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INFLUENCE OF BIOTIC AND ABIOTIC FACTORS ON THE EPIDEMIOLOGY OF BEECH BARK DISEASE IN THE GREAT SMOKY MOUNTAINS NATIONAL PARK

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Robert Nicholas Klein Jr.
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DEDICATION

This thesis is dedicated to my parents

Robert and Mary Klein.

A small consideration for a lifetime of love and support.

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ABSTRACT

Beech trees (*Fagus grandifolia* Ehrhart) in North America are threatened by beech bark disease (BBD), a destructive insect-pathogen complex thought to have originated in Europe. The disease complex is initiated when boles of American beech become infested with colonies of the beech scale, *Cryptococcus fagisuga* Lindinger, and culminates when one of two species of the fungus *Nectria* Fries invade the tree. The disease results in dieback of the crown and general tree decline, and often results in tree mortality. Ten long term study plots were established in the Great Smoky Mountains National Park (GSMNP) in 1994 to monitor the spread and impact of BBD in the park.

Monitoring efforts were continued in 1996 and1997. Beech trees within the plots were evaluated for mortality and sampled on the north and south tree-side with a 1089-cm^2 sample square. Sampled areas were rated for abundance of fungal sexual fruiting structures, and trees were also given an overall rating. Signs of the fungi were observed within 9 of the 10 plots and just outside the border of the other. Total prevalence of the fungi in the combined plots was 9% in 1996 and 11.4% in 1997. Total mortality due to disease rose from nearly 16% in 1996 to nearly 18% in 1997. BBD is both firmly established and on the rise in GSMNP.

The role of a common beech bark fungus, *Ascodichaena rugosa* Butin, in the occurrence and spread of the beech scale and BBD was evaluated in

the 10 study plots. Beech trees were rated for abundance of signs of *A. rugosa* in the same manner as they were rated for abundance of *Nectria* species. These ratings were tested for association with abundance ratings of the beech scale (Wiggins 1997) and *Nectria* spp. using Fisher's Exact Test and analyzed for direction with Kendall's tau-b statistic (P=0.05). Significant negative associations between abundances of *A. rugosa* and the beech scale were observed in north, south, and overall ratings during 1996 and 1997. Significant negative associations between abundance of *A. rugosa* and the incidence of BBD were observed in north and overall ratings during 1996 and 1997. These results indicate that *A. rugosa* may limit the spread and development of infestations of the insect, in effect also reducing the occurrence and severity of BBD.

Several biotic and abiotic variables were measured and tested for relationship to incidence of disease and mortality due to disease. Linear models were constructed accounting for significant portions of the variation in incidence and abundance of *Nectria* spp. and the mortality due to disease (P=0.05). R-square values for the models exceeded 0.30 only for mortality due to disease. Crown class and evergreen basal area were negatively correlated and elevation and number of stems positively correlated with disease mortality. It was concluded that a larger sample size was required to make biologically significant conclusions concerning the impact of these variables on disease.

TABLE OF CONTENTS

CHAPTER PAGE

1. INTRODUCTION....1

2. THE ADVANCE OF BEECH BARK DISEASE IN THE GREAT SMOKY MOUNTAINS NATIONAL PARK....21
   - Introduction....21
   - Materials and Methods....23
     - Data Collection....23
     - Measurements of Disease Progression....27
   - Results....28
     - Current Status of Disease in Permanent Plots....28
     - Disease Progression: 1994-1997....35
   - Discussion....39

3. THE ROLE OF *ASCODICHAENA RUGOSA* BUTIN IN THE EPIDEMIOLOGY OF BEECH BARK DISEASE IN THE GREAT SMOKY MOUNTAINS....44
   - Introduction....44
   - Materials and Methods....48
     - Incidence and Abundance of *Ascodichaena rugosa*....48
     - The Association Between Abundances of *Ascodichaena rugosa* and *Cryptococcus fagisuga*....49
     - The Association Between *Aschodichaena rugosa* Abundance Ratings and Incidence of Beech Bark Disease....49
     - Comparison of *Nectria* spp. and *Ascodichaena rugosa* Abundance on North and South Tree Sides....50
   - Results....51
     - Incidence and Abundance of *Ascodichaena rugosa*....51
     - The Association Between Abundances of *Ascodichaena rugosa* and *Crytococcus fagisuga*....53
     - The Association Between *Ascodichaena rugosa* Abundance Ratings and Incidence of Beech Bark Disease....58
     - Comparison of *Nectria* spp. and *Ascodichaena rugosa* Abundance on North and South Tree Sides....59

CHAPTER Page

Discussion....63

4. INFLUENCE OF SITE AND STAND FACTORS ON INCIDENCE AND PREVALENCE OF INFECTION, PERITHECIAL ABUNDANCE, AND *NECTRIA*-RELATED MORTALITY....68
Introduction....68
Materials and Methods....72
Biotic and Abiotic Variables....72
Measurement of Variables: Fractional Interception of PAR and Canopy Cover....74
Measurement of Variables: Evergreen Basal Area....76
Statistical Analysis....76
Results....78
Discussion....80

REFERENCES....86

VITA....96

LIST OF TABLES

TABLE PAGE

2-1. Summary of permanent plots established for long-term monitoring of beech bark disease in Great Smoky Mountains National Park.....25

2-2. Rating scale for relative abundance of *Nectria* spp. perithecia on bark of *Fagus grandifolia*.....26

2-3. Mean abundance ratings for *Nectria* spp. perithecia in ten permanent plots for spring 1996, fall 1996, and spring 1997 on (a) the north side, and (b) the south side of American beech trees.....31

2-4. Mean overall tree abundance ratings for *Nectria* spp. perithecia in ten permanent plots for spring 1996, fall 1996, and spring 1997.....34

3-1. Contingency tables testing for association between abundance of *Ascodichaena rugosa* and abundance of *Cryptococcus fagisuga* on the north-facing tree side for (a) fall 1996, and (b) spring 1997.....55

3-2. Contingency tables testing for association between abundance of *Ascodichaena rugosa* and abundance of *Cryptoccus fagisuga* on the south-facing tree side for (a) fall 1996, and (b) spring 1997.....56

3-3. Contingency tables testing for association between abundance of *Ascodichaena rugosa* and abundance of *Cryptococcus fagisuga* on the overall tree for (a) fall 1996, and (b) spring1997.....57

3-4. Contingency tables testing for association between abundance of *Ascodichaena rugosa* and incidence of *Nectria* spp. on the north-facing tree side for (a) fall 1996, and (b) spring 1997.....60

TABLE PAGE

3-5. Contingency tables testing for association between abundance of *Ascodichaena rugosa* and incidence of *Nectria* spp. on the overall tree for (a) fall 1996, and (b) spring 1997.....61

