	0	3.0	0	2.5	2	2.0	2	2.0	1	0	0
286	0050.65	-02.60	058	5	5	0	3.0	0	3.0	0	3.0	0	3.0	0	2.5	2	2.0	2	2.0	0	1	0
211	0078.80	01.79	033	6	5	1	2.5	1	2.5											1	0	0
213	0078.80	02.05	058	7	8	1	2.5	2	2.0	2	1.0	2	0.0	2	0.0	2	0.0	2	0.0	1	0	1
210	0079.20	01.83	040	6	5	1	2.5	2	2.5	2	1.5	2	0.0	2	0.0	2	0.0	2	0.0	1	0	1
212	0079.20	02.17	036	6	5	1	2.5	1	2.0	1	1.0	1	0.0	1	0.0	1	0.0	1	0.0	1	0	1
440	0233.41	01.65	045	5	6	0	3.0	1	2.5	2	2.0	5	2.0	5	0.0	5	0.0	5	0.0	0	1	1
441	0233.36	01.50	044	4	5	0	3.0	1	2.5	2	2.0	5	2.0	4	0.0	4	0.0	4	0.0	0	1	1
442	0239.56	-02.10	053	6	6	0	3.0	0	3.0											1	0	0
218	0115.73	-01.79	N/A	N/A	N/A					0	3.0											
444	0354.26	-03.25	089	9	8	0	3.0	1	2.5	1	2.5	1	2.5	3	2.0	8	1.5	9	1.0	0	5	1
445	0366.76	-02.80	067	7	10	0	3.0	0	3.0											1	0	0
450	0514.87	03.70	050	6	8	0	3.0	0	3.0	1	2.0	5	1.0	6	0.0	6	0.0	6	0.0	1	0	0
231	0674.29	00.56	069	6	6	0	3.0	0	3.0	1	2.5	0	3.0	0	2.5	1	2.0	1	2.0	1	0	0
232	0674.37	00.67	059	6	5	0	3.0	0	3.0	0	3.0	0	3.0	0	2.5	0	2.5	0	2.5	1	0	0
278	0712.60	05.37	091	8	10	0	3.0	0	3.0	1	2.5	1	2.5	1	2.5	7	1.5	8	0.0	0	0	0

	Plant Coordinate					Census Date																
						5.22.12		6.10.12		6.26.12		7.10.12		7.24.12		8.07.12		8.20.12				
ID	x	y	Ht	Wh	LW	DW	D.S.	DW	D.S.	DW	D.S.	DW	D.S.	DW	D.S.	DW	D.S.	DW	D.S.	Br	Ca	DR
279	0725.46	00.43	098	9	10	0	3.0	1	2.5	6	2.0	8	1.5	9	1.0	9	0.0	9	0.0	1	2	1
248	0961.02	02.01	041	5	6	0	3.0	0	3.0	1	2.5			1	2.0	1	2.0	1	2.0	1	0	0
247	0965.42	01.97	067	8	8	0	3.0	0	3.0	1	2.5	1	2.5	1	2.5	3	2.0	3	2.0	0	3	0
456	0990.32	01.11	120	7	11	0	3.0	0	3.0	0	3.0	0	3.0	0	3.0	1	2.5	2	2.0	1	0	0
253	0943.97	01.79	090	8	8	0	3.0	0	3.0	1	2.5	3	2.0	3	2.0	3	2.0			1	0	0
257	1255.50	-01.20	N/A	N/A	N/A															1	0	0
258	1255.50	-01.42	059	6	6	0	3.0	0	3.0	0	3.0	0	2.5							1	0	0
457	1256.78	-01.19	068	6	8	0	3.0	0	3.0	0	3.0	0	3.0	1	2.0	2	1.5	6	0.0	1	0	0
458	1260.50	-04.39	N/A	N/A	N/A															1	0	0
460	1261.14	-04.95	040	6	6	0	3.0	0	3.0											1	0	0
466	1289.24	-02.40	063	7	9	0	3.0	0	3.0	0	3.0	0	2.5	0	2.5					1	0	0
289	0966.71	01.80	N/A	N/A	N/A															1	0	0
476	0438.97	03.08	042	5	5			1	2.5											1	0	0
475	1023.49	02.04	086	7	8	0	3.0	0	3.0	0	3.0	0	2.5	0	2.5	0	2.5			1	0	0
393	0011.89	02.35	053	6	6	0	3.0	0	3.0	0	3.0	0	3.0	1	2.5	1	2.5	1	2.5	0	2	0
392	N/A	N/A	049	6	5	0	3.0	0	3.0	0	3.0	0	3.0							1	0	0
391	0013.72	02.16	069	7	7	0	3.0	0	3.0	0	3.0	1	2.5	1	2.5	1	2.5	1	2.5	0	2	1
390	0047.73	03.02	072	10	8	0	2.5	1	2.5	1	3.0	1	2.5	1	2.5	1	2.0	1	2.0	0	2	1
389	0045.81	-03.00	070	5	5	2	2.5	2	2.5	2	2.5	2	2.0	2	2.0	5	1.5	5	1.5	0	1	1
388	0049.99	-03.23	053	6	6	0	3.0	0	3.0	0	3.0	0	2.5	1	2.5	5	1.5	5	1.5	0	0	1
387	0076.50	02.77	051	6	7	1	2.5	1	2.5	1	1.5	6	0.0	6	0.0	6	0.0	6	0.0	1	0	0
277	0234.16	01.60	N/A	N/A	N/A																	
385	N/A	N/A	049	6	8	0	3.0	0	3.0	0	3.0	0	3.0							1	0	0
384	N/A	N/A	045	5	6	0	3.0	0	3.0	0	3.0	0	3.0							1	0	0
383	N/A	N/A	043	6	8	0	3.0	0	3.0	0	3.0	0	3.0							1	0	0
382	N/A	N/A	048	7	8	0	3.0	0	3.0	0	3.0	0	3.0							1	0	0
381	0347.50	-05.41	050	6	6	0	3.0	1	2.5											1	0	0

	Plant Coordinate					Census Date																
						5.22.12		6.10.12		6.26.12		7.10.12		7.24.12		8.07.12		8.20.12				
ID	x	y	Ht	Wh	LW	DW	D.S.	DW	D.S.	DW	D.S.	DW	D.S.	DW	D.S.	DW	D.S.	DW	D.S.	Br	Ca	DR
380	0476.80	01.15	069	6	11	0	3.0	0	3.0	0	3.0	0	3.0	3	1.5	6	0.0	6	0.0	1	0	0
379	0515.20	04.45	048	6	6	0	3.0	1	2.5	1	2.0	6	0.0	6	0.0	6	0.0	6	0.0	1	0	0
378	0674.25	01.02	071	5	5	0	3.0	0	3.0	0	2.5	0	2.5	0	2.5	0	2.0	2	2.0	0	0	0
376	1322.19	-04.73	049	6	7	0	3.0	0	3.0	0	3.0	0	2.5								0	0
375	1263.46	02.35	051	6	8	0	3.0	1	2.5	1	2.5	1	2.5	1	2.5	1	2.5	1	2.5	1	1	0
374	1262.94	03.50	045	6	6	0	3.0	0	3.0	0	3.0	0	3.0	0	2.5	2	2.0	1	2.0	1	0	0
373	1261.99	03.53	044	7	8	0	3.0	0	3.0	0	2.5	0	3.0	0	2.5	1	2.0	3	2.0	0	0	0
371	1130.13	08.00	035	5	6	0	3.0	0	3.0											1	0	0
370	0943.97	02.00	063	6	6	0	3.0	0	3.0	0	3.0									1	0	0
369	0809.78	00.50	047	8	8	0	3.0	0	3.0											1	0	0
368	1130.13	08.75	052	6	8	0	3.0	0	3.0											1	0	0
386	N/A	N/A	051	7	6	0	3.0													1	0	0
488	0223.91	05.83	072	6	7			1	2.5	1	3.0	1	2.5							1	0	0
493	0321.38	01.60	058	8	5			0	3.0	0	3.0	0	2.5	0	2.5	2	2.0	2	2.0	0	0	0
492	0321.69	01.87	062	8	6			1	2.5	1	3.0	1	2.5	1	2.5	2	2.0	2	2.0	0	0	0
489	0336.01	-05.23	066	8	7			1	2.5	1	2.5	4	2.0	8	1.5	8	1.0	8	1.0	0	2	1
490	0405.57	-02.32	061	6	5			0	3.0	0	3.0			1	2.5	1	2.0	1	2.0	0	1	0
491	0443.27	-07.70	039	6	5			0	3.0	0	3.0									1	0	0
372	0443.27	-05.16	033	5	5			0	3.0											1	0	0
367	0772.12	02.48	079	7	8			1	2.5	1	1.5	7	1.0	7	1.0	7	1.0	7	1.0	0	1	1
363	0303.08	-01.50	031	5	5			0	3.0											1	0	0
362	0990.36	01.99	084	6	10			0	3.0	0	3.0	0	2.5	0	2.5					1	0	0
355	1255.59	-00.88	064	6	6			0	3.0	0	3.0	0	3.0							1	0	0
356	1255.53	-01.20	057	5	5			0	3.0	0	3.0	1	2.5	3	2.0	5	0.0	5	0.0	0	0	0
353	1273.73	01.94	057	6	5			0	3.0	0	3.0									1	0	0
354	1271.87	03.36	055	5	5			0	3.0	0	2.5									1	0	0
352	1273.21	04.61	053	5	5			0	3.0	0	2.5									1	0	0

	Plant Coordinate					Census Date																
						5.22.12		6.10.12		6.26.12		7.10.12		7.24.12		8.07.12		8.20.12				
ID	x	y	Ht	Wh	LW	DW	D.S.	DW	D.S.	DW	D.S.	DW	D.S.	DW	D.S.	DW	D.S.	DW	D.S.	Br	Ca	DR
351	1280.46	03.29	029	7	6			0	3.0	0	2.5									1	0	0
350	1280.60	03.40	028	4	5			0	3.0	0	2.5									1	0	0
349	1280.60	03.42	031	6	5			0	3.0	0	2.5									1	0	0
348	1280.90	03.63	035	6	7			0	3.0	0	2.5									1	0	0
345	1322.07	-04.83	037	5	6			0	3.0	0	3.0	0	3.0							1	0	0
346	1321.43	-04.46	035	5	5			0	3.0	0	3.0	0	3.0							1	0	0
344	0034.31	-02.96	056	6	6					0	3.0									1	0	0
343	0034.11	-03.26	060	7	6					0	3.0									1	0	0
338	0037.06	-02.46	071	7	6					0	3.0	1	2.5	7	1.0	7	1.0	7	0.0	0	1	1
342	0039.71	01.61	085	7	6					0	3.0									1	0	0
341	0039.61	01.60	085	8	7					0	3.0	0	2.5	8	1.0	8	1.0	8	0.0	0	0	1
339	0043.81	-01.45	070	5	5					0	3.0	0	2.0	4	1.5	4	1.0	4	1.0	0	1	1
340	0045.81	-05.50	069	6	6					0	3.0	0	3.0	0	2.5	5	1.5	5	1.5	1	1	1
337	0043.91	-07.38	068	6	6					0	3.0	1	2.5	0	2.5	4	2.0	4	2.0	0	1	1
336	0042.81	-00.79	054	6	5					1	2.5			1	0.0	1	0.0	1	0.0	1	0	1
335	N/A	N/A	063	5	6					4	2.0									1	0	0
332	N/A	N/A	055	5	6					0	3.0									1	0	0
333	N/A	N/A	051	6	6					6	1.0	6	0.0	6	0.0	6	0.0	6	0.0	0	0	1
334	N/A	N/A	085	7	5					0	3.0	1	2.5	3	2.0	7	1.5	7	1.5	0	0	0
330	0343.47	05.00	070	7	7					1	2.5	1	2.5	2	2.5	6	1.5	6	1.5	0	1	0
331	0343.52	05.15	070	6	5					0	3.0	0	3.0	0	3.0	0	2.0	0	2.0	0	0	0
329	0383.54	-02.56	065	7	9					1	2.5	1	2.5	1	2.5	2	2.5	2	2.0	1	0	0
328	0512.70	01.70	064	6	3					1	2.5	2	2.5	4	2.0	6	0.0	6	0.0	0	0	0
327	N/A	N/A	067	6	6					6	1.0	6	0.0	6	0.0	6	0.0	6	0.0	0	0	1
326	0679.15	00.75	091	7	5					2	2.5	2	2.0	1	2.5	2	2.0	2	2.0	0	1	0
325	0883.63	03.13	145	8	14					1	2.5	1	2.5							1	0	0
324	0962.60	03.40	080	5	6					0	3.0	0	2.5							1	0	0

	Plant Coordinate					Census Date																
						5.22.12		6.10.12		6.26.12		7.10.12		7.24.12		8.07.12		8.20.12				
ID	x	y	Ht	Wh	LW	DW	D.S.	DW	D.S.	DW	D.S.	DW	D.S.	DW	D.S.	DW	D.S.	DW	D.S.	Br	Ca	DR
323	0941.62	01.48	097	6	6					1	2.5	1	2.5	1	2.5	1	2.5	1	2.5	0	2	1
265	1335.49	05.65	065	7	6					0	3.0	0	3.0	0	3.0	0	3.0	0	3.0	0	1	0
322	N/A	N/A	066	6	6					0	3.0	1	2.5	0	2.5	0	2.5	0	2.5	1	0	0
321	N/A	N/A	086	6	7					1	2.5	0	2.5	1	2.0	1	2.0	1	2.0	1	0	0
320	N/A	N/A	098	7	7					1	2.5	1	2.5	1	2.0					1	0	0
319	N/A	N/A	066	5	7					0	3.0	0	3.0							1	0	0
318	N/A	N/A	066	6	6					1	2.5	1	2.5							1	0	0
317	0953.67	-02.47	070	5	6					0	3.0	0	2.5	0	2.5	2	2.0	3	2.0	1	0	0
316	0977.82	-01.06	092	5	6					0	3.0	0	3.0							1	0	0
315	N/A	N/A	056	4	6					0	3.0	0	3.0							1	0	0
314	1275.03	-05.19	062	6	6					0	3.0	0	3.0	1	2.5	6	1.5	6	0.0	0	1	0
313	1313.10	-05.46	039	4	5					0	3.0	0	3.0							1	0	0
312	1314.23	-06.10	060	5	5					0	3.0									1	0	0
311	1320.20	-08.01	049	5	5					0	3.0	0	2.0							1	0	0
310	1320.15	-10.10	071	6	6					0	3.0	0	3.0							1	0	0
309	1320.19	-05.50	065	5	5					0	3.0	1	2.5	1	2.5					1	0	0
303	1325.99	03.94	044	4	5					0	3.0	1	2.5							1	0	0
304	1326.03	03.80	046	5	6					0	3.0	1	2.5							1	0	0
305	1326.01	04.18	041	4	5					0	3.0	1	2.5	1	2.5	1	2.5	1	2.5	1	0	0
306	1326.00	04.20	043	5	5					0	3.0	1	2.5							1	0	0
307	1325.99	04.76	063	5	6					0	3.0	0	3.0	1	3.0	1	3.0	1	3.0	1	0	0
308	1327.05	05.60	046	5	5					0	3.0	1	2.5							1	0	0
276	0687.55	-01.98	084	7	7					2	2.0	6	1.5	6	1.0	7	0.0	7	0.0	0	1	0

APPENDIX E

Non-Reproductively Mature Lily Demographic and Disease Severity Data Collected in 2011. Key to symbols: "Plot" = plot number; "L/Wh" = maximum number of leaves within a whorl; "Wh" = number of whorls; "SJ" = ("0" = seedling, "1" = juvenile); "D.S." = disease severity scale value. D.S. data for each sampling event are located below the corresponding census date.

