This is to certify that the

dissertation entitled

Double-stranded RNA mediated recovery of
American chestnut populations:
A demographic analysis

presented by

Anita L. Davelos

has been accepted towards fulfillment
of the requirements for

Ph.D. degree in Botany and Plant Pathology

Andrew M. Jarosz
Major professor

Date 26 Aug 99

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DOUBLE-STRANDED RNA MEDIATED RECOVERY OF AMERICAN CHESTNUT POPULATIONS: A DEMOGRAPHIC ANALYSIS

By

Anita L. Davelos

A DISSERTATION

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

Department of Botany and Plant Pathology

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ABSTRACT

DOUBLE-STRANDED RNA MEDIATED RECOVERY OF AMERICAN CHESTNUT POPULATIONS: A DEMOGRAPHIC ANALYSIS

By

Anita L. Davelos

Cryphonectria parasitica, the chestnut blight pathogen, can cause serious reductions of the survival and reproduction in American chestnuts (*Castanea dentata*). This pathogenic fungus can be infected with a cytoplasmic hyperparasite (a double-stranded (ds) RNA) which debilitates the pathogen and reduces its virulence. In host populations infected with hyperparasitized fungi, trees can respond to infection and recover. However, no work has determined if recovery of individual trees translates into recovery at the population level. The main objectives of this study are: (1) evaluate the effects of *C. parasitica* on trees of varying size; (2) determine how disease alters host demographics; and (3) evaluate the extent of dsRNA mediated recovery in American chestnut populations.

Inoculation and natural infection studies revealed that small individuals succumb to infection regardless of the virulence of the inoculant. The presence of dsRNA appears to delay death for medium to large sized branches.

Matrix projection models compared the finite rate of population increase (λ) and size distributions of healthy, recovering, and non-recovering populations of the American chestnut. Disease reduced population growth rates of non-recovering. Further,

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retrogressions in size of large individuals are observed in diseased populations but not in healthy ones resulting in an increased frequency of small to mid-sized trees in non-recovering populations. Transition matrices for recovering populations contained characteristics of both healthy and non-recovering populations, and population growth rates tended to be slightly lower than growth rates found in healthy populations.

Sensitivity and elasticity analyses indicate that dsRNA should be introduced onto 1-10 cm dbh trees in non-recovering populations to have the largest impact on population growth rates. The *G/L/F* (Growth/Longevity/Fecundity) elasticity ratio, used to detect stressed populations in conservation biology, did not detect the effects of chestnut blight epidemics. Since pathogen infections do not materially affect survivorship, epidemics have little effect on the *G/L/F* portrayal of a population.

Studies on the effects of disease on seedling survival and growth revealed that the disease status of the adult population generally affects emergence and survival of seedlings with a trend for recovering populations to perform best. Disease status does not influence final seedling size; rather, final seedlings size was influenced by population.

This study emphasizes the need to examine not only effects of infection on individuals but also on populations. Matrix projection models are an effective tool for predicting population growth rates and examining the relative contributions of different life history stages to population growth rates. This information allows effective management strategies to be developed.

To my father

ACKNOWLEDGMENTS

To my advisor, Andy Jarosz, I express my deepest gratitude for his encouragement, guidance, good humor, and willingness to challenge me throughout the course of this work. He has been a wonderful colleague and supportive friend. I also thank Andy for exposing me to some fine dining experiences, especially Dinghy's and the Cabbage Shed, and for not giving me the "one more plot look" more often.

I thank my other committee members, Dennis Fulbright, Richard Lenski, Bryan Epperson, and Jim Hancock, for being generous with their time and constructive comments. In particular, I wish to thank Dennis for being an excellent resource and for his enthusiasm. Helen Alexander, Bill MacDonald, Michael Milgroom, and Tobin Peever also provided guidance and support for this work.

For making my experience as a graduate student at Michigan State University an extremely positive experience, I express my appreciation to the faculty, staff, and students of the Department of Botany and Plant Pathology and the Ecology, Evolutionary Biology, and Behavior Program.

This study could not have been completed without the help of many people in the field. For dealing with often unpleasant conditions with a minimum of grumbling I thank Andy Jarosz, Jennifer Schaupp, Dennis Fulbright, Richard Leschen, Ngoc Kieu, Emily Lyons, Jim Bier, Mario Mandujano, Amanda Posto, Katy Kampf, Lissa Leege, Tim Tibbetts, and many undergraduates. I also wish to acknowledge the property owners, particulary the Rau and Millard families, for allowing me to conduct my research on their

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land. I also thank Ed Lawler for conducting the greenhouse experiment. For their hospitality, I thank Jim and Norma Rice.

This work was supported by the National Science Foundation, the Association for Women in Science, Sigma Xi, the College of Natural Sciences, the Department of Botany and Plant Pathology, and the Ecology, Evolutionary Biology, and Behavior Program.

I am grateful to my parents for their love, patience, and support. I especially thank my father for inspiring in me a love of reading and curiosity about the world around me. I thank my cousins, Pericles Georges, Margaret Sinclair, and Helen Georges, for their love and encouragement and always providing me with a good meal.

Finally, I could not have completed this work without my many wonderful friends. My gratitude goes to Steve Buehne, Lissa Leege, Richard Leschen, Natalie Panshin, Anne Plovanich-Jones, Amanda Posto, Susan Simmons, and Anna Wiese for their support and encouragement. My deepest affection and appreciation go to Erika Barthelmess, Chris Haufler, Andy Jarosz, Emily Lyons, Judy Mongold, Paco Moore, Scott Nunes, Amanda Rinehart, Jennifer Schaupp, Denise Searles, Sue Stoltzfus, Carmen Storm, and Sara Taliaferro for their unconditional love, encouragement, and support.

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CHAPTER 1

INTRODUCTION

Pathogens have the potential to influence plant community diversity and plant species distributions through their effects on plant fitness and the outcome of intra- and inter-specific competition (Burdon 1982; Burdon 1987). Both survivorship and reproduction, the components of host fitness, can be reduced by pathogens. For example, infection with a rust fungus decreased survival of groundsel both over winter and during summer (Paul & Ayres 1986a; Paul & Ayres 1987). Other studies of natural systems have demonstrated pathogen mediated decreases in host survival for both herbaceous plants (Alexander & Burdon 1984; Parker 1986; Wennström & Ericson 1990; Jarosz & Burdon 1992; Roy & Bierzychudek 1993; Thrall & Jarosz 1994) and trees (Geils & Jacobi 1993; Burdon et al. 1994). Disease has also been shown to decrease reproduction, whether measured as flower number, fruit or cone production, seed production, or seed biomass (Schaffer et al. 1983; Alexander & Burdon 1984; Clay 1984; Parker 1986; Paul & Ayres 1986b; Parker 1987; Paul & Ayres 1987; Wennström & Ericson 1991; Jarosz & Burdon 1992; Roy & Bierzychudek 1993; García-Guzmán et al. 1996; Marr 1997). The genetic composition of plant populations can also be altered by pathogens through reduction of susceptible genotypes and increase of resistant genotypes (Burdon et al. 1981; Carlsson et al. 1990; but see Parker 1991) and potentially, the increased fitness of rare genotypes (Antonovics & Ellstrand 1984).

Many studies on the effects of disease on plant community structure have focused on introduced pathogens in forest systems. One well known example is the change in composition

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composition of eastern deciduous forests in the U.S. after the introduction of chestnut blight (Day & Monk 1974). Dramatic changes in species abundances, including the devastation of dominant species, were also seen as the result of *Phytophthora cinnamomi* infection of the eucalyptus forests of Australia (Weste 1980; Weste 1981; Dickman 1992). Community structure can also be altered by endemic pathogens. Elimination of eucalyptus from forest areas infected with *Armillaria* root rot (Kile 1983) and the increase in abundance of rare species in hemlock and Douglas fir forests in infection centers caused by an unspecialized root rot fungus (Holah et al. 1993) are examples of indigenous pathogens changing plant community composition.

With the wide attention given to the effects of disease on individuals and plant communities, it is surprising that little work exists addressing the effects of infection on host population size and persistence. However, there is a small body of work which suggests pathogens can have adverse effects on host populations. For example, there is a negative relationship between population size in subsequent years and proportion of diseased individuals and large population size in previous years in the *Silene-Microbotryum* interaction (Antonovics et al. 1994). While these results suggest a negative effect of infection on host population growth rates, the intrinsic rate of increase for both disease-free and diseased populations was not explicitly measured. The potential difference in growth rate for diseased and healthy populations has important implications for the persistence of individual host populations in areas where disease is common.

The effects of a pathogen on host population and community structure is not easily quantified because pathogens tend to affect some life history stages more than others. For example, damping-off disease results in up to 74% mortality of seedlings in

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three tree species while older individuals are virtually immune (Augspurger 1984). In contrast, the anther-smut pathogen, *Microbotryum violaceum*, can infect both seedlings and adults (Alexander & Antonovics 1988; Alexander 1989) but appears to only have severe fitness consequences for adults by sterilizing infected plants. Further, size of an individual rather than life history stage may influence the impact of disease. In the *Linum-Melampsora* host-pathogen system, larger individuals are more likely to become infected (Jarosz & Burdon 1992). However, small individuals of *Podophyllum peltatum* experienced higher disease incidence and severity, and mortality than large plants when infected with the pathogen, *Puccinia podophylii* (Parker 1988). How these differential effects of disease on various life history stages alter host population growth rates has not been investigated. High seedling mortality due to disease might have a small effect on population growth rates if seedlings succumb to other factors, such as competition, in the absence of disease. In contrast, a simple reduction in seed reproduction, not even mortality, of a few large individuals could severely decrease population growth and persistence.

Matrix projection models have been used to examine how perturbations to particular life history stages might affect population size for endangered or rare plant and animal species (e.g. Crouse et al. 1987; Lande 1988; Menges 1990; Kephart & Paladino 1997). These methods should also be useful in examining the influence of disease on host population growth rate and structure. Sensitivity analyses, which calculate the effect of small perturbations in a projection matrix element on population growth, have been particularly useful in guiding conservation efforts. Perhaps the best example of this method's utility is seen in conservation efforts in the loggerhead sea turtle, *Caretta*

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caretta (Crouse et al. 1987). Early conservation efforts were targeted at increasing the success of egg clutches that were laid on beaches. However, sensitivity analyses indicated that this did little to increase sea turtle populations because the survival of hatchlings after their release into the sea was exceedingly low. Sensitivity analyses further indicated that slight increases in the survivorship of older juvenile and adult age classes would have a much greater effect on increasing population size. This led to the use of turtle exclusion fishing nets as a primary mode of conservation of this species (Crowder et al. 1994; Crowder et al. 1995). Further, Silvertown et al. (1993, 1996) suggested using elasticity values, which measure each matrix element's proportional contribution to a population's growth rate, to determine if a population is at risk. They proposed that species have characteristic elasticity ratios with regard to growth (*G*), survival (*L*) and fecundity (*F*). The *G/L/F* ratio is determined largely by life history, and as a consequence annuals, herbaceous perennials and trees have characteristic *G/L/F* ratios. Stressed populations will have *G/L/F* ratios that deviate significantly from the ratios exhibited by species or populations having a similar life history (Silvertown et al. 1996).

I propose to extend the use of matrix projection analyses to evaluate the effects of pathogens on host populations. At one level, matrix projection models can determine the extent to which pathogens actually influence the growth and size of host populations. In this context, it would be possible to answer the question of whether high pathogen pressure (i.e., high levels of disease incidence and severity) influences the size or density of plant populations. The matrix projection method will be particularly useful in situations where pathogen pressure varies (either naturally or by manipulation) over space

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or time, allowing direct comparisons between populations. Among population comparisons can be used to estimate the decrease in host population growth due to disease, and it can also be used to identify growth stages that are most affected by pathogen infection.

Matrix projections may also be used for the development of disease management programs in a manner similar to their current use in developing species recovery strategies for rare and endangered species. A number of introduced pathogens have had large effects on some species in North America, Europe and other continents. Among the most dramatic in their effects are introduced pathogens that infect tree species, which are common within our native forests and urban landscapes. In North America, introduced pathogens such as *Cryphonectria parasitica* on American chestnut (Roane et al. 1986), *Ophiostoma ulmi* on elms (Brasier 1990), and *Discula destructiva* on native dogwoods (Hibben 1990, Daughtrey & Hibben 1994) have had large effects on the structure of both the host species population and the community. There is interest in controlling these diseases using biological agents, the most likely being double-stranded RNA (hereafter, dsRNA) elements that infect many fungal pathogens (Nuss & Koltin 1990). DsRNA hyperparasites have been found in *C. parasitica* (Biraghi 1950a,b; Day et al. 1977), *O. ulmi* (Brasier 1990) and *D. destructiva* (Yao & Tainter 1996, Yao et al. 1997); Brasier (1998) has proposed that they be introduced specifically for the purpose of controlling these pathogens. However, the natural spread of these hyperparasites and subsequent biocontrol have met with varying degrees of success.

Ophiostoma ulmi, the causal agent of Dutch elm disease, was introduced into North America and Europe. Many elms survived the spread of this pathogen. A new

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more virulent form of the pathogenic fungus, *O. novo-ulmi*, has evolved and appears to be displacing the original less virulent form and having a much bigger impact on the survival of elms (Brasier 1987). Reduced virulence in both species is reported to be the result of infection of the pathogen by cytoplasmic intracellular hyperparasites (Brasier 1990). These hyperparasites (d-factors) are not widespread in either species and fungal strains infected with d-factors are replaced by hyperparasite-free *O. novo-ulmi* (Brasier 1990).

A second example of an introduced pathogen with the potential to be controlled by a dsRNA hyperparasite is the focus of this dissertation. The pathogenic fungus causing chestnut blight, *Cryphonectria parasitica*, was introduced accidentally into the U.S. around 1904 in New York (Roane et al. 1986) and spread rapidly throughout the range of the American chestnut, *Castanea dentata*, devastating populations. Infected branches and trunks are girdled and killed by the fungus. A severe *C. parasitica* epidemic occurred in Europe around 1938 (Roane et al. 1986). Subsequently, dsRNA hyperparasites spread throughout the pathogen populations in Europe (Biraghi 1950a,b). Infection by the dsRNA hyperparasite reduced pathogen virulence (also termed hypovirulence) (Jaynes & Elliston 1982), producing recovery of chestnut populations in Europe (Heiniger & Rigling 1994). Hyperparasite infection debilitates *C. parasitica* and reduces pathogen virulence on chestnut trees by reducing canker growth rate (Anagnostakis & Waggoner 1981), asexual conidia production (Elliston 1985), and sexual reproduction (Anagnostakis 1984, 1988). Reduced canker expansion rates will, in some cases, allow the tree to produce enough wound callus tissue to wall of the fungus, resulting in

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resulting in a superficial, non-lethal canker (healing canker). Trees producing non-lethal cankers are said to be recovering.

Before the spread of the blight, chestnuts were a dominant overstory species in eastern U.S. forests (Roane et al. 1986). Today, although few trees reach reproductive status and populations are maintained by sprouts from the rootstock (Paillet 1984, 1988), chestnuts are a dominant understory component of plant communities within their natural range (Keever 1953, Russell 1987). Michigan is outside the natural range of the American chestnut. However, chestnut populations have become established through naturalization of planted trees (Brewer 1995). Trees began dying of blight in Michigan in the late1920s (Baxter & Strong 1931). In the late 1950s, healing cankers began to appear and the presence of dsRNA in isolates of the fungus were discovered in the late 1970s (Day et al. 1977). Michigan appears to be the only area in the U.S. where dsRNA induced hypovirulence has spread naturally (Fulbright et al. 1983). For this reason, Michigan provides an unique opportunity to study this interaction because of the presence of recovering populations, non-recovering populations, and uninfected reproducing populations of the American chestnut (Brewer 1995). Matrix projection models will allow demographic parameters (population growth rate, sensitivity, and elasticity) of these different population types to be compared.

Not only the influence of *C. parasitica* infection but also that of the potential biocontrol of *C. parasitica* by dsRNA on American chestnut populations can be evaluated. This matrix model approach may have general application to examining the effects of decreases in pathogen virulence. Indeed, there is a strong theoretical basis for this idea. The observation that pathogens with a large negative effect on plant fitness

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(high virulence) could destroy their own resource led researchers to postulate that pathogens should evolve reduced virulence (Harper 1977). Implicit in this argument is that reductions in virulence will lead to recovery of the plant population, which in turn restores the resource base for the pathogen. There is empirical evidence for this phenomenon from the interaction of Myxoma virus and rabbits. A virulent strain of Myxoma was introduced into both Australia and Great Britain to control rabbit populations (Fenner 1983). Rabbit populations were reduced initially but increased with time. The loss of virus effectiveness in controlling rabbit populations was found to be the result of the evolution of decreased virulence in the virus along with increased resistance and immunity in the rabbits (Fenner & Ratcliffe 1965; Anderson & May 1982; May & Anderson 1983).

However, there does not appear to be any evolutionary tendency for the evolution of reduced virulence in natural plant-pathogen interactions (Jarosz & Davelos 1995). Theoreticians have also pointed out that reduced virulence will not usually be favored by individual selection, and may only be favored in situations where group selection is important (May & Anderson 1990). Models incorporating ecological factors, such as host density, predict evolutionary trajectories towards intermediate levels of pathogen virulence (Lenski & May 1994). These models also found that reductions in virulence need not result in appreciable increases in host population density.

In this dissertation, the impact of dsRNA mediated reductions in pathogen virulence on the biology of American chestnuts will be investigated using matrix projection analyses. Population growth rate, sensitivity, and elasticity (demographic parameters

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Chapter 5.

parameters derived from matrix projection models) will be used to evaluate the effects of *C. parasitica* and dsRNA on chestnut populations.

The specific questions addressed are:

1. Does the pathogen have differential effects on individuals of different size?
2. How does disease affect chestnut demographics?
3. Do dsRNA epidemics result in full recovery of chestnut populations?

A further general objective is to determine the application of the *G/L/F* elasticity ratio of Silvertown et al. (1993, 1996) to the evaluation of disease processes. The interaction of branch size and reductions in pathogen virulence, caused by dsRNA infection, on branch survival will be examined in Chapter 2. The result of spread of dsRNA at the host population level will be the focus of Chapter 3. Whether chestnut populations infected with hyperparasitized forms of the pathogen have the same finite rate of increase as disease-free populations will be examined. The implications of the demographic study for management and conservation of the American chestnut is discussed in Chapter 4. Greenhouse and field experiments examining seedling survival and growth are present in Chapter 5. Chapter 6 contains conclusions and future directions.

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CHAPTER 2

EFFECTS OF BRANCH SIZE AND PATHOGEN VIRULENCE ON CANKER DEVELOPMENT AND BRANCH MORTALITY

INTRODUCTION

Although most models of reductions in pathogen virulence, measured as a negative effect on host fitness, focus on evolutionary changes in the parasite (e.g. Lenski & May 1994), changes in virulence can also result from infection of a pathogen by a hyperparasite. The relative importance of evolutionary versus ecological reductions in virulence has not been explored empirically and they may result in very different numerical and genetic dynamics of both host and pathogen populations. Hyperparasites of fungal pathogens of plants are found commonly (Hollings 1982). Other groups of organisms can also be adversely affected by intracellular elements. For example, a plasmid which confers antibiotic resistance reduced growth of its bacterial host in the absence of antibiotic (Lenski & Bouma 1987).

For cytoplasmic hyperparasites to be useful in biological control, we must not only understand how they alter virulence but also how they spread within a pathogen population. Many theoretical models have explored the relationship between pathogen virulence and transmission between hosts (Anderson & May 1982, Ewald 1983, May & Anderson 1983, Frank 1996) with increased virulence related to increased transmission; hyperparasites may show similar dynamics. An empirical example with a parasite (bacteriophage) and its bacterial host demonstrated that without horizontal spread (high transm ssio

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transmission) the bacteriophage evolved toward a mutualistic relationship with its host (Bull et al.1991).

Double-stranded RNA (dsRNA) hyperparasites have been examined as potential biological controls of plant pathogenic fungi through their ability to reduce pathogen virulence. Despite their presence in many groups of fungi, dsRNAs have not been found consistently to reduce pathogen virulence (Nuss & Koltin 1990). This lack of effect may result from dsRNA transmission being necessarily dependent upon pathogen transmission because dsRNA is only transmitted over a distance via fungal spores. In contrast to the conventional wisdom of increased virulence leading to increased transmission, for these types of cytoplasmic hyperparasites increased virulence could lead to decreases in pathogen, and therefore hyperparasite, transmission. A recent theoretical model on the evolution of hyperparasites stresses that a balance between pathogen transmission and hyperparasite debilitation (i.e., virulence of the hyperparasite on the pathogen) will need to be maintained for successful biological control of a pathogen by a hyperparasite (Taylor et al. 1998). In the absence of other evolutionary pressures, the model also predicts that hyperparasites should evolve toward mutualism (i.e., low virulence or debilitation) with their pathogen host thereby having a detrimental effect on the plant host population. In other words, biological control of a pathogen by a cytoplasmic element may not be effective in the long term.

However, dsRNAs may indeed be useful biological control agents for the chestnut blight fungus, *Cryphonectria parasitica* (Murrill) Barr. The accidental introduction of *C. parasitica*, a pathogenic ascomycete, into the U.S. was first reported in 1904 in New York (Merkel 1905) . The fungus spread rapidly throughout the range of the American chestnut, *Castanea dentata* (Marsh.) Borkh., devastating populations. Infected branches and trunks are girdled and killed by the fungus. Chestnut blight was first documented in Europe in 1938 and by 1967 infection had spread to most chestnut-growing areas (Heiniger & Rigling 1994). Infection of *C. parasitica* by a dsRNA hyperparasite was found to reduce pathogen virulence (also termed hypovirulence) (Jaynes & Elliston 1982), producing recovery of chestnut populations in Europe (Heiniger & Rigling 1994). Hyperparasite infection debilitates *C. parasitica* and reduces pathogen virulence on chestnut trees by reducing canker growth rate (Anagnostakis & Waggoner 1981), asexual conidia production (Elliston 1985), and sexual reproduction (Anagnostakis 1984, 1988). A superficial, non-lethal canker (healing canker) may result from wound callus tissue, which develops in response to pathogen infection in some cases, reducing canker expansion rates and walling off the fungus. Trees producing non-lethal cankers are said to be "recovering" because infected stems are not always killed by infection, allowing for continued growth and seed production.

Introductions of dsRNA have not resulted in spread of the hyperparasite and recovery of the American chestnut (MacDonald & Fulbright 1991). Michigan is the only area in the U.S. where dsRNA induced hypovirulence has spread naturally (Fulbright et al. 1983). In Michigan populations, the proportion of dsRNA containing isolates and tree recovery (presence of at least one callused canker on a tree) are positively correlated (Davelos et al. 1997). However, dsRNA-containing strains are found in cankers below surviving and dying branches with equal frequency (Davelos et al. 1995). A preliminary survey of two recovering Michigan populations found that infected branches which survive have a larger diameter than those that die (Davelos, Fulbright, & Jarosz, unpub.).

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These observations suggest that infections by both dsRNA-free and dsRNA-containing isolates of *C. parasitica* kill small branches, while large branches are more likely to recover from infections by dsRNA-containing isolates of *C. parasitica*.