3-6. Contingency tables testing for association between abundance of *Ascodichaena rugosa* and incidence of *Nectria* spp. on the south-facing tree side for (a) fall 1996, and (b) spring 1997.....62

4-1. Category values and definitions for independent plot variables.....73

4-2. Linear models accounting for significant portions of variability in (a) incidence of *Nectria* spp., and (b) overall abundance ratings for *Nectria* species.....79

4-3. Linear model accounting for a significant portion of the variability in mortality due to disease.....81

LIST OF FIGURES

FIGURE PAGE

1-1. The ranges of American beech (*Fagus grandifolia* Ehrh.) and beech bark disease in North America....4

2-1. Frequency of *Nectria* spp. in permanent plots; (a) percent incidence, and (b) percent prevalence....29

2-2. Spring 1997 mortality figures for beech in permanent plots; (a) percent mortality, and (b) percent mortality due to disease....34

2-3. Change in mean percent incidence and mean percent prevalence of *Nectria* spp. by year for the period 1994-1997....36

2-4. Change in mean north tree side, south tree side, and overall tree ratings for abundance of *Nectria* spp. during the period 1994-1997....37

2-5. Change in mean percent mortality and mean percent mortality due to disease for the period 1994-1997....38

3-1. Frequency of *Ascodichaena rugosa* in ten permanent plots; (a) percent incidence, and (b) percent prevalence....52

3-2. Mean overall abundance ratings of *Ascodichaena rugosa* on *Fagus grandifolia* from ten permanent plots....54

LIST OF PLATES

PLATE PAGE

1-1. The beech scale, *Cryptoccocus fagisuga*, on the bark of American beech: (a) infestation of the bole with colonies of the scale, and (b) an individual insect with the woolly covering brushed aside.....14

1-2. Perithecia of *Nectria* spp. on the bark of American beech: (a) red perithecia on the bole, and (b) a magnified view of sporulating perithecia atop red stromata.....16

1-3. Symptomatic cracking and flaking of American beech bark due to beech bark disease in Great Smoky Mountains National Park.....17

3-1. Stroma and fruiting bodies of *Ascodichaena rugosa* on the bark of American beech: (a) horizontal and elliptical patches of stroma and fruiting bodies (pycnidia) on the bole, and (b) a magnified view of sporulating pycnidia atop black stromata.....46

CHAPTER 1

INTRODUCTION

The American beech, *Fagus grandifolia* Ehrhart, is a common tree of eastern North America, ranging from southeastern Canada south to the panhandle of Florida and west to Wisconsin, Missouri, and Texas (Preston 1989). It is a deciduous member of the Fagaceae, an important family of hardwoods which also includes chestnuts (*Castanea* Miller), chinkapins [(*Castanopsis* (D. Don) Spach], and oaks [*Quercus* (Tourn.) L.], among others.

The genus *Fagus* Linnaeus consists of 10 tree species found in temperate regions of the northern hemisphere and is characterized by smooth, pale bark and alternate, simple, and toothed leaves (Elias 1980). The winter buds of *Fagus* species are terminal, distinctly elongated, sharply pointed, and imbricately scaly (Elias 1980). Beeches are monoecious, bearing male and female flowers separately, and the fruits are three-sided nuts, usually borne in pairs within prickly burs (Elias 1980).

Fagus grandifolia is the only species of *Fagus* native to North America, where it grows as a medium sized tree, averaging 18-25 m in height and 60-90 cm in diameter (Preston 1989). The tree is a shade tolerant species that grows best in cool, moist sites with deep, humus-rich soil (Elias 1980,

Hamilton 1955). American beech is commonly found in a wide variety of low elevation forest types throughout the eastern United States, but can be found at elevations greater than 1800 m in the southern Appalachians (Elias 1980, Stupka 1964). This tree is considered a climax species throughout most of its range, and is a dominant species in several forest types including mixed mesophytic, beech-maple, and northern hardwood (Halls 1977, Hamilton 1955).

American beech is an unusual tree that can reproduce vegetatively via root sprouts as well as sexually, by the production of seed (Houston and Houston 1994, Ward 1961). Prolific root sprouting by mature trees often leads to pure stands, or groves, of beech. It has been suggested that this root sprouting may be a response to wounding of the roots by human activity or environmental stresses (Hamilton 1955). Beechnuts, the sexual seed of the beech, are known to be widely dispersed by migratory blue jays (*Cyanocitta cristata* L.) (Houston 1996, Johnson and Adkisson 1985).

Beechnuts are produced in late fall and provide an important source of food for a variety of wildlife. The nuts, which are high in crude fat and calcium, are a favorite food of squirrels, and are also favored by black bears, deer, chipmunks, mice, and ruffed grouse (Halls 1977, Tubbs and Houston 1990). The winter buds, as well as the nuts, are a preferred food source for wild turkeys (Halls 1977). Production of beechnuts is particularly important to wildlife in the northeast, as beech is the only nut producer in

the northern hardwood forests (Tubbs and Houston 1990). American beech often provides an irreplaceable resource for all manner of wildlife, both in terms of food and cover.

Although beech has historically been favored as a source of firewood and charcoal, it was long considered a poor timber species because of difficulties associated with drying the wood, such as its tendency to crack (Baker and McMillen 1955, Shigo 1972, Webber 1955). Improvements in drying and utilization of the wood have resulted in the increased use of beech as a timber species (Shigo 1972). The abundance of beech in eastern North America has made it an important source of timber for flooring, furniture, handles, and pulp (Houston 1975, Preston 1989). The wood also has medicinal value for humans and animals. Creosote derived from beech wood is applied as a nontoxic internal or external treatment for certain human and animal disorders (Tubbs and Houston 1990).

Beech trees in North America are now threatened by beech bark disease throughout much of their natural range (Figure 1-1) (Houston 1994b). Beech bark disease is a destructive insect-pathogen complex affecting the boles of American and European beech trees, frequently causing high rates of defect and mortality (Ehrlich 1934, Houston et al. 1979a, Rutherford 1996). The disease is initiated when large colonies of beech scale, *Cryptococcus fagisuga* Lindinger, become established and begin feeding on the outer bark of living trees (Ehrlich 1934). This infestation is



Figure 1-1. The ranges of American beech (*Fagus grandifolia* Ehrh.) and beech bark disease in North America.

typically followed by invasion of the bark by one of two Ascomycetous fungi in the genus *Nectria* Fries (Cotter 1977, Ehrlich 1934, Lohman and Watson 1943). The beech scale is indigenous to the European continent and is widespread in western Europe (Ehrlich 1932). This insect has been recognized as a pest of European beech (*Fagus sylvatica* Linnaeus) since the early to mid-1800's, and tree mortality was believed to be associated with infestations of the scale followed by fungal infection as early as 1911 (Boodle and Dallimore 1911). It is now widely accepted that *Nectria coccinea* (Persoon ex Fries) Fries is the pathogen in Europe (Houston et al. 1979a). Beech bark disease is currently recognized as widespread, though of relatively low impact, in the beech forests of Great Britain, France, and Germany (Parker 1983, Perrin 1983a, Lang 1983).