					Census Date					
					6.11.11	6.24.11	7.07.11	7.24.11	8.06.11	8.20.11
n	Plot	L/Wh	Wh	SJ	D.S.	D.S.	D.S.	D.S.	D.S.	D.S.
001	1			0	2.5	2.5	2.0	2.0	2.0	0.0
002	1			0	2.0	2.5	2.0	2.0	2.0	0.0
003	1			0	2.0	2.5	2.0	2.0	0.0	0.0
004	1			0	2.5	2.5	1.5	1.0	0.0	0.0
005	1			0	2.5	2.5	2.0	1.0	0.0	0.0
006	1			0	2.5	2.0	2.0	1.5	0.0	0.0
007	1			0	2.0	2.0	2.0	1.0	0.0	0.0
008	1			0	2.0	2.0	1.5	1.5	0.0	0.0
009	1			0	2.0	2.0	1.5	0.0	0.0	0.0
010	1			0	2.5	1.5	2.0	0.0	0.0	0.0
011	1			0	2.0	1.5	2.0	0.0	0.0	0.0
012	1			0	2.0	1.5	1.5	0.0	0.0	0.0
013	1			0	2.5	1.5	1.5	0.0	0.0	0.0
014	1			0	2.0	1.0	1.0	0.0	0.0	0.0
015	1			0	2.0	1.0	0.0	0.0	0.0	0.0
016	1			0	1.5	0.0	0.0	0.0	0.0	0.0
017	1			0	2.0	0.0	0.0	0.0	0.0	0.0
018	1			0	2.0	0.0	0.0	0.0	0.0	0.0
019	1			0	2.0	0.0	0.0	0.0	0.0	0.0
020	1			0	2.0	0.0	0.0	0.0	0.0	0.0
021	1			0	2.5	0.0	0.0	0.0	0.0	0.0
022	1			0	2.0	0.0	0.0	0.0	0.0	0.0
023	1	4	1	1	2.5	1.0	1.5	1.5	1.5	0.0
024	1	4	1	1	2.0	2.0	1.5	1.5	0.0	0.0
025	1	3	1	1	2.0	2.0	1.5	0.0	0.0	0.0
026	1	2	1	1	2.5	1.5	1.5	0.0	0.0	0.0
027	1	5	1	1	2.0	2.0	1.5	0.0	0.0	0.0
028	1	5	1	1	2.0	2.0	1.5	0.0	0.0	0.0
029	1	3	1	1	2.0	1.5	0.0	0.0	0.0	0.0
030	1	2	1	1	2.0	1.5	0.0	0.0	0.0	0.0
031	1	6	1	1	2.0	1.5	0.0	0.0	0.0	0.0
032	1	3	2	1	1.5	2.0	0.0	0.0	0.0	0.0

					Census Date					
					6.11.11	6.24.11	7.07.11	7.24.11	8.06.11	8.20.11
n	Plot	L/Wh	Wh	SJ	D.S.	D.S.	D.S.	D.S.	D.S.	D.S.
033	2			0	2.5	2.0	0.0	0.0	0.0	0.0
034	2			0	2.5	2.0	0.0	0.0	0.0	0.0
035	2			0	2.5	0.0	0.0	0.0	0.0	0.0
036	2			0	2.0	0.0	0.0	0.0	0.0	0.0
037	2			0	2.5	0.0	0.0	0.0	0.0	0.0
038	2			0	2.5	0.0	0.0	0.0	0.0	0.0
039	2			0	2.5	0.0	0.0	0.0	0.0	0.0
040	2			0	2.5	0.0	0.0	0.0	0.0	0.0
041	2			0	0.0	0.0	0.0	0.0	0.0	0.0
042	2	1	4	1	0.0	0.0	0.0	0.0	0.0	0.0
043	2	4	4	1	1.5	1.5	0.0	0.0	0.0	0.0
044	3			0	1.5	1.5	0.0	0.0	0.0	0.0
045	3			0	3.0	3.0	2.5	0.0	0.0	0.0
046	3			0	3.0	3.0	0.0	0.0	0.0	0.0
047	3			0	2.5	2.5	0.0	0.0	0.0	0.0
048	3			0	1.5	0.0	0.0	0.0	0.0	0.0
049	3			0	1.0	0.0	0.0	0.0	0.0	0.0
050	3			0	1.0	0.0	0.0	0.0	0.0	0.0
051	3	2	5	1	2.5	2.0	1.5	1.5	0.0	0.0
052	3	1	5	1	2.5	2.5	2.0	0.0	0.0	0.0
053	3	1	5	1	2.0	1.0	1.5	0.0	0.0	0.0
054	3	1	5	1	2.5	2.0	0.0	0.0	0.0	0.0
055	3	1	4	1	2.0	1.5	0.0	0.0	0.0	0.0
056	3	1	4	1	2.0	1.0	0.0	0.0	0.0	0.0
057	4			0	1.5	0.0	0.0	0.0	0.0	0.0
058	4			0	2.5	1.0	0.0	0.0	0.0	0.0
059	4			0	2.5	0.0	0.0	0.0	0.0	0.0
060	4			0	1.5	0.0	0.0	0.0	0.0	0.0
061	4	1	4	1	1.0	0.0	0.0	0.0	0.0	0.0
062	4	2	4	1	0.0	0.0	0.0	0.0	0.0	0.0
063	4	1	4	1	0.0	0.0	0.0	0.0	0.0	0.0
064	4	2	5	1	0.0	0.0	0.0	0.0	0.0	0.0
065	5			0	2.5	2.5	0.0	0.0	0.0	0.0
066	5			0	3.0	2.0	0.0	0.0	0.0	0.0
067	5			0	2.0	0.0	0.0	0.0	0.0	0.0
068	5			0	1.0	0.0	0.0	0.0	0.0	0.0
069	5	1	3	1	2.5	2.0	2.0	0.0	0.0	0.0
070	5	1	5	1	1.5	0.0	0.0	0.0	0.0	0.0

					Census Date					
					6.11.11	6.24.11	7.07.11	7.24.11	8.06.11	8.20.11
n	Plot	L/Wh	Wh	SJ	D.S.	D.S.	D.S.	D.S.	D.S.	D.S.
071	5	2	4	1	2.0	1.5	2.0	1.5	0.0	0.0
072	6			0	2.5	2.5	2.5	1.5	0.0	0.0
073	6			0	2.5	2.5	2.5	0.0	0.0	0.0
074	6			0	2.5	2.5	2.5	0.0	0.0	0.0
075	6			0	2.5	2.5	2.5	0.0	0.0	0.0
076	6			0	2.5	2.5	2.5	0.0	0.0	0.0
077	6			0	2.5	2.5	2.0	0.0	0.0	0.0
078	6			0	2.5	2.5	2.0	0.0	0.0	0.0
079	6			0	2.5	2.0	2.0	0.0	0.0	0.0
080	6			0	2.5	2.0	2.0	0.0	0.0	0.0
081	6			0	2.5	2.0	1.0	0.0	0.0	0.0
082	6			0	2.5	2.0	1.0	0.0	0.0	0.0
083	6			0	2.5	2.0	1.0	0.0	0.0	0.0
084	6			0	2.5	2.5	2.5	0.0	0.0	0.0
085	6			0	2.5	2.5	2.0	0.0	0.0	0.0
086	6			0	2.5	2.5	0.0	0.0	0.0	0.0
087	6			0	2.5	1.5	0.0	0.0	0.0	0.0
088	6			0	2.5	1.5	0.0	0.0	0.0	0.0
089	6			0	2.5	0.0	0.0	0.0	0.0	0.0
090	6			0	2.5	0.0	0.0	0.0	0.0	0.0
091	6			0	2.5	0.0	0.0	0.0	0.0	0.0
092	6			0	1.5	0.0	0.0	0.0	0.0	0.0
093	6			0	1.0	0.0	0.0	0.0	0.0	0.0
094	6			0	1.0	0.0	0.0	0.0	0.0	0.0
095	6			0	1.0	0.0	0.0	0.0	0.0	0.0
096	6			0	1.0	0.0	0.0	0.0	0.0	0.0
097	6			0	2.0	0.0	0.0	0.0	0.0	0.0
098	6			0	2.0	0.0	0.0	0.0	0.0	0.0
099	6	4	5	1	3.0	2.5	2.5	2.5	0.0	0.0
100	6	2	5	1	3.0	2.5	2.0	2.0	0.0	0.0
101	6	2	4	1		2.5	2.0	1.5	0.0	0.0
102	6	1	4	1		2.5	2.0	2.0	0.0	0.0
103	6	2	4	1		2.5	1.5	0.0	0.0	0.0
104	6	1	6	1			2.0	0.0	0.0	0.0
105	6	1	4	1			2.0	0.0	0.0	0.0
106	6	1	3	1			2.0	0.0	0.0	0.0
107	6	4	5	1			2.5	0.0	0.0	0.0
108	6	4	5	1			2.0	0.0	0.0	0.0

					Census Date					
					6.11.11	6.24.11	7.07.11	7.24.11	8.06.11	8.20.11
n	Plot	L/Wh	Wh	SJ	D.S.	D.S.	D.S.	D.S.	D.S.	D.S.
109	7			0	2.5	2.5	2.0	0.0	0.0	0.0
110	7			0	2.0	2.0	0.0	0.0	0.0	0.0
111	7			0	3.0	2.5	0.0	0.0	0.0	0.0
112	7			0	3.0	2.0	0.0	0.0	0.0	0.0
113	7			0	2.0	1.5	0.0	0.0	0.0	0.0
114	7			0	1.5	2.0	0.0	0.0	0.0	0.0
115	7			0	2.5	0.0	0.0	0.0	0.0	0.0
116	7			0	2.5	0.0	0.0	0.0	0.0	0.0
117	7			0	3.0	0.0	0.0	0.0	0.0	0.0
118	7			0	3.0	0.0	0.0	0.0	0.0	0.0
119	7	1	3	1	1.0	0.0	0.0	0.0	0.0	0.0

APPENDIX F

Non-Reproductively Mature Lily Demographic and Disease Severity Data Collected in 2012. Key to symbols: "Plot" = plot number; "L/Wh" = maximum number of leaves within a whorl; "Wh" = number of whorls; "SJ" = ("0" = seedling, "1" = juvenile); "D.S." = disease severity scale value. D.S. data for each sampling event are located below the corresponding census date.

					Census Date					
					5.07.12	5.22.12	6.03.12	6.13.12	6.26.12	7.10.12
n	Plot	L/Wh	Wh	SJ	D.S.	D.S.	D.S.	D.S.	D.S.	D.S.
1	1			0	3.0	2.0	0.0	0.0	0.0	0.0
2	1			0	2.5	2.5	0.0	0.0	0.0	0.0
3	1			0	3.0	2.5	0.0	0.0	0.0	0.0
4	1			0	3.0	2.5	0.0	0.0	0.0	0.0
5	1			0	2.5	2.5	0.0	0.0	0.0	0.0
6	1			0	3.0	2.5	0.0	0.0	0.0	0.0
7	1			0	3.0	3.0	3.0	3.0	3.0	0.0
8	1			0	3.0	0.0	0.0	0.0	0.0	0.0
9	1			0	3.0	0.0	0.0	0.0	0.0	0.0
10	1	6	1	1	3.0	2.0	0.0	0.0	0.0	0.0
11	1	4	2	1	2.5	2.5	2.5	0.0	0.0	0.0
12	2			0	2.5	0.0	0.0	0.0	0.0	0.0
13	2			0	2.5	0.0	0.0	0.0	0.0	0.0
14	2			0	2.5	1.0	0.0	0.0	0.0	0.0
15	2			0	3.0	1.0	0.0	0.0	0.0	0.0
16	2			0	2.0	0.0	0.0	0.0	0.0	0.0
17	2			0	2.0	0.0	0.0	0.0	0.0	0.0
18	2			0	3.0	0.0	0.0	0.0	0.0	0.0
19	2			0	2.5	0.0	0.0	0.0	0.0	0.0
20	2			0	3.0	0.0	0.0	0.0	0.0	0.0
21	2			0	2.0	0.0	0.0	0.0	0.0	0.0
22	2			0	2.0	0.0	0.0	0.0	0.0	0.0
23	2			0	3.0	0.0	0.0	0.0	0.0	0.0
24	2			0	3.0	0.0	0.0	0.0	0.0	0.0
25	2			0	2.5	0.0	0.0	0.0	0.0	0.0
26	2			0	2.5	0.0	0.0	0.0	0.0	0.0
27	2	6	2	1	2.5	2.0	1.0	0.0	0.0	0.0
28	2	2	1	1	2.5	2.0	0.0	0.0	0.0	0.0
29	2	6	1	1	3.0	1.5	0.0	0.0	0.0	0.0
30	2	6	1	1	2.5	1.5	0.0	0.0	0.0	0.0
31	2	3	1	1	3.0	0.0	0.0	0.0	0.0	0.0
32	2	3	1	1	2.5	0.0	0.0	0.0	0.0	0.0