This study focuses on the interaction between dsRNA-induced changes in *C. parasitica* virulence and American chestnut branch size. Specifically, the following questions were addressed: (1) How does the interaction between pathogen virulence and branch size affect canker development and branch mortality; (2) Is the pattern of canker development and subsequent branch mortality similar for cankers initiated from artificial inoculations and from natural infections; (3) Is presence/absence of dsRNA predictive of canker morphology in an inoculation study; and (4) Is branch size predictive of morphology of naturally occurring cankers?

METHODS

Study Site

A number of naturalized populations of *C. dentata* have become established in Michigan from trees planted by early settlers (Brewer 1995). Blight was first reported in Michigan chestnut populations in the late 1920s (Baxter & Strong 1931). By the late 1950s, healing cankers began to appear and the presence of dsRNA in isolates of the fungus was discovered in the late 1970s (Elliston et al. 1977; Day et al. 1977). The site that was selected for this study, County Line, has been infected with chestnut blight since 1958 and healing cankers began to appear in the early 1970s (Brewer 1995). Studies of vegetative compatibility group diversity, RFLP variation, and DNA fingerprints of the *C. parasitica* population at County Line revealed that a single clonal lineage predominates (Milgroom 1995; Milgroom & Lipari 1995; Davelos et al. 1996; Liu et al 1996; Davelos et al. 1997

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et al. 1997). The dsRNA population at this site has also been found to consist of a single type (Paul & Fulbright 1988; Peever et al. 1997). The dsRNA found at County Line cross-hybridizes to Cryphonectria hypovirus 3-GH2 (Paul & Fulbright 1988; Peever et al. 1997) and is therefore a member of the virus family Hypoviridae (Hillman et al. 1995).

Cryphonectria parasitica Isolates

A single spore isolate of the most common *C. parasitica* vegetative compatibility genotype found at the County Line (CL) site, which also contained dsRNA, was selected for the field inoculation experiment. This genotype was identified from a population level survey (Davelos, Schaupp, Jarosz, & Fulbright, unpub.). The complimentary dsRNA-free strain was generated from a single spore isolate of the hyperparasitized strain; dsRNA is not present in all conidia of a culture (Fulbright 1984, Russin & Shain 1985, Enebak et al. 1994). A vigorously growing isolate which was putatively dsRNA-free was selected from among the conidial offspring. This isolate was screened essentially as described in Fulbright et al. (1983) to confirm the absence of dsRNA.

Virulence of both the dsRNA-free and the dsRNA-containing isolates was determined by comparing these strains with known controls (virulent dsRNA-free isolate Ep155 and debilitated dsRNA-containing isolate GH2) using a bark inoculation test (Lee et al. 1992). The pair of isolates selected for the field experiment produced lesions on excised bark that differed significantly in area from each other 5 days after inoculation (dsRNA-free CL isolate: 3.8 cm^2; dsRNA-containing CL isolate: 1.9 cm^2; $t = 4.43$, $df = 2$, $P < 0.05$). Lesions produced by the dsRNA-containing isolate did not differ in area from those produced by the GH2 control (GH2: 2.1 cm^2; $t = 0.74$, $df = 2$, N.S.); lesions

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produced by the dsRNA-free isolate also did not differ in area from those produced by the virulent control (Ep155: 5.3 cm^2; $t = 3.45$, $df = 2$, N.S.).

Inoculation Experiment

The effect of the interaction between branch size and pathogen virulence on branch mortality was explicitly tested by inoculating branches of varying size at the County Line site. In July 1997, a total of 60 uninfected branches on separate trees were selected: 20 small (≤ 2 cm in diameter; mean diameter: 1.6 cm), 20 medium (2-4 cm in diameter; mean diameter: 3.0 cm) and 20 large (≥4 cm in diameter; mean diameter: 5.3 cm). Half of the branches in a size class were inoculated with the dsRNA-containing *C. parasitica* isolate; the other half were inoculated with the complimentary dsRNA-free isolate. Branches were assigned randomly to treatment groups. Mean branch size did not differ significantly between treatments within a size class. Inoculations followed the agar-disk cork-borer method of Griffin et al. (1978). Canker size (length and width) and morphology (non-callused or callused) were monitored bi-monthly through October 1997 and again in April, July, and September 1998. If a branch was girdled by a canker, i.e., if the branch above the canker was dead, only canker width and morphology were recorded.

Natural Infections

To investigate how branch size affects branch mortality and canker morphology of naturally infected branches, uninfected branches on 100 trees were selected randomly at the County Line site. These branches were wounded with a sterilized aluminum nail to promote natural infection because *C. parasitica* is thought to enter trees through wounds in the bark (Anagnostakis 1982). Wounding was done in July 1997 because infection is most likely

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most likely to occur at wound sites created in summer (Steven Jakobi, pers comm). Branch diameter at the wound site was noted. These branches were monitored in September 1997, April 1998, and September 1998 for the appearance of cankers. The whole branch from the wound site distal was inspected for the appearance of new cankers. When cankers were found, they were classified as non-callused or callused. The diameter of the branch immediately below each new canker was measured.

Statistical Analyses

All analyses were performed with the SAS statistical package, Release 6.12 (SAS Institute, Inc. 1997). To examine differences in canker expansion rates for the inoculation experiment, two response variables were used: canker area (½ width X ½ length X π) and canker width / branch circumference. The former measure was selected for comparison to previously published studies on canker expansion rates. The latter measurement was chosen because of its biological relevance; a value of 1.0 indicates that a branch has been completely girdled by a canker. These repeated measures data were analyzed with profile analysis using multivariate analysis of variance (MANOVA) with PROC GLM. Profile analysis examines the shape of the response curves (Time*Size*dsRNA), the levels of the main effects (Time*Size and Time*dsRNA), and the flatness of the response curves (Time) (i.e., whether or not the slopes of the curves differ from zero) (von Ende 1993). Area and width/circumference at each survey were the response variables and branch size class, presence or absence of dsRNA in the inoculation, and the interaction between them were the main effects included in the models. Pillai's trace is the test statistic reported because it is the most robust to violations

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violations of assumptions (Scheiner 1993). Contingency table analyses for the relationship between branch survival to September 1998 and either presence/absence of dsRNA or branch size class were performed using PROC FREQ. Comparisons among all six branch survivorship curves were made using Peto and Peto's logrank test (1972) following the methods in Pyke and Thompson (1986 & 1987). Significant differences between pairs of curves were examined using the same methods with Bonferroni corrections applied to the significance levels for multiple comparisons. The relationship between canker morphology (non-callused or callused) and branch size and presence/absence of dsRNA was examined with logistic regression using PROC CATMOD. Branch size and dsRNA were the main effects and canker morphology was the dependent variable. This relationship was investigated both at the end of the 1997 survey (before any deaths had occurred) and at the end of the 1998 survey.

For analyses in the natural infection study, PROC GLM was used with branch diameter as the independent variable and infection status, branch survival, or canker morphology as dependent variables. Data were transformed (square root or log) when needed to improve normality of residuals and homogeneity of variances. *A posteriori* tests were performed using the Tukey-Kramer method. For analyses with survival or canker morphology as the dependent variable, logistic regression (PROC CATMOD) was used with branch size as a main effect for both models. Canker morphology and the interaction between canker morphology and branch size were also included as main effects in the branch survival analyses.

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RESULTS

Inoculation Experiment

Four branches were eliminated from the study due to cankers developing below the inoculation site or because the inoculation was unsuccessful. For the response variable canker area, the level of the response for presence/absence of dsRNA was marginally significantly different (Time*dsRNA) with cankers produced by dsRNA-free isolates being larger and the slope of the curves was significantly different from zero (Time) (Table 2-1; Figure 2-1). However, branch size class (Time*Size) (Figure 2-2) and the interaction between dsRNA and branch size class (Time*dsRNA*Size) did not differ significantly for canker area.

For the response variable canker width/branch circumference, the level of the response differed significantly for branch size class (Time*Size) and the slope of the curves was significantly different from zero (Time) (Table 2-2; Figure 2-3). Although the level of responses did not differ significantly for presence/absence of dsRNA, the ratio of width/circumference was higher consistently for dsRNA-free isolates (Figure 2-4).

Survivorship of the branch distal to the inoculation site until the final survey (September 1998) differed significantly among branch size class ($\chi^2 = 23.14$, df = 2, $P < 0.001$; Table 2-3) with large branches having the highest survival (95%), medium branches being intermediate (68%), and small branches having the lowest survival (18%). Survivorship to the final survey (September 1998) was not affected by presence/absence of dsRNA in the isolate used for inoculation ($\chi^2 = 0.69$ df = 1, N.S.; Table 2-3). When the pattern of survival over time was examined, significant differences were found for

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survivorship curves among the six combinations of dsRNA presence/absence and branch size categories (Log Rank = 105.09, df = 5, $P < 0.001$; Table 2-3; Figure 2-5). A general trend was found for survivorship to decrease with decreasing branch size class. In general, branches inoculated with dsRNA-containing isolates have higher survivorship than those branches inoculated with dsRNA-free isolates. However, this latter trend was reversed when considering small branches separately.

Canker morphology (non-callused or callused) may be influenced by the presence or absence of dsRNA and branch size. This relationship was examined explicitly from results of the inoculation experiment. At the end of the 1997 survey, canker morphology was predicted by presence or absence of dsRNA in the inoculating isolate ($\chi^2 = 4.97$, df = 1, $P < 0.05$; Table 2-4a). However, by the end of 1998, branch size category significantly influenced canker morphology ($\chi^2 = 16.18$, df = 2, $P < 0.005$; Table 2-4b) with larger branches having the highest proportion of callused cankers. In contrast, dsRNA became uninformative ($\chi^2 = 0.01$, df = 1, N.S.; Table 2-4a) with cankers being classified into either category with equal probability. Further, there was no relationship between canker ratings at the end of 1997 and at the end of 1998 ($\chi^2 = 3.50$, df = 1, N.S.) with about 37% of cankers in each category in 1997 being classified into the other category in 1998.

Natural Infections

Whole branch.--None of 100 uninfected branches wounded in July 1997 had detectable infections at the wound site by September 1997. By April 1998, 90 branches remained in the survey; the remainder were dropped from the study because they had died from causes other than disease or a canker had developed below the wound site. Of the branches

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branches remaining in the survey, 32% became infected at or distal to the wound site by April 1998; an additional 50% of the remaining uninfected branches developed a canker by September 1998 (Table 2-5). The total percentage of branches infected at some point in time during the study was 66%. Of the 59 branches that became infected, 20 branches developed more than one canker and 33 branches developed cankers at the wound site. The size of the branch appeared to influence the likelihood of a canker developing somewhere on the branch. For newly infected branches in both April and September 1998, the diameter of the branch at the wound site was significantly larger than those branches that remained disease-free (Table 2-5).

Individual cankers.--The size of a branch where a canker develops could influence the mortality of the branch distal to the canker. To examine this relationship between branch size at the canker and branch mortality, individual cankers from the natural infection study were analyzed. A total of 36 cankers were found on the 29 branches infected by April 1998 with 53% of branches alive distal to these cankers. By September 1998, 50 new cankers were found with 86% of branches alive distal to these cankers. There were no significant differences in branch size at the canker between branches that survived and those that died early in 1998 (Table 2-6). However, by September there was a distinct trend for surviving branches to be larger than those that died (Table 2-6). Branch size at the canker was also related to canker morphology. Again there was no trend by April 1998 but by September, the diameter of branches with cankers which were classified as callused were significantly larger than those with cankers in the non-callused category (Table 2-6).

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With logistic regression one can investigate whether branch size is predictive of canker morphology. In general, branch diameter immediately below a canker was predictive of canker morphology (Table 2-7) with larger branches tending to have callused cankers. Further, logistic regression can test whether canker morphology and branch size are predictive of survival. Canker morphology was not related to survivorship of a branch (results not presented) but branch diameter immediately below a canker was marginally predictive of survival (Table 2-7); there was not an interaction between canker morphology and branch size.

DISCUSSION

The importance of American chestnut branch size in determining the outcome of infection of a branch by chestnut blight is supported by both the inoculation experiment and the natural infection study. Branch size influences canker morphology and branch survival although some of these effects develop over time. DsRNA reduces canker growth rates and may also delay mortality of medium sized branches and potentially of large branches.

The results showing that the presence of dsRNA reduces canker growth rates regardless of branch size is consistent with the findings of other researchers. After 12 weeks significant differences among treatments were found when dsRNA-free and dsRNA-containing isolates were paired in all combinations and inoculated onto chestnut stems (Kuhlman 1978). Further, field inoculations of dsRNA-free and dsRNA-containing strains from both Italy and the US showed significant differences in mean canker area between strains with and without dsRNA after 6 months (Elliston 1985). The measure canker width/branch circumference appears to be a good indicator of how infection

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infection is likely to influence branch survival. The closer the ratio is to 1, the more likely the branch is to die, regardless of branch size or presence/absence of dsRNA.

Classification of cankers into callused or non-callused does not appear to accurately predict the type of fungal isolate which initiated infection, especially after the first season of infection. Branch size appears to be a more important influence on canker development. This result is supported by both the inoculation experiment and the natural infection study. In the inoculation experiment, a canker classified as non-callused is just as likely to contain a dsRNA-containing isolate as a dsRNA-free one. Although isolates from natural cankers were not screened for dsRNA, branch size was found to be predictive of canker morphology especially as time since infection increased. A study by McManus et al. (1989) found no correlation between canker morphology and presence/absence of dsRNA in naturally occurring cankers. This observation came with the caveat that the cankers were several years old and therefore might contain a mixture of pathogen isolates both with and without dsRNA which could confound the interpretation of the relationship. Although it is possible that the inoculations in this study subsequently became colonized with other strains of the pathogen, the result of no relationship between canker morphology and presence/absence of dsRNA is consistent with the findings of McManus et al. (1989). This outcome regarding the lack of importance of dsRNA and the role of branch size in canker morphology has implications for field surveys of chestnut populations where the degree of recovery is rated by the presence of callused cankers. If a population is dominated by small trees, many non-callused cankers might be found and the population would be considered non-recovering. However, dsRNA could be present in these isolates, which could have implications for the long-t

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the long-term prognosis of the population. Conversely, the presence of callused cankers within populations containing large trees may not indicate that the chestnut population is recovering due to the spread of dsRNAs.

The effect of branch size at time of infection on survivorship was most striking in the inoculation study, where it was found that small branches were more likely to die regardless of the type of inoculum used. The finding that dsRNA alone is not related to branch survivorship emphasizes that the presence of dsRNA in a pathogen population may be necessary but not sufficient for biological control of disease. Although the presence of dsRNA may enable large branches to survive, small branches still succumb to infection by both dsRNA-containing and dsRNA-free isolates. This trend for disease to have differential impacts on small versus large individuals has also been found in another empirical study. In the *Podophyllum peltatum-Puccinia podophylli* plant-pathogen interaction, small ramets had higher disease incidence and severities than large ramets, resulting in higher mortality for small individuals (Parker 1988).

The length of time a branch has been infected appears to play an important role in branch survivorship. No branch deaths were observed in either study until the second season. Further, because many branches in the large and medium size classes were still alive at the end of the study, the ultimate influence of dsRNA on branch survivorship cannot be determined. However, there is a trend for the presence of dsRNA to delay branch death for medium sized branches. Because there was little mortality of branches in the large size class, no effect of dsRNA was observed; however, the presence of dsRNA is expected to delay death for branches in this size category as well.

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Overall, branch size at the time of infection appears to have a large influence on branch survivorship, at least in the short term. Indeed, higher survival and more superficial (i.e., callused) cankers were observed on chestnuts in managed clearcuts than those in forest clearcut sites (Griffin et al. 1991). Although the effects of tree size and the presence of dsRNA were not explicitly examined in the study, trees in the managed clearcut were probably larger due to lack of competition from other hardwoods. This larger size could have contributed to the higher survival and presence of callused cankers. A similar trend was found for European chestnuts; sprouts with smaller diameter at breast height were more likely to succumb to infection by chestnut blight or competition (Bissegger et al. 1997).

These findings on the influence of branch size on survivorship have important implications not only for individual trees but also for American chestnut populations as a whole if one assumes that effects on trees are similar to effects observed for branches. Significant reductions in fitness of smaller trees and saplings due to infection by either dsRNA-free or dsRNA-containing strains of chestnut blight could affect population growth and persistence. The presence of dsRNA appears to at least delay death of large trees. In the meantime, these trees may reproduce adding new recruits to the population. However, if these small individuals never attain reproductive size due to the negative effects of infection, then the population will not persist through time although individual large trees are growing and reproducing. Therefore, the presence of a sufficiently debilitating dsRNA in the pathogen population may not be the only requirement for recovery of American chestnut populations. The size of an individual tree at the time of

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infection could also be an important factor in determining the long term survival of American chestnut populations.

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Table 2-1. Profile analysis of canker area for the inoculation experiment. F-values are for Pillai's trace.

Effect	F	Numerator df	Denominator df	Prob > F
Time*dsRNA*Size	0.69	14	88	0.7787
Time*Size	1.33	14	88	0.2076
Time*dsRNA	2.22	7	43	0.0507
Time	20.95	7	43	0.0001

Table 2-2. Profile analysis of canker width/branch circumference for the inoculation experiment. F-values are for Pillai's trace.

Effect	F	Numerator df	Denominator df	Prob > F
Time*dsRNA*Size	0.92	16	86	0.5479
Time*Size	3.79	16	86	0.0001
Time*dsRNA	0.58	8	42	0.7896
Time	87.75	8	42	0.0001

Table 2-3. Branch survival for the inoculation experiment. Number of branches alive or dead in September 1998 for each category of branch size at the inoculation site and presence/absence of dsRNA for inoculations performed at County Line in July 1997. Different letters indicate significant differences in survivorship curves (Pyke & Thompson 1986,1987 with Bonferroni corrections for multiple tests).

		STATUS		
DSRNA	BRANCH SIZE	ALIVE	DEAD	SURVIVORSHIP CURVES
ABSENT	SMALL (≤ 2 cm)	3	6	c
	MEDIUM (2 - 4 cm)	4	5	bc
	LARGE (≥ 4 cm)	9	1	ab
	Total	16	11	
PRESENT	SMALL (≤ 2 cm)	0	8	d
	MEDIUM (2 - 4 cm)	9	1	ab
	LARGE (≥ 4 cm)	10	0	a
	Total	19	9	

Table 2-4. Canker morphology (non-callused or callused) for presence/absence of dsRNA and branch size class for the inoculation experiment at County Line. D[illegible] included in the total

Table 2-4. Canker morphology (non-callused or callused) for presence/absence of dsRNA and branch size class for the inoculation experiment at County Line. Dead branches are included in the totals.

a. Canker morphology and presence/absence of dsRNA.

	CANKER MORPHOLOGY (end of 1997)		CANKER MORPHOLOGY (end of 1998)	
DSRNA	NON-CALLUSED	CALLUSED	NON-CALLUSED	CALLUSED
ABSENT	19	11	14	14
PRESENT	10	19	14	14

b. Canker morphology and branch size class

b. Canker morphology and branch size class.

	CANKER MORPHOLOGY (end of 1997)		CANKER MORPHOLOGY (end of 1998)	
BRANCH SIZE	NON-CALLUSED	CALLUSED	NON-CALLUSED	CALLUSED
SMALL (≤ 2 cm)	13	6	15	2
MEDIUM (2 - 4 cm)	10	10	11	8
LARGE (≥ 4 cm)	6	14	2	18

Note: Some branches were removed from the study between 1997 and 1998 due to the development of cankers below the inoculation site.

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Table 2-5. Average branch diameter at the wound site in the natural infection study. Date of survey, number of new branches found with at least one canker, the average diameter of a branch at the wound site (i.e., general branch size) for newly infected versus uninfected branches for the natural infection study at County Line. Means ± standard errors are presented.

		Average diameter of branch at wound site		
Survey date	Newly infected branches	Infected branches	Uninfected branches	
September 1997	0	-	-	
April 1998	29	2.6 ± 0.19	1.7 ± 0.08	$F_{1,87} = 26.31, P < 0.0001$
September 1998	30	1.9 ± 0.12	1.5 ± 0.10	$F_{1,57} = 7.15, P < 0.01$

Table 2.6. *Survival, canker morphology, and average diameter of branch below canker for natural infection study.* Date canker found,

and diameter of branch below canker for surviving versus

Table 2-6. Survival, canker morphology, and average diameter of branch below canker for natural infection study. Date canker found, date branch distal to canker survived until, proportion of branches surviving, and diameter of branch below canker for surviving versus dead branches and for callused versus non-callused canker morphology for individual cankers in the natural infection study at County Line. Raw means ± standard errors are presented.

			Average diameter of branch below canker			Average diameter of branch below canker		
Date infected	Survival up to	Proportion surviving	Surviving	Dead		Callused	Non-callused	
Before Apr. 1998	Apr. 1998	0.53	1.6 ± 0.35	0.7 ± 0.12	$F_{1,34} = 3.78$, N.S.	1.6 ± 0.54	1.0 ± 0.19	$F_{1,34} = 1.61$, N.S.
After Apr. 1998 & before Sept. 1998	Sept. 1998	0.86	2.2 ± 0.13	1.5 ± 0.26	$F_{1,48} = 4.77$, $P < 0.05$	2.4 ± 0.17	1.7 ± 0.13	$F_{1,48} = 10.89$, $P < 0.001$
Anytime before Sept. 1998	Sept. 1998	0.80	2.1 ± 0.15	1.3 ± 0.20	$F_{1,67} = 6.67$, $P < 0.025$	2.4 ± 0.20	1.5 ± 0.14	$F_{1,67} = 17.29$, $P < 0.0001$

branch size, and survival for natural infection study. Date canker found, date

Table 2-7. Relationship between canker morphology, branch size, and survival for natural infection study. Date canker found, date branch distal to canker survived until, relationship between branch diameter below canker and canker morphology and relationship between branch size and survival for individual cankers in the natural infection study at County Line.