The disease was first observed in North America in Halifax County, Nova Scotia, Canada around 1920 (Ehrlich 1934). The name 'beech bark disease' was subsequently given to the affliction by John Ehrlich in 1932 in his report of this initial outbreak (Ehrlich 1934). By 1929, the beech scale had entered the United States and was found in several counties in eastern Massachusetts (Ehrlich 1932). The scale was found in 1932, together with a species of *Nectria*, to be associated with dead and dying trees in south-central Maine (Ehrlich 1934). The scale, and the disease, spread quickly into the Canadian Maritime Provinces of Prince Edward Island, Cape Breton Island, and New Brunswick (Magasi and Newell 1983).

Since that time, the causal organisms have spread steadily south and west. The beech scale was observed during 1958 in northeastern Pennsylvania, and the fungus was found there in 1970 (Drooz 1959, Towers et al. 1971). The disease was known in Quebec in 1965, and by 1985 there was a report of the beech scale in Ontario (Bisessar et al. 1985, Lachance 1983). These sites represent the expanding contiguous range of beech bark disease in North America.

The expanding disease is distinguishable as three distinct 'zones' in North America, the advance front, the killing front, and the aftermath stage (Shigo 1972). In this model, the advance front is characterized by a rapid buildup of beech scale populations, and the absence of signs of the fungus. The killing front follows, where high rates of mortality are associated with signs of the *Nectria* spp.. Finally, in the aftermath stage, root sprouts of susceptible trees proliferate into areas where the disease organisms are now endemic, thus perpetuating the disease (Houston 1975).

Isolated outbreaks of the disease, beyond the contiguous range, have also been found as far west as West Virginia and as far south as Tennessee and North Carolina (Houston 1994b, Mielke et al. 1982). The beech scale has been found as far west as Ohio, though there remain no reports of the disease from this site (D. Balser personal communication, Mielke et al. 1985). It has been proposed that such long distance dispersal of the

were infested with scale or that had been artificially wounded. Since infection of the bark occurred in both of these cases, he concluded that the role of the insect was only to create infection sites for the fungus through mechanical shrinkage and rupture of the bark.

Later research by Lonsdale (1980) showed that mean lesion length caused by infection of European beech with *N. coccinea* increased with higher levels of scale infestation. It is particularly interesting that, in these experiments, the fungus was inoculated through artificial wounds. In contrast to Ehrlich, Lonsdale concluded that the role of *C. fagisuga* in predisposing trees to infection by *Nectria* was greater than, though not exclusive of, the formation of entry courts for the fungus.

The exact role of this predisposition is of great interest in understanding the etiology of beech bark disease, and further work on this has been conducted with the European counterparts of the disease complex. Lonsdale and Sherriff (1983) were able to isolate pathogenic strains of *N. coccinea* from insect colonies, the bark surface surrounding the colonies, and from necrotic areas associated with insect feeding, in spots where infection had not yet occurred. They suggested that these findings represented creation, by the insect, of a pre-infection niche for the fungus, which was able to undergo latent colonization of these areas. It is important to note, however, that neither Stone (1967) nor Lonsdale (1983)

were able to isolate *Nectria coccinea* var. *faginata* Lohman, Watson, and Ayers or *N. coccinea*, respectively, from individual scale insects.

Working within the European system, Perrin (1983b) found that *C. fagisuga* produces endohydrolase and endo-polymethyl-transeliminase (PMTE) *in vivo* during the spring. These enzymes are indicative of the first steps in the degradation of pectin, which is a major component of the plant cell wall. Further, production of the pectinases exo-polygalacturonase (PG) and exo-polygalacturonate transeliminase (PMTE) by *N. coccinea* were significantly higher on scale-infested bark than healthy bark after 6 and 14 days, respectively (Perrin 1983b).

The beech bark disease pathogens in North America have been identified as *Nectria coccinea* var. *faginata* and *Nectria galligena* Bresadola (Cotter 1977, Lohman and Watson 1943). It is generally accepted that these two species of *Nectria* are the principal fungi causing the disease; however, *Nectria ochroleuca* (Schwein.) Berk. may be involved in isolated situations (Houston 1996, Manion 1991). *N. galligena* and *N. ochroleuca* are both native to the North American continent, with *N. galligena* commonly causing perennial target cankers on many hardwoods (Houston 1996, Sinclair et al. 1987). The primary pathogen of the complex is *Nectria coccinea* var. *faginata*, to which Ehrlich referred as an unnamed variety of *N. coccinea* (Ehrlich 1934).

Nectria coccinea var. *faginata* was first formally described by Lohman and Watson (1943). The origin of this fungus is uncertain. It differs from the European *N. coccinea* by having broader ascospores (perfect stage), larger conidia (imperfect stage), and also by cultural characteristics and host specificity (Ehrlich 1934, Lohman and Watson 1943). In addition, Cotter (1977) found that *N. coccinea* var. *faginata* differed from *N. coccinea* by lacking a central pore in the ascal apex. This pore has traditionally been an important character in the separation of *Nectria* species, prompting Cotter to suggest that *N. coccinea* var. *faginata* may be a distinct species (Cotter 1977). *Nectria coccinea* var. *faginata* is known only from North America and has only been found infecting *F. grandifolia* that have been infested with the beech scale.

Two possibilities for the origin of *N. coccinea* var. *faginata* have been suggested; 1) the fungus is native to North America and has been favored by the introduction of the beech scale, or 2) the fungus is an uncommon species in Europe that was introduced and found a more favorable substrate and environmental conditions in North America (Ehrlich 1934, Lohman and Watson 1943).

A survey to determine the range of *N. coccinea* var. *faginata* in the United States found that the fungus was absent beyond the range of *C. fagisuga* and that is was predominantly found in association with the latter stages of the disease (Cotter 1977). A recent study by Houston (1994a) has

shown that *N. galligena* is more frequently associated with diseased trees in the early stages of an outbreak and *N. coccinea* var. *faginata* replaces it as the disease progresses. These patterns are typical of an exotic pathogen and indicate that *N. coccinea* var. *faginata* was probably introduced (Houston 1994a). Also, Southern blot hybridizations were conducted with nuclear and mitochondrial DNA from *N. coccinea* var. *faginata* and seven other *Nectria* species, including *N. coccinea* var. *coccinea* from Europe. Probes from the nuclear and mitochondrial DNA of *N. coccinea* var. *faginata* consistently hybridized well with DNA from *N. coccinea* var. *coccinea*, indicating that *N. coccinea* var. *faginata* is most closely related to the European pathogen and suggesting that *N. coccinea* var. *faginata* originated in Europe (Mahoney 1991).