					Census Date					
					5.07.12	5.22.12	6.03.12	6.13.12	6.26.12	7.10.12
n	Plot	L/Wh	Wh	SJ	D.S.	D.S.	D.S.	D.S.	D.S.	D.S.
33	3			0	3.0	3.0	2.0	0.0	0.0	0.0
34	3			0	3.0	3.0	3.0	0.0	0.0	0.0
35	3			0	2.5	3.0	3.0	0.0	0.0	0.0
36	3			0	3.0	1.0	2.0	0.0	0.0	0.0
37	3			0	2.5	1.5	0.0	0.0	0.0	0.0
38	3			0	2.5	0.0	0.0	0.0	0.0	0.0
39	3			0	2.5	0.0	0.0	0.0	0.0	0.0
40	3			0	3.0	0.0	0.0	0.0	0.0	0.0
41	3			0	3.0	0.0	0.0	0.0	0.0	0.0
42	3			0	3.0	0.0	0.0	0.0	0.0	0.0
43	3			0	3.0	0.0	0.0	0.0	0.0	0.0
44	3			0	3.0	0.0	0.0	0.0	0.0	0.0
45	3	7	2	1	2.0	3.0	0.0	0.0	0.0	0.0
46	3	5	1	1	2.0	3.0	0.0	0.0	0.0	0.0
47	3	4	1	1	2.5	0.0	0.0	0.0	0.0	0.0
48	3	4	1	1	2.0	0.0	0.0	0.0	0.0	0.0
49	3	4	1	1	1.5	0.0	0.0	0.0	0.0	0.0
50	4			0	2.0	3.0	3.0	3.0	3.0	0.0
51	4			0	2.0	3.0	1.0	3.0	0.0	0.0
52	4			0	3.0	3.0	1.0	3.0	0.0	0.0
53	4			0	3.0	3.0	3.0	3.0	0.0	0.0
54	4			0	2.5	3.0	3.0	3.0	0.0	0.0
55	4			0	2.5	3.0	3.0	2.0	0.0	0.0
56	4			0	2.0	2.5	3.0	2.0	0.0	0.0
57	4			0	2.5	3.0	3.0	3.0	0.0	0.0
58	4			0	3.0	3.0	3.0	3.0	0.0	0.0
59	4			0	2.5	2.0	3.0	3.0	0.0	0.0
60	4			0	3.0	3.0	3.0	3.0	0.0	0.0
61	4			0	3.0	2.5	3.0	0.0	0.0	0.0
62	4			0	3.0	3.0	3.0	0.0	0.0	0.0
63	4			0	3.0	3.0	2.0	0.0	0.0	0.0
64	4			0	3.0	0.0	0.0	0.0	0.0	0.0
65	4			0	2.5	0.0	0.0	0.0	0.0	0.0
66	4			0	2.5	0.0	0.0	0.0	0.0	0.0
67	4			0	2.5	0.0	0.0	0.0	0.0	0.0
68	4	5	2	1	3.0	3.0	3.0	2.5	3.0	0.0
69	4	4	1	1	3.0	2.5	3.0	3.0	0.0	0.0
70	4	5	3	1	3.0	3.0	3.0	3.0	0.0	0.0

					Census Date					
					5.07.12	5.22.12	6.03.12	6.13.12	6.26.12	7.10.12
n	Plot	L/Wh	Wh	SJ	D.S.	D.S.	D.S.	D.S.	D.S.	D.S.
71	4	6	1	1	3.0	3.0	2.5	3.0	3.0	2.5
72	4	6	2	1	3.0	3.0	3.0	3.0	0.0	0.0
73	4	4	1	1	3.0	3.0	2.5	0.0	0.0	0.0
74	4	5	1	1		2.5	2.0	0.0	0.0	0.0
75	4	4	1	1		3.0	0.0	0.0	0.0	0.0
76	5			0	2.5	2.0	1.0	1.0	0.0	0.0
77	5			0	2.5	2.0	3.0	0.0	0.0	0.0
78	5			0	2.5	3.0	3.0	0.0	0.0	0.0
79	5			0	2.5	0.0	3.0	0.0	0.0	0.0
80	5			0	2.5	0.0	3.0	0.0	0.0	0.0
81	5			0	3.0	0.0	3.0	0.0	0.0	0.0
82	5			0	3.0	0.0	2.5	0.0	0.0	0.0
83	5			0	3.0	0.0	3.0	0.0	0.0	0.0
84	5			0	2.5	0.0	0.0	0.0	0.0	0.0
85	5			0	2.5	0.0	0.0	0.0	0.0	0.0
86	5	5	2	1	3.0	3.0	2.0	2.0	2.5	0.0
87	5	4	2	1	2.5	3.0	3.0	0.0	3.0	0.0
88	5	5	4	1	3.0	3.0	3.0	0.0	0.0	0.0
89	5	4	2	1		3.0	0.0	0.0	0.0	0.0
90	5	4	2	1		3.0	0.0	0.0	0.0	0.0
91	6			0	2.5	0.0	0.0	0.0	0.0	0.0
92	6			0	2.5	0.0	0.0	0.0	0.0	0.0
93	6			0	2.5	0.0	0.0	0.0	0.0	0.0
94	6			0	3.0	0.0	0.0	0.0	0.0	0.0
95	6			0	2.5	0.0	0.0	0.0	0.0	0.0
96	6			0	2.5	0.0	0.0	0.0	0.0	0.0
97	6			0	2.5	0.0	0.0	0.0	0.0	0.0
98	6			0	2.0	0.0	0.0	0.0	0.0	0.0
99	6			0	2.5	0.0	0.0	0.0	0.0	0.0
100	6			0	2.5	0.0	0.0	0.0	0.0	0.0
101	6	5	1	1	3.0	0.0	0.0	0.0	0.0	0.0
102	6	5	3	1	2.5	0.0	0.0	0.0	0.0	0.0
103	6	5	1	1	3.0	0.0	0.0	0.0	0.0	0.0
104	6	4	2	1	3.0	0.0	0.0	0.0	0.0	0.0
105	7			0	2.5	2.5	3.0	0.0	0.0	0.0
106	7			0	2.5	2.5	3.0	0.0	0.0	0.0
107	7	5	2	1	3.0	3.0	3.0	3.0	3.0	0.0
108	7	4	1	1	3.0	3.0	3.0	3.0	1.0	0.0

					Census Date					
					5.07.12	5.22.12	6.03.12	6.13.12	6.26.12	7.10.12
n	Plot	L/Wh	Wh	SJ	D.S.	D.S.	D.S.	D.S.	D.S.	D.S.
109	7	5	1	1	3.0	3.0	3.0	3.0	0.0	0.0
110	7	4	1	1	3.0	3.0	3.0	3.0	2.5	0.0
111	7	3	1	1	3.0	3.0	2.5	3.0	0.0	0.0
112	7	4	1	1	3.0	3.0	3.0	3.0	2.0	0.0
113	7	4	1	1	3.0	3.0	3.0	3.0	2.5	0.0
114	7	5	2	1	3.0	3.0	3.0	3.0	2.0	0.0
115	7	5	1	1	3.0	3.0	3.0	3.0	0.0	0.0
116	7	5	3	1	3.0	3.0	3.0	3.0	0.0	0.0
117	7	3	1	1	3.0	3.0	3.0	3.0	3.0	0.0
118	7	6	1	1	3.0	3.0	3.0	3.0	3.0	0.0
119	7	6	3	1	3.0	3.0	3.0	3.0	0.0	0.0
120	7	6	4	1				3.0	0.0	0.0

APPENDIX G

Morphological Diagnosis of *P. inconspicua*: Measurements of Conidial Length.

Identification Number of Plant Sampled											
	213	**222**	**235**	**260**	**272**	**274**	**282**	**295**	**434**	**442**	**498**
n	Length (μm)	Length (μm)	Length (μm)	Length (μm)	Length (μm)	Length (μm)	Length (μm)	Length (μm)	Length (μm)	Length (μm)	Length (μm)
1	101.25	90.82	93.07	101.25	81.05	105.24	64.73	83.72	91.94	56.03	50.58
2	89.64	84.91	92.42	69.28	77.96	88.01	78.99	101.86	85.87	68.97	52.97
3	72.23	85.83	105.52	92.12	87.66	93.48	82.11	94.31	71.28	67.67	61.39
4	98.86	80.17	85.83	96.24	87.10	84.51	42.43	67.93	83.88	65.41	64.42
5	92.16	65.76	88.88	123.52	85.81	94.92	51.09	91.52	76.16	52.64	66.16
6	78.22	83.52	99.82	105.23	72.68	67.93	73.88	95.59	92.84	64.06	68.94
7	83.00	72.29	104.65	105.81	82.71	65.97	64.59	93.48	87.96	47.89	68.99
8	95.55	90.51	79.02	109.40	74.87	100.84	66.92	96.64	89.79	98.19	73.94
9	87.43	93.66	93.58	108.96	87.43	98.12	69.14	100.5	75.15	45.09	75.00
10	96.26	92.55	82.82	97.69	55.82	101.26	80.55	89.84	84.61	57.93	75.56
11	98.16	73.80	90.69	88.82	90.34	102.05	84.02	88.26	78.74	62.21	78.80
12	115.86	75.96	82.89	104.36	78.77	87.35	87.49	90.22	84.43	43.81	79.30
13	103.22	59.98	85.78	88.37	85.02	106.09	69.25	75.34	64.09	61.02	83.44
14	92.56	65.99	80.84	81.48	104.92	88.66	65.44	65.73	89.38	101.65	84.56
15	95.31	74.25	82.85	70.58	68.07	97.73	62.21	99.39	92.20	106.86	87.22
16	92.79	54.61	90.84	67.51	83.69	81.98	63.26	94.41	62.08	112.32	91.90
17	98.40	92.64	79.42	95.70	89.52	86.96	55.82	102.92	88.38	80.17	92.88
18	99.34	80.14	80.88	99.32	82.31	97.15	66.22	96.92	62.62	105.58	94.70
19	92.44	93.40	84.73	87.65	87.75	93.98	76.31	106.84	64.54	91.61	101.81
20	100.83	88.78	98.84	83.44	85.87	82.17	79.62	76.47	76.65	61.10	113.03
	Mean	Mean	Mean	Mean	Mean	Mean	Mean	Mean	Mean	Mean	Mean
	94.17	79.98	89.17	93.84	82.47	91.22	69.2	90.59	80.13	72.51	78.28
	St.Dev	St.Dev	St.Dev	St.Dev	St.Dev	St.Dev	St.Dev	St.Dev	St.Dev	St.Dev	St.Dev
	9.15	11.51	7.94	14.21	9.63	10.78	11.19	11.12	10.26	21.65	15.52
	Min.	Min.	Min.	Min.	Min.	Min.	Min.	Min.	Min.	Min.	Min.
	72.23	54.61	79.02	67.51	55.82	65.97	42.43	65.73	62.08	43.81	50.58
	Max.	Max.	Max.	Max.	Max.	Max.	Max.	Max.	Max.	Max.	Max.
	115.86	93.66	105.52	123.52	104.92	106.09	87.49	106.84	92.84	112.32	113.03

APPENDIX H

Morphological Diagnosis of *P. inconspicua*: Measurements of Conidial Width.

Identification Number of Plant Sampled											
	213	**222**	**235**	**260**	**272**	**274**	**282**	**295**	**434**	**442**	**498**
n	Width (µm)	Width (µm)	Width (µm)	Width (µm)	Width (µm)	Width (µm)	Width (µm)	Width (µm)	Width (µm)	Width (µm)	Width (µm)
1	5.08	5.54	2.79	6.39	4.61	5.23	3.63	3.67	3.49	2.61	5.85
2	4.96	6.49	4.41	4.64	5.66	6.12	3.70	3.89	3.80	2.27	5.67
3	4.55	8.12	4.01	4.36	3.70	4.15	4.22	4.35	6.03	4.41	4.15
4	5.13	5.96	3.80	6.39	5.23	3.92	5.23	3.20	4.54	2.79	5.13
5	5.55	7.12	3.34	5.55	4.55	4.87	5.05	5.37	5.52	4.35	5.40
6	5.28	6.16	3.34	4.95	4.10	4.95	5.74	4.51	3.31	4.54	5.85
7	2.67	5.51	4.54	5.60	4.35	5.87	3.39	3.96	4.33	3.63	4.61
8	4.54	6.16	4.23	4.91	3.95	4.82	5.85	5.07	4.23	4.01	3.80
9	5.67	4.22	3.24	7.18	3.92	5.51	4.10	4.23	5.37	2.47	5.51
10	3.91	6.88	5.32	5.13	5.52	3.78	4.95	6.75	5.67	2.94	4.22
11	4.94	5.20	4.55	4.72	5.35	3.08	4.87	4.36	6.30	4.13	4.68
12	4.22	2.53	6.52	6.40	3.57	3.91	4.94	5.78	4.22	4.15	5.87
13	4.68	3.24	6.52	5.28	3.91	3.31	5.40	4.68	3.63	4.64	5.23
14	4.61	4.86	4.55	5.13	3.91	3.19	4.22	5.85	3.63	4.35	5.87
15	5.23	5.85	6.69	5.55	3.95	2.36	4.87	5.03	4.64	2.90	5.66
16	3.63	5.52	4.86	3.58	3.51	3.80	5.23	5.13	4.61	3.70	5.67
17	4.94	4.68	4.82	4.68	4.13	5.13	4.33	4.41	5.24	4.23	4.23
18	3.21	4.79	7.66	6.30	4.01	3.63	3.20	6.12	4.91	3.24	4.96
19	4.72	4.51	4.55	4.33	3.63	3.70	4.06	4.35	4.10	4.36	3.89
20	4.22	4.87	5.05	6.99	3.91	3.91	4.88	4.59	5.37	4.41	4.94
	Mean	Mean	Mean	Mean	Mean	Mean	Mean	Mean	Mean	Mean	Mean
	4.59	5.41	4.74	5.40	4.27	4.26	4.59	4.76	4.65	3.71	5.06
	St.Dev	St.Dev	St.Dev	St.Dev	St.Dev	St.Dev	St.Dev	St.Dev	St.Dev	St.Dev	St.Dev
	0.75	1.27	1.24	0.93	0.65	0.98	0.74	0.86	0.86	0.76	0.69
	Min.	Min.	Min.	Min.	Min.	Min.	Min.	Min.	Min.	Min.	Min.
	2.67	2.53	2.79	3.58	3.51	2.36	3.20	3.20	3.31	2.27	3.8
	Max.	Max.	Max.	Max.	Max.	Max.	Max.	Max.	Max.	Max.	Max.
	5.67	8.12	7.66	7.18	5.66	6.12	5.85	6.75	6.3	4.64	5.87

APPENDIX I

Morphological Diagnosis of *P. inconspicua*: Measurements of Conidial Attenuation.
Key: "M/A" = Width at "Mid-Point" or "Apex"; "Att." = attenuation.