Date infected	Survival up to	Diameter and canker morphology	Diameter and survival
Before Apr. 1998	Apr. 1998	$\chi^2 = 1.24$, df = 1, N.S.	$\chi^2 = 3.02$, df = 1, $P < 0.10$
After Apr. 1998 & before Sept. 1998	Sept. 1998	$\chi^2 = 7.67$, df = 1, $P < 0.0025$	$\chi^2 = 3.74$, df = 1, $P < 0.10$
Anytime before Sept. 1998	Sept. 1998	$\chi^2 = 10.99$, df = 1, $P < 0.001$	$\chi^2 = 5.68$, df = 1, $P < 0.025$

Canker Area versus Time

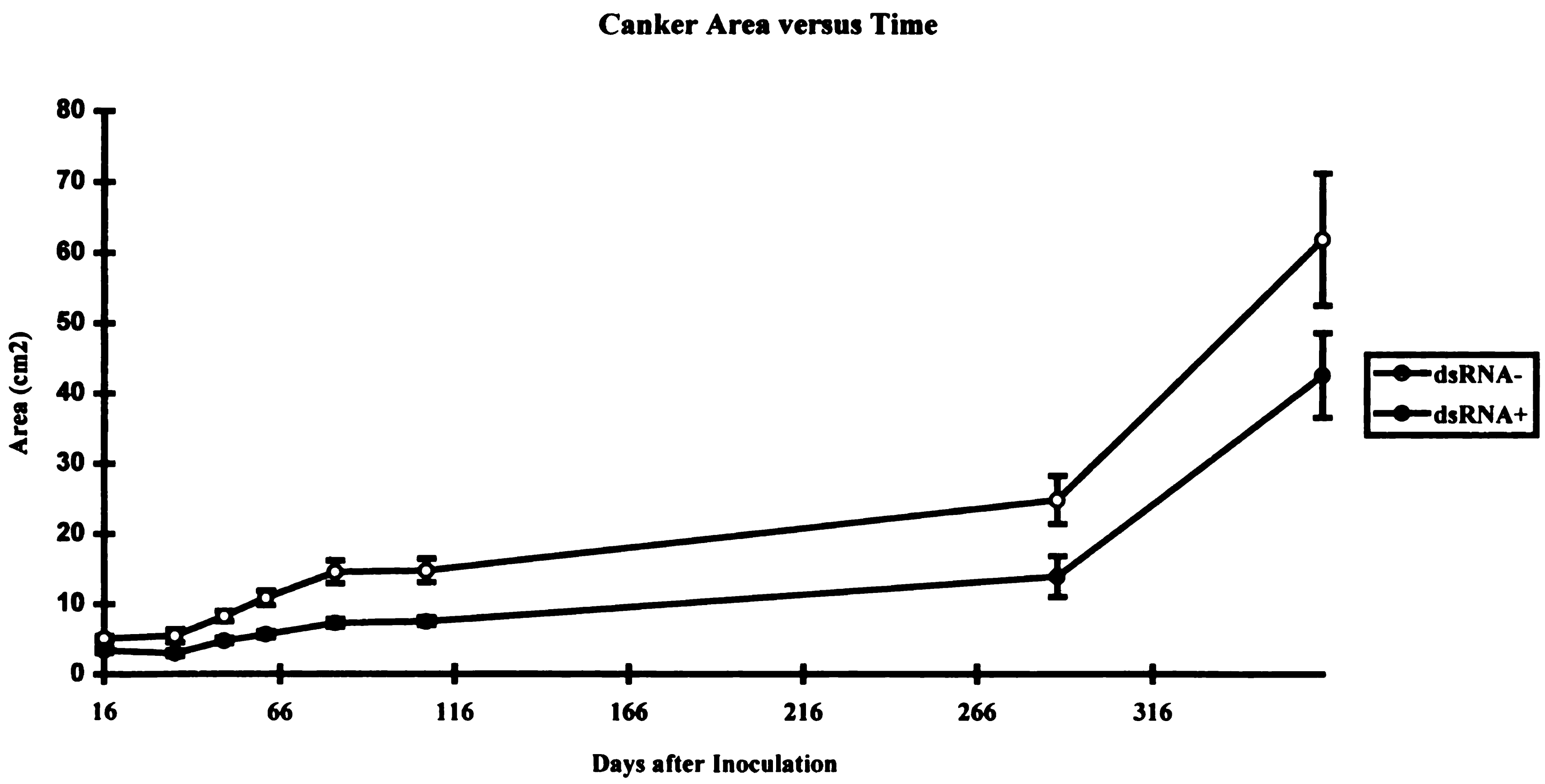

Figure 2-1. Mean canker area (cm^2) ± standard errors for branches inoculated with isolates with and without dsRNA versus days since inoculation for the inoculation experiment at County Line.

Canker Area versus Time

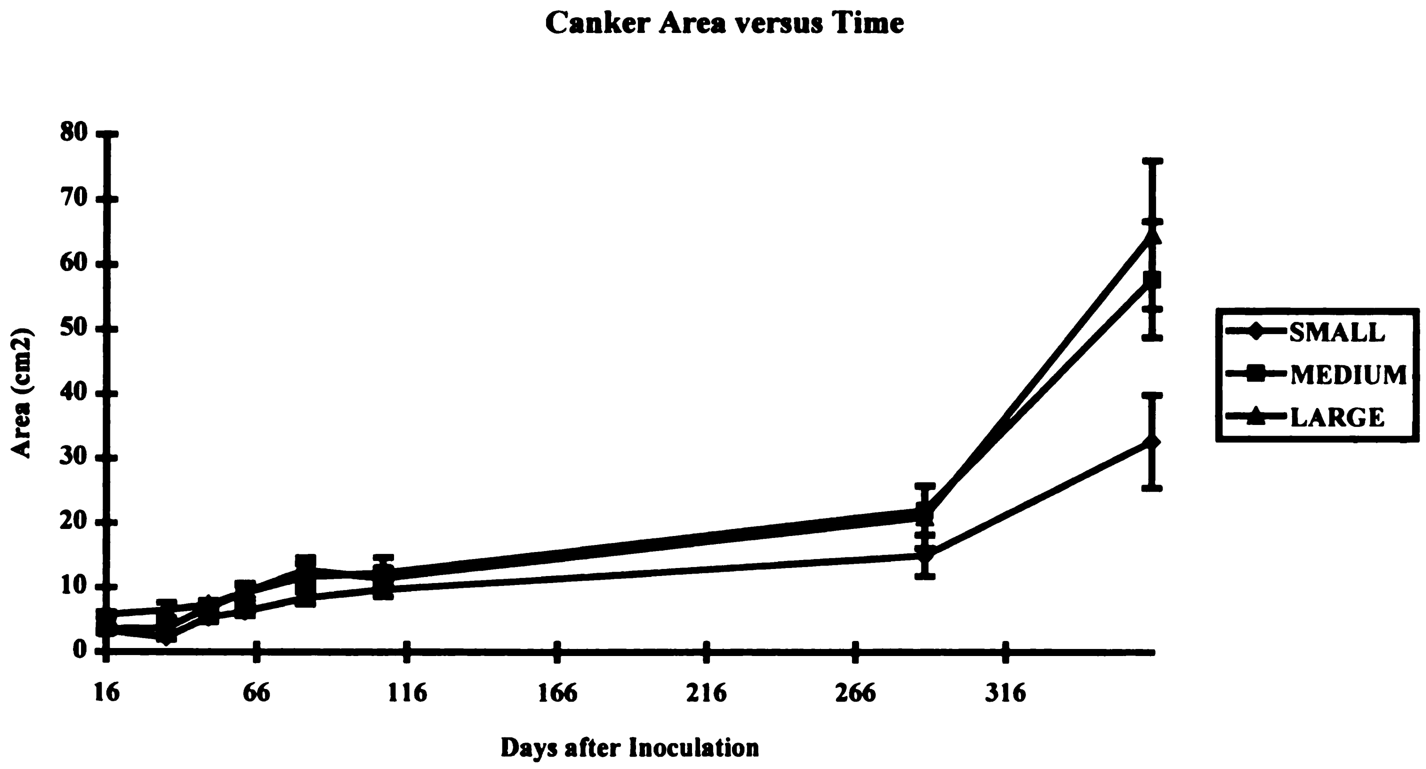

Figure 2-2. Mean canker area (cm^2) ± standard error for each branch size class versus days since inoculation for the inoculation experiment at County Line.

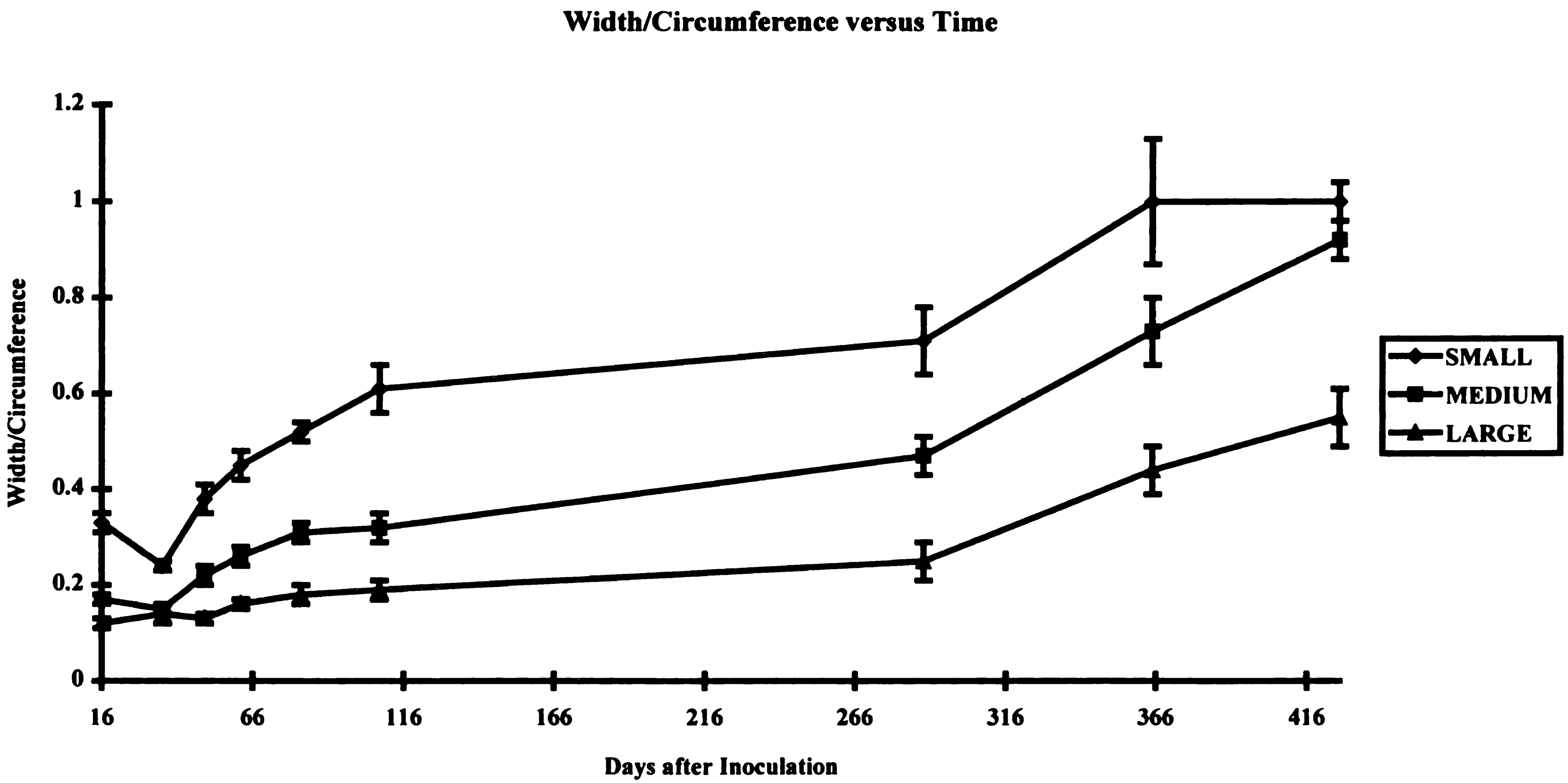

Figure 2-3. Mean canker width/branch circumference ± standard errors for each branch size class versus days since inoculation for the inoculation experiment at County Line.

Width/Circumference versus Time

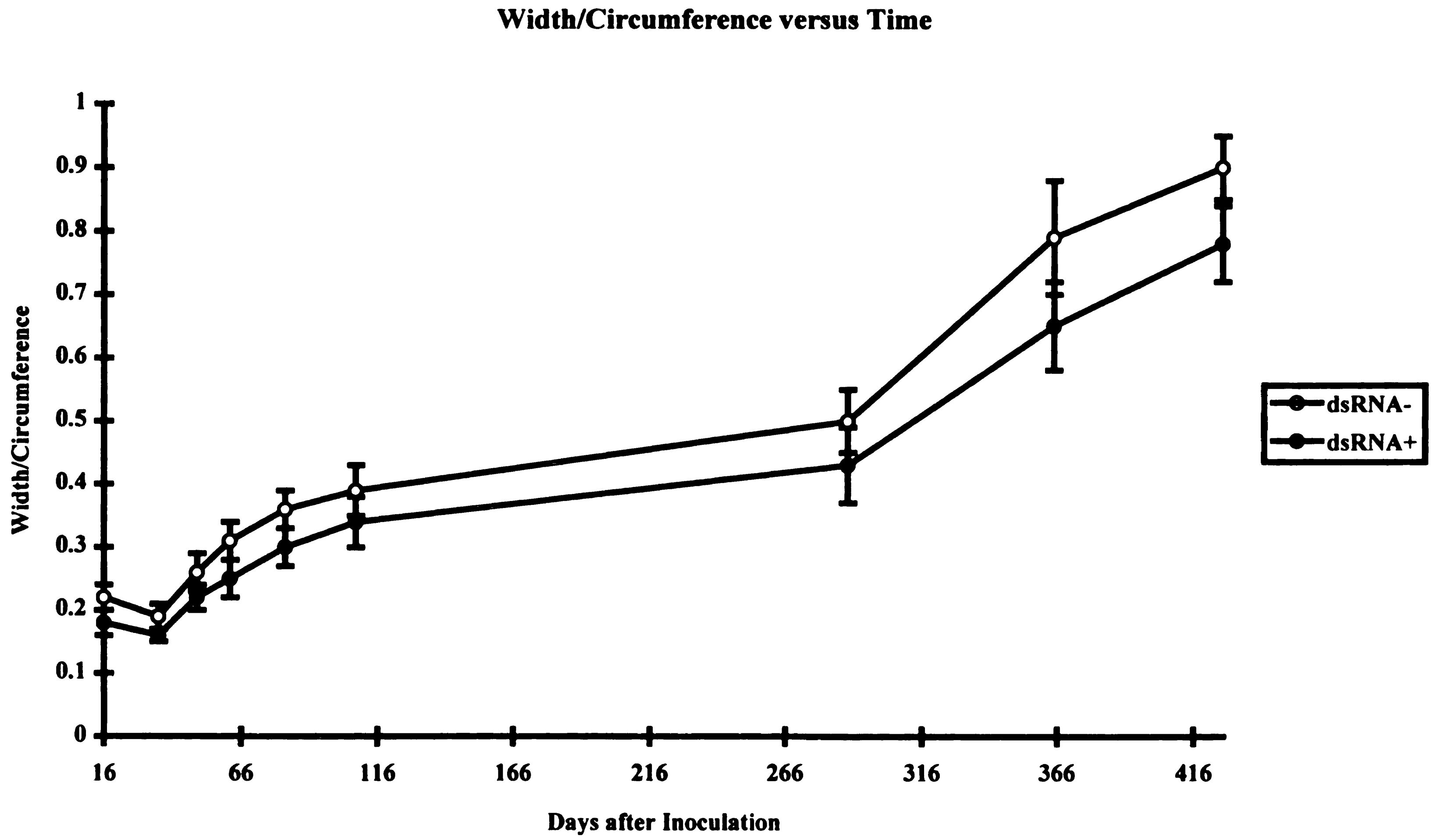

Figure 2-4. Mean canker width/branch circumference ± standard errors for branches inoculated with isolates with and without dsRNA versus days since inoculation for the inoculation experiment at County Line.

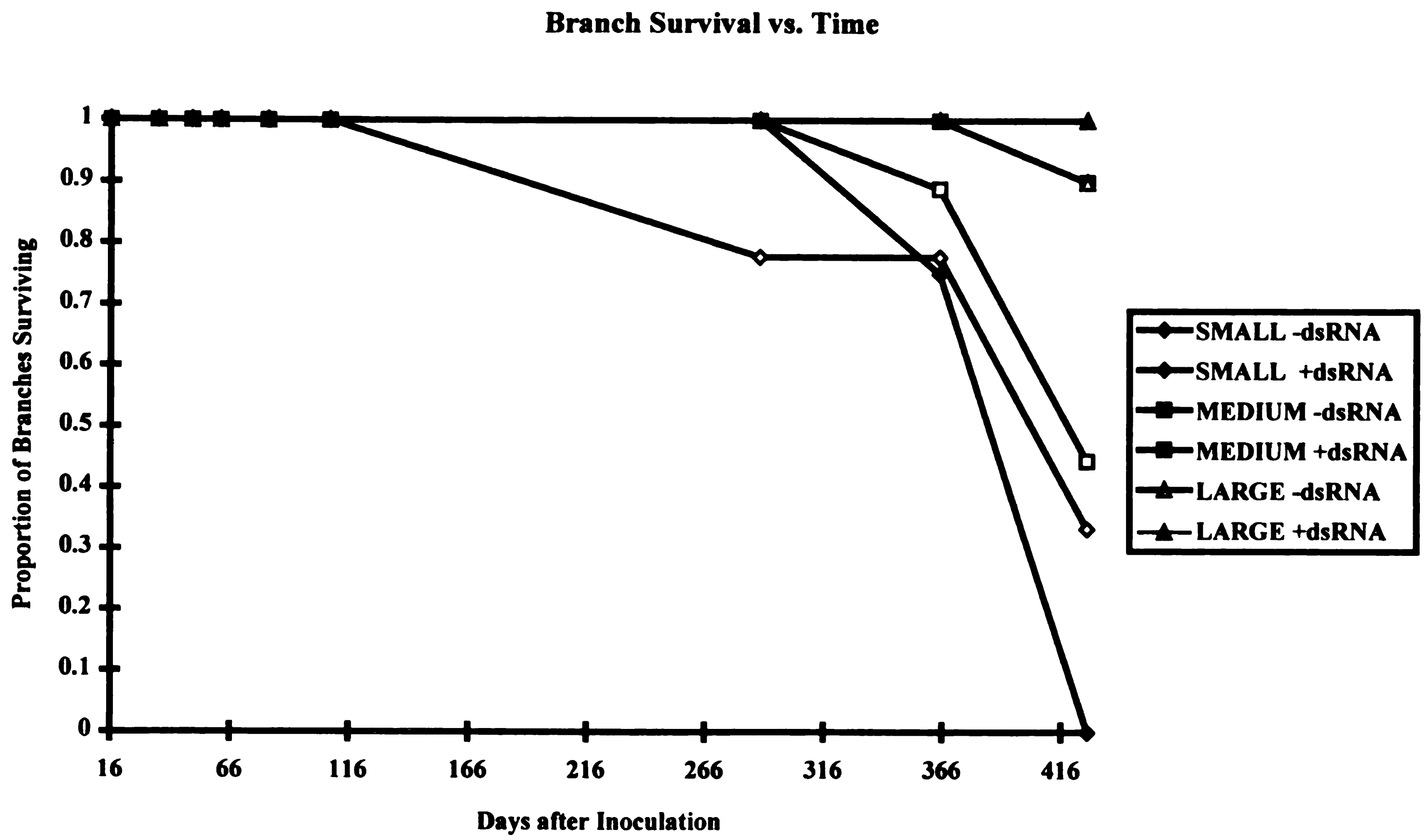

Figure 2-5. Proportion of branches surviving during the inoculation experiment.

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CHAPTER 3

EFFECTS OF *CRYPHONECTRIA PARASITICA* AND DOUBLE-STRANDED RNA INFECTIONS ON AMERICAN CHESTNUT DEMOGRAPHY

INTRODUCTION

Pathogens can influence plant community diversity and species distributions through their effects on fitness and the outcome of intra- and inter-specific competition (Burdon 1982; Burdon 1987). Both host survivorship and reproduction can be reduced by pathogens (Alexander & Burdon 1984; Clay 1984; Parker 1986; Paul & Ayres 1986a&b; Parker 1987; Paul & Ayres 1987; Wennström & Ericson 1990; Wennström & Ericson 1991; Jarosz & Burdon 1992; Roy & Bierzychudek 1993; Thrall & Jarosz 1994; García-Guzmán et al. 1996; García-Guzmán & Burdon 1997; Marr 1997). However, the focus of most empirical studies has been on the individual rather than on population-level effects.

Matrix projection models can be used to determine population growth rates and stable stage distributions for age or size structured populations given information on survival and reproductive rates. The finite rate of population increase (λ), a measure of population growth rate, indicates whether a population is growing or declining in size (Caswell 1989; Silvertown & Lovett Doust 1993). The finite rate of population increase has been shown to vary with habitat and over space and time for a variety of plant species (e.g., Menges 1990; Kalisz & McPeek 1992; Horvitz & Schemske 1995; Kephart & Paladino 1997; Leege 1997). Matrix models have rarely been used to evaluate the impact

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of disease on λ (Emery 1998). However, estimates of changes in population size over time for populations of *Silene latifolia* (= *S. alba*) infected with *Microbotryum violaceum* (=*Ustilago violacea*) show that populations with a high proportion of diseased individuals increase in size at a slower rate (Antonovics et al. 1994). Further, the population expansion rate of *Silene dioica* populations was reduced by infection with *M. violaceum* (Carlsson & Elmqvist 1992). This potential difference in population growth rate has important implications for the persistence of host populations in areas where disease is common. Stable stage distributions, the fixed ratio of the number of individuals between stages, can be compared to the observed population structure to predict whether or not the size structure of a population will change over time (Silvertown & Lovett Doust 1993). For example, if disease causes greater mortality for small versus large individuals, the size distribution of infected populations could shift towards larger individuals. Matrix population models have been used to examine how disease may alter the stage distributions of infected plant populations (Antonovics & Alexander 1989; Frantzen 1994).

The effects of pathogens on population level processes of their hosts can be investigated using matrix projection models. By comparing uninfected host populations to populations infected with pathogens of different levels of virulence, defined here as the negative effects of infection, the influence of pathogen virulence on population growth rates and stable stage distributions can be directly assessed. To examine how infection may affect host demographic processes, I focused on the interaction between the American chestnut, *Castanea dentata*, and its pathogen, *Cryphonectria parasitica*, the caus

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causal agent of chestnut blight. This pathogenic fungus itself can be chronically infected with a cytoplasmic hyperparasite, a double-stranded (ds) RNA, which debilitates the pathogen by reducing growth and sporulation, effectively reducing its virulence. In host populations infected with hyperparasitized fungi, trees may respond to infection by producing wound callus tissue and walling off the fungus leading to recovery of the infected individual. However, no work has been conducted to determine if tree recovery is associated with recovery at a population level, i.e. successful recruitment of seedlings into the population. It is possible that seedlings could become established in populations, yet never reach reproductive status because they are killed by blight. Indeed, in a population of American chestnuts maintained by root sprouts, stems are small in size and had high mortality (64%) over a six year period (Parker et al. 1993). Even if the pathogen's virulence level is reduced, the small stems of young trees could be girdled and killed by the fungus. Further, infection could have a more dramatic effect on the growth of small versus large trees. This could have important implications for the long-term persistence of chestnut populations if infection prevents small trees from reaching reproductive size even if large trees are relatively unaffected.

The objective of this study was to compare demography of healthy, non-recovering, and recovering American chestnut populations. The presence of large infected, yet surviving, trees has been thought of as chestnut population recovery (Fulbright et al. 1983); however, this may not represent recovery in a demographic sense. I investigated the extent of recovery by comparing the finite rate of increase (λ) in healthy and recovering chestnut populations. The effects that virulent forms of *C. parasitica*

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(i.e., not infected with dsRNA) have on host demography also were determined using λ. The finite rate of increase was calculated from transition matrices constructed from measurements of growth of adult trees, seed production, and seedling establishment and growth at each population site. Further, differences among size distributions and the observed and stable stage distributions for each population were examined.