Both *N. galligena* and *N. coccinea* var. *faginata* are members of the phylum Ascomycota, subgroup Pyrenomycetes, order Hypocreales, and family Nectriaceae (Alexopoulos et al. 1996). The ascocarps are small (generally around 0.25-0.4 mm in width x 0.2-0.35 mm in length), bright red perithecia that are usually produced in clusters above orange-red erumpent stroma (Lohman and Watson 1943). Each ascus contains eight oval to elliptical two-celled ascospores. The perithecia are produced in the fall and ascospores are discharged during periods of rain (Ehrlich 1934). The ascospores are known to be disseminated by wind, water, and insects (Ehrlich 1934, Houston 1994b, Shigo 1964).

Each species also has a *Cylindrocarpon* Wollenweber imperfect stage by which asexual production of micro- and macroconidia occurs (Houston 1994b). The imperfect stage of *N. coccinea* var. *faginata* is *Cylindrocarpon faginatum* Booth, and it produces three- to nine-septate, elongated, and slightly curved macroconidia on white sporodochia that are erumpent on the bark surface during the summer months (Booth 1966, Ehrlich 1934). Microconidia are usually unicellular and are borne on short hyphal branches soon after germination of the ascospores (Ehrlich 1934). Lonsdale and Sherriff (1983) have suggested that the ability to produce macroconidia may aid *Nectria* spp. in saprophytic survival on beech bark. The imperfect stage of *N. galligena* is *Cylindrocarpon candidium* (Link) Wollenweber, which also produces microconidia on simple conidiophores and macroconidia on white to cream sporodochia (Houston 1994b, Lohman and Watson 1943). The microconidia are transported in water films, while the macroconidia are disseminated by wind (Ehrlich 1934, Houston 1994b).

Nectria coccinea var. *faginata* and *N. galligena* appear similar on infected beech bark. No characters reliably separate the two in the field, and identification relies on often times subtle cultural differences and ascospore length. The cultural differences lie in the coloration of the aerial mycelia when grown on a malt extract agar medium. Under these conditions, the mycelium of *N. galligena* appears light cinnamon to buff while that of *N. coccinea* var. *faginata* appears dark brown to tawny

(Lohman and Watson 1943). Positive identification usually relies on the average length of 25 ascospores, with mean lengths of greater than 14.3 μm indicating *N. galligena* and those less than 13.3 μm indicating *N. coccinea* var. *faginata* (Cotter 1977). Another difference between the two is that *N. galligena* is homothallic while *N. coccinea* var. *faginata* is heterothallic (Booth 1959, Cotter 1977).

Early indications of beech bark disease outbreaks are the appearance of the white, woolly secretions of the beech scale colonies in small cracks on the surface of the bark (Plate 1-1). Wound cavities resulting from the feeding activity of another scale insect on beech, *Xylococculus betulae* (Pergande) Morrison, can also be colonized by the beech scale (Shigo 1964). The number of insect colonies typically increases over a period of a few years, often forming long strips or even covering the entire bole (Shigo 1972, Sinclair et al. 1987). This massive infestation of the bark can generally weaken the tree and cause localized shrinking and fissuring of the phellem (Ehrlich 1934, Shigo 1972).

The *Nectria* spp. fungi typically enter through these wounds, and are able to grow inter- and intracellularly into the phellogen, phelloderm, cortex, phloem, and cambium (Ehrlich 1934). The first evidence of invasion by *Nectria* spp. are blackening of the wax coating of *C. fagisuga*, and red-brown areas of slime flux (Sinclair et al. 1987). As the fungus invades and kills the bark, the beech scale can no longer survive on the bark, and the