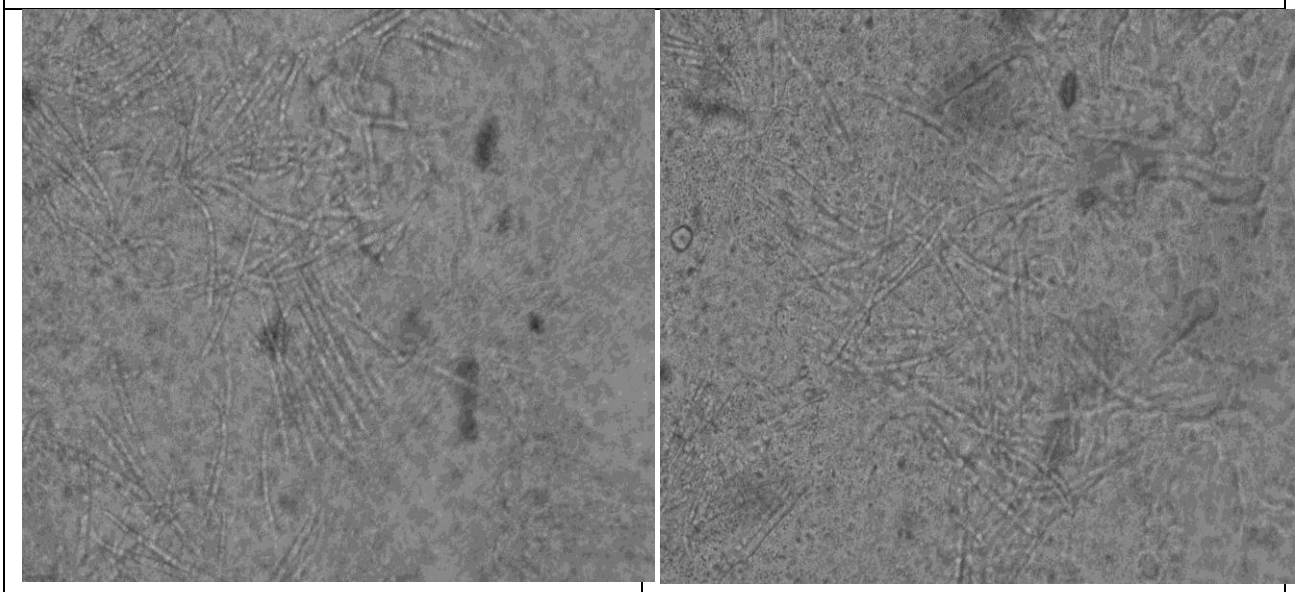
Identification Number of Plant Sampled												
		213	**222**	**235**	**260**	**272**	**274**	**282**	**295**	**434**	**442**	**498**
n	M/A	Att. (μm)	Att. (μm)	Att. (μm)	Att. (μm)	Att. (μm)	Att. (μm)	Att. (μm)	Att. (μm)	Att. (μm)	Att. (μm)	Att. (μm)
1	Mid-Point	6.26	3.78	6.10	5.28	3.70	4.61	3.31	2.77	3.70	3.39	3.24
	Apex	2.36	3.06	2.79	2.77	1.95	2.47	3.26	2.34	2.18	2.29	3.69
2	Mid-Point	4.68	3.63	5.66	8.01	4.06	5.05	3.34	3.70	3.92	2.90	5.51
	Apex	4.41	2.29	4.35	3.91	2.47	2.36	2.34	1.65	3.31	1.75	2.93
3	Mid-Point	4.33	5.52	3.70	5.55	3.49	4.68	4.22	3.63	3.45	4.10	5.12
	Apex	2.29	3.26	2.61	2.77	2.05	1.89	2.34	1.75	2.61	2.61	3.57
4	Mid-Point	5.20	8.73	6.17	5.35	2.68	4.41	5.23	3.20	3.20	3.45	6.47
	Apex	3.57	2.59	3.95	2.90	2.18	1.75	3.57	2.18	2.34	2.79	3.20
5	Mid-Point	5.24	6.57	4.41	6.49	4.61	6.20	4.82	5.37	3.63	2.92	5.95
	Apex	2.68	3.08	3.08	2.99	2.79	1.89	2.77	2.77	1.62	2.47	3.20
6	Mid-Point	3.34	4.41	5.05	6.26	4.23	3.31	3.67	6.30	4.91	2.67	3.94
	Apex	2.62	2.27	3.51	2.61	2.75	1.95	2.05	2.62	2.36	2.29	2.89
7	Mid-Point	3.96	6.40	5.96	6.69	4.51	3.95	3.51	4.27	3.96	2.77	3.94
	Apex	3.70	2.08	3.06	3.34	3.51	1.65	2.05	2.67	2.79	2.47	6.80
8	Mid-Point	5.20	4.41	5.35	6.77	4.79	4.68	3.08	4.23	3.67	3.89	3.94
	Apex	2.18	2.34	2.05	3.06	2.62	1.75	2.77	3.26	2.29	2.05	3.07
9	Mid-Point	5.20	5.51	4.01	6.39	6.44	2.61	4.22	4.36	3.51	3.89	4.86
	Apex	1.83	2.99	2.05	3.91	2.18	2.05	1.75	1.75	2.29	1.89	3.69
10	Mid-Point	5.32	4.06	4.55	5.13	4.41	4.13	3.91	5.54	3.39	3.24	4.22
	Apex	2.08	2.67	2.61	3.49	1.97	2.99	2.29	3.89	2.08	2.79	2.75
11	Mid-Point	4.36	6.96	5.19	4.68	4.23	3.78	4.91	3.24	5.66	4.01	6.42
	Apex	2.79	2.94	2.47	2.62	2.27	2.29	2.47	2.18	2.59	1.65	2.59
12	Mid-Point	3.89	5.03	4.54	6.39	3.91	2.79	4.72	4.22	6.88	4.88	4.78
	Apex	2.29	3.49	2.92	3.92	2.36	1.97	2.99	3.63	2.99	2.08	3.24
13	Mid-Point	5.07	6.37	4.33	6.17	4.91	4.23	4.36	3.70	2.99	3.58	5.95
	Apex	2.18	4.54	1.75	3.08	2.47	2.61	2.92	2.05	2.27	1.65	3.20
14	Mid-Point	4.52	4.86	3.96	7.71	3.19	3.70	4.15	5.13	4.82	3.26	4.71
	Apex	2.36	2.77	1.89	2.34	1.89	2.47	2.34	2.61	2.47	1.89	2.46
15	Mid-Point	5.60	6.62	5.80	6.69	4.61	3.92	3.45	4.06	5.24	3.26	4.12
	Apex	2.29	1.95	2.53	3.49	2.77	2.05	1.75	2.60	3.24	2.27	2.46
16	Mid-Point	3.70	7.79	4.95	6.57	5.13	3.20	4.72	3.63	4.59	3.26	5.51
	Apex	2.29	3.80	2.27	2.99	2.29	2.79	2.27	2.29	3.67	1.65	3.07
17	Mid-Point	5.23	7.00	4.35	5.67	3.95	5.67	5.19	6.37	5.55	5.80	5.74
	Apex	2.29	2.99	2.18	3.24	1.95	2.34	2.92	2.67	2.47	1.97	2.64
18	Mid-Point	5.60	5.66	4.01	6.37	4.87	3.24	3.39	4.79	6.66	3.34	5.35
	Apex	2.27	3.49	3.70	3.24	2.68	2.05	2.77	2.99	3.89	3.21	3.20
19	Mid-Point	5.32	6.37	5.85	6.40	5.44	4.68	4.41	4.41	3.26	3.21	6.87
	Apex	2.18	3.63	1.83	2.99	2.94	1.97	2.77	2.75	1.83	2.08	4.74
20	Mid-Point	5.96	5.60	5.58	7.49	3.91	4.79	4.33	4.15	4.23	3.78	6.30
	Apex	2.34	2.59	2.79	3.20	1.65	1.95	2.62	3.06	2.34	2.18	5.02

APPENDIX J

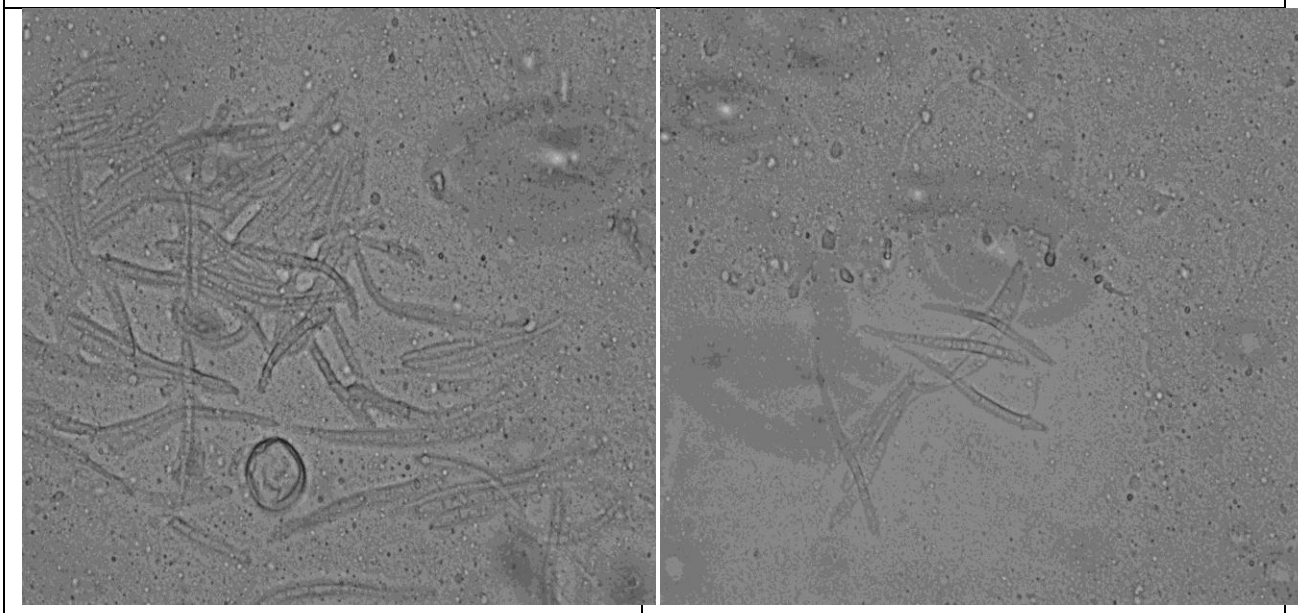
Photographs of Conidia Obtained from Diseased Hosts within the Field and Used for the Morphological Diagnosis of *P. inconspicua*.

Key: Plant Identification Number (PIN) = plant sample was obtained from. All photographs were taken at 200x magnification.