Another result of this study is the examination of the role of changes in pathogen virulence on host population level processes. A trend towards evolution of reduced virulence should be found because pathogens with high virulence could destroy their resource base (Harper 1977). However, in a review of empirical studies of plant-pathogen interactions trends favoring reduced virulence were not found commonly (Jarosz & Davelos 1995). Further, a model by Lenski and May (1994) indicates reductions in pathogen virulence need not result in an increase in host population size, i.e. recovery. The study presented here represents a situation in which reductions in virulence are due to the ecological process of infection of the pathogen by an intra-cellular hyperparasite rather than an evolutionary change in the pathogen population. Demographic methods may be an effective tool for evaluating the use of hyperparasites as potential biological controls.

METHODS

Study System

For a description of the introduction of *C. parasitica* into the U.S. and the effects of dsRNA on the pathogen, see Chapter 2. Before the spread of chestnut blight throughout the eastern U.S., chestnuts were a dominant overstory species (Day & Monk

1974; Karban 1978; Russell 1987). Today, although few trees reach reproductive status and populations are maintained by sprouts from the rootstock (Paillet 1984, 1988, 1993), chestnuts are a dominant understory component of plant communities within their natural range (Keever 1953, Russell 1987; Stephenson et al. 1991).

Study Sites

For the history of American chestnuts and chestnut blight in Michigan, see Chapter 2. Michigan appears to be the only area in the U.S. where dsRNA induced hypovirulence has spread naturally (Fulbright et al. 1983). For this reason, Michigan provides an unique opportunity to study this interaction because of the presence of healthy, non-recovering, and recovering populations of the American chestnut. I have defined recovering populations as those dominated by trees with healing cankers; non-recovering populations have less than 30 percent of trees with healing cankers. Six sites in Michigan were selected for study: two healthy populations (Leelanau and Missaukee Healthy), two non-recovering populations (Stivers and Missaukee Diseased) and two recovering populations (County Line and Frankfort) (Figure 3-1).

Population Projection Matrices

Stage classes.--Individuals were classified into size-based categories to construct transition matrices. Lefkovitch (1965) stage-based matrices were used instead of Leslie (1945) age-based matrices because individuals of the same age could be very different sizes due to disease or other environmental factors. Therefore, size class is likely to be more predictive of an individual's fate than age, at least in the short term (Caswell 1989). Lefkovitch matrices have non-zero elements in the main diagonal, which represent survival within a stage from year to year, and in the top row of the matrix, which

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represent production of offspring. The subdiagonal represents growth from one stage to the next, not an increase in chronological age (Silvertown & Lovett Doust 1993). Elements above the main diagonal represent reductions in size, also termed retrogressions. As in other demographic studies of woody plants, I based stage classes on plant size measured as height and stem diameter at breast height (dbh) (e.g., Huenneke & Marks 1987; Enright & Watson 1991; Pascarella & Horvitz 1998).

Eight classes were constructed for each population (Table 3-1; Figure 3-2). Stage 1 includes only first year seedlings; often the seed remained attached to the new seedling at the start of the season so they were identified easily. Beyond this stage, some small plants were derived from these first year seedlings; others were the result of infection of larger plants or size suppression by herbivore browsing. Infection by chestnut blight can drastically reduce plant size by girdling and killing chestnut stems leaving only small sprouts from the root collar surviving. Significant differences in survival probability between true seedlings and other small individuals were found (see Chapter 5, Table 5-1). Therefore, separate classes were used for small plants derived from seedlings (Stage 2), and for those that result from disease or herbivory (Stage 3). Transitions for plants larger than 50 cm in height were based solely on size, not on their history.

Population sampling.-- Transition probabilities between size classes of some juvenile and all potentially reproducing trees were obtained from yearly measurement of diameter at breast height (dbh) for the largest stem of all individuals. Size reductions (retrogressions) from these larger size classes occur when the largest stem dies and the surviving sprouts are smaller in size.

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Estimates of natural recruitment and transition probabilities for small individuals were obtained by setting up permanent plots in each of the six study populations in May 1996. Plots were 9 m X 9 m and were placed haphazardly in the population. This type of plot placement allowed different microsites to be sampled. In a site in Wisconsin with few diseased trees, Paillet and Rutter (1989) found 1-5 seedlings/yr/hectare became established in chestnut-dominated woodlands; higher rates of establishment were found in other microsites, such as field edges. The number of plots per population ranged from 8 - 16; enough plots were used to cover approximately half the area of a given population. Plots were divided into quarters and the corners were marked permanently with flags. All seedlings within a survey plot were marked. In each quadrat, up to two resprouts, individuals sprouting from a root collar with the largest living stem shorter than 100 cm in height, and two damaged individuals, shorter than 100 cm in height but clearly not first year seedlings, were marked (maximum of eight each per plot). If an individual within these categories had multiple stems, one stem was selected randomly to be followed. Surveys were conducted in early (May/June), middle (July/August), and late (September/October) season. New seedlings were marked and followed as they appeared. Height was measured for each plant. If a plant was dead for two successive surveys it was removed from the census.

Reproduction by an individual tree was determined by counting the number of branches with burrs for each tree. Three branches were selected haphazardly and the number of burrs on these branches was counted. This method allowed the total number of burrs produced on a given tree to be estimated. Since nearly all burrs contain 3 seeds days

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(Jaynes 1978; Paillet & Rutter 1989), number of burrs was used as the measure of reproduction for an individual.

Projection matrices.--To calculate demographic parameters for each population, the following matrix model was used:

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

where $\mathbf{n}(t)$ is a vector of the number of individuals in each size class at time t, $\mathbf{n}(t+1)$ is the vector for the population at the next time interval, and **A** is a matrix that shows how individuals in each stage class at one time may become or contribute to each stage class one time unit later, in which columns refer to the stage at time t and rows refer to the stage at time t +1 (Figure 3-2). To calculate demographic parameters, the elements of **A**, fecundities and transition probabilities, are assumed to be constant parameters (Caswell 1989; Silvertown & Lovett Doust 1993). The vector $\mathbf{n}(t+1)$ is calculated iteratively by multiplying the transition matrix, **A**, by the vector $\mathbf{n}(t)$ until a stable stage distribution is reached at which point λ, the finite rate of population increase, can be estimated. For this study, **A** is an 8 X 8 matrix whose elements a_{ij} represent the transitions or contributions of individuals in the jth class at time t to the ith class at time t + 1 (Figure 3-2). These are given by the survivorship, growth, and fecundity of individuals between time t and t +1 (Caswell 1989). In this study, the time interval is one year. The finite rate of population increase (λ) and stable stage distributions were calculated for each population using RAMAS/stage (Ferson 1994). Further, the minimum λ for each population was calculated. This value is equivalent to the average survivorship for all individuals in the population (Silvertown et al. 1996).

Because there appears to be no seed bank in these American chestnut populations (Davelos & Jarosz, unpublished data), including a seed to seedling stage in the transition matrix would introduce a time-lag into the population projection (Caswell 1989; Enright & Watson 1991). Therefore, the average number of seedlings produced per tree in each size class was calculated to fill in the seedling row (stage 1) of the matrix (see Table 3-2). Approximately half the area of a population was covered with natural recruitment plots; hence, the number of seedlings found in the plots was doubled to obtain an estimate of total seedling production for a population. The average number of seedlings per individual in a stage was determined using the following formula:

$$\frac{\text{(no. of burrs in stage/no. of burrs for population) X no. of seedlings for population}}{\text{no. of trees in stage}}$$

Since a maximum of 8 each of resprouts or damaged plants were followed in the plot census, the total number of small non-seedling individuals is underestimated. Therefore, to determine the number of small non-seedling individuals within each population, the number of resprouts and damaged individuals within each survey plot was counted in 1998. This number was doubled to estimate the total number of small non-seedlings for a population. The observed ratio from the census of individuals in stages 3 and 4 for 1996 and 1997 was multiplied by this estimate to give the total number of individuals in these stages for 1996 and 1997. Further, at the start of the census in 1996, second year seedlings could not be identified. To determine the number of individuals in stage 2 in 1996, the observed ratio of stage 2 to stage 3 from 1997 was used to estimate what proportion of 1996 stage 3 individuals should be assigned to stage 2.

Some biologically important transitions were not observed in all populations. For example, in some populations, no individuals were observed to grow from stage 2 to stage 4. Further, for both non-recovering populations, the forward transition for stage 7 individuals to stage 8 was not observed in either year, i.e. no growth of trees in the 10-20 cm dbh size class to the greater than 20 cm dbh size class was observed. In these cases, estimates of transition probabilities were made by calculating the average probability of that transition across all populations. If the transition did not occur in any other population (e.g., death in the largest size class), the total number of individuals across all populations in a stage was determined. A transition probability was estimated by assuming there was one more individual in that class and that individual was assumed to make the transition; the probability of that individual transitioning (either growing to the next size class or dying) was used as an estimate for that matrix element. For the 1996-1997 transition matrices, stage 2 probabilities from 1997-1998 were used since these individuals (second year seedlings) could not be identified with certainty in 1996 when the census began.

To examine how these estimated transition probabilities affected population growth rates, simulations were performed in which the transition probabilities were altered and the effects on λ were investigated. For the transition from stage 2 to stage 4, the transition probability was increased by 10, 100, and 1000 percent. For the transition of stage 7 to stage 8 in non-recovering populations and for death in stage 8, the effect of reductions in these probabilities by 1/10, 1/50 and 1/1000 was investigated.

Statistical Analyses

Population growth.--Finite rates of population increase were determined for each population for two census periods. These data were analyzed with profile analysis using multivariate analysis of variance (MANOVA) with PROC GLM. Profile analysis examines the levels of the main effects (Time*Disease), and the flatness of the response curves (Time) (i.e., whether or not the slopes of the curves differ from zero) (von Ende 1993). The disease status of a population--healthy, non-recovering, or recovering--was the main effect included in the model. Roy's greatest root is the test statistic reported because it has the greatest power of the tests for significant differences among groups (Scheiner 1993).

Size distributions.--Comparisons of the observed proportion of individuals in each size class among years, size distributions at each site to their projected stable stage distributions, and stable stage distributions in different years were made with the log likelihood ratio, *G*, using PROC FREQ in SAS. This test is more robust than χ^2 for evaluating goodness of fit (Sokal & Rohlf 1981). The stable stage distributions were generated using RAMAS/stage (Ferson 1994). These distributions are given in relative frequencies; therefore, to make comparisons to the observed data, the relative frequency distributions were converted to expected number of individuals in each stage.

Cross-sectional area.--To examine how disease might affect the average size of individuals within a population, the mean cross sectional area at breast height for trees greater than 0.2 cm dbh was calculated for each population. These data were analyzed using repeated measures analysis as described above.

RESULTS

Patterns in Transition Matrices

Transition matrices for each of the six study populations for 1996-1997 and 1997-1998 are presented in Table 3-2a-l. Survival within stage 1 (first year seedlings) was generally lower in healthy populations as compared to infected populations. For stage 2 individuals (seedlings at least 2 years of age), survivorship was high. However, growth from stage 2 to stage 4 was observed in only one population for one census period (Missaukee Diseased, 1997-1998). Survival within stage 3 was generally intermediate between that observed for stage 1 and 2; the transition from stage 3 to 4 was relatively high in non-recovering populations.

Retrogressions (size reductions) were observed in all populations for trees in stages 4 to 6, and the pattern of retrogressions (all transitions down to stage 3 were possible) for these stages was similar among populations. In general, the magnitude of growth transitions (growth to stage 5) was greater than the magnitude of retrogressions (size reduction to stage 3) for individuals in stage 4. For stage 5 individuals, in half the matrices, growth transitions had a greater magnitude than retrogressions; in the other half, the reverse pattern was observed with retrogressions being of larger magnitude than growth transitions. In contrast, in 9 out of 12 cases, the magnitude of retrogressions in stage 6 was greater than the magnitude of the growth transitions. Further, at the Frankfort site, for all transitions in these three stages across two years, the magnitude of retrogressions was greater than that of growth transitions.

Populations differed in the pattern of size reductions for stages 7 and 8. No retrogressions were found for these size classes in either healthy population across two years, while all populations infected with *C. parasitica* had some large trees (> 10 cm dbh) which were reduced in size. The severity of size reductions was greatest at the two non-recovering sites (Stivers and Missaukee Diseased). In particular, the proportion of stage 7 and 8 trees moving to smaller size classes at the Stivers site was extremely high in 1996-1997.

The pattern of reproduction also changed when disease was present. At the two healthy sites, only stage 8 trees reproduced. In contrast, trees in stage 7 produced seed at all four diseased populations across two years and stage 6 trees produced seed at three of these sites in at least one year.

Population Growth Rates

Finite rates of population increase (λ) ranged from 0.978 to 1.000 based on 1996-1997 matrices and from 0.992 to 1.021 based on 1997-1998 transition probabilities (Table 3-3). There were no significant differences in λ among population types, and λ did not change over time within a population (i.e., slopes were not significantly different from zero) (Table 3-4). However, the relative ranking of the populations based on the 1996-1997 transition matrices suggests that disease is having an effect on the growth of chestnut populations. Indeed, for estimates of population growth based on 1996-1997 transition matrices, there was a significant correlation between population type and λ (r_s = 0.956, $P < 0.0014$). Healthy populations have the highest population growth rates and the two populations classified as non-recovering have the lowest values, with the recovering populations being intermediate. The results from 1997-1998 indicate there is year to year variability in estimates of the finite rate of population increase both within and among populations (Table 3-3). The highest value of λ was found for a recovering population but again the lowest population growth rate was observed for a non-recovering population. There was no correlation between population type and λ based on 1997-1998 transition matrices ($r_s = 0.667$, N.S.).

Minimum values of λ ranged from 0.823 to 0.971 for 1996-1997 and from 0.855 to 0.967 for 1997-1998 (Table 3-3). The observed values of population growth rates were closer to the minimum value for non-recovering populations than for both healthy and recovering populations. Indeed, observed population growth rates for non-recovering populations were generally less than 5 percent above the minimum value while the observed growth rates for the other population types ranged from 5.1 to 21.5 percent higher than the minimum value of λ (Table 3-3).

Reducing the transition probability of stage 7 to stage 8 to near zero (0.00005) for the two non-recovering populations (Stivers and Missaukee Diseased) did not qualitatively alter results based on 1996-1997 transition matrices (Table 3-3). However, this change did affect the relative ranking of population growth rate for the Stivers population in 1997-1998. When this low transition probability was used, both non-recovering populations had relatively lower population growth rates than the other population types. However, this change did not alter the results of the repeated measures analysis (results not presented). Because the death of an individual in the largest size class (dbh > 20.0 cm) was not observed, the probability of an individual of this size dying was estimated at 0.003 as described in the methods. Decreasing the probability of death of trees in this largest size class (stage 8) did not qualitatively alter estimates of the finite rate of population increase (the greatest observed increase in λ was 0.3 percent) and did not affect the repeated measures analysis (results not presented). Further, increasing the transition probability for stage 2 to stage 4 did not change the relative rankings of populations (a maximum increase in λ of 2 percent was observed) and again did not affect the repeated measures analysis (results not presented).

Size Distributions

In five out of six sites, observed population structures differed within a site between 1996 and 1997 (Table 3-5; Figure 3-3). In contrast, four out of the six sites did not differ for observed population structures between 1997 and 1998. For the healthy Leelanau site, the observed population structure was not significantly different among years. At four of the six sites in 1996-1998, stage 6 individuals were found in the highest frequency. At one of the other two sites, Missaukee Healthy, stage 3 individuals were found in the greatest proportion across the three years. At the County Line site, a different stage class had the highest frequency in each year (stage 6 in 1996, stage 3 in 1997, and stage 2 in 1998). In three out of the four populations where *C. parasitica* was present, the proportion of individuals in stage 7 was greater than that in stage 8. In contrast, for healthy populations, there was a higher frequency of individuals in the largest size class (stage 8) than in the next largest class (stage 7). This pattern was also seen at the Missaukee Diseased site (Figure 3-3).

Observed population structures differed from calculated stable stage distributions for all sites in all years, and calculated stable stage distributions differed significantly between years (Table 3-5; Figure 3-3). These results indicate that the study populations are not at equilibrium. In four out of six populations, the stage with the highest frequency was the same for both calculated stable distributions. In all cases, the stable stage with the highest frequency was either stage 2, 6, or 8. In five out of eight year-by-population combinations, stage 6 or 8 represents the greatest proportion of the population at a stable stage distribution in populations with disease. At the recovering County Line site, the calculated stable distribution for both censuses predicts stage 2 individuals will dominate the population. In contrast, for the recovering Frankfort population, the largest size class (stage 8) will dominate the population at a stable distribution. At the non-recovering Missaukee Disease site, stage 6 trees will constitute the highest proportion of individuals within the population at a stable stage distribution. This pattern was also the result based on one census period at the other non-recovering site, Stivers .

Cross Sectional Area

Cross sectional area at breast height differed both with time and among population types (Table 3-6). Both healthy and recovering populations increased in cross sectional area with time while the non-recovering populations decreased slightly in size over time (Figure 3-4). Individuals from healthy populations were larger in size and trees in non-recovering populations had the smallest cross sectional area.

DISCUSSION

While the significant impact of *Cryphonectria parasitica* on American chestnut populations is clear from observations of Eastern hardwood forests, this study suggests that the negative effects of *C. parasitica* are not uniform across all chestnut size classes. Instead there are specific changes in the dynamics of the largest trees within diseased populations. No retrogressions of trees in size classes 7 or 8 were ever observed in the two healthy populations (Table 3-2a-d), but they were observed in both of the non-recovering populations (Table 3-2i-l).

However, the size reductions of large trees did not translate into a major decline in the growth rate of non-recovering chestnut populations (Table 3-3). Two explanations might account for this lack of effect. First, infections decrease plant size by girdling branches and main trunks, but only rarely kill trees since the pathogen does not enter and kill the root system. Once the main trunk is killed, the stump sprouts from the root collar commonly grow for a few years before succumbing to *C. parasitica* infections. Therefore, mortality rates are not appreciably increased in diseased populations and average survivorship is still very high. The average survivorship sets a minimum value for population growth estimates (Silvertown et al. 1996). Thus, although growth rates appear to be only slightly depressed in populations infected with *C. parasitica*, they are actually quite close to the minimum possible value set by average survivorship (Table 3-3).

The other explanation for the lack of depressed population growth rates in non-recovering populations is that smaller trees (stages 6 and 7) produce burrs, a phenomenon not observed in healthy populations. This pattern could be the result of selection for reproduction rather than growth of infected individuals, physiological stress from disease, or a greater light availability in diseased populations due to increased canopy openings from the die-back of larger trees. Since reproduction appears to be related to light availability(Paillet & Rutter 1989), the latter scenario seems most likely.

Recovering populations did not share all the characteristics of healthy populations, but instead retained some features of diseased populations. For example, retrogressions of stage 7 and 8 trees and reproduction by stage 6 and 7 trees were found in both recovering populations. The retrogressions of large trees were offset by increased probabilities of growth. As a consequence, the number of stage 7 and 8 trees increased steadily over the two years (Frankfort +10; County Line +8), while the number of stage 7 and 8 trees decreased in the two non-recovering populations (Missaukee Diseased -8; Stivers -6) and increased only slightly in the healthy populations (Missaukee Healthy +2; Leelanau +3).

Population growth rate estimates fell into a rather narrow range and they did not differ statistically. However, the ranking of populations within each year was consistent with the hypotheses that: 1) disease was reducing chestnut population growth rates and 2) dsRNA was allowing chestnut populations to recover to some degree. The only possible exception was the ranking for the Stivers non-recovering population in 1997-1998 where the growth rate estimate was highly dependent on the transition probability from stage 7 to 8. No tree was observed to grow from stage 7 to 8 in either non-recovering population in either year. The actual probability for this transition is likely to be near zero. Since the total number of stage 7 trees is low in both populations, this transition had to be estimated. Initially, I estimated this transition by using the average growth rate across all six populations (i.e., making the assumption that this transition probability did not differ across populations). Growth from 1997-1998 in the Stivers population was the second highest using this estimate. If the true value of this transition is actually close to zero (i.e., 0.00005), then the growth rate estimate for Stivers was reduced to 0.992, a value similar to the other non-recovering population for both census periods (Table 3-3).

The observed structure of all populations differed significantly from the predicted stable stage distributions. This result indicates that the environment has not been stable for a long enough period to attain equilibrium. The expectation of equilibrium has been challenged recently by a number of researchers (Ehrlén 1995; Pascarella & Horvitz 1998; Valverde & Silvertown 1998). Temporal changes in the environment may be relatively predictable for species that exploit temporary environments such as light gaps (Pascarella & Horvitz 1998; Valverde & Silvertown 1998) or more episodic for environmental disturbances such as hurricanes (Pascarella & Horvitz 1998) or herbivory (Ehrlén 1995).

There are many explanations for the lack of equilibrium in Michigan chestnut populations. All populations were established in the last century after the introduction of chestnuts into Michigan by settlers. When farms were abandoned in the mid to late 1800's, chestnuts began invading feral lands and have continued to establish naturalized populations that are just now beginning to contain a significant number of large trees (Brewer 1995). The introduction of *C. parasitica* into these chestnut populations altered the dynamics; the predicted stable stage distribution of non-recovering populations would be expected to contain relatively few large individuals compared to the distributions predicted for healthy populations (Figure 3-3). When dsRNAs invade the *C. parasitica* pathogen population, they further alter chestnut population dynamics so that the expected stable stage distribution will contain a larger percentage of stage 7 and 8 individuals. The net effect is that most chestnut populations have not experienced a stable environment through time, and the observed population structure deviates quite significantly from the expected stable stage distribution based on the current environment.

It has been suggested that pathogens can affect many aspects of the ecological and evolutionary dynamics of plant populations, including alterations in population size (Burdon 1987). However, very few studies have documented that disease is actually reducing population growth rates (Carlsson & Elmqvist 1992; Antonovics et al. 1994; Emery 1998). The results of this study indicate that *C. parasitica* epidemics do have the potential to reduce growth rates within infected chestnut populations. This reduction leads to a gradual decrease in chestnut population size. In addition, infections result in significant size reductions of surviving individuals (Figure 3-4). For chestnuts, this has resulted in a change in the species' place in the community; it is now restricted to the forest understory (Keever 1953; Russell 1987; Stephenson et al. 1991) while trees in healthy populations are a major component of the forest overstory.

DsRNA hyperparasites have been suggested as potential biocontrol agents for chestnut blight by reducing pathogen virulence (Van Alfen et al. 1975; MacDonald & Fulbright 1991; Nuss 1992). When dsRNAs do spread within the pathogen population, extant trees recover in the sense that they begin to attain larger size. The hypothesis that recovery of extant trees translates into increased population growth rates was supported by the results of this study. Indeed, recovering populations exhibit increased growth rates that approach the rates found in healthy populations. The following scenario is proposed for *C. dentata* populations in Michigan: Healthy populations have growth rates that are at or slightly above one, i.e., they are near equilibrium (Figure 3-5). When *C. parasitica* epidemics begin, the growth rate of chestnut populations gradually declines to near the minimum value as the larger trees retrogress but survive and seed production declines to near zero. Growth rates remain near the minimum λ until the dsRNA hyperparasite successfully spreads through the pathogen population. DsRNA spread allows extant trees to increase in size until reproduction again becomes significant. It is not certain that recovering populations attain population growth rates that are similar to rates found in healthy populations over a range of temporal and spatial conditions. It is also unclear whether dsRNAs will provide long-term biological control of the *C. parasitica* pathogen population, since selection may favor less debilitating forms of dsRNA (Taylor et al. 1998). The effects of these different forms of dsRNA on American chestnut population processes is unknown.