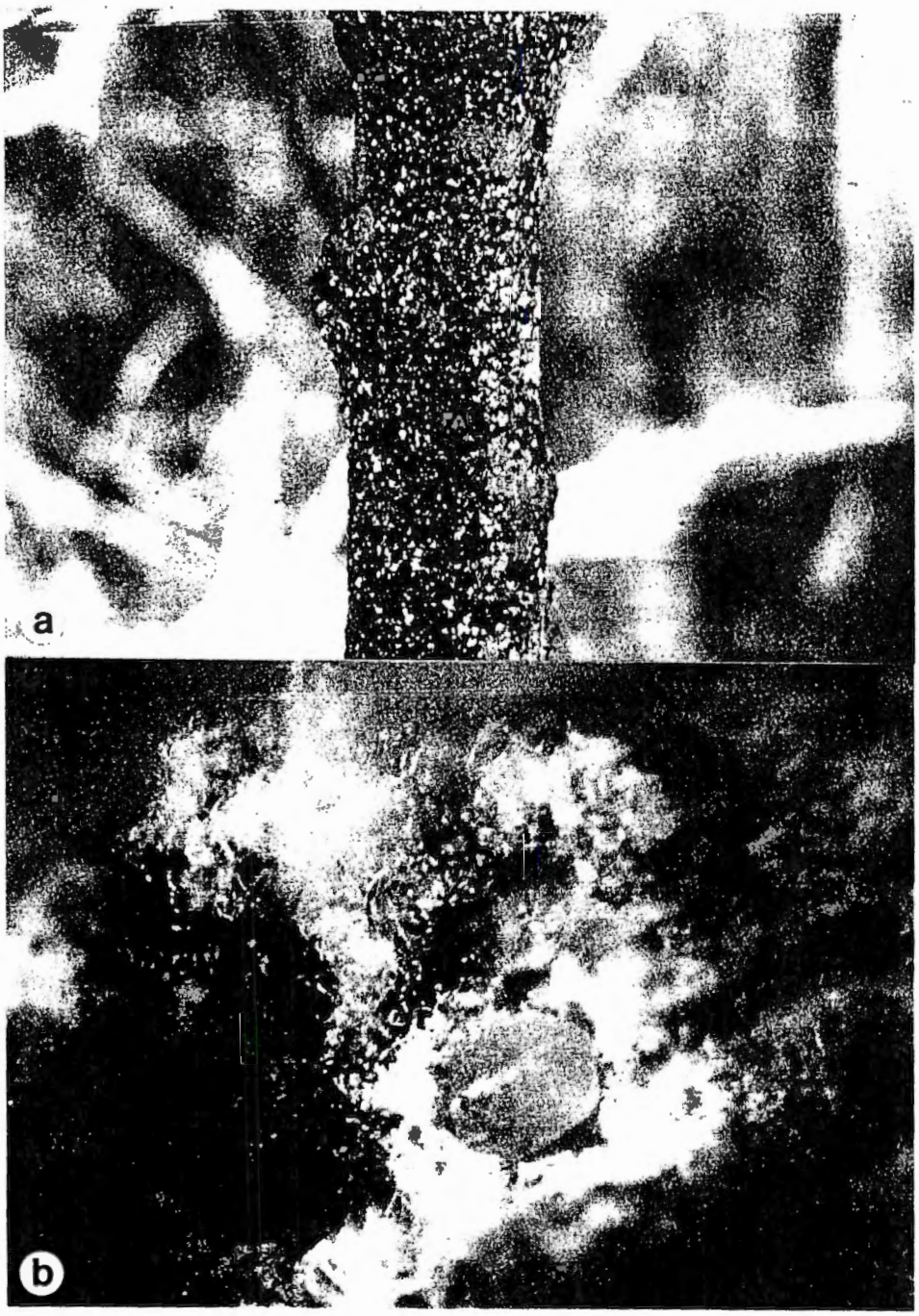


Plate 1-1. The beech scale, *Cryptoccocus fagisuga*, on the bark of American beech: (a) infestation of the bole with colonies of the scale, and (b) an individual insect with the woolly covering brushed aside.

wax covering the dead colonies frequently becomes colonized and blackened by a fungus, *Cladosporium cladosporioides* (Fresen.) G.A. De Vries (Lonsdale 1983). The slime flux results from cambial necrosis caused by the invading species of *Nectria* (Sinclair et al. 1987).

Infected trees respond to the fungal infection with the production of hyperplastic tissue with a layer of suberized callus (Ehrlich 1934). In most cases, this defense fails, and the fungus continues to grow outward, forming necrotic lesions, or small cankers. The phellem becomes ruptured as the hyphae at the center of the lesion begin to aggregate into a stroma, upon which the sporodochia are formed. Later in the season, the red perithecia of *Nectria* emerge atop this same stroma (Plate 1-2). As secondary infections occur, the ability of the tree to wall off the invasion usually decreases. Over the following two to three years, the expanding mycelia from separate infections may coalesce, forming large strips or patches of dead bark that shrink and dry, causing the dead bark to flake off of the tree (Plate 1-3).

The widespread invasion of the phloem and the vascular cambium by the fungus reduces the ability of the tree to effectively translocate water and nutrients. At this point, the leaves become curled and chlorotic, and there is a general dieback in the crown. On trees where the fungus has infected large areas surrounding the trunk, girdling occurs and the tree soon dies. On other trees, infection is limited to strips that result in serious defect of the wood (Houston and O'Brien 1983).



Plate1-2. Perithecia of *Nectria* spp. on the bark of American beech: (a) red perithecia on the bole, and (b) a magnified view of sporulating perithecia atop red stromata.



Plate1-3. Symptomatic cracking and flaking of American beech bark due to beech bark disease in Great Smoky Mountains National Park.

the beech scale (Houston 1983a). In general, this resistance is rare, occurring in less than 1% of inspected trees, and often in discrete groups (Houston 1994b).

The amounts of individual amino acids and amino nitrogen in bark tissue may be a limiting factor to colonization by the beech scale. Wargo (1988) found that higher populations of the insect occupied trees with higher levels of both individual amino acids and amino nitrogen in the bark. It is believed that low amino nitrogen in the bark tissues may be a mechanism of resistance to the beech scale (D. R. Houston, personal communication). Efforts are currently underway to propagate American beech that are resistant to beech bark disease (Barker et al. 1995).

Beech bark disease was first observed in Great Smoky Mountains National Park (GSMNP) in 1993 (Houston 1994b). Since that time, two studies have been conducted to determine the incidence and life cycles of the beech scale and *Nectria* spp. in ten permanently established plots within the park (Rutherford 1996, Vance 1995). These studies also included attempts to find some suitable biological control candidates, though none were found.

The current investigations were undertaken to continue beech bark disease research in GSMNP. The objectives of this research were to: 1) continue monitoring incidence of beech bark disease and mortality of *F. grandifolia*, 2) examine the role that a common beech bark fungus,

Ascodichaena rugosa Butin, may play in the epidemiology of beech bark disease, and 3) determine the effects of certain site and stand factors on incidence and relative population size of the causal fungi and beech tree mortality. This disease is still new to the southeastern United States, and it is important to learn as much as we can about this occurrence as quickly as possible. The American beech plays both an important and unique role in our eastern deciduous forests, which are among the most celebrated in the world.

These data will provide important monitoring information to resource management personnel in the park, aiding in our understanding of what is happening and what could occur in the high elevation beech gaps of GSMNP. They will also provide evaluations of several beech bark disease concepts that were developed in the northeastern United States and Europe. Finally, this research will provide further documentation on the course of a significant forest disease that is new to the southeastern United States.

CHAPTER 2

THE ADVANCE OF BEECH BARK DISEASE IN GREAT SMOKY MOUNTAINS NATIONAL PARK

i. Introduction

Great Smoky Mountains National Park (GSMNP) is one of the largest forested wilderness areas in the eastern United States, and is the most heavily visited park in the United States National Park system. The park covers an area of approximately 2075 square kilometers along the Tennessee-North Carolina state line and is known for its incredible biological diversity. The park is comprised of a unique combination of plant communities, one of which, the cove hardwoods forest, is considered "among the most beautiful in the world" (Whittaker 1956). The public affairs office at GSMNP estimates that more than 9.2 million people visited the park in 1996. Visitors, along with park employees, contribute $772 million annually to the economies of Tennessee and North Carolina (N. Gray, personal communication).

The American beech, *Fagus grandifolia* Ehrhart, is a dominant tree species in several important communities within GSMNP, including the cove hardwoods, upper cove forests, eastern hemlock, and northern hardwood forests (Stupka 1964, Whittaker 1956). *Fagus grandifolia* occurs

In the spring of 1994, nine permanent plots were established, and a previously established plot was added, to monitor the incidence of beech bark disease and its causal organisms in GSMNP (Rutherford 1996, Vance 1995). At that time, nearly 47% of the trees in the combined plots harbored beech scale (*Cryptococcus fagisuga* Lindinger) populations, and the perithecia of *Nectria* Fries spp. were found on 10% of trees (Vance 1995). By spring 1995, incidence of beech scale populations had increased slightly, to 49%, while incidence of *Nectria* spp. perithecia had increased to almost 19% (Rutherford 1996, Vance 1995). Tree mortality increased from 17% to 20% over this same time period (Rutherford 1996).