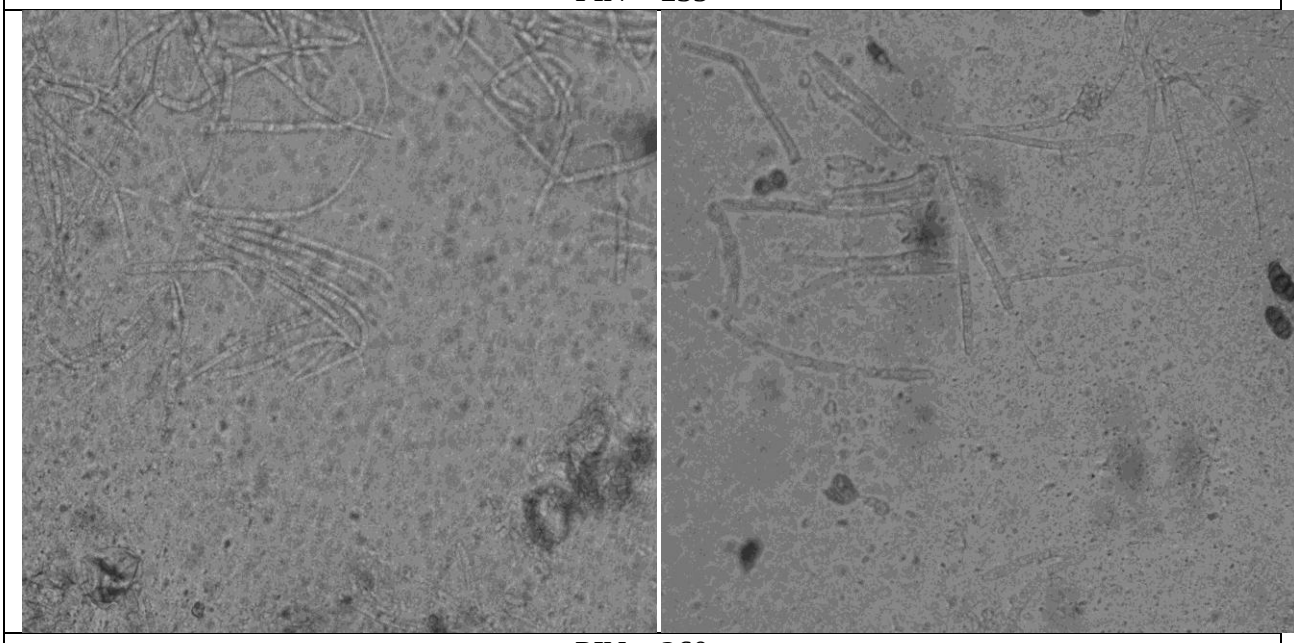
PIN = 213



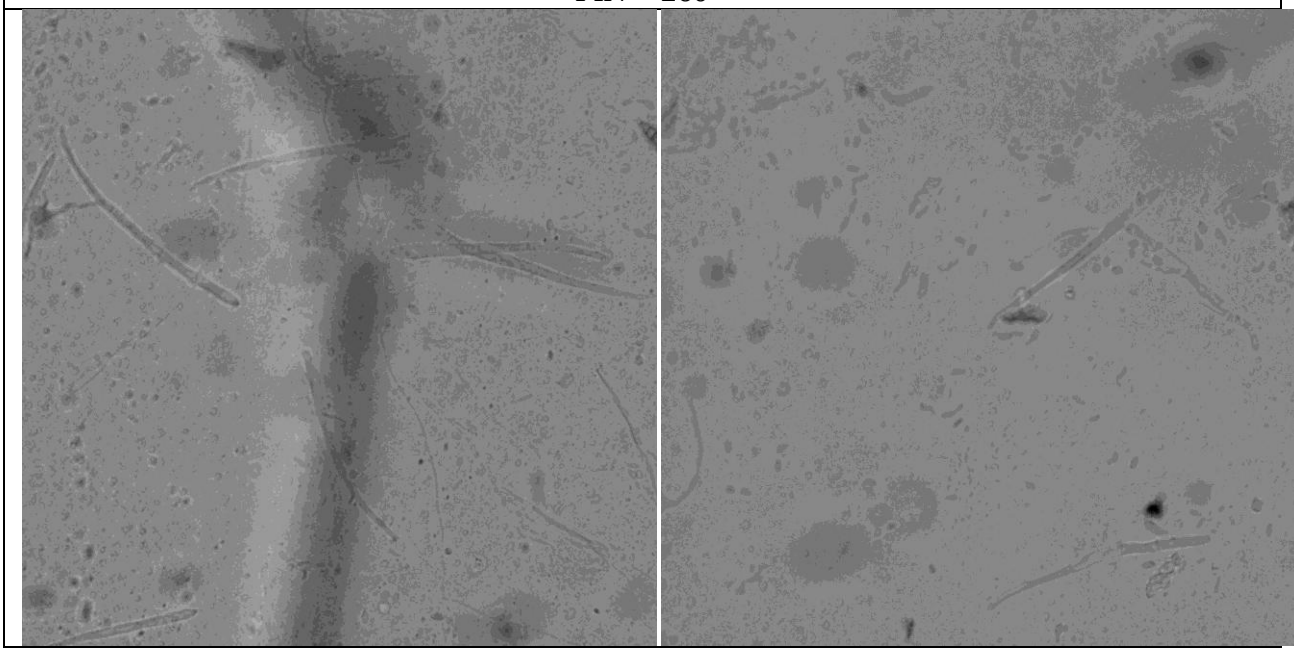
PIN = 222



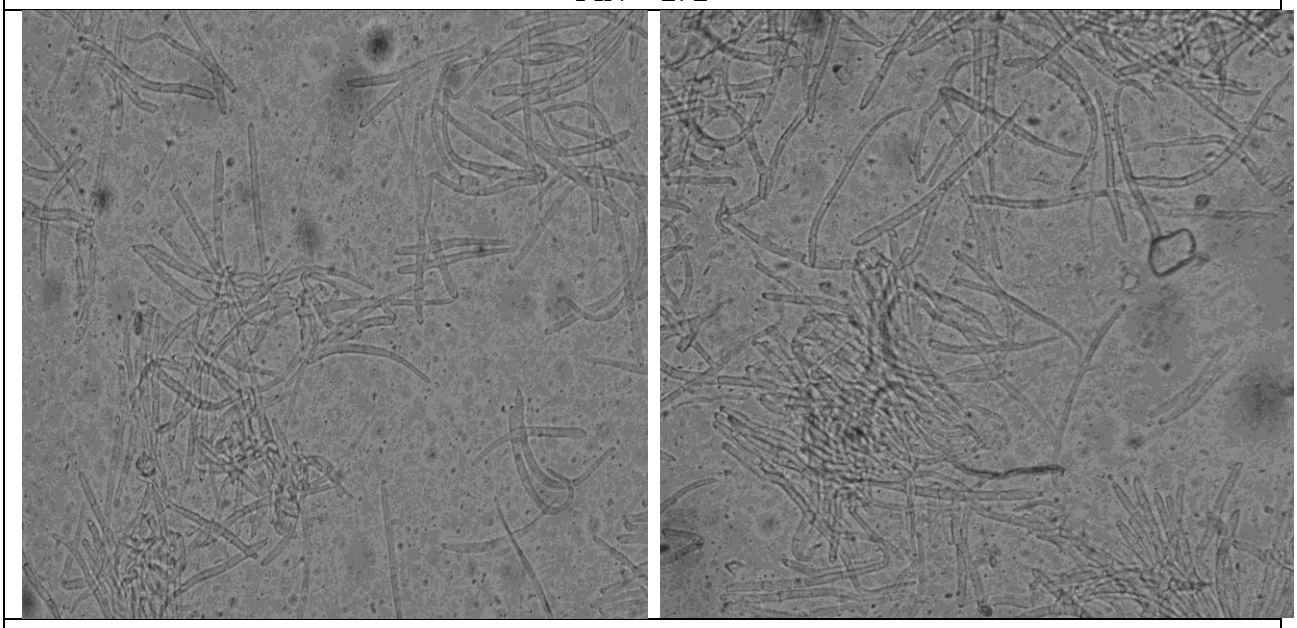
PIN = 235



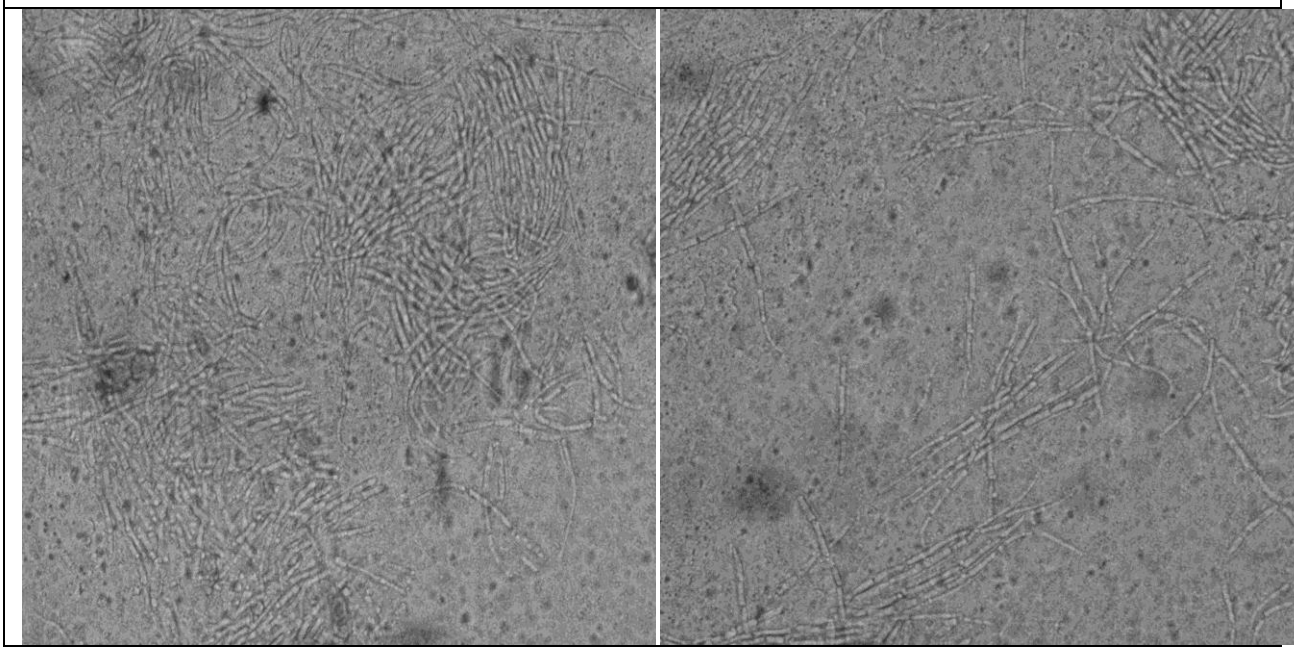
PIN = 260



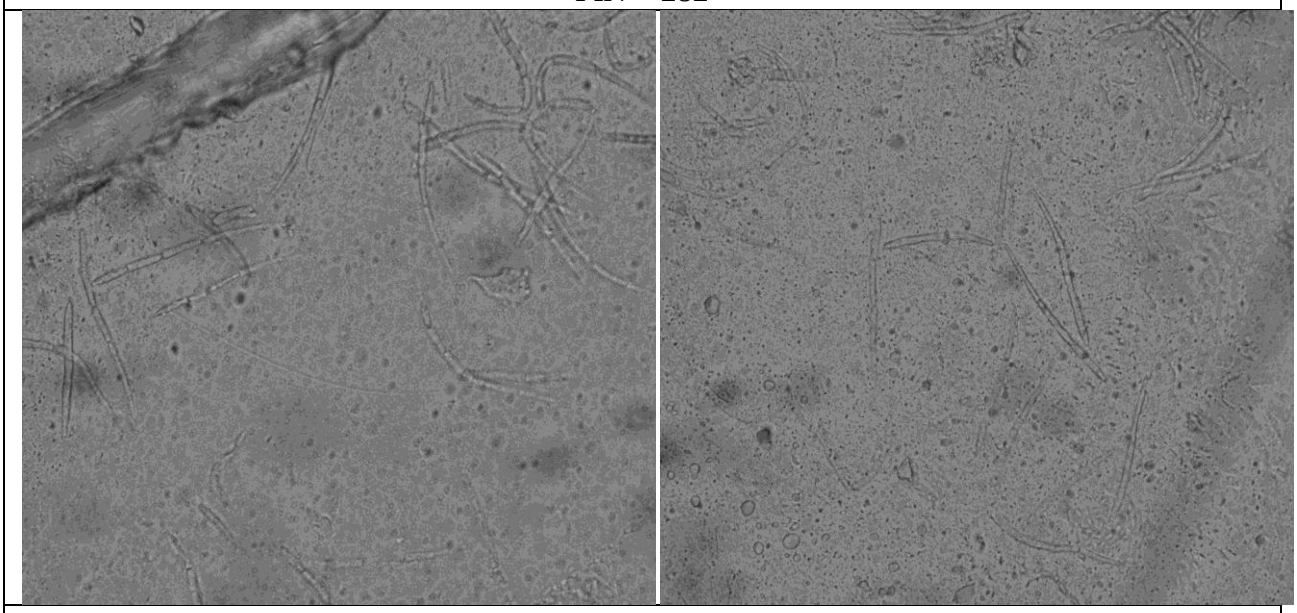
PIN = 272



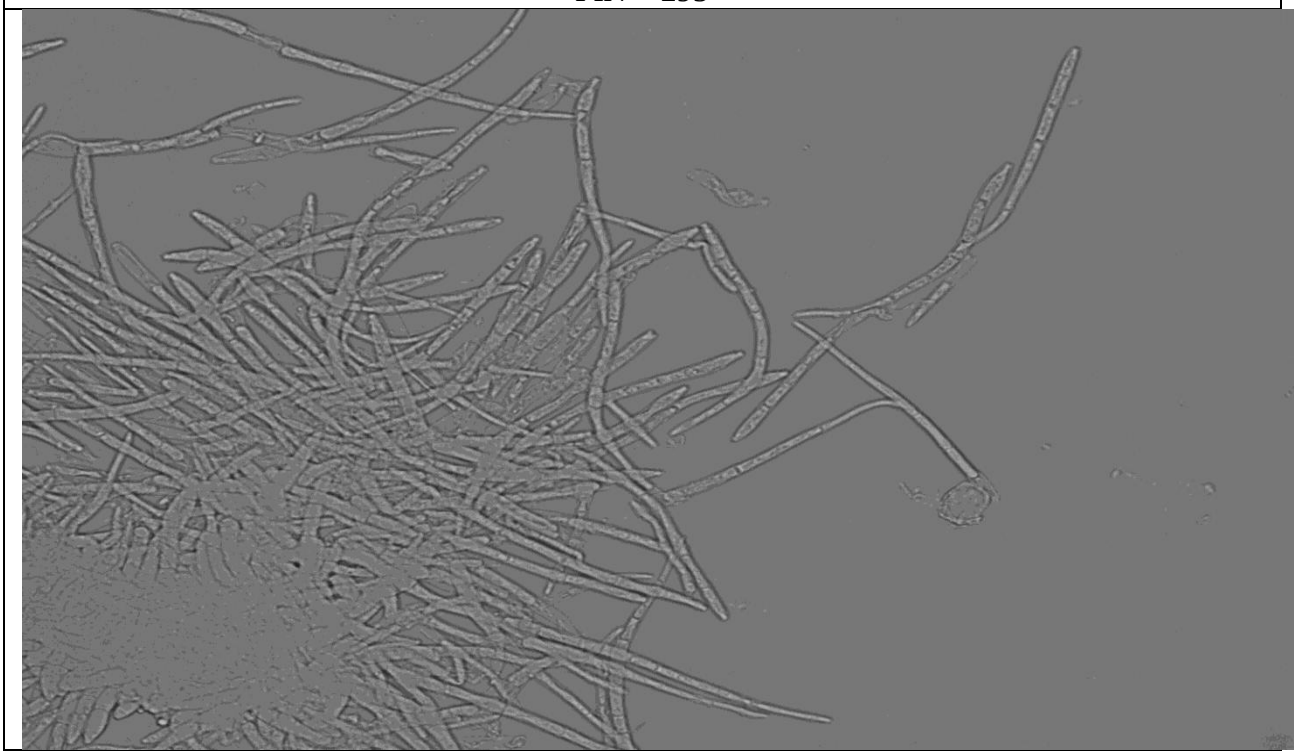
PIN = 274



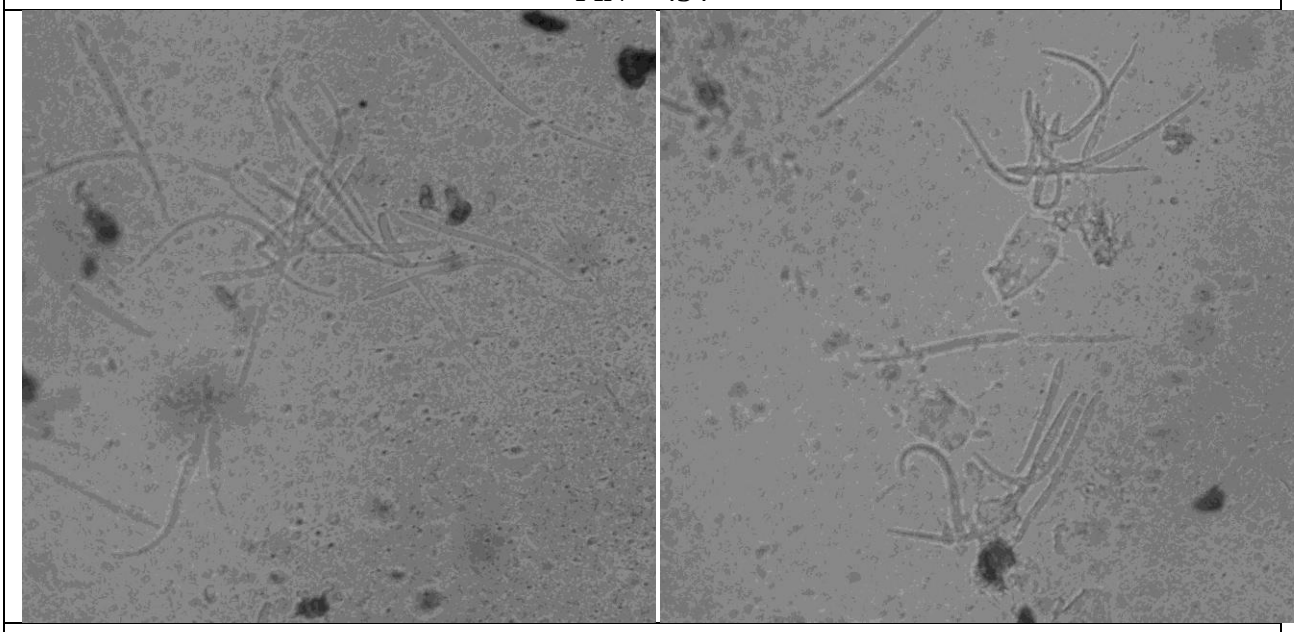
PIN = 282