Table 3-1. Stage classes used to describe populations of American chestnut.

Stage	Size		Category
	Height (cm)	dbh (cm)	
1	≤ 50		First year seedlings
2	≤ 50		Second year or older seedlings
3	≤ 50		Disease or herbivore damaged
4	> 50 and ≤ 100		Small juveniles
5	> 100	≤ 1	Juveniles
6	> 100	> 1 and ≤ 10	Potentially reproductive
7	> 100	> 10 and ≤ 20	Potentially reproductive
8	> 100	> 20	Potentially reproductive

Table 3-2. Transition matrices for six American chestnut populations. Stages represent the following size classes: Stage 1, first year seedlings; Stage 2, Second year or older seedlings, 0-50 cm tall; Stage 3, Others, 0-50 cm tall; Stage 4, 50.1-100 cm tall; Stage 5, greater than 1.0 m in height and dbh less than 1 cm; Stage 6, 1.1-10.0 cm dbh; Stage 7, 10.1-20.0 cm dbh; and Stage 8, > 20.0 cm dbh. N is the number of individuals in a stage in at the beginning of a census period. Probabilities in italics represent survival or remaining in a given stage from year to year. Probabilities below the diagonal represent growth and probabilities above the diagonal represent reductions in size. Probabilities within a column do not always sum to one due to mortality of individuals within a stage. Transition probabilities followed by an * are estimates.

TABLE 3-2a. Transition matrix for Missaukee Healthy 1996-1997.

STAGE	STAGE IN 1996							
IN 1997	1	2	3	4	5	6	7	8
1								__2.25__
2	__0.47__	___0.87*___						
3			___0.71___	__0.17*__	__0.003*__	__0.0007*__		
4		__0.01*__	__0.06*__	___0.62___	__0.097__	__0.0693__		
5				__0.21*__	___0.87___	__0.02__		
6					__0.03__	___0.90___		
7						__0.01*__	___0.95___	
8							__0.05*__	___0.997*___
N	106	46	315	19	144	101	10	16

TABLE 3-2b. Transition matrix for Missaukee Healthy 1997-1998.

STAGE	STAGE IN 1997							
IN 1998	1	2	3	4	5	6	7	8
1								__0.25__
2	__0.39__	___0.79___						
3			___0.79___	__0.21__	__0.06__	__0.05__		
4		__0.01*__	__0.02__	___0.46___		__0.06__		
5				__0.29__	___0.90___	__0.02__		
6					__0.01__	___0.85___		
7						__0.01__	___0.80___	
8							__0.20__	___0.997*___
N	36	50	355	45	125	95	10	16

TABLE 3-2c. Transition matrix Leelanau 1996-1997.

STAGE IN 1997	STAGE IN 1996							
	1	2	3	4	5	6	7	8
1								0.52
2	0.38	_0.87*_						
3			_0.75_	0.13*	0.005*	0.0002*		
4		0.01*	0.06	_0.47_	0.155	0.0198		
5				0.20	_0.57_			
6					0.24	_0.95_		
7						0.02	_0.94_	
8							0.06	_0.997*_
N	16	6	64	22	37	132	17	27

TABLE 3-2d. Transition matrix for Leelanau 1997-1998.

STAGE IN 1998	STAGE IN 1997							
	1	2	3	4	5	6	7	8
1								0.93
2	0.57	_0.99_						
3			_0.69_	0.33		0.02		
4		0.01*	0.06*	_0.50_	0.04	0.01		
5				0.17	_0.91_	0.04		
6					0.04	_0.92_		
7						0.01	_0.94_	
8							0.06	_0.997*_
N	14	6	65	30	26	133	18	28

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TABLE 3-2e. Transition matrix for Frankfort 1996-1997.

STAGE	STAGE IN 1996							
IN 1997	1	2	3	4	5	6	7	8
1						0.004	0.13	0.17
2	0.62	_0.87*_						
3			_0.71_	0.12	0.0057*	0.0005*		
4		0.01*	0.04	_0.62_	0.1843	0.0495		
5				0.04	_0.69_	0.06	0.03	
6					0.08	_0.86_	0.07	
7					0.004	0.03	_0.87_	
8							0.03	_0.997*_
N	58	39	209	58	263	388	72	17

TABLE 3-2f. Transition matrix for Frankfort 1997-1998.

STAGE	STAGE IN 1997							
IN 1998	1	2	3	4	5	6	7	8
1							0.27	0.06
2	0.86	_0.60_						
3			_0.71_	0.22	0.05	0.02		
4		0.01*	0.06	_0.41_	0.06	0.02		
5				0.14	_0.78_	0.05	0.01	
6				0.02	0.09	_0.87_	0.03	
7						0.02	_0.91_	
8							0.05*	_0.997*_
N	14	36	241	125	206	359	76	20

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TABLE 3-2g. Transition matrix for County Line 1996-1997.

STAGE	STAGE IN 1996							
IN 1997	1	2	3	4	5	6	7	8
1						0.001	0.04	1.08
2	0.88	_0.87*_						
3			_0.83_	0.17*	0.0075*	0.0001*		
4		0.01*	0.06	_0.62_	0.2425	0.0099		
5				0.21*	_0.73_	0.04		
6					0.02	_0.91_	0.04	
7						0.04	_0.89_	0.12
8							0.07	_0.877*_
N	166	87	138	27	64	181	102	69

TABLE 3-2h. Transition matrix for County Line 1997-1998.

STAGE	STAGE IN 1997							
IN 1998	1	2	3	4	5	6	7	8
1						0.001	0.15	1.13
2	0.74	_0.94_						
3			_0.91_	0.06				
4		0.01*	0.07	_0.67_	0.02			
5				0.27	_0.89_	0.01		
6					0.09	_0.97_		
7						0.02	_0.92_	0.01
8							0.08	_0.987*_
N	78	146	208	62	53	169	107	68

TABLE 3-2i. Transition matrix for Stivers 1996-1997.

STAGE IN 1997	STAGE IN 1996							
	1	2	3	4	5	6	7	8
1						0.007	0.16	1.13
2	0.71	_0.87_*						
3			_0.71_	0.15*	0.0015*	0.0001*		
4		0.01*	0.21	_0.53_	0.0485	0.0099		
5				0.26	_0.73_	0.09		
6					0.20	_0.88_	0.55	0.40
7						0.002	_0.40_	
8						0.003	0.05*	_0.597_*
N	14	10	125	61	440	637	11	5

TABLE 3-2j. Transition matrix for Stivers 1997-1998.

STAGE IN 1998	STAGE IN 1997							
	1	2	3	4	5	6	7	8
1						0.001	0.09	0.58
2	0.83	_0.99_						
3			_0.73_	0.02	0.02	0.002		
4		0.01*	0.17	_0.45_	0.05	0.01		
5				0.33	_0.65_	0.08		
6				0.02	0.25	_0.90_	0.17	
7						0.01*	_0.78_	
8							0.05*	_0.997_*
N	12	10	103	103	394	664	6	5

TABLE 3-2k. Transition matrix for Missaukee Diseased 1996-1997.

STAGE IN 1997	STAGE IN 1996							
	1	2	3	4	5	6	7	8
1							0.004	0.36
2	0.65	*0.87**						
3			*0.82*	0.18*	0.0027*	0.0001*		
4		0.01*	0.11	*0.64*	0.0873	0.0099	0.09	
5				0.12	*0.79*	0.01	0.14	
6					0.11	*0.94*		0.08
7						0.03	*0.72*	
8							0.05*	*0.917**
N	68	30	59	54	111	135	22	39

TABLE 3-2l. Transition matrix for Missaukee Diseased 1997-1998.

STAGE IN 1998	STAGE IN 1997							
	1	2	3	4	5	6	7	8
1							0.02	0.15
2	0.57	*0.90*						
3			*0.83*	0.07				
4		0.10	0.09	*0.53*	0.03	0.01		
5				0.30	*0.88*	0.06	0.05	0.03
6					0.09	*0.89*	0.19	0.03
7						0.01	*0.71*	
8						0.01	0.05*	*0.937**
N	14	42	78	71	99	141	21	36

Table 3-3. Finite rate of increase for American chestnut populations.

	1996-1997			1997-1998		
	Observed	Minimum	Percentage above minimum	Observed	Minimum	Percentage above minimum
<u>HEALTHY</u>						
Missaukee Healthy	1.000	0.823	21.5	0.997	0.855	16.6
Leelanau	0.999	0.910	9.8	1.007	0.931	8.2
<u>RECOVERING</u>						
Frankfort	0.997	0.909	9.7	0.997	0.915	9.0
County Line	0.994	0.946	5.1	1.021	0.965	5.8
<u>NON-RECOVERING</u>						
Stivers (7-8 transition: 0.05)	0.982	0.971	1.1	1.007	0.967	4.1
(7-8 transition: 0.00005)	0.982		1.1	0.992		2.6
Missaukee Diseased (7-8 transition: 0.05)	0.978	0.929	5.3	0.992	0.956	3.8
(7-8 transition: 0.00005)	0.973		4.7	0.991		3.7

Table

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Time

Time

Table 3-4. Repeated measures analysis of finite rate of population increase for American chestnut populations. F-values are for Roy's greatest root.

	F	Num df	Den df	P
Time	5.1579	1	3	0.1078
Time*Disease	0.9989	2	3	0.4651

Table 3-5. Comparisons of observed population structures and calculated stable stage distributions for six populations of American chestnut.

	Observed 1996 vs. Observed 1997	Observed 1997 vs. Observed 1998	Observed 1996 vs. Observed 1998	Observed 1997 vs. Stable 1996-1997	Observed 1998 vs. Stable 1997-1998	Stable 1996-1997 vs. Stable 1997-1998
MH	50.60***	25.00***	129.21***	766.25***	1218.25***	397.83***
L	3.36	8.49	7.79	465.01***	339.85***	103.80***
CL	75.66***	7.54	87.00***	203.98***	221.02***	34.06***
FF	64.47***	5.08	54.94***	1843.33***	1321.90***	136.68***
ST	17.74*	14.10*	50.95***	76.23***	1656.55***	1353.83***
MD	46.40***	4.07	60.70***	30.36***	74.95***	81.50***

* P < 0.05, *** P < 0.001

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Table 3-6. Repeated measures analysis of cross sectional area at breast height for American chestnut populations. F-values are for Roy's greatest root.

	F	Num df	Den df	P
Time	254.0145	2	2	0.0039
Time*Disease	493.5203	2	3	0.0001

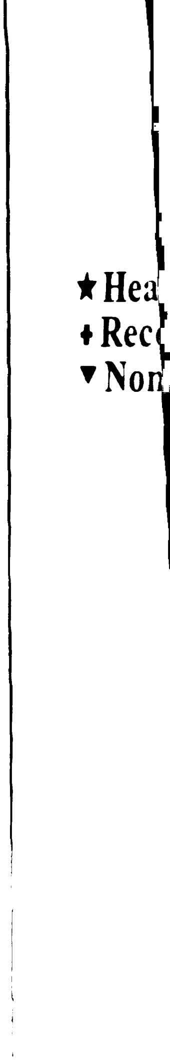

Figure 3.

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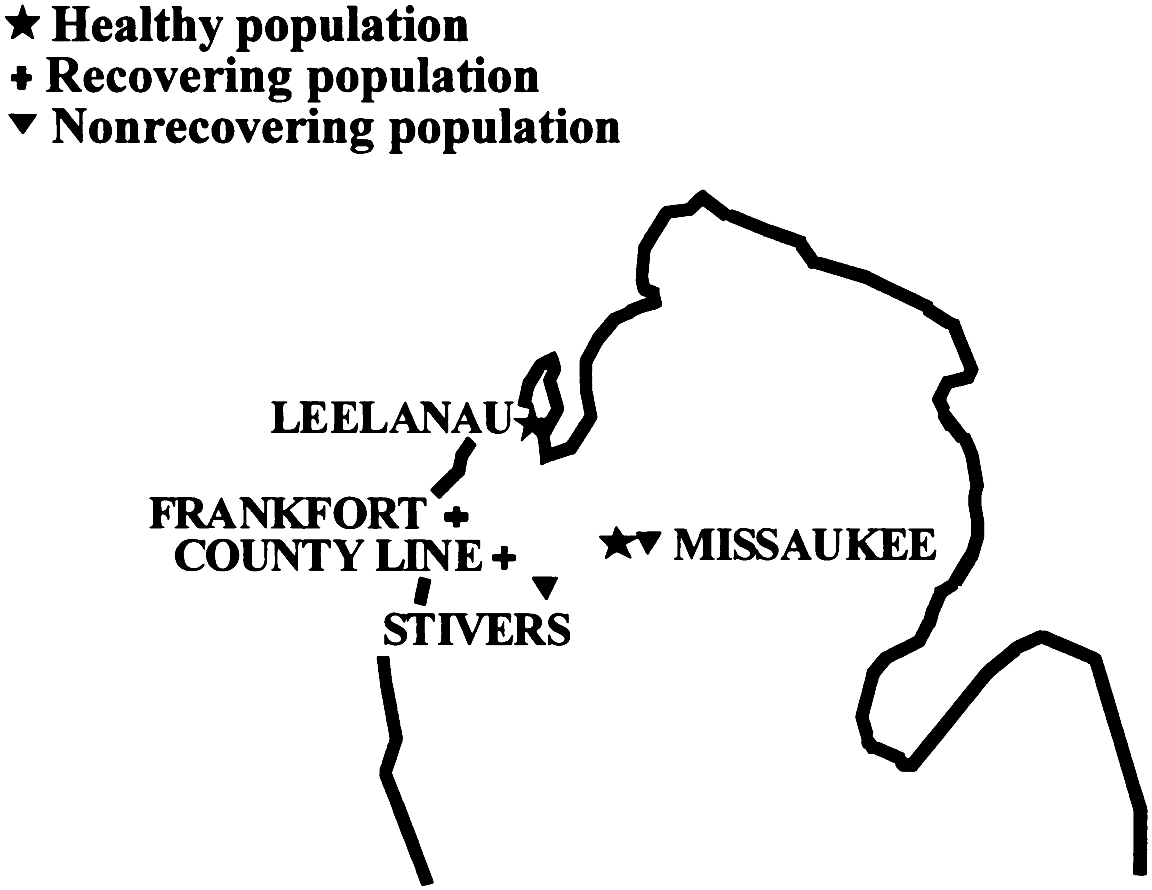
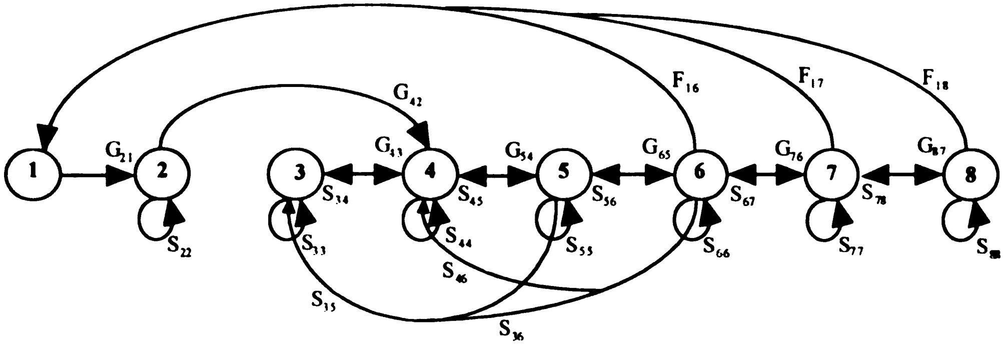


Figure 3-1. Map of American chestnut populations used in this study. Recovering populations are infected predominantly with dsRNA-containing chestnut blight (reduced virulence) and non-recovering populations are infected predominantly with dsRNA-free pathogens (virulent).

Figure 3-2. Life-cycle for a recovering population of American chestnut and its correspondence with the basic population projection matrix (**A**).



a. Circles represent stage classes, arrows show the possible transitions between stages, and letters indicate the connection between each transition and its corresponding matrix entry.

Stage at time $t + 1$	Stage at time t							
	1	2	3	4	5	6	7	8
1	0	0	0	0	0	F_{16}	F_{17}	F_{18}
2	G_{21}	S_{22}	0	0	0	0	0	0
3	0	0	S_{33}	S_{34}	S_{35}	S_{36}	0	0
4	0	G_{42}	G_{43}	S_{44}	S_{45}	S_{46}	0	0
5	0	0	0	G_{54}	S_{55}	S_{56}	0	0
6	0	0	0	0	G_{65}	S_{66}	S_{67}	0
7	0	0	0	0	0	G_{76}	S_{77}	S_{78}
8	0	0	0	0	0	0	G_{87}	S_{88}

b. Matrix elements represent fecundity (F), growth (G), and survival (S).

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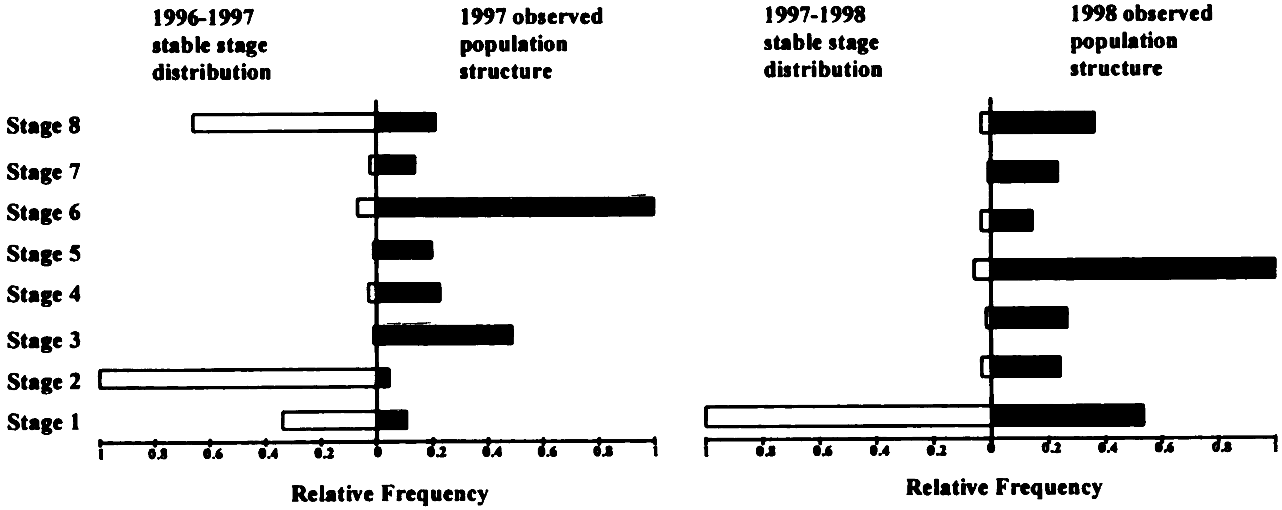
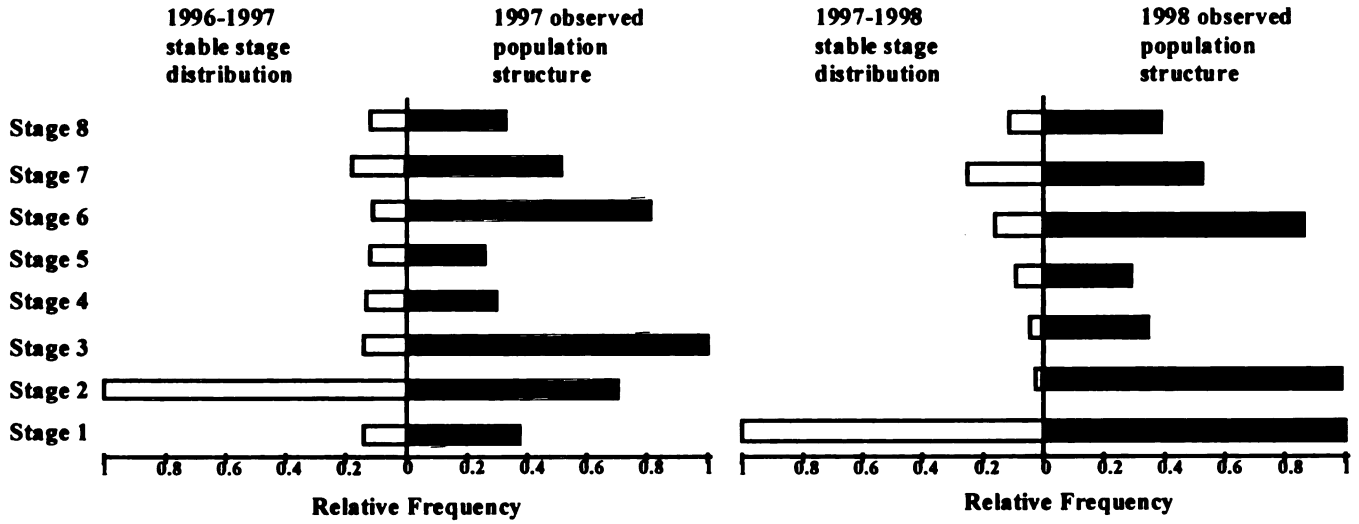
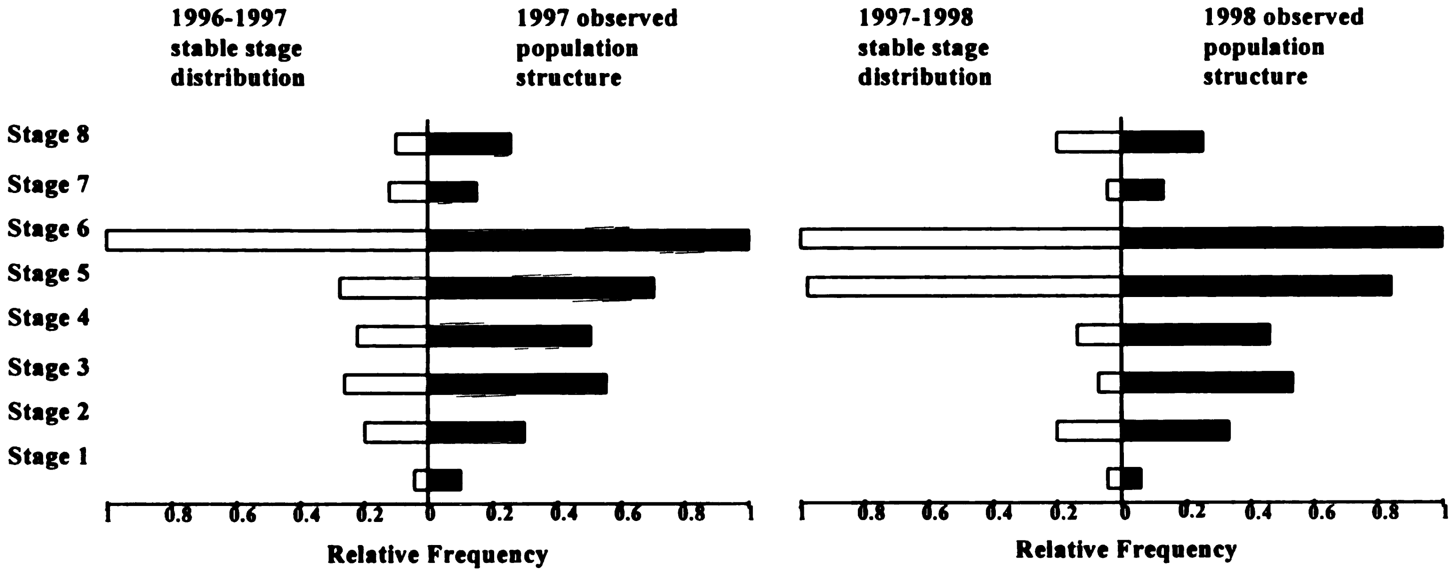
Figure 3-3. Population structures observed in 1997 and 1998 and calculated stable stage distributions for the census periods 1996-1997 and 1997-1998 for (a) a healthy population, (b) a recovering population, and (c) a non-recovering population.