The current research was undertaken to continue monitoring the advance of beech bark disease in GSMNP. The objectives of this study were to: 1) monitor the incidence, prevalence, and abundance of *Nectria* spp. in the ten permanent study plots, 2) document the mortality due to beech bark disease in the plots, and 3) compare these data with data from previous beech bark disease studies in the park.

ii. Materials and Methods

Data Collection

Research was conducted in ten permanent plots that were established in the spring of 1994 for long term monitoring of beech bark disease in GSMNP (Rutherford 1996, Vance 1995). Plots were selected for varying

levels of beech scale infestation, and site data including elevation, aspect, and percent slope were recorded (Table 2-1) (Rutherford 1996). Within each plot, all beech trees greater than 3.5 cm in diameter at breast height (DBH) were mapped and tagged with aluminum markers at a height of 137 cm (Vance 1995). Each plot was 400 m^2 after correction for slope, and was marked at the corners, perimeter midpoints, and center, with painted-orange rebar markers.

Beech trees in the plots were all evaluated for mortality, and rated for relative abundance of perithecia formed by *Nectria* spp.. Rating techniques followed Rutherford (1996) and Vance (1995). A rating square, constructed from 1.5 cm diameter PVC and measuring 1089 cm^2, was centered 122 cm above the base of the tree. Ratings were made on the north and south sides of each tree within the rating square, and ratings of the overall abundance were made by inspecting the entire lower 2 m of each tree trunk. The scale used to rate the abundance of perithecia on the bark also follows Rutherford (1996) and Vance (1995), and is given in Table 2-2. Each plot was rated three times for tree mortality and abundance of *Nectria* spp., once during the spring of 1996, again in the fall of 1996, and finally, in the spring of 1997. Data from plot ratings conducted during 1994-1995 (Rutherford 1996) were combined with the 1996-1997 data to broaden the scope of the study and allow for analysis of disease progression over a longer period of time.

Table 2-1. Summary of permanent plots established for long-term monitoring of beech bark disease in Great Smoky Mountains National Park.

Plot	Location	n[a]	Elevation (m)	Slope	Aspect
A	Gregory Bald*	45	1420	25.5%	358.0
B	Jenkin's Knob*	54	1341	16.0%	10.0
C	Forney Ridge*	77	1670	44.5%	258.5
D	Trillium Gap	55	1451	38.0%	277.0
E	Deep Creek	31	1378	43.0%	178.0
F	Indian Gap	55	1596	58.0%	193.0
G	Chimney Tops Trail	20	1109	21.0%	315.5
H	Sweat Heifer	32[b]	1778	19.0%	133.0
I	Fork Ridge	35	1451	52.0%	171.0
J	Newfound Gap*	49	1596	63.5%	63.5

[a] n = Number of tagged beech trees in each permanent plot.
* Indicates presence of *Nectria* spp. at time of plot establishment (Spring 1994).

Sources:

Rutherford, B. A. 1996. Identification, life cycle, and presence of *Nectria* species associated with beech bark disease in the Great Smoky Mountains National Park and the effectiveness of *Nematogonum ferrugineum* as a biocontrol. Knoxville: University of Tennessee. 44 pp. M. S. Thesis.

Vance, R. A. 1995. Incidence and life history of beech scale, initiator of beech bark disease, in the Great Smoky Mountains National Park. Knoxville: University of Tennessee. 72 pp. M. S. Thesis.

Table 2-2. Rating scale for relative abundance of *Nectria* spp. perithecia on bark of *Fagus grandifolia.*

Rating	Relative Abundance
0	none
1	low and scattered
2	low and uniform
3	moderate and scattered
4	moderate and uniform
5	high and scattered
6	high and uniform

Source:

Rutherford, B. A. 1996. Identification, life cycle, and presence of *Nectria* species associated with beech bark disease in the Great Smoky Mountains National Park and the effectiveness of *Nematogonum ferrugineum* as a biocontrol. Knoxville: University of Tennessee. 44 pp. M. S. Thesis.

Measurements of Disease Progression

Several variables were calculated from the data and used to analyze the status and progression of beech bark disease in ten permanent plots during 1996-1997 and the progression of the disease in the pooled plots from 1994-1997. These included mean abundance ratings, percent incidence, percent prevalence, percent mortality, and percent mortality due to disease.

Means were calculated for north side, south side, and overall perithecial abundance. The total number of beech trees, live or dead, with signs of infection by *Nectria* spp. on the boles, was summed and divided by the total number of trees rated to determine the percent incidence. Percent prevalence was calculated as the number of live trees showing signs of infection by *Nectria* spp. divided by the total number of live trees at that time.

Percent mortality was calculated as the number of dead trees at each rating divided by the total number of tagged trees at the establishment of the plots in spring 1994. An additional variable, mortality due to disease, was determined to exclude tree mortality due to other causes, such as natural succession, windthrow, or ice damage. The variable was calculated as number of dead trees that had received an overall *Nectria* spp. rating of greater than zero.

Means for all variables were calculated for each plot by every year and season combination for the years 1994-1997, and separated by least significant difference (LSD). Means and mean separations for 1996 and 1997 data values were extracted from the analysis and observed separately to provide results from the current study.

Pooled plot means for each variable were then calculated for each year 1994-1997. The pooled yearly means were separated by Duncan's multiple range test. All significance was determined at the P=0.05 level of probability.

iii. Results

Current Status of Disease in Permanent Plots

Nectria spp. were observed in 6, 9, and 9 of the 10 permanent plots during the spring 1996, fall 1996, and spring 1997 ratings, respectively. Percent incidence of the fungi increased significantly in four of the plots (F, G, I, and J) between spring 1996 and fall 1996 sampling, while a significant decrease was recorded for only one plot (C) during the same time period (Figure 2-1a). Between the fall 1996 and spring 1997 sampling dates, the only change observed was a decrease in plot J (Figure 2-1a). Five of the ten plots experienced neither significant changes nor significant mean