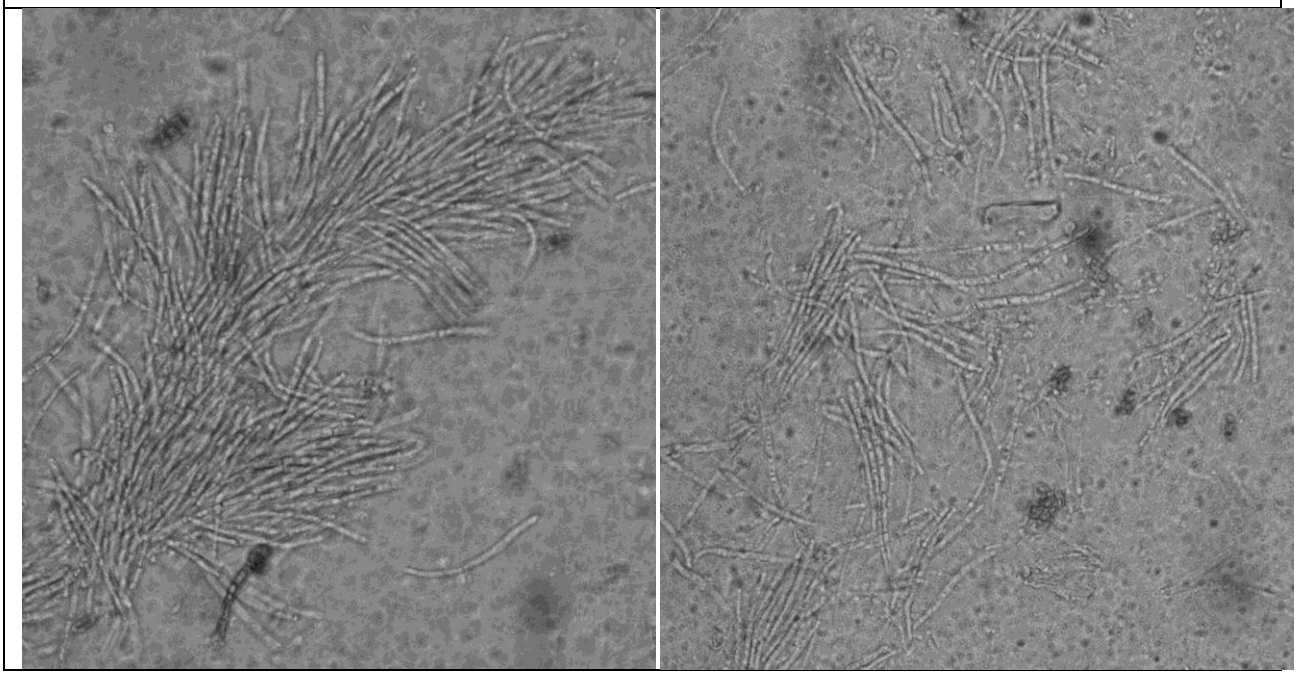
PIN = 295



PIN = 434



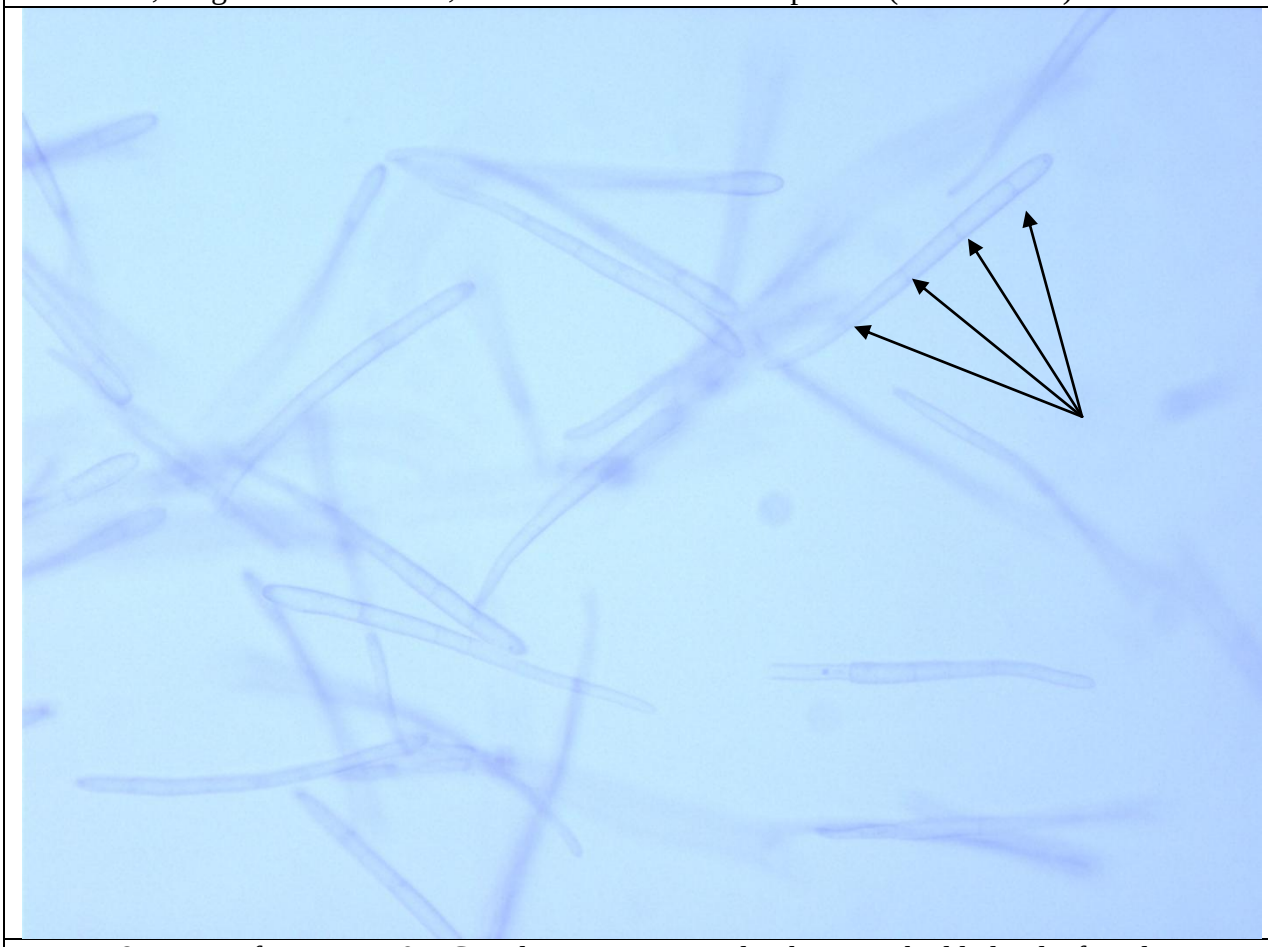
PIN = 442



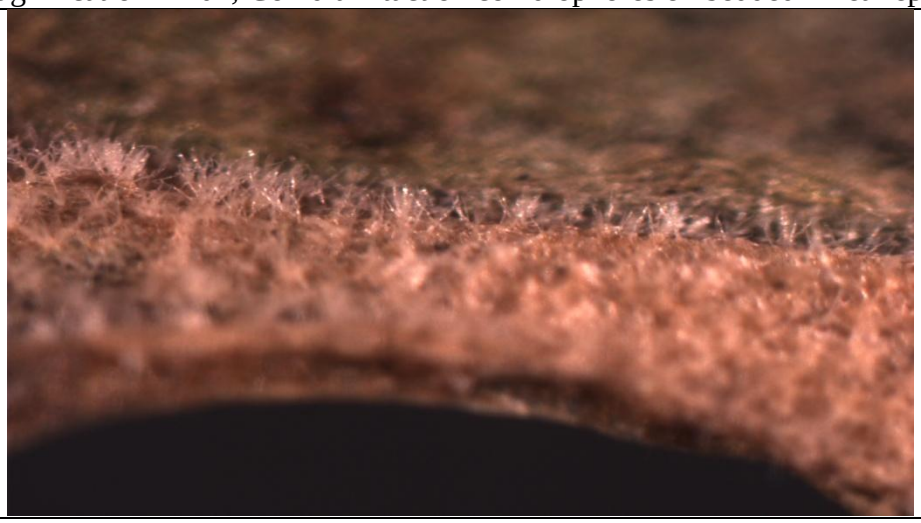
APPENDIX K

Photographs Depicting the Diagnostic Morphological Characteristics of *P. inconspicua* Conidia.

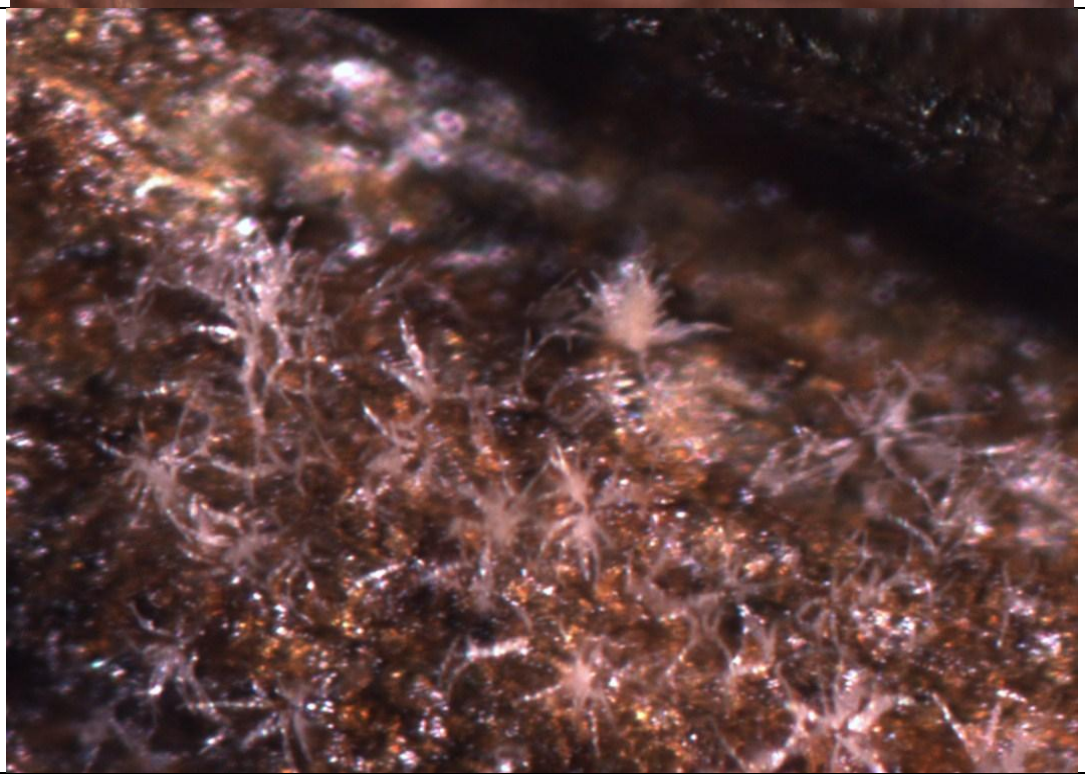
PIN = 294; Magnification = 400x; Fusiform structure and septation (black arrows) are shown.