1996-1997
stable stage
distribution
1997 observed
population
structure
1997-1998
stable stage
distribution
1998 observed
population
structure
Stage 8
Stage 7
Stage 6
Stage 5
Stage 4
Stage 3
Stage 2
Stage 1
1
0.8
0.6
0.4
0.2
0
0.2
0.4
0.6
0.8
1
Relative Frequency
Relative Frequency

(a) Leelanau



(b) County Line



(a) Missaukee Diseased

Mean cross sectional area versus census date

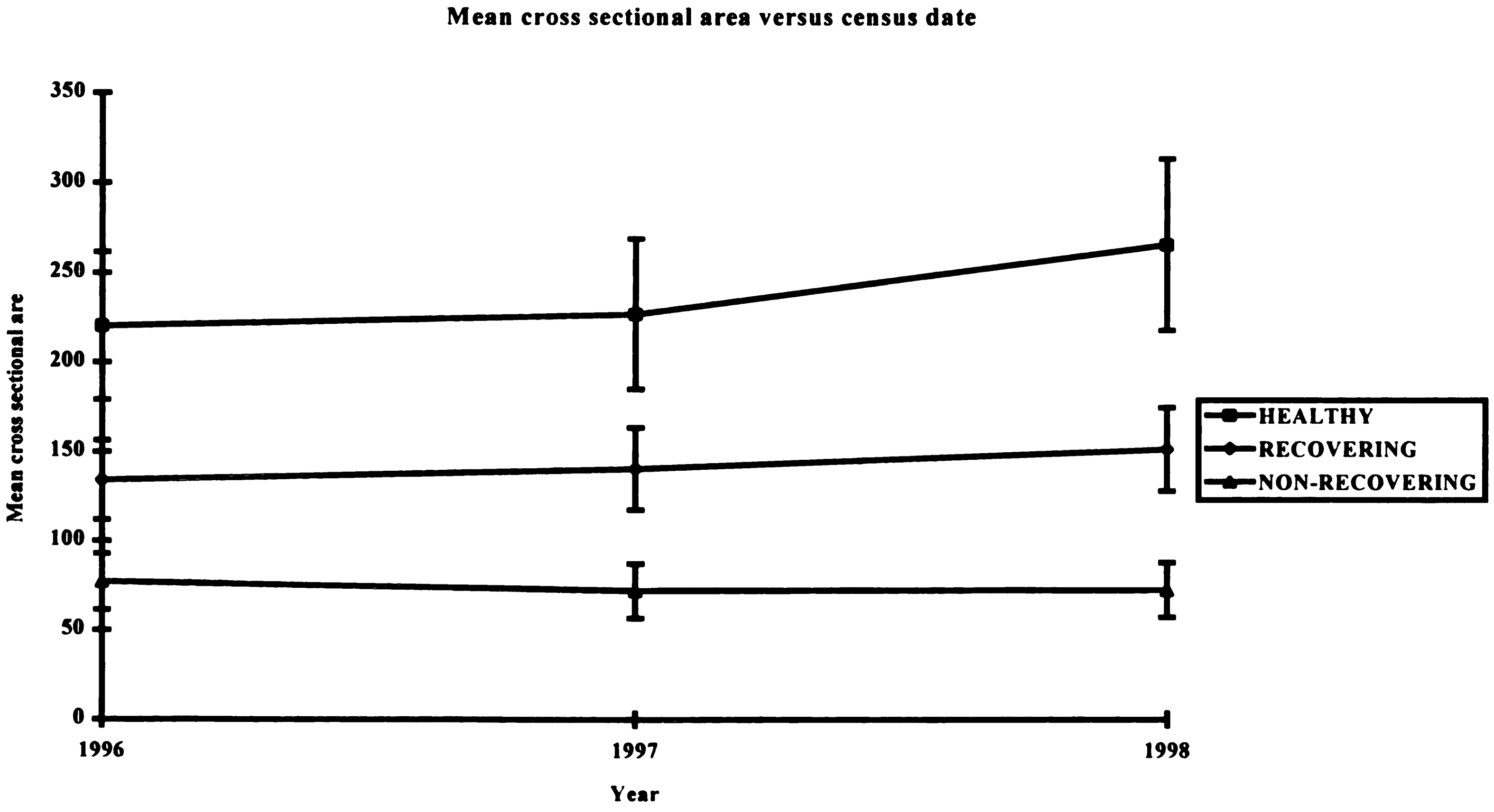


Figure 3-4. Mean cross sectional area (cm^2) ± standard error of American chestnut trees in each population type (healing, recovering, non-recovering) versus census date.

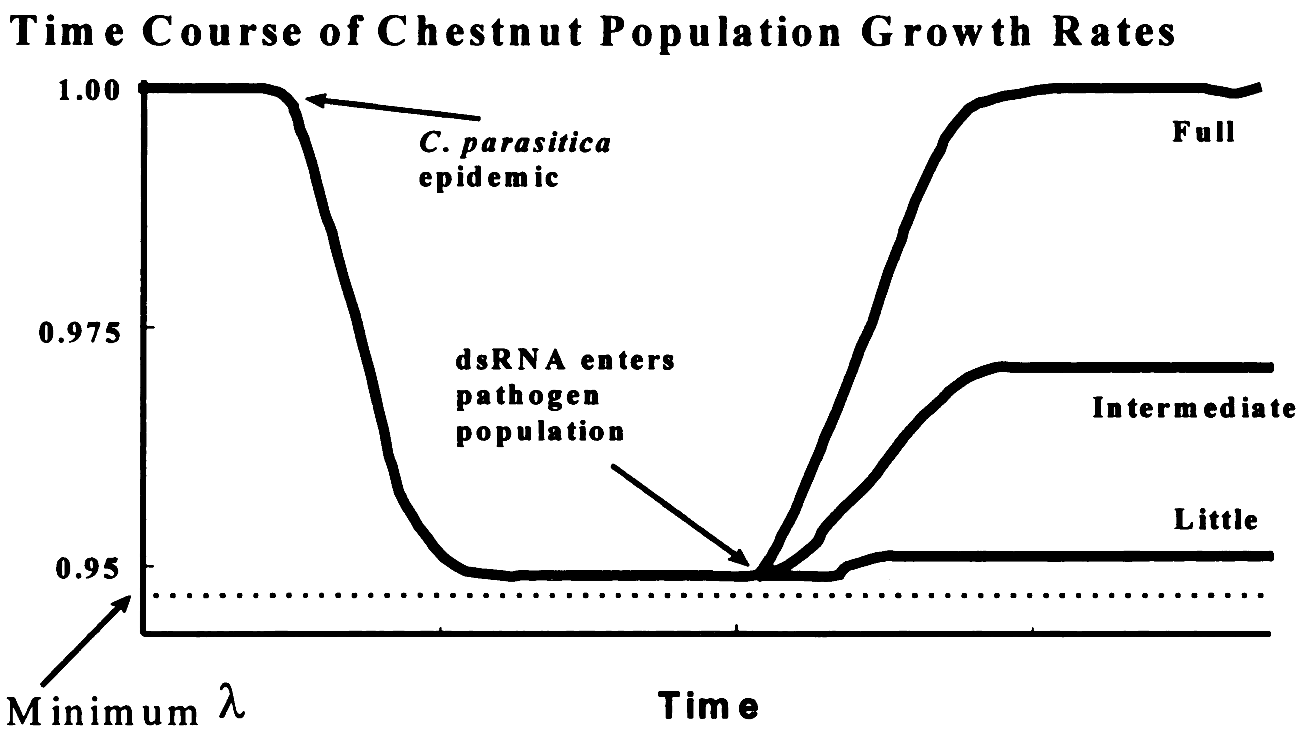


Figure 3-5. Hypothetical time course of population growth rates for American chestnut populations.

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CHAPTER 4

DOUBLE-STRANDED RNA MEDIATED RECOVERY OF THE AMERICAN CHESTNUT: WHAT CAN WE LEARN FROM HOST DEMOGRAPHY?

INTRODUCTION

Information on demographic parameters not only provide insights into the ecological and evolutionary dynamics of populations but also allows for effective management of rare and endangered species. Matrix population models may be the best method for making predictions about population dynamics and identifying critical life history stages of threatened species (Schemske et al. 1994). Indeed, these models have been used to evaluate population viability and to determine which life history stage should be the focus of management for a variety of endangered and rare plant and animal species (e.g. Crouse et al. 1987; Lande 1988; Menges 1990; Byers & Meagher 1997; Kephart & Paladino 1997).

Although the finite rate of population increase (λ) is an important demographic parameter which indicates whether a population is increasing or decreasing in size through time, other demographic parameters that indicate which particular stage or age class contributes most to population growth rate are more useful for management decisions. Matrix models are constructed by assigning individuals to age or stage classes and then determining the probability of an individual making a transition to another class (Caswell 1989). Sensitivity values measure the relative effect of small changes in transition probabilities on λ and elasticities represent the proportional contribution to λ

for each transition probability (Caswell 1989; Silvertown & Lovett Doust 1993). These parameters may be used to make recommendations about which life history stages of endangered or rare species to protect or manage (e.g. Crouse et al. 1987; Lande 1988; Kephart & Paladino 1997). Further, Silvertown et al. (1996) propose using elasticities to examine particular regions (growth, survival, and fecundity) of the transition matrix and their impact on population growth rates as a means of identifying populations that differ from the pattern normally found in a species.

Sensitivity and elasticity values could also be used to develop disease management programs. This approach may be particularly useful in evaluating the effectiveness of potential biological control agents of pathogens. By comparing these demographic parameters among healthy populations, diseased populations, and populations where biological control has been attempted, the success of treatment can be evaluated. Further, which particular life history stage of the plant should be treated to have the greatest impact on population growth rates can be determined.

Based on estimates of the finite rate of population increase, the introduction of double-stranded RNA (hereafter, dsRNA) into populations of *Cryphonectria parasitica*, the pathogenic fungus causing chestnut blight, may promote the recovery of American chestnut populations (see Chapter 3). Examination of other demographic parameters, specifically elasticities and sensitivities, may provide an alternate means of evaluating the effectiveness of dsRNA as a treatment for chestnut blight and provide insight into how best to deploy this management strategy.

The specific goals of this study were to (1) determine which size class would have the biggest impact on population growth as measured by sensitivity analysis thereby providing an indication of where management efforts should be concentrated; and (2) compare elasticities among American chestnut populations to evaluate the effectiveness of dsRNA mediated recovery; Do recovering populations have the same patterns of growth, survival, and fecundity as healthy populations or as non-recovering populations?

METHODS

Study System and Study Sites

For a description of the interaction between the American chestnut (*Castanea dentata*), chestnut blight, and dsRNA, see Chapters 2 and 3. Study sites utilized in this project are detailed in Chapter 3. Estimates of population growth rates revealed that recovering populations typically exhibit rates of growth intermediate between healthy and non-recovering populations (see Table 3-3).

Population Projection Matrices

Stage classes and projection matrices.--Construction of size classes and a description of the matrix model are presented in Chapter 3.

Data analyses.--Sensitivity and elasticity values were calculated for each population using RAMAS/stage (Ferson 1994). Sensitivity values ($s_{ij} = \delta\lambda/\delta a_{ij}$) measure the effect of a small change in a transition probability on λ relative to the same magnitude of change in other probabilities (Caswell 1989; Silvertown & Lovett Doust 1993; Valverde & Silvertown 1998). Elasticity values ($e_{ij} = a_{ij}/\lambda \text{ X } \delta\lambda/\delta a_{ij}$) represent the proportional contribution to λ for each transition probability (Caswell 1989; Silvertown & Lovett Doust 1993). Within a given transition matrix, elasticities sum to one which allows comparisons among populations to be made (de Kroon et al. 1986, Caswell 1989; Silvertown et al. 1996).

Silvertown et al. (1993) divide elasticity matrices into three regions and sum elasticities within each region. The *G* region (elements below the main diagonal of the transition matrix) represents the effect of growth on λ, the *L* region (non-fecundity elements above and on the main diagonal) measures the impact of survival within a stage and retrogression to a smaller stage, and the *F* region represents the effect of reproduction (top row of matrix). The *G/L/F* ratio for each population can be plotted on demographic triangles to investigate their relative positions and potential trajectories.

Some biologically important transitions were not observed in all populations and therefore were estimated as described in Chapter 3. To examine how these estimated transition probabilities affected sensitivities and elasticities, simulations were performed in which the transition probabilities were altered and the effects on sensitivities and elasticities were investigated. For the transition from stage 2 to stage 4, the transition probability was increased by 1000 percent to match the highest transition probability observed within a population (see Chapter 3). For the transition of stage 7 to stage 8 in non-recovering populations and for death in stage 8, the effect of reductions in these probabilities by 1/1000 was investigated. This transition in non-recovering populations and death in stage 8 were not observed and were estimated (see Chapter 3). Since these values were essentially observed to be zero, the effects of reducing the estimated probabilities to near zero was investigated.

RESULTS

Sensitivities

Sensitivity analysis describes how small perturbations in transition probabilities would impact the finite rate of population increase (λ); a large sensitivity for a transition indicates a small change in that transition would have a relatively large effect on λ (Silvertown & Lovett Doust 1993). The highest sensitivity values within a stage were often the transition representing growth to the next stage (Table 4-1a-l). Reproduction generally had the lowest sensitivity values (< 0.005). The only exceptions to this pattern were in the non-recovering populations where reproduction was relatively more important for stage 6 trees at Stivers for both the 1996-1997 and 1997-1998 censuses and for the stage 8 trees in the Missaukee Diseased site for 1997-1998. For diseased populations, the highest sensitivities were for a stage 6 transition, either growth to a larger stage (7 or 8) or survival in stage 6. In the recovering County Line population, sensitivity for growth (from stage 2 to 4 or from stage 7 to 8) was most important. However, for the other recovering site, Frankfort, and both healthy sites, the highest sensitivity overall was for survival in the largest size class (stage 8). For the Frankfort site in both census periods and for 1997-1998 at the Missuakee Healthy site, this transition overwhelmingly had the highest potential impact on population growth rate (sensitivity > 0.999).

Sensitivity values in the non-recovering populations indicated that stage 6 trees were very important, since this stage had the top five sensitivity values found in the matrix. The only exception was Stivers in 1997-1998, where the highest value was for growth from stage 6 to 7 but the growth transition from stage 2 to 4 was second highest and survival of stage 8 trees was third highest.

Decreasing the death rate by a factor of 1/1000 in stage 8 did not alter the general pattern of sensitivities for any population in any census period and had only a minor impact of the magnitude of sensitivities (data not shown). Changing these transitions did not materially change the pattern or magnitude of sensitivities for most populations. In those populations where changes occurred, the sensitivity values for some of the growth and fecundity transitions increased in value; however, there was no consistent pattern in which specific transitions changed in magnitude.

Elasticities

Elasticity analysis describes the proportional impact of a transition on the finite rate of population increase (Silvertown & Lovett Doust 1993). Across all populations, survival within a stage (diagonal elements of the matrices) had the greatest contribution to λ (Table 4-2a-l). However, which particular stage was most important differed among populations and among census periods. For the 1996-1997 census period, one of the larger size classes (stages 6 to 8) had the greatest elasticity value within each population. For both healthy populations and overwhelmingly for the recovering Frankfort site, survival in the largest size class (stage 8) had the largest proportional impact on population growth rate. At the remaining recovering site, County Line, survival in stage 7 was most important. In contrast, for the non-recovering populations, survival in stage 6 had the highest elasticity. For the 1997-1998 census period, the elasticity for survival in the largest size class was greatest for five of the six populations. The one exception was the non-recovering Missaukee Diseased site where again survival in stage 6 had the greatest proportional contribution to λ.

The relative contribution to λ of survivorship (*L*), growth (*G*), and fecundity (*F*) can be assessed by summing elasticities across all stages (Silvertown et al. 1993). Survivorship made the greatest contribution to λ in all populations across both years, while fecundity never contributed more than 0.01 for any population-by-year combination (Table 4-3). Growth was relatively more important in the chestnut populations where *C. parasitica* was present. The only exception to this pattern was at Frankfort where growth elasticity values, based on observed transitions, were extremely low. Indeed, growth values for Frankfort were one to two orders of magnitude lower than growth values for any other population.

A possible explanation for the extremely low values for growth at Frankfort may be associated with the lack of observed retrogressions of stage 8 trees (see Table 3-2e-f). Stage 8 retrogressions were observed in all other chestnut populations where *C. parasitica* was present. The absence of retrogressions at Frankfort is most likely due to the small number of stage 8 trees (17 in 1996 and 20 in 1997), which meant that retrogressions might not have been observed by chance alone. If the stage 8 retrogression values are estimated from the average stage 8 retrogressions observed in the remaining 5 populations, then growth elasticity values increase by more than 150 fold and the *G/L/F* ratio becomes very similar to those observed in the two healthy populations (Table 4-3).

Silvertown et al. (1993) included retrogressions in the *L* region. Because retrogression appears to be a characteristic of diseased populations (see Chapter 3), I examined the sum of the elasticities for retrogressions alone. In populations with disease, there is an order of magnitude difference in the corresponding elasticities for populations with disease versus healthy populations (Table 4-4). Again, the recovering Frankfort population is an exception to this pattern.

All populations matrices contained one or more transition values that were not observed over the two years of the study, but were estimated because they must occur for biological reality. For example, no stage 8 tree died in any population over the two census periods, yet it is reasonable to assume that these trees do occasionally die. The mortality rate for stage 8 trees was estimated by assuming that the next sampled stage 8 tree would have died during the study. This "death" was added to the existing stage 8 trees and a grand mortality rate (0.003) was calculated across all populations and used in the matrix for each individual population. It is possible that the mortality rate of stage 8 trees is significantly lower than this value. Reanalyzing the matrices with a lower stage 8 mortality (0.000003) resulted in only slight increases in the contribution of survivorship (L) to λ (data not shown). Growth from stage 2 to stage 4 was observed only at the Missaukee Diseased site for the 1997-1998 census. In the other matrices, this transition was estimated as the grand average across all population by year combinations (0.01). Increasing the stage 2 to 4 growth rate to the value observed at the Missaukee Diseased site from 1997-1998 resulted in a slight increase in the importance of growth (G) to λ (data not shown).

Finally, growth from stage 7 to 8 was not observed in either of the non-recovering populations. As a conservative estimate of this transition, the average stage 7 to 8 transition (0.05) observed across the other four populations was used. However, the presence of *C. parasitica* in these non-recovering populations may actually reduce this transition to near zero. Reducing this transition in non-recovering populations resulted in only slight increases in the importance of survivorship (*L*) to λ in these populations (data not shown).

DISCUSSION

Elasticity values and the sensitivity analyses indicate that survival of the largest trees has the greatest impact on population growth in both healthy populations. This result is not surprising given that chestnuts are normally long-lived trees. Indeed, other studies of woody plants have revealed similar patterns of population growth being most sensitive to survivorship of large individuals (e.g. Piñero et al. 1984; Huenneke & Marks 1987). The blight pathogen, *C. parasitica*, affects demographic patterns within infected chestnut populations. The most noticeable changes are seen in the largest stages of these diseased, non-recovering populations. Transitions that were not observed in healthy populations had a significant impact within diseased populations. Stage 7 and 8 trees retrogressed to smaller stages, and stage 6 and 7 trees were observed to set seed (Chapter 3). In addition, the growth transition from stage 7 to 8 was not observed in either of the diseased population. These changes resulted in a slight decrease in population growth rate so that λ values were near the minimum possible for a population, given its survival schedule (see Table 3-3). Sensitivity and elasticity values also indicated a major change in population demographics. The most striking change was seen for the stage 8 trees at Missaukee Diseased and at Stivers in 1996-97 where stage 8 trees were no longer having the largest effect on population growth (Tables 4-1i,k & l and 4-2i,k & l). At these diseased sites, stage 6 trees were most important. These results are in general accord with known dynamics of *C. parasitica* epidemics, in which infections rarely kill trees, but instead cause a reduction in size. This trend has also been found in diseased chestnut populations within the natural range where large trees are extremely rare, but chestnuts are still a major component of the forest understory (Stephenson et al. 1991; Parker et al. 1993).

Silvertown et al. (1996) proposed that a graphical presentation of elasticity values for growth (*G*), survivorship (*L*) and fecundity (*F*) can be used to identify populations at risk. This concept was proposed as an aid for plant population conservation; populations with altered *G/L/F* ratios normally have reduced population growth rates (Silvertown et al. 1996). When this ratio is combined with sensitivity analyses decisions can be made to determine which growth stages should have highest priority with regard to conservation efforts. Demographic data have been used to identify unique features of rare species (Byers & Meagher 1997) and aid conservation efforts (Crouse et al. 1987).

One major objective of this study was to determine whether *G/L/F* ratios could be used in a more general context to highlight altered population demographics. There was no clear separation between healthy, non-recovering and recovering populations when they were graphically plotted for *G/L/F* ratios and population growth (λ) (Figure 4-1). The lack of any clear separation can be explained by the fact that infections do not materially increase tree mortality within infected populations and survivorship (*L*) remains unchanged because of the way it has been traditionally calculated. The *L* component of the *G/L/F* ratios includes retrogressions (Silvertown et al. 1993). Retrogressions are uncommon for woody plants, and thus including retrogressions into *L* does not normally influence the *G/L/F* ratios. As discussed above, the increase in retrogressions of large trees within diseased chestnut population is one of the major effects of *C. parasitica* epidemics. Thus, one problem of this graphical analysis is the fact that potentially important alterations in demographics can be hidden within the *L* variable.

It is doubtful that diseased populations would appear to be aberrant even if retrogressions were discounted from the calculation of *L*. Retrogressions contribute only between 1 and 8% to population growth (Table 4-4). Further, sensitivity values are usually low for retrogressions, except for those involving stage 6 trees (Table 4-1i-l). The inability of this graphical method to detect altered demographics is due to the long-lived nature of chestnuts. The *G/L/F* ratios for all long-lived plants are always overwhelmingly dominated by *L*. A survey of 21 woody plant species found that L contributed greater than 78% to the population growth rate (Silvertown et al. 1993). As a result, long-lived perennials are constrained to a very restricted corner of the *G/L/F* space unless the factors altering demographics cause large increases in mortality. This study demonstrates that significant changes in the demographics of long-lived species can occur without increasing mortality. The net effect is that the *G/L/F* graphical method should be used with caution. While the method may be useful for a generalized evaluation of some species, it will not always detect significant changes in population demographics.