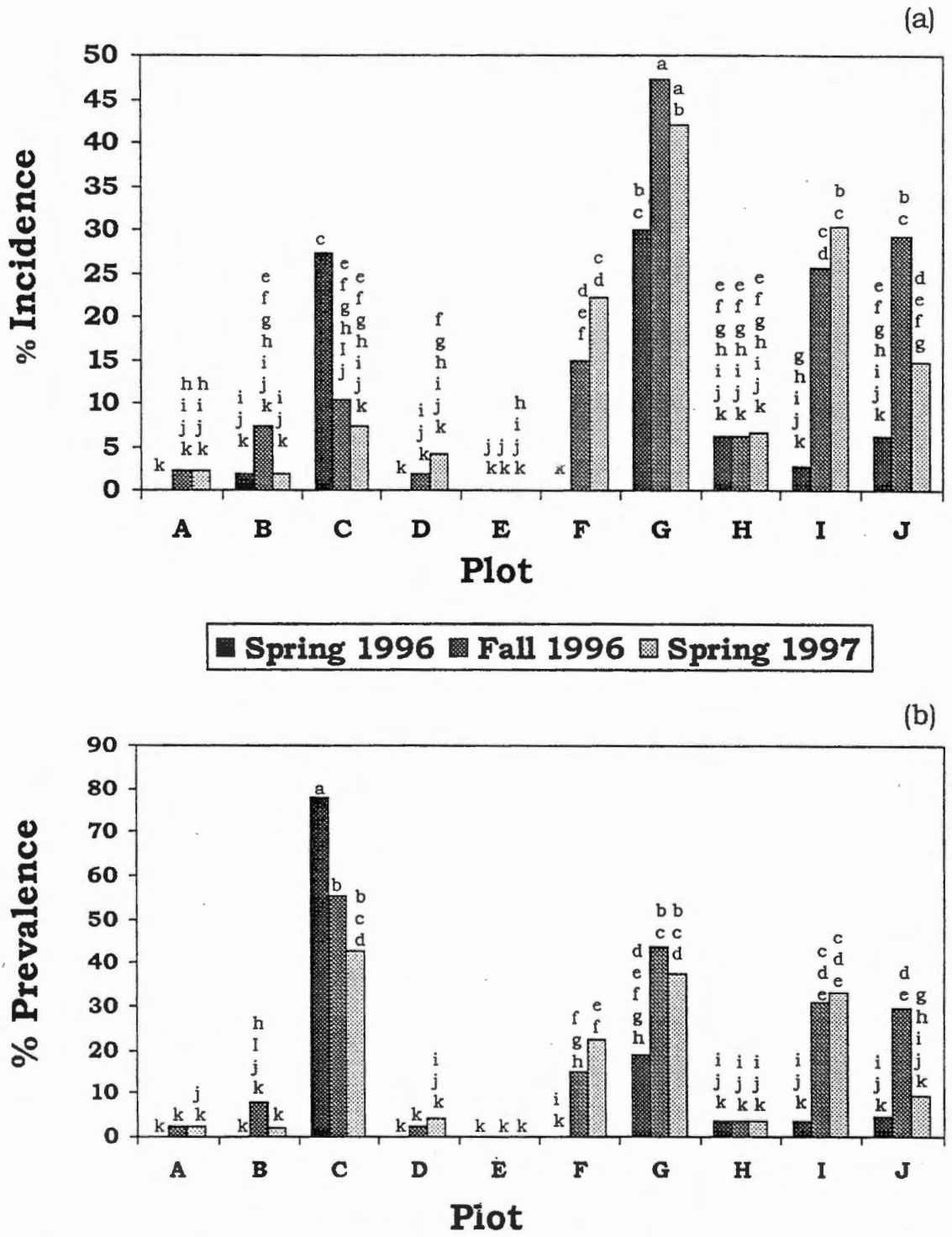


Figure 2-1. Frequency of *Nectria* spp. in permanent plots; (a) percent incidence, and (b) percent prevalence. Columns that share a letter do not differ significantly.

Note: Mean separations were adapted from LSD (P=0.05) analysis of 1994-1997 plot ratings.

incidences greater than zero during this study. Significant differences in incidence of *Nectria* spp. were observed among plots throughout the study.

The same patterns that were observed for plot values of percent incidence of *Nectria* spp. were observed for percent prevalence (Figure 2-1b). A single notable exception was the difference in magnitude between incidence and prevalence in plot C. The percent prevalence was nearly three times as great as the percent incidence at the first sampling date, and nearly five and six times as great at the second and third sampling dates, respectively (Figure 2-1). Significant differences in prevalence of *Nectria* spp. were observed among plots throughout the study (Figure 2-1b).

Mean north side and south side abundance ratings are shown in Tables 2-3a and 2-3b. No significant differences were observed on the north or south sides within nine of the plots during the entire course of the study. In fact, only one significant change in a plot (J) was observed on the north side ratings, between the spring 1996 and fall 1996 sampling dates (Table 2-3a). The mean south side abundance rating in Plot G was significantly greater in fall 1996 than in spring 1996, but by spring 1997 the mean south side rating had dropped back below the spring 1996 level (Table 2-3b). This pattern of higher mean ratings in fall 1996 followed by reduced ratings in spring 1997 was repeated eight times when north side and south side ratings are combined, though in most cases, the changes themselves were non-significant.

Table 2-3. Mean abundance ratings for *Nectria* spp. perithecia in ten permanent plots for spring 1996, fall 1996, and spring 1997 on (a) the north side, and (b) the south side of American beech trees. Cell values that share a letter do not differ significantly. LSD (P=0.05).

(a)

	Mean North Abundance Rating		
Plot	Spring 1996	Fall 1996	Spring 1997
A	0.00 h	0.00 h	0.00 h
B	0.00 h	0.00 h	0.00 h
C	0.25 bcde	0.19 cdefg	0.09 efgh
D	0.00 h	0.00 h	0.00 h
E	0.00 gh	C 00 gh	0.00 gh
F	0.00 h	0.11 defgh	0.04 gh
G	0.25 bcdefgh	0.47 ab	0.47 ab
H	0.03 fgh	0.13 cdefgh	0.00 gh
I	0.03 fgh	0.20 bcdefgh	0.27 bcdef
J	0.00 h	0.52 ab	0.34 bc

(b)

	Mean South Abundance Rating		
Plot	Spring 1996	Fall 1996	Spring 1997
A	0.00 f	0.00 f	0.00 f
B	0.02 f	0.00 f	0.00 f
C	0.07 f	0.01 f	0.00 f
D	0.00 f	0.00 f	0.00 f
E	0.00 f	0.00 f	0.00 f
F	0.00 f	0.11def	0.02 f
G	0.30 bcd	0.53 a	0.16 cdef
H	0.00 f	0.09 def	0.00 f
I	0.00 f	0.06 ef	0.03 f
J	0.00 f	0.23 cdef	0.02 f

Significant changes in mean overall abundance ratings were observed in five of the plots (C, F, G, I, and J), and mean overall ratings at each sampling date were significantly different in two of those plots (I and J) (Table 2-4). The pattern of increased mean rating values in fall followed by reduced values in the spring was observed in four of the plots (B, G, H, and J), though only in plots G and J were these changes statistically significant (Table 2-4).

Significant differences were observed in north and overall rating means among plots for every sampling period during the study (Tables 2-3a and 2-4). Variation was less pronounced for south side rating means among plots, with only one plot (G) showing a statistical difference between spring 1996 and fall 1996, and no significant differences among plots in spring 1997 (Table 2-3b).

The percent mortality and percent mortality due to disease for each plot at the final sampling period (spring 1997) are shown in Figures 2-2a and 2-2b. Five of the plots (C, E, G, I, and J) had greater than or equal to 20% mortality (Figure 2-2a), with the greatest mortality (90.9%) in plot C. In contrast, only plot C had greater than 20% mortality due to disease (84.4%), and three of the plots (A, D, and E) had no mortality due to disease (Figure 2-2b). A comparison of percent mortality and percent mortality due to disease reveals that four of the five plots having greater or equal to 20% mortality also had some occurrence of mortality due to disease. Significant

Table 2-4. Mean overall tree abundance ratings for *Nectria* spp. perithecia in ten permanent plots for spring 1996, fall 1996, and spring 1997. Cell values that share a letter do not differ significantly. LSD (P=0.05).

	Mean Overall Abundance Rating		
Plot	**Spring 1996**	**Fall 1996**	**Spring 1997**
A	0.00 hi	0.02 hi	0.02 hi
B	0.02 hi	0.07 ghi	0.02 hi
C	0.33 ef	0.18 fghi	0.07 ghi
D	0.00 i	0.02 hi	0.04 hi
E	0.00 hi	0.00 hi	0.00 hi
F	0.00 i	0.22 efgh	0.22 efgh
G	0.75 abc	1.11 a	0.63 bcd
H	0.13 fghi	0.19 efghi	0.07 ghi
I	0.03 hi	0.31 ef	0.61 c
J	0.06 hi	0.67 c	0.34 def

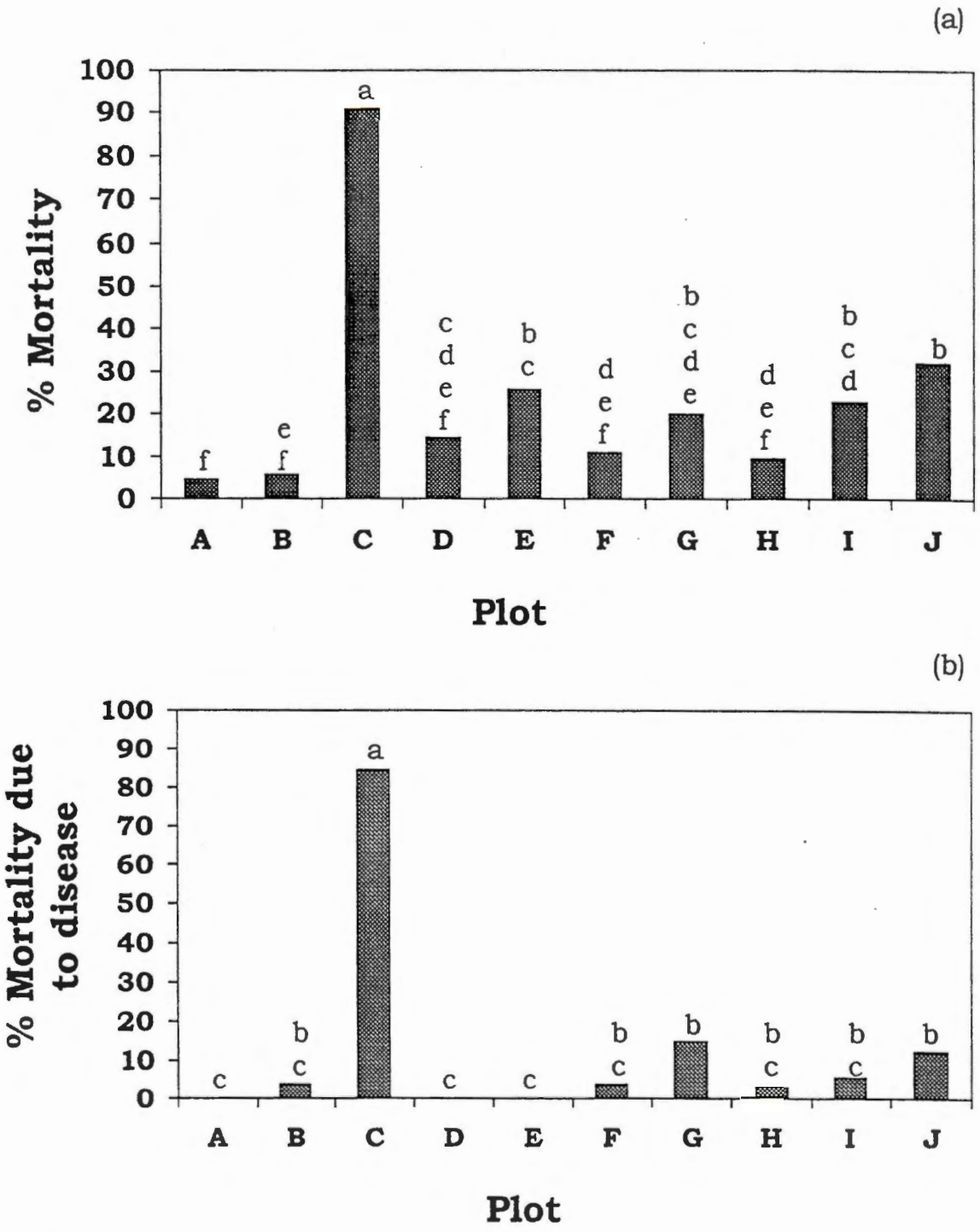


Figure 2-2. Spring 1997 mortality figures for beech in permanent plots; (a) percent mortality, and (b) percent mortality due to disease. Columns that share a letter do not differ significantly.
Note: Mean separations were adapted from LSD (P=0.05) analysis of 1994-1997 plot ratings.

variations in percent mortality and percent mortality due to disease were observed among the plots (Figures 2-2a and 2-2b).

Disease Progression: 1994-1997

Total mean percent incidence of *Nectria* spp. in 1997 (11.5%) was not statistically different than that of 1994 (11.6%) (Figure 2-3). A significant rise in incidence occurred during 1995, when perithecia of *Nectria* spp. were observed on 16% of all rated beech trees. This increase was followed by a sharp decrease to 10% in 1996. Incidence of *Nectria* spp. rebounded by spring 1997, increasing slightly though not significantly. Percent prevalence showed a significant and steady increase over the four year span, rising from just below 6% in 1994 to 11.4% by 1997 (Figure 2-3).

Mean overall and north side abundance ratings for 1996 and 1997 did not differ statistically from their respective means of 1994 (Figure 2-4). Mean south side ratings were significantly lower in both 1996 and 1997 than in 1994. North, south, and overall ratings all followed a similar pattern of a sharp, significant increase between 1994 and 1995 followed by an equally sharp decline in 1996 (Figure 2-4).

Percent mortality and percent mortality due to disease both showed significant increases from 1994 to 1995 (Figure 2-5). Mortality jumped from nearly 17% in spring 1994 to just over 28% in spring 1997, while mortality due to disease increased from around 7% in 1994 to nearly 18% by 1997. The largest increase in mortality occurred between 1996 and 1997, while