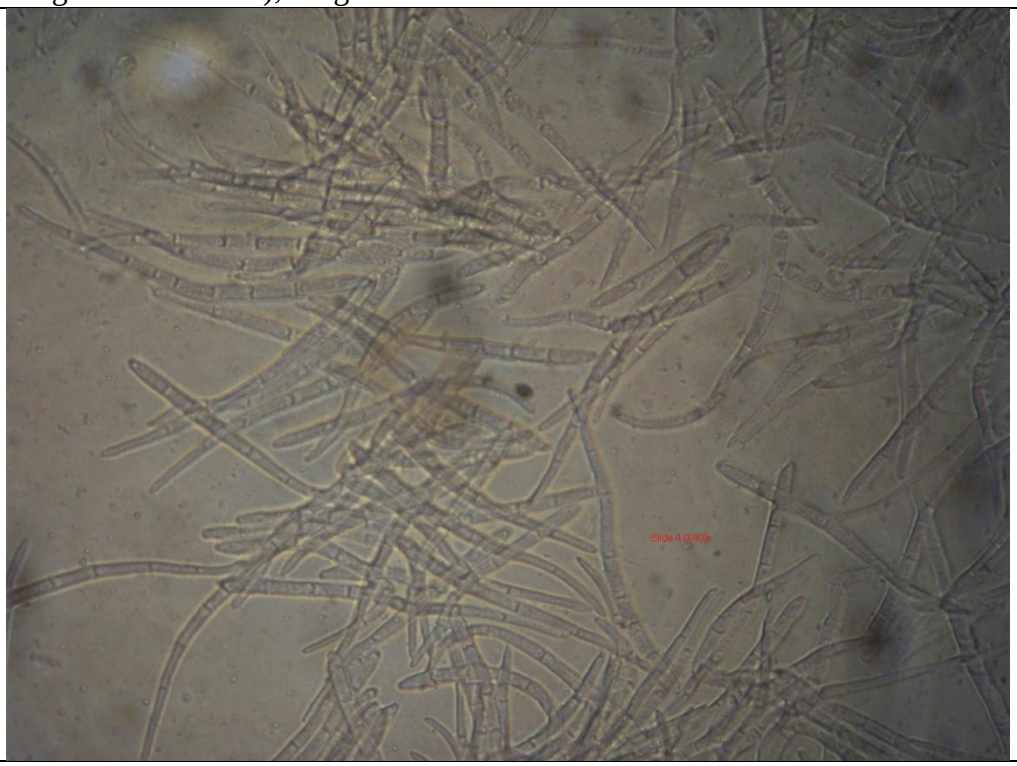
PIN = 294; Magnification = 20x; Conidia intact on conidiophores embedded in leaf epidermis.



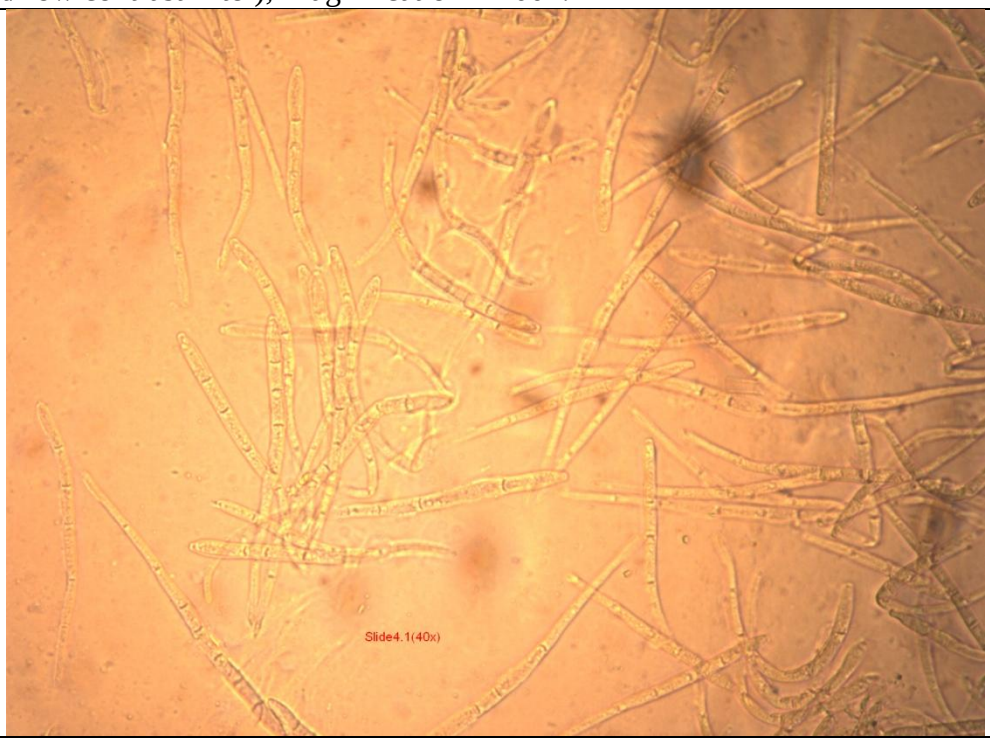
Photographs of diseased lily leaf with *P. inconspicua* conidia intact on conidiophores embedded in leaf epidermis. Magnification = 90x.



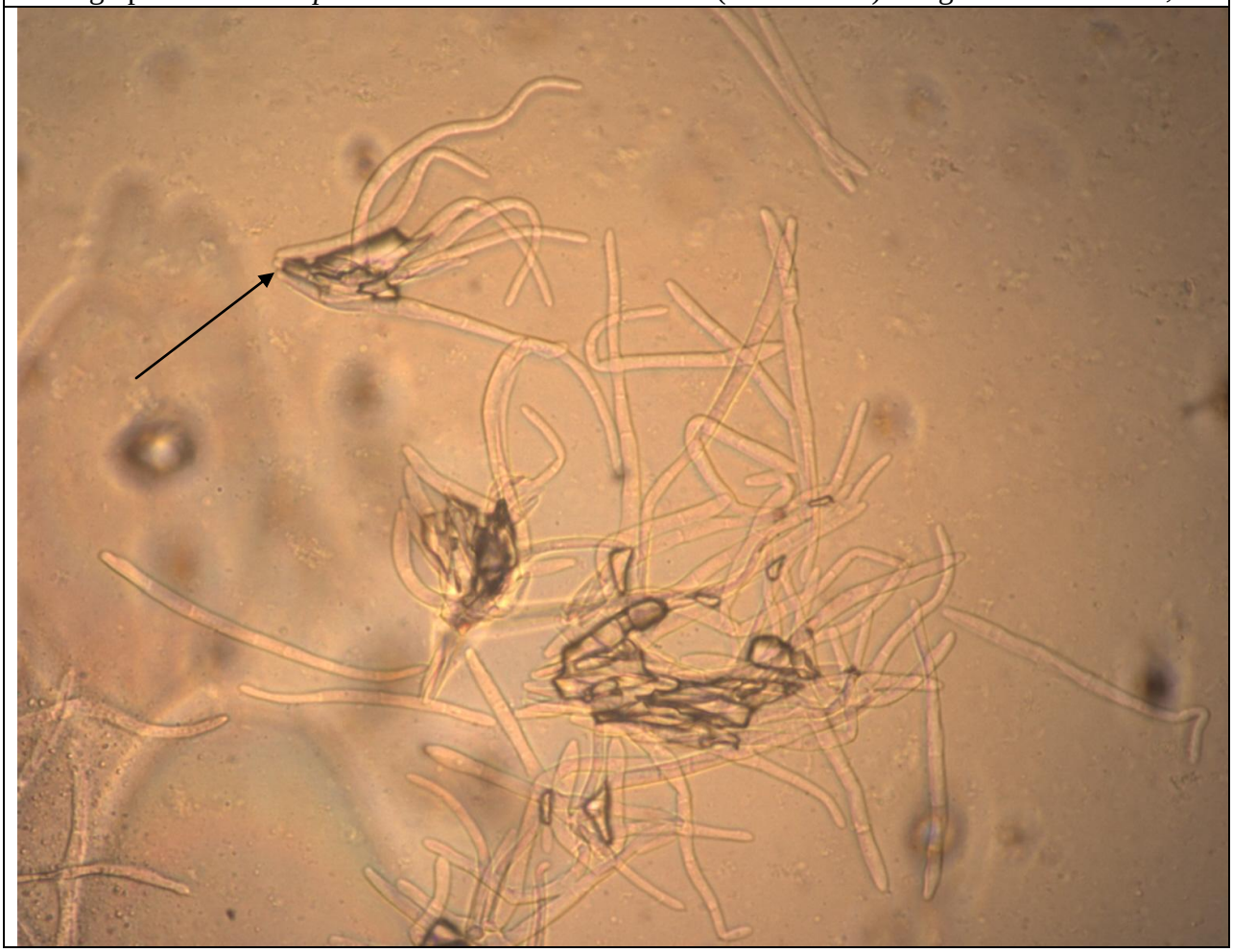
Photograph of *P. inconspicua* conidia depicting characteristic fusiform structure and septation. (Low light/high contrast filter); Magnification = 400x



Photograph of *P. inconspicua* conidia depicting characteristic fusiform structure and septation. (High light/ low contrast filter); Magnification = 400x.



Photograph of *P. inconspicua* conidia intact on stromata (black arrow). Magnification = 400x;



APPENDIX L

Photographs of *L. grayi* plants suffering from the lily leaf spot disease caused by *P. inconspicua*.

Photographs of seedling and juvenile *L. grayi* exhibiting symptoms of the lily leaf spot disease.

Photographs of adult *L. grayi* plants Moderately to Severely Diseased by *P. inconspicua*.

Photographs of adult *L. grayi* plants Moderately to Severely Diseased by *P. inconspicua*.

APPENDIX M

Photographs of Healthy and Diseased *L. grayi* Seed Capsules Used within the Study of Seed Viability.

Healthy Seed Capsules	Diseased Seed Capsules
	
	
	

APPENDIX N

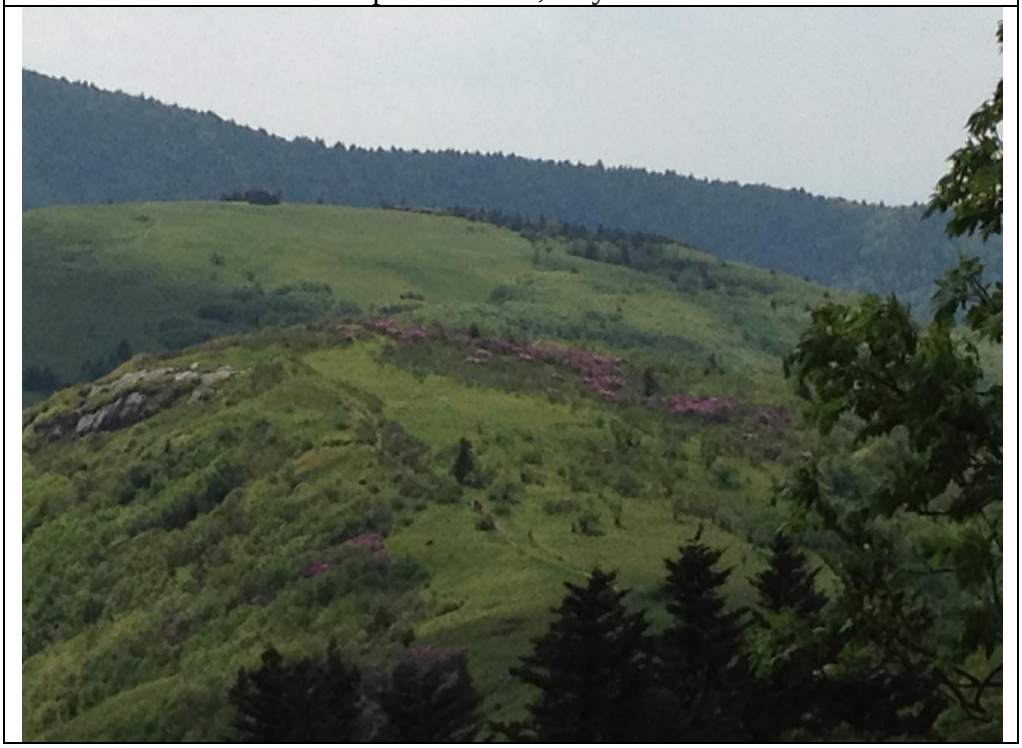
Photographs of *L. grayi* in Flower.

APPENDIX O

Photographs of the Grassy Balds on Roan Mountain.

View of the Appalachian Trial on the North side of Round Bald, June 2012.

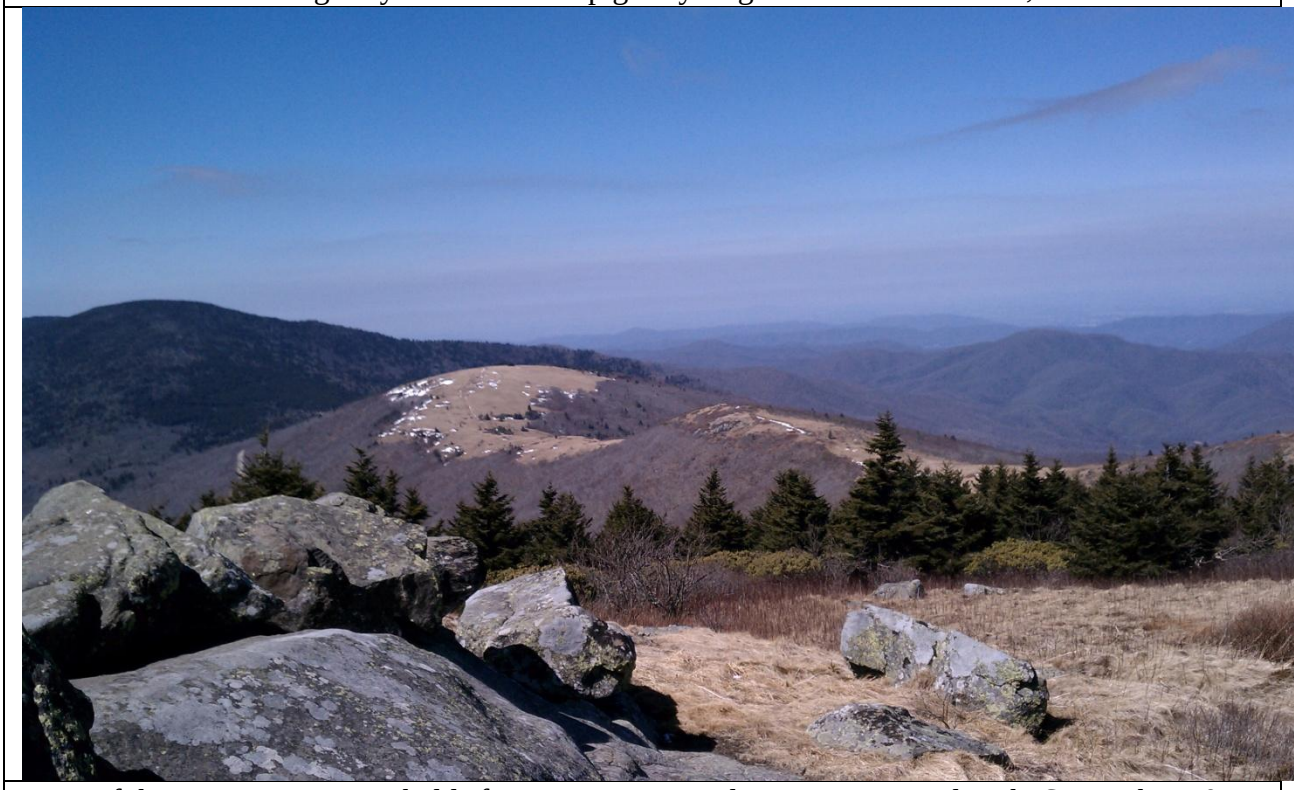
View of Jane Bald from atop Alder Bald, July 2011.



View of the north side of Jane Bald, July 2011.

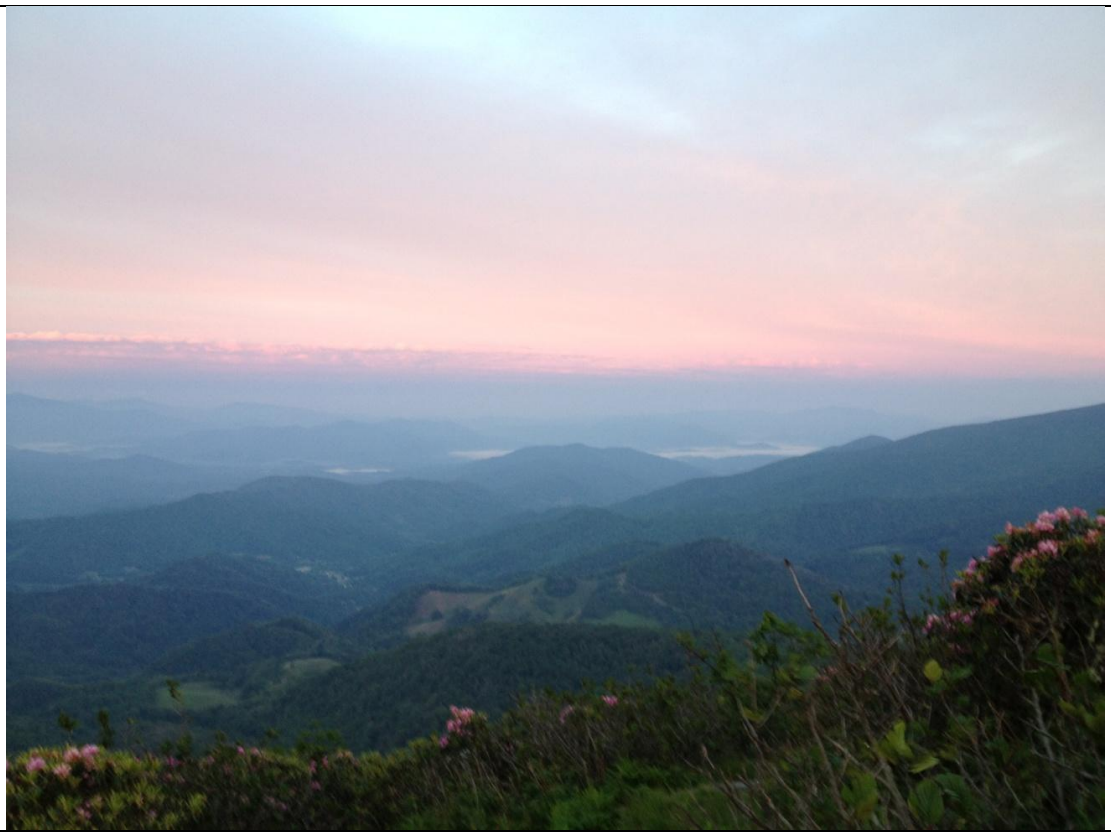
View of Alder Bald from atop Grassy Ridge near the memorial rock, July 2012.

View of the Roan Mtn grassy balds from atop grassy ridge near memorial rock, March 2011.

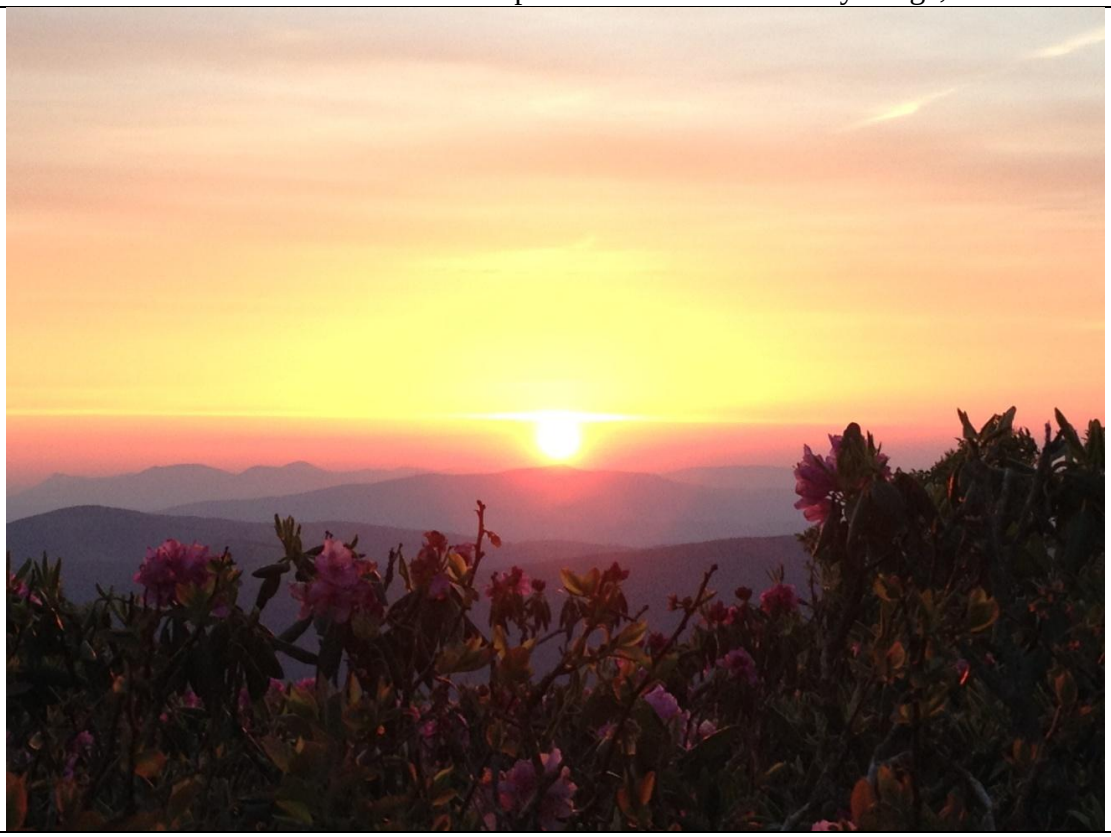


View of the Roan Mtn grassy balds from atop grassy ridge near memorial rock, September 2011.

South-east view of North Carolina from atop the heath bald on Grassy Ridge, June 2012



North-east view of North Carolina from atop the heath bald on Grassy Ridge, June 2012.



VITA

RUSSELL JACKSON INGRAM

Personal Data:

Date of Birth: November 26, 1984
Place of Birth: Augusta, GA
Marital Status: Single

Education:

East Tennessee State University, Johnson City, TN; Biology, M.S., 2013
Augusta State University, Augusta, GA; Biology, B.S., 2010; Study Abroad Program, University of Salamanca, Salamanca, Spain, 2009;Study Abroad Program, Endemic Species of the Southern Cape, Capetown, South Africa, 2007
Oak Ridge Military Academy, Oak Ridge, North Carolina; H.S.D., 2003

Professional Experience:

Graduate Teaching Assistant, East Tennessee State University, Johnson City, TN, 2011- 2013
Greenhouse Manager, East Tennessee State University, Johnson City, TN, 2012- 2013
Herbarium Assistant, East Tennessee State University, Johnson City, TN, 2011
Undergraduate Teaching Assistant, Augusta State University, Augusta, Ga, 2008
Greenhouse Manager, Augusta State University, Augusta, GA, 2007-2010

Honors and Awards:

Dr. Denise Pav Scholarship Award, $500,
East Tennessee State University, Johnson City, TN, 2013
School of Graduate Studies Research Grant Award, $800,
East Tennessee State University, Johnson City, TN, 2012
Marcia Davis Research Award for Graduate Research in Conservation of Natural Resources, $500, East Tennessee State University, Johnson City, TN, 2011
Dean's List, Augusta State University, Augusta, GA, 2009
Best Poster Presentation Award, Phi Kappa Phi 10th Annual Student Research and Fine Arts Conference, $100, Augusta State University, Augusta, GA, 2009
Dean's List, Augusta State University, Augusta, GA, 2005

Research Grants:

Appalachian Trial Conservancy Research Grant, $1300, 2012
East Tennessee State University School of Graduate Studies Research Grant, $800, 2012
Appalachian Trial Conservancy Research Grant, $1300, 2011

Professional Presentations:

Ingram, R., F. Levy. 2013. Demography and disease of *Lilium grayi* (Gray's lily) on Roan Mountain TN/NC. Proceedings. American Society of Plant Biologists Southern Section Meeting. April 6-8. Oral Presentation. Little Rock, AR.

Ingram, R., F. Levy. 2013. Demography and disease of *Lilium grayi* (Gray's lily) on Roan Mountain TN/NC. Proceedings. American Phytopathological Society Southern Division Meeting. February 8-10. Oral Presentation. Baton Rouge, LA.

Ingram, R., F. Levy. 2012. The plight of Gray's lily on Roan Mtn. Appalachian Trail Conservancy and the U.S. Forest Service. Oral Presentation. USDA Forest Service, Asheville, NC.

Ingram, R., F. Levy. 2012. Demography and disease of Gray's lily on Roan Mountain. Southeastern Population Ecology and Evolution Genetics Conference. October 12-14. Oral Presentation. Clemson, SC.

Ingram, R., F. Levy. 2011. Demography and disease of Gray's lily on Roan Mountain. East Tennessee State University Department Seminar Series. Oral Presentation. Johnson City, TN

Ingram, R., S. Bennetts. 2010. Indications of genetic differences in plant development between two populations of *Phacelia dubia* var. *Georgiana.* Annual Meeting of the Association of Southern Biologists. April 7-10. Poster. Asheville, NC.

Ingram, R., S. Bennetts. 2010. A novel adaptation in *Phacelia dubia* var. *Georgiana (Hydrophyllaceae).* Phi Kappa Phi 11th Annual Student Research and Fine Arts Conference. March 10. Poster. Augusta State University. Augusta, GA.

Ingram, R., D. Wear. 2009. The effects of a summer prescription burn on the community structure of gopher tortoise forage. Proceedings. Gopher Tortoise Council Annual Meeting. October 2-3. Oral Presentation. Gainesville, FL.

Ingram, R., D. Wear. 2009. The effects of a summer prescription burn on the community structure of gopher tortoise forage. Phi Kappa Phi 10th Annual Student Research and Fine Arts Conference. March 11. Poster. Augusta State University. Augusta, GA

Ingram, R., D. Wear. 2008. The effects of a summer prescription burn on the community structure of gopher tortoise forage. Student Research and Scholarship Brown Bag Series. Spring. Oral Presentation. Augusta State University. Augusta, GA

Research Publications:

Ingram, R., F. Levy. 2013. Cause and Impacts of the Early Season Collapse of *Lilium grayi* (Gray's lily), on Roan Mountain, TN/NC. Manuscript in preparation.

Ingram, R., F. Levy. 2013. Demography and disease of *Lilium grayi* (Gray's lily) on Roan Mountain TN/NC. Proceedings. American Society of Plant Biologists Southern Section Meeting. April 6-8. Little Rock, AR.

Ingram, R., F. Levy. 2013. Demography and disease of *Lilium grayi* (Gray's lily) on Roan Mountain TN/NC. Proceedings. American Phytopathological Society Southern Division Meeting. February 8-10. Baton Rouge, LA.

Ingram, R., F. Levy. 2012. Demography and disease of Gray's lily on Roan Mountain. Southeastern Population Ecology and Evolution Genetics Conference. October 12-14. Clemson, SC. Abstracts in program.

Articles:

Georgia Botanical Society Newsletter "Gray's Lily on Roan Mtn.", September 2012 Issue

Media Coverage:

Johnson City Press, Newspaper article, "The Plight of Gray's Lily on Roan Mtn." July 2012

Fox, Carol, ETSU Website Homepage Highlighted Research, "Consider the Lilies of the Field: Russell Ingram does just that with his research on the Roan Balds." June 2012

Professional Associations:

American Phytopathological Society, Member
American Society of Plant Biologists, Member
Georgia Botanical Society, Member