This study also sought to evaluate the extent of recovery in chestnut populations where the dsRNA hyperparasite has invaded the *C. parasitica* pathogen population.

Recovery, as indicated by the presence of non-lethal cankers, was first noted in the 1970s at the County Line and Frankfort sites (Brewer 1995). A recent survey *of C. parasitica* from County Line and Frankfort found that dsRNA had successfully spread to the majority of the pathogen population with 89% and 94%, respectively, of the sampled cankers containing dsRNA (Davelos et al. 1997). Population growth rates of the recovering populations tend to be similar to those of healthy populations (Chapter 3, Table 3-3). Recovering populations also retain a number of transitions that are found in diseased populations. For example, retrogressions of stage 7 and 8 trees are found in non-recovering and recovering populations, but not healthy populations. In addition, stage 6 and 7 trees were observed to reproduce at recovering and non-recovering sites, but not at healthy sites. Recovery seems to be due to the reduced magnitude of retrogressions and increased growth of large trees at recovering sites.

The *G/L/F* ratios were again inconclusive for the recovering populations. Ratios for County Line were very similar to those ratios found at the non-recovering sites, and the Frankfort site had ratios that were unique but closest to the healthy sites (Table 3-3). If estimates for stage 8 retrogressions were used at Frankfort, then Frankfort's *G/L/F* ratios became very similar to ratios found at the healthy sites. A detailed examination of the elasticity and sensitivity matrices indicated that the recovering sites had largely recaptured much of the important dynamics found at healthy sites. Similar to healthy sites, the highest elasticity and sensitivity values were found for survivorship of stage 8 trees. These data indicate that the dsRNA hyperparasite can help to mediate recovery of chestnut populations.

The pattern of sensitivity and elasticity values at the non-recovering sites can also be used to aid efforts to introduce dsRNAs for the purpose of saving chestnut populations (MacDonald & Fulbright 1991). The high sensitivity and elasticity values for stage 6 trees argue that these trees will have the most immediate effect on population growth, and initial efforts to introduce dsRNAs should concentrate on this class of trees. Knowledge about the contribution of life history stages to population growth and stability is crucial for species conservation efforts where limited resources need to be utilized effectively (Crouse et al. 1987). These results emphasize the need for a careful examination and interpretation of all demographic parameters when developing management strategies.

Table 4-1. Sensitivity matrices for six American chestnut populations for two census periods. A large value indicates a small change in that transition would have a relatively large effect on λ (Silvertown & Lovett Doust 1993). Values for survival within a stage are italicized.

TABLE 4-1a. Sensitivities for Missaukee Healthy 1996-1997.

STAGE IN 1997	STAGE IN 1996							
	1	2	3	4	5	6	7	8
1								**0.00121**
2	**0.00581**	***0.02093***						
3			***0.00267***	**0.0044**	**0.00808**	**0.00241**		
4		**0.27307**	**0.0129**	***0.02131***	**0.03909**	**0.01168**		
5				**0.03504**	***0.06428***	**0.01920**		
6					**0.13085**	***0.03909***		
7						**0.27307**	***0.05414***	
8							***0.05461***	***0.79486***

TABLE 4-1b. Sensitivities for Missaukee Healthy 1997-1998.

STAGE	STAGE IN 1997							
IN 1998	1	2	3	4	5	6	7	8
1								**0.00008**
2	**0.00005**	***0.0001***						
3			***0.00001***	**0.00000**	**0.00001**	**0.00000**		
4		**0.00203**	**0.00008**	***0.00004***		**0.00001**		
5				**0.00007**	***0.00023***	**0.00002**		
6					**0.00212**	***0.00014***		
7						**0.00203**	***0.0001***	
8							**0.0001**	***0.99936***

TABLE 4-1c. Sensitivities for Leelanau 1996-1997.

STAGE IN 1997	STAGE IN 1996							
	1	2	3	4	5	6	7	8
1								**0.00329**
2	**0.0045**	***0.01328***						
3			***0.00058***	**0.00109**	**0.00051**	**0.00249**		
4		**0.17112**	**0.00241**	***0.00451***	**0.00210**	**0.01033**		
5				**0.01122**	***0.00523***			
6					**0.00798**	***0.0392***		
7						**0.08556**	***0.02906***	
8							**0.02852**	***0.90643***

TABLE 4-1d. Sensitivities for Leelanau 1997-1998.

STAGE IN 1998	STAGE IN 1997							
	1	2	3	4	5	6	7	8
1								0.00475
2	0.00776	*0.27669*						
3			*0.00273*	0.00246		0.00251		
4		0.44216	0.01437	*0.01295*	0.02846	0.01324		
5				0.03378	*0.07422*	0.03453		
6					0.14964	*0.06962*		
7						0.44216	*0.06701*	
8							0.07369	*0.49237*

TABLE 4-1e. Sensitivities for Frankfort 1996-1997.

STAGE IN 1997	STAGE IN 1996							
	1	2	3	4	5	6	7	8
1						0.00000	0.00000	0.00011
2	0.00003	*0.00014*						
3			*0.00000*	0.00001	0.00000	0.00000		
4		0.00183	0.00002	*0.00006*	0.00001	0.00001		
5				0.0005	*0.00008*	0.00005	0.00001	
6					0.00027	*0.00018*	0.00004	
7						0.00072	*0.00017*	
8							*0.00061*	*0.99935*

TABLE 4-1f. Sensitivities for Frankfort 1997-1998.

STAGE	STAGE IN 1997							
IN 1998	1	2	3	4	5	6	7	8
1							**0.00000**	**0.00043**
2	**0.00003**	***0.00006***						
3			***0.00001***	**0.00001**	**0.00001**	**0.00001**		
4		**0.00258**	**0.00005**	***0.00006***	**0.00005**	**0.00004**		
5				**0.00016**	***0.00014***	**0.00013**	**0.00003**	
6				**0.00035**	**0.00030**	***0.00028***	**0.00006**	
7						**0.00140**	***0.00032***	
8							**0.00051**	***0.99910***

TABLE 4-1g. Sensitivities for County Line 1996-1997.

STAGE IN 1997	STAGE IN 1996							
	1	2	3	4	5	6	7	8
1						**0.00145**	**0.00229**	**0.0015**
2	**0.00194**	***0.01378***						
3			***0.00904***	**0.00837**	**0.00778**	**0.00743**		
4		**0.17085**	**0.02469**	***0.02286***	**0.02126**	**0.02031**		
5				**0.03393**	***0.03157***	**0.03015**		
6					**0.15582**	***0.14884***	**0.23444**	
7						**0.27712**	***0.43649***	***0.28571***
8							***0.51287***	***0.3357***

TABLE 4-1h. Sensitivities for County Line 1997-1998.

STAGE	STAGE IN 1997							
IN 1998	1	2	3	4	5	6	7	8
1						**0.01385**	**0.00355**	**0.00829**
2	**0.0134**	***0.12203***						
3			***0.01264***	**0.02345**				
4		**0.9918**	**0.02010**	***0.03728***	**0.08850**			
5				**0.04329**	***0.10277***	**0.18038**		
6					**0.13024**	***0.22859***		
7						**0.49521**	***0.12707***	**0.29657**
8							**0.15421**	***0.35990***

TABLE 4-1i. Sensitivities for Stivers 1996-1997.

STAGE	STAGE IN 1996							
IN 1997	1	2	3	4	5	6	7	8
1						**0.02980**	**0.00051**	**0.00007**
2	**0.00051**	***0.00326***						
3			***0.02066***	**0.03563**	**0.16409**	**0.35700**		
4		**0.03655**	**0.02679**	***0.04619***	**0.21274**	**0.46284**		
5				**0.05980**	***0.27539***	**0.59915**		
6					**0.29453**	***0.64080***	**0.01101**	**0.00143**
7						**0.67812**	***0.01165***	
8							***0.01293***	***0.00168***

TABLE 4-1j. Sensitivities for Stivers 1997-1998.

STAGE	STAGE IN 1997							
IN 1998	1	2	3	4	5	6	7	8
1						**0.03845**	**0.00169**	**0.00820**
2	**0.00596**	***0.28593***						
3			***0.00200***	**0.00865**	**0.01908**			
4		**0.49490**	**0.00326**	***0.01412***	**0.03112**	**0.08076**		
5				**0.02165**	***0.04773***	**0.12387**		
6				**0.02743**	**0.06047**	***0.15693***	**0.00690**	
7						**0.60842**	***0.02677***	
8							**0.09517**	***0.46161***

TABLE 4-1k. Sensitivities for Missaukee Diseased 1996-1997.

STAGE	STAGE IN 1996							
IN 1997	1	2	3	4	5	6	7	8
1							**0.00192**	**0.00156**
2	**0.00088**	***0.00526***						
3			***0.04854***	**0.04184**	**0.05278**	**0.18772**		
4		**0.05705**	**0.06994**	***0.06028***	**0.07605**	**0.27046**	**0.03139**	
5				**0.10728**	***0.13533***	**0.48129**	**0.05586**	
6					**0.17023**	***0.60542***		**0.05715**
7						**0.52625**	***0.06108***	
8							***0.10269***	***0.08351***

TABLE 4-1l. Sensitivities for Missaukee Diseased 1997-1998.

STAGE IN 1998	STAGE IN 1997							
	1	2	3	4	5	6	7	8
1							**0.00554**	**0.03338**
2	**0.00898**	***0.05557***						
3			***0.00852***	**0.01974**				
4		**0.05118**	**0.01535**	***0.03555***	**0.24743**	**0.25037**		
5				**0.05015**	***0.34907***	**0.35321**	**0.01252**	**0.07548**
6					**0.35227**	***0.35645***	**0.01264**	**0.07617**
7						**0.45764**	***0.01622***	
8						**0.81175**	***0.02878***	***0.17346***

Table 4-2. Elasticity matrices for six American chestnut populations for two census periods. Within a given population for each census period, elasticities sum to 1. Values for survival within a stage are italicized.

TABLE 4-2a. Elasticities for Missaukee Healthy 1996-1997.

STAGE IN 1997	STAGE IN 1996							
	1	2	3	4	5	6	7	8
1								**0.00273**
2	**0.00273**	***0.01821***						
3			***0.00189***	**0.00075**	**0.00002**	**0.00000**		
4		**0.00273**	**0.00077**	***0.01320***	**0.00379**	**0.00081**		
5				**0.00735**	***0.05654***	**0.00038**		
6					**0.00392**	***0.03516***		
7						**0.00273**	***0.05141***	
8							***0.00273***	***0.79213***

TABLE 4-2b. Elasticities for Missaukee Healthy 1997-1998.

STAGE	STAGE IN 1997							
IN 1998	1	2	3	4	5	6	7	8
1								0.00002
2	0.00002	*0.00008*						
3			*0.00001*	0.00000	0.00000	0.00000		
4		0.00002	0.00000	*0.00002*		0.00000		
5				0.00002	*0.00020*	0.00000		
6					0.00002	*0.00012*		
7						0.00002	*0.00008*	
8							*0.00002*	*0.99934*

TABLE 4-2c. Elasticities for Leelanau 1996-1997.

STAGE	STAGE IN 1996							
IN 1997	1	2	3	4	5	6	7	8
1								**0.00171**
2	**0.00171**	***0.01156***						
3			***0.00044***	**0.00014**	**0.00000**	**0.00000**		
4		**0.00171**	**0.00014**	***0.00212***	**0.00033**	**0.00020**		
5				**0.00225**	***0.00299***			
6					**0.00192**	***0.03728***		
7						**0.00171**	***0.02735***	
8							***0.00171***	***0.90472***

TABLE 4-2d. Elasticities for Leelanau 1997-1998.

STAGE	STAGE IN 1997							
IN 1998	1	2	3	4	5	6	7	8
1								**0.00440**
2	**0.0044**	***0.27230***						
3			***0.00187***	**0.00081**		**0.00005**		
4		**0.00440**	**0.00086**	***0.00644***	**0.00113**	**0.00013**		
5				**0.00571**	***0.06714***	**0.00137**		
6					**0.00595**	***0.06367***		
7						**0.00440**	***0.06262***	
8							**0.00440**	***0.48798***

TABLE 4-2e. Elasticities for Frankfort 1996-1997.

STAGE IN 1997	STAGE IN 1996							
	1	2	3	4	5	6	7	8
1						**0.00000**	**0.00000**	**0.00002**
2	**0.00002**	***0.00013***						
3			***0.00000***	**0.00000**	**0.00000**	**0.00000**		
4		**0.00002**	**0.00000**	***0.00003***	**0.00000**	**0.00000**		
5				**0.00002**	***0.00005***	**0.00000**	**0.00000**	
6					**0.00002**	***0.00016***	**0.00000**	
7						**0.00002**	***0.00015***	
8							***0.00002***	***0.99933***

TABLE 4-2f. Elasticities for Frankfort 1997-1998.

STAGE IN 1998	STAGE IN 1997							
	1	2	3	4	5	6	7	8
1							0.00000	0.00003
2	0.00003	*0.00004*						
3			*0.00001*	0.00000	0.00000	0.00000		
4		0.00003	0.00000	*0.00002*	0.00000	0.00000		
5				0.00002	*0.00011*	0.00001	0.00000	
6				0.00001	0.00003	*0.00024*	0.00000	
7						0.00003	*0.00029*	
8							*0.00003*	*0.99908*

TABLE 4-2g. Elasticities for County Line 1996-1997.

STAGE	STAGE IN 1996							
IN 1997	1	2	3	4	5	6	7	8
1						0.00000	0.00009	0.00163
2	0.00172	*0.01207*						
3			*0.00755*	0.00143	0.00006	0.00000		
4		0.00172	0.00149	*0.01426*	0.00519	0.00020		
5				0.00717	*0.02318*	0.00121		
6					0.00314	*0.13627*	0.00943	
7						0.01115	*0.39085*	*0.03449*
8							*0.03612*	*0.29958*

TABLE 4-2h. Elasticities for County Line 1997-1998.

STAGE	STAGE IN 1997							
IN 1998	1	2	3	4	5	6	7	8
1						**0.00001**	**0.00052**	**0.00918**
2	**0.00971**	***0.11231***						
3			***0.01127***	**0.00138**				
4		**0.00971**	**0.00138**	***0.02446***	**0.00173**			
5				**0.01144**	***0.08956***	**0.00177**		
6					**0.01148**	***0.21711***		
7						**0.00970**	***0.11447***	**0.00290**
8							**0.01208**	***0.34782***

TABLE 4-2i. Elasticities for Stivers 1996-1997.

STAGE IN 1997	STAGE IN 1996							
	1	2	3	4	5	6	7	8
1						0.00021	0.00008	0.00008
2	0.00037	*0.00288*						
3			*0.01494*	0.00544	0.00025	0.00004		
4		0.00037	0.00573	*0.02492*	0.0105	0.00466		
5				0.01583	*0.20466*	0.0549		
6					0.05997	*0.57409*	0.00616	0.00058
7						0.0069	*0.00474*	
8							*0.00066*	*0.00102*

TABLE 4-2j. Elasticities for Stivers 1997-1998.

STAGE IN 1998	STAGE IN 1997							
	1	2	3	4	5	6	7	8
1						0.00004	0.00015	0.00472
2	0.00491	*0.28102*						
3			*0.00145*	0.00017	0.00038			
4		0.00491	0.00055	*0.00631*	0.00154	0.0008		
5				0.00709	*0.03080*	0.00984		
6				0.00054	0.01501	*0.14022*	0.00117	
7						0.00604	*0.02073*	
8							*0.00472*	*0.45688*

TABLE 4-2k. Elasticities for Missaukee Diseased 1996-1997.

STAGE IN 1997	STAGE IN 1996							
	1	2	3	4	5	6	7	8
1							**0.00001**	**0.00058**
2	**0.00058**	***0.00468***						
3			***0.04068***	**0.00770**	**0.00015**	**0.00002**		
4		**0.00058**	**0.00786**	***0.03943***	**0.00678**	**0.00274**	**0.00289**	
5				**0.01316**	***0.10926***	**0.00492**	**0.00799**	
6					**0.01914**	***0.58161***		**0.00467**
7						**0.01613**	***0.04494***	
8							**0.00525**	***0.07827***

TABLE 4-21. Elasticities for Missaukee Diseased 1997-1998.

STAGE	STAGE IN 1997							
IN 1998	1	2	3	4	5	6	7	8
1							**0.00011**	**0.00505**
2	**0.00516**	***0.05041***						
3			***0.00713***	**0.00139**				
4		**0.00516**	**0.00139**	***0.01899***	**0.00748**	**0.00252**		
5				**0.01516**	***0.30963***	**0.02136**	**0.00063**	**0.00228**
6					**0.03196**	***0.31977***	**0.00242**	**0.00230**
7						**0.00461**	***0.01161***	
8						**0.00818**	***0.00145***	***0.16383***

Table 4-3. Sums of elasticities within the G (growth), L (survival), and F (fecundity) regions of transition matrices for six populations of American chestnut.

a. 1996-1997 census

Population	Growth (G)	Survival (L)	Fecundity (F)
Healthy			
Missaukee Healthy	0.02296	0.97429	0.00273
Leelanau	0.01115	0.98713	0.00171
Recovering			
Frankfort (Observed)	0.00012	0.99985	0.00002
(With stage 8 retrogressions)	0.02323	0.97664	0.00010
County Line	0.06251	0.93577	0.00172
Non-recovering			
Stivers	0.08983	0.90978	0.00037
Missaukee Diseased	0.06270	0.93673	0.00059

b. 1997-1998 census

Population	Growth (G)	Survival (L)	Fecundity (F)
Healthy			
Missaukee Healthy	0.00012	0. 99985	0.00002
Leelanau	0.03012	0. 96551	0.00440
Recovering			
Frankfort (Observed)	0.00018	0. 99980	0.00003
(With stage 8 retrogressions)	0.02961	0. 97026	0.00014
County Line	0.06550	0. 92478	0.00971
Non-recovering			
Stivers	0.04377	0. 95131	0.00491
Missaukee Diseased	0.07307	0.92175	0.00516

Table 4-4. Sum of the elasticities for retrogressions for six American chestnut populations for each census period.

	1996-1997	1997-1998
Missaukee Healthy	0.00575	0.00000
Leelanau	0.00067	0.00349
Frankfort	0.00000	0.00001
County Line	0.05201	0.00778
Stivers	0.08253	0.01390
Missaukee Diseased	0.03786	0.04038

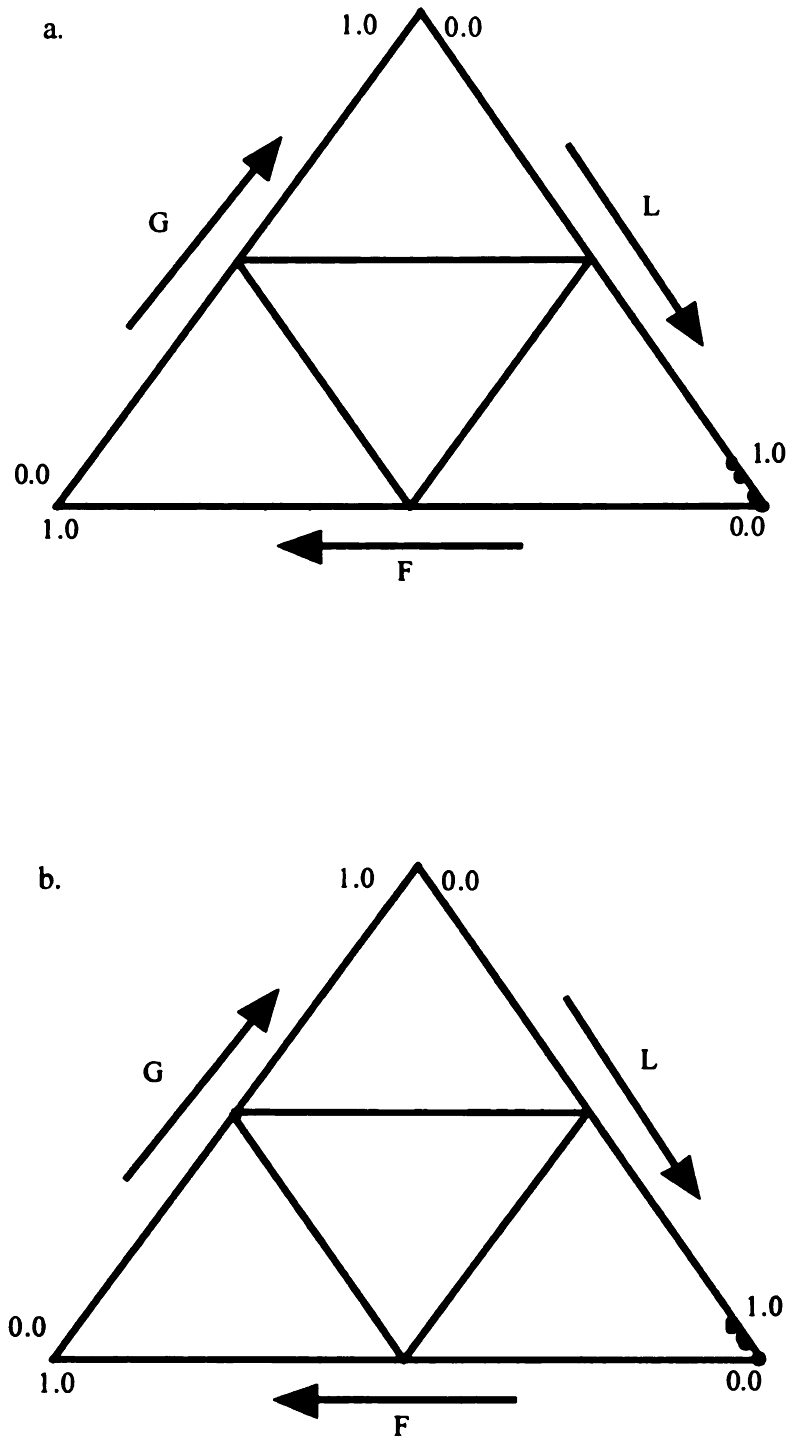

Figure 4-1. Plot of G/L/F elasticities for six populations of American chestnut. a. 1996-1997 census. b. 1997-1998 census.

CHAPTER 5

SEEDLING GROWTH AND SURVIVAL IN POPULATIONS OF AMERICAN CHESTNUT THAT DIFFER IN DISEASE STATUS

INTRODUCTION

Pathogen infection has been shown to decrease plant reproduction, whether measured as flower number, fruit production, seed production, or seed biomass (Alexander & Burdon 1984; Clay 1984; Parker 1986; Paul & Ayres 1986a; Paul & Ayres 1986b; Parker 1987; Paul & Ayres 1987; Wennström & Ericson 1990; Wennström & Ericson 1991; Jarosz & Burdon 1992; Roy & Bierzychudek 1993; Thrall & Jarosz 1994; García-Guzmán et al. 1996; Marr 1997). Clearly reductions in reproduction by seed have negative fitness consequences for an individual plant. Moreover, this negative effect on individuals may translate into effects at a population level by decreasing recruitment and population expansion rates. One extreme example is anther smut of *Silene alba*. Infections completely sterilize the host, and no seed is produced (Alexander et al. 1996). As a consequence, population growth rates are depressed when the smut fungus, *Microbotryum violaceum*, is found within a host population (Antonovics et al. 1994). A similar trend of reduction in the rate of population expansion was observed for populations of *Silene dioica* infected by *M. violaceum* (Carlsson & Elmqvist 1992).

However, pathogens may also cause more subtle changes in reproductive effort through effects on seed quality, which also affect growth and competitive ability in the next generation. For example, seed from wheat infected with *Erysiphe graminis* (a fungal pathogen that causes a foliar disease and does not infect the seed produced by a plant)

The chestnut blight pathogen, *Cryphonectria parasitica*, clearly has a negative impact on reproduction of diseased American chestnuts because the main trunk is often killed as a result of infection. However, when trees are infected with a strain of the pathogen hyperparasitized by a double-stranded RNA and therefore reduced in virulence (hypovirulent), infected stems may survive to reproductive size. Whether pathogen virulence has any prolonged effects on the next generation was examined by studying populations infected with either virulent or hypovirulent forms of *C. parasitica*. Survival and size of naturally recruited seedlings were compared to individuals which were similar in size to seedlings due to infection or herbivory from healthy populations and infected chestnut populations. Greenhouse and field experiments investigated whether infection resulted in any adverse effects in seed size and subsequent seedling development of American chestnuts (i.e. seed quality). Further, the effects of herbivores on seedling establishment and growth were investigated with a caging experiment.

METHODS

Study System and Study Sites

For a description of the interaction between the American chestnut (*Castanea dentata*), chestnut blight, and dsRNA, see Chapters 2 and 3. The six sites (two healthy, two non-recovering, and two recovering) utilized in this project are the same as those used in the demographic study (see Chapter 3 for details).

Natural Recruitment

A series of 9 m X 9 m plots were placed haphazardly with the number of plots varying from site to site so that approximately one-half of a population's area was monitored. Seedlings and other small chestnut plants less than 1 m tall, resprouts and herbivore damaged individuals, were marked and censused repeatedly for survivorship and growth from June 1996 to October 1998 (see Chapter 3 for details).

Field Experiments

Bulk seed collections were made at the six study sites in fall 1996. Seeds were stored overwinter at 4 °C. In April 1997, two treatments were initiated at all sites. One treatment consisted of planting 45 seeds in four unprotected (open) plots placed haphazardly at each site. The other treatment was a cage experiment in which four plots of 45 seeds each were protected from mammalian herbivores and seed predators by wood and wire mesh cages. Only 30 seeds per plot and three plots per treatment were used at the Frankfort site. Seeds were planted back into their site of origin. Height, basal diameter, number of leaves, and length and width of the first, third, and fifth leaves were recorded monthly from June to September. Leaves were marked with paint for identification. Only proportion emerging, final height, and survivorship to the end of the season will be discussed here.

Greenhouse Experiment

One hundred seeds from each site (50 from Frankfort) were reserved for a greenhouse study. The mass of each seed was recorded prior to planting in a four-inch plot filled with a standard potting mix. Pots were placed randomly on greenhouses benches and were re-randomized every third week. Plant measures as described above were made weekly for 11 weeks.

Statistical Analyses

Differences in emergence and survival among disease status types were examined using PROC GLM with arcsine-transformed proportions as the dependent variable and population as the unit of replication. Plant type (seedling or other) was also included in models of survivorship for the natural recruitment study. Type III sums of squares were interpreted throughout. To examine size differences, the effect of disease status was analyzed using a nested ANOVA with populations nested within a disease status type. Since disease status did not have a significant effect on seed mass and final height, one-way ANOVA's were used to examine differences among sites. All analyses were performed with the SAS statistical package (SAS Institute, Inc. 1997) except for correlation analyses which were performed with Minitab (Minitab, Inc. 1994).

RESULTS

Natural Recruitment

Preliminary analyses revealed no significant differences in survival or size of resprouts or herbivore damaged individuals (results not presented). Therefore, these two classes of plants were grouped together into an "other" class for comparison to true seedlings. There was a trend for survival of American chestnut seedlings to be lower than that of other small individuals, both within a season and overwinter (Table 5-1). Survivorship was generally lower within the 1997 season and overwinter 1997-1998 than for the other census periods. There was no trend for greater survivorship either within a season or overwinter. Overwinter survival was generally lower in 1996-1997 than within the 1996 season. This pattern was reversed for 1997 with overwinter survival greater than within season survivorship. Overall, the Missaukee Healthy site had low survival for both seedlings and other small individuals.

Survival did not vary with disease status, either within a season or overwinter (Table 5-2). However, there was a trend for populations infected with chestnut blight to have higher survivorship than healthy populations. The only exception to this trend was within the 1998 season where healthy populations showed higher survival than recovering ones. There was a distinct pattern of seedlings having lower survival than other small plants (Table 5-2). Indeed, for two out of three within season periods (1997 and 1998), seedlings had significantly lower survival than resprouts and damaged individuals.

Final seedling height did not vary with disease status for any census period (results not presented). End of season seedling height varied significantly among sites in 1996 and 1998 ($F_{5,220} = 9.49$, $P < 0.0001$ and $F_{5,78} = 6.22$, $P < 0.0001$, respectively; Table 5-3). There was no significant difference in end of season seedling height in 1997 ($F_{5,78} = 3.84$, N.S.; Table 5-3). In all years, seedlings from County Line, a recovering site, were smallest. For two out of three years, seedlings from the non-recovering Stivers population were largest.

No consistent correlation was found between final seedling height and survival. In 1996, there was a non-significant negative trend ($r_s = -0.368$, df = 4, N.S.) while non-significant positive trends were evident in 1997 and 1998 ($r_s = 0.45$, df = 4, N.S. and $r_s = 0.633$, df = 4, N.S., respectively).

Greenhouse Experiment

There was no effect of disease status on mean seed mass ($F_{2,3} = 0.28$, $P = 0.771$), but there was a significant site effect ($F_{5,554} = 60.51$, $P < 0.0001$; Table 5-4) with the highest (Stivers) and lowest (Missaukee Diseased) average mass being found in non-recovering sites. Mean seed mass and emergence were positively but not significantly correlated ($r_s = 0.66$, df = 4, N.S.). Disease status did not affect the proportion of seedlings emerging ($F_{2,3} = 0.11$, N.S.; Table 5-4) nor the relationship between mean seed mass and emergence ($r = -0.50$, df = 1, N.S.).

Individuals from only four sites survived until the end of the experiment in sufficient numbers to be included in analyses of final height. Therefore, only among site differences could be examined. Significant differences among these four sites in final seedling height were found ($F_{3,103} = 7.45$, $P < 0.0001$; Table 5-4) with Frankfort seedlings being largest and seedlings from the Missaukee Healthy site being smallest. There was no relationship between final seedling height and either mean seed mass ($r_s = 0.00$, df = 2, N.S.) or emergence ($r_s = 0.00$, df = 2, N.S.).

Field Experiments

The proportion of seedlings emerging did not differ for disease type ($F_{2,3} = 0.23$, N.S.) or for sites nested within disease type ($F_{3,6} = 3.26$, N.S.). However, there was a significant difference in proportion of seedlings emerging between open and caged plot treatments ($F_{6,34} = 16.74$, $P < 0.0001$) with caged plots usually displaying higher frequencies of emergence (Table 5-5). When emergence data from open and caged plots were combined to give an overall emergence rate for a site, greenhouse and field seedling emergence proportions were significantly positively correlated ($r_s = 0.93$, df = 4, $P <$

0.01). For within season survival, there were no significant differences in emergence for disease type, site, or plot treatment ($F_{2,3} = 5.54$, N.S.; $F_{3,6} = 3.57$, N.S.; and $F_{6,34} = 1.43$, N.S.; respectively).

Significant differences in final height of seedlings for sites nested with disease type were found ($F_{2,5} = 10.09$, $P < 0.0176$; Table 5-5). However, there were no differences in final seedling height for either treatment (caged versus open) or population disease type ($F_{5,23} = 1.07$, N.S. and $F_{2,2} = 0.82$, N.S., respectively). Final size in the field and greenhouse studies were positively but not significantly correlated ($r_s = 0.72$, df = 2, N.S.).

The Missaukee Diseased population was not included in the following statistical analyses because no seed germinated in either the open or caged plots at this site. Survival of chestnut seedlings in open plots was not correlated with survival of first year naturally recruited seedlings ($r_s = 0.235$, df = 3, N.S.). Naturally recruited seedlings at the non-recovering Stivers location had 100% survival (highest among naturally recruited seedlings) while seedlings in the open plot experiment only had a 41% survival probability (lowest for the open plot experiment). However, final height of open plot seedlings and naturally recruited seedlings was positively and significantly correlated ($r_s = 0.915$, df = 3, $P < 0.05$).

DISCUSSION

The large among site differences in seed mass and emergence were not correlated with the disease status of the *Castanea dentata* populations. Thus, effects of *Cryphonectria parasitica* infection on *C. dentata* adults may not carry-over into their offspring, contrasting with the "Ghost of infection past" effects found in other studies

(Jarosz et al. 1989). From a practical standpoint, this means that the effects of infection can be evaluated exclusively from the performance of infected adult trees. Infected trees are known to display significant reductions in growth, survivorship and number of seeds produced (Griffin et al. 1983). There is a trend for populations infected with the virulent form of the pathogen to have relatively lower finite rates of increase, while populations infected with hyperparasitized forms of C. parasitica have intermediate growth rates between healthy and non-recovering American chestnut populations (see Chapter 3).

Seedling survival of naturally recruited chestnut seedlings varied among sites and among years. True seedlings generally had lower survival than other older individuals of the same size. This finding may result from these older individuals having a larger more established root system (Paillet 1984, 1993). Surprisingly, post-emergent survival may have been affected by the disease status of a population. For example, the relatively low survival observed at the Missaukee Healthy site could be due the vigor of the adult trees at the site. The closed canopy may effectively shade the seedlings and there may be increased competition from the high density of seedlings and other small individuals (pers. obs.).

There were some enhancements to seedling performance in chestnut populations where *C. parasitica* was present. Most notable was a trend for increased survivorship within recovering populations. There are several possibilities to explain why this pattern might be seen. This class of population may be closer to optimal conditions for seedling growth. The trees are clearly recovering from the infection and growing to the point where they are producing seed, which may be provisioned adequately for future growth, as indicated by relatively large seed mass (Table 5-4). At the same time, average tree size of adults is smaller than trees found at either healthy population studied here (see Figure 3-3). Smaller adult tree size due to disease may indirectly increase seedling performance because of increased light levels to the forest floor (i.e., disease causes a more open canopy in recovering populations). Alternatively, adult-seedling competition may be reduced because of the smaller adult size.

The lack of correlation between survival of naturally recruited seedlings and seedlings in the open plot treatment of the field experiment could be due to the differences in sample size. Up to 5.5 times the number of seedlings were included in the experiment as were found in the natural recruitment plots. This discrepancy could be the result of not accurately censusing all naturally recruited seedlings at the start of the season. Naturally recruited seedlings suffering from severe herbivore damage are particularly difficult to identify and may die before being included in the census. However, the strong correlation between the final size of naturally recruited and open plot seedlings indicates that the open plot seedlings are representative of what is occurring naturally within the populations. Therefore, the comparison of the open and caged experiments is valid. The increased size and survivorship of caged individuals indicates that protection of chestnut seedlings from mammalian herbivores might be an effective management strategy to increase the transition of stage 2 to stage 4 individuals, an important transition in some diseased populations as indicated by sensitivity and elasticity analyses (see Chapter 4).

TABLE 5-1. Proportion of seedlings or other individuals less than 1 m in height, which are clearly not seedlings but are similar in size due to herbivory or infection by chestnut blight, from natural recruitment plots surviving in six American chestnut populations.

SITE	WITHIN 1996	OVERWINTER 1996-1997	WITHIN 1997	OVERWINTER 1997-1998	WITHIN 1998
HEALTHY					
MISSAUKEE HEALTHY					
SEEDLINGS	0.71	0.75	0.61	0.65	0.94
OTHERS	0.98	0.89	0.87	0.89	0.91
LEELANAU					
SEEDLINGS	1.00	1.00	0.56	0.80	0.88
OTHERS	0.96	0.86	0.98	0.93	1.00
RECOVERING					
COUNTY LINE					
SEEDLINGS	0.94	0.96	0.92	0.91	0.90
OTHERS	1.00	0.99	0.93	0.97	0.99
FRANKFORT					
SEEDLINGS	1.00	0.86	0.78	0.84	0.85
OTHERS	1.00	0.96	0.80	0.91	0.87
NON-RECOVERING					
STIVERS					
SEEDLINGS	0.64	0.86	0.92	1.00	0.93
OTHERS	0.95	1.00	0.94	0.86	1.00
MISSUAKEE DISEASED					
SEEDLINGS	0.88	0.81	0.76	0.97	0.94
OTHERS	0.98	1.00	0.94	0.93	1.00

TABLE 5-2. Proportion of seedlings or other small individuals from natural recruitment plots surviving in six American chestnut populations. F values are reported for each period.

DISEASE STATUS	WITHIN 1996	OVERWINTER 1996-1997	WITHIN 1997	OVERWINTER 1997-1998	WITHIN 1998
HEALTHY	0.86	0.85	0.78	0.83	0.94
RECOVERING	0.98	0.96	0.88	0.92	0.91
NON-RECOVERING	0.93	0.92	0.86	0.95	0.97
DISEASE (df = 2,8)	2.38, N.S.	0.34, N.S.	1.06, N.S.	2.17, N.S.	2.26, N.S.

PLANT TYPE	WITHIN 1996	OVERWINTER 1996-1997	WITHIN 1997	OVERWINTER 1997-1998	WITHIN 1998
SEEDLINGS	0.85	0.87	0.79	0.86	0.90
OTHERS	0.98	0.96	0.89	0.92	0.95
TYPE (df = 1,8)	3.05, N.S.	1.91, N.S.	5.49, P < 0.05	0.24, N.S.	7.43, P < 0.05

TABLE 5-3. Mean final height of naturally recruited first year seedlings for six populations of American chestnut. Means ± standard errors are presented. Different letters indicate significant differences in means.

SITE	1996	1997	1998
HEALTHY			
MISSAUKEE HEALTHY	15.52 ± 0.56 ab	12.75 ± 0.42 a	15.00 ± 2.25 ab
LEELANAU	16.11 ± 1.27 a	17.36 ± 0.91 a	14.36 ± 1.14 ab
RECOVERING			
COUNTY LINE	11.90 ± 0.42 b	12.03 ± 0.71 a	11.72 ± 0.38 b
FRANKFORT	16.38 ± 0.78 a	15.21 ± 1.50 a	15.38 ± 0.69 ab
NON-RECOVERING			
STIVERS	17.57 ± 1.47 a	16.67 ± 2.03 a	17.83 ± 1.42 a
MISSAUKEE DISEASED	14.11 ± 0.78 ab	15.14 ± 1.98 a	15.13 ± 0.72 ab

TABLE 5-4. Mean seed mass, proportion of seedlings emerging, and mean final height in a greenhouse experiment for six populations of American chestnut. Means ± standard errors are presented. Different letters indicate significant differences in means. Final size for County Line is not presented because only one individual survived to the end of the experiment.

SITE	MEAN SEED MASS	PROPORTION EMERGING	MEAN FINAL HEIGHT
HEALTHY			
MISSAUKEE HEALTHY	0.93 ± 0.07 cd	0.30	17.80 ± 1.30 c
LEELANAU	0.60 ± 0.07 de	0.10	24.93 ± 3.82 ab
RECOVERING			
COUNTY LINE	1.44 ± 0.06 b	0.02	
FRANKFORT	1.20 ± 0.15 bc	0.44	26.24 ± 1.65 a
NON-RECOVERING			
STIVERS	1.98 ± 0.10 a	0.70	20.51 ± 0.70 bc
MISSAUKEE DISEASED	0.31 ± 0.02 e	0.00	

TABLE 5-5. Proportion of seedlings emerging, within season survival, and mean final height in field experiments for six populations of American chestnut. Means ± standard errors are presented. Means followed by the same letter are not significantly different. *A posteriori* tests were performed to compare site means after the non-significant disease status main effect was removed from the analysis.

SITE	PROPORTION EMERGING	WITHIN SEASON SURVIVAL	MEAN FINAL HEIGHT
MISSAUKEE HEALTHY	0.11	0.56	11.15 ± 0.64 b
OPEN	0.09	0.65	9.68 ± 1.03
CAGED	0.13	0.50	12.50 ± 0.60
LEELANAU	0.03	0.67	16.08 ± 2.55 a
OPEN	0.03	0.60	15.67 ± 0.88
CAGED	0.02	0.75	16.50 ± 5.62
COUNTY LINE	0.07	0.96	7.10 ± 0.71 c
OPEN	0.04	0.88	4.86 ± 1.11
CAGED	0.09	1.00	8.08 ± 0.80
FRANKFORT	0.34	0.92	13.44 ± 0.43 ab
OPEN	0.03	1.00	11.17 ± 2.74
CAGED	0.65	0.92	13.56 ± 0.43
STIVERS	0.26	0.67	13.56 ± 0.54 ab
OPEN	0.18	0.41	12.18 ± 1.00
CAGED	0.33	0.82	13.92 ± 0.62
MISSAUKEE DISEASED	0.00	0.00	N.D.

N.D. = No seeds germinated from this population.

CHAPTER 6

CONCLUSIONS

The presence of double-stranded RNA in populations of the chestnut blight pathogen, *Cryphonectria parasitica*, allows recovery of American chestnuts, both at an individual and population level. While dsRNA appears necessary for recovery to occur, the mere presence of dsRNA in isolates of the pathogen is not sufficient for recovery of individuals or populations. The effects of dsRNA may be altered by the size of the individual infected and spatial and temporal variation among populations.

The importance of American chestnut branch size in determining the outcome of infection by chestnut blight was supported by both an inoculation experiment and a natural infection study. Branch size influenced canker morphology and branch survival although some of these effects only developed over time. DsRNA reduced canker growth rates and appeared to delay mortality for medium and large size branches. Classification of cankers into callused or non-callused did not accurately predict the type of fungal isolate which initiated infection, especially after the first season of infection when branch size was a more important influence on canker development. The finding that dsRNA alone is not related to branch survivorship emphasizes that the presence of dsRNA in a pathogen population may be necessary but not sufficient for biological control of disease. Although the presence of dsRNA may enable large branches to survive, small branches still succumb to infection by both dsRNA-containing and dsRNA-free isolates. Overall, branch size at the time of infection appears to have a large influence on branch survivorship, at least in the short term. Therefore, the presence of a sufficiently debilitating dsRNA in the pathogen population may not be the only requirement for recovery of American chestnut populations. The size of an individual tree at the time of infection could also be an important factor in determining the long term survival of American chestnut populations.

Investigations on the influence of the interaction of pathogen virulence and branch size on mortality and canker development should be expanded to include dsRNAs with a range of debilitating effects. In addition, efforts should be made to examine natural canker establishment, colonization and subsequent spread of dsRNA within the cankers, and ultimate branch fate.

Matrix projection models of population growth rate and size class distributions revealed that chestnut blight is having an impact on the structure and growth of infected American chestnut populations. In the presence of disease, large trees may be reduced in size, a trend which was not observed in healthy populations during either census period. Indeed, retrogressions (reductions in size) were not found in any of the 21 woody plant species examined in a recent review (Silvertown et al. 1993). Further, reproduction of individuals in smaller size classes was found in infected populations. Population growth rates (λ) varied among sites and among years. However, the relative rankings, with non-recovering populations having the lowest growth rates over two census periods, indicate that disease is indeed having a negative impact on chestnut population growth rates. The intermediate rankings of recovering populations indicates that the presence of dsRNA can promote ecological recovery of American chestnut populations. Observed population structures differed significantly from the predicted stable stage distributions. The pattern of diseased populations being dominated by intermediate-sized individuals at stable stage, while healthy populations are dominated by small individuals, was further indication that disease is influencing American chestnut populations.

There are several avenues of investigation that might be pursued further. First, demographic studies of healthy, recovering, and non-recovering American chestnut populations should be continued to examine temporal variation in estimates of the finite rate of population increase (λ). When transition matrices are available that represent a range of conditions (i.e. good versus bad years), more accurate long-term predictions of the fate of the populations can be made. Models can be constructed in which transition matrices representing different conditions are selected at random and the trajectories of the populations can be predicted. Further, following populations for a longer period of time will allow the trajectory and pattern of population growth rates after the introduction of *C. parasitica* and then dsRNA to be determined (see Figure 3-4).

Matrix projection models can also produce sensitivity and elasticity analyses to examine the contribution of particular stages and transitions to populations growth rates. The sensitivity analyses reveal that, in general, for healthy and recovering populations of American chestnut, changes in the transition probability for survival in the largest stage would have the greatest impact on population growth. An exception to this finding is that for non-recovering populations the highest sensitivities were generally found for growth of stage 6 (1 - 10 cm dbh) individuals. This result may have important management implications. If one wanted to treat chestnut trees and introduce dsRNA into populations, these findings suggest that trees in this size class should be treated. Elasticity analyses revealed that survival within a stage has the largest proportional contribution to the finite rate of population increase. Silvertown et al. (1993) divide elasticity matrices into three regions (*G*, *L*, and *F*) and sum elasticities within each region. For species such as the American chestnut, which appear to occupy a very narrow range of *G/L/F* space, evaluating successful management strategies may be problematic with this method. These results emphasize the need for examination and interpretation of a combination of demographic parameters to evaluate management strategies for successful treatment and conservation of American chestnut populations.

While the use of *G/L/F* ratios in conservation management strategies is promising (Silvertown et al. 1996), the interpretation of the resulting patterns among populations within a species should be refined to reflect the narrow range some species such as the American chestnut occupy in *G/L/F* space. Further, the influence of environmental factors such as herbivory and disease as opposed to successional stage should be examined to determine the potential trajectories of populations under these more subtle yet still important disturbances.

Cryphonectria parasitica infections of adult chestnut trees do not appear to have any prolonged adverse effects on the performance of offspring. Therefore, the effects of infection can be evaluated exclusively from the performance of infected adult trees. Survival of naturally recruited chestnut seedlings varied among sites and among years. True seedlings generally had lower survival than other older individuals of the same size. However, there was a trend for increased seed mass and enhanced germination and survivorship of seedlings within recovering populations. Disease status did not affect final seedling size whereas final seedling size did vary with population. The increased size and survivorship of individuals in a cage experiment versus those seedlings in open plots indicate that protection of chestnut seedlings from mammalian herbivores might be an effective management strategy to increase the transition probabilities of small individuals, an important transition in some diseased populations as indicated by sensitivity and elasticity analyses.

Studies on the effects of infection on reproduction could be refined to account for the relative health of the seed parent and the subsequent effects on seed quality and seedling performance. Further, genetic versus environmental components of seedling performance could be teased apart by using maternal half-sib families in greenhouse and field reciprocal transplant studies.

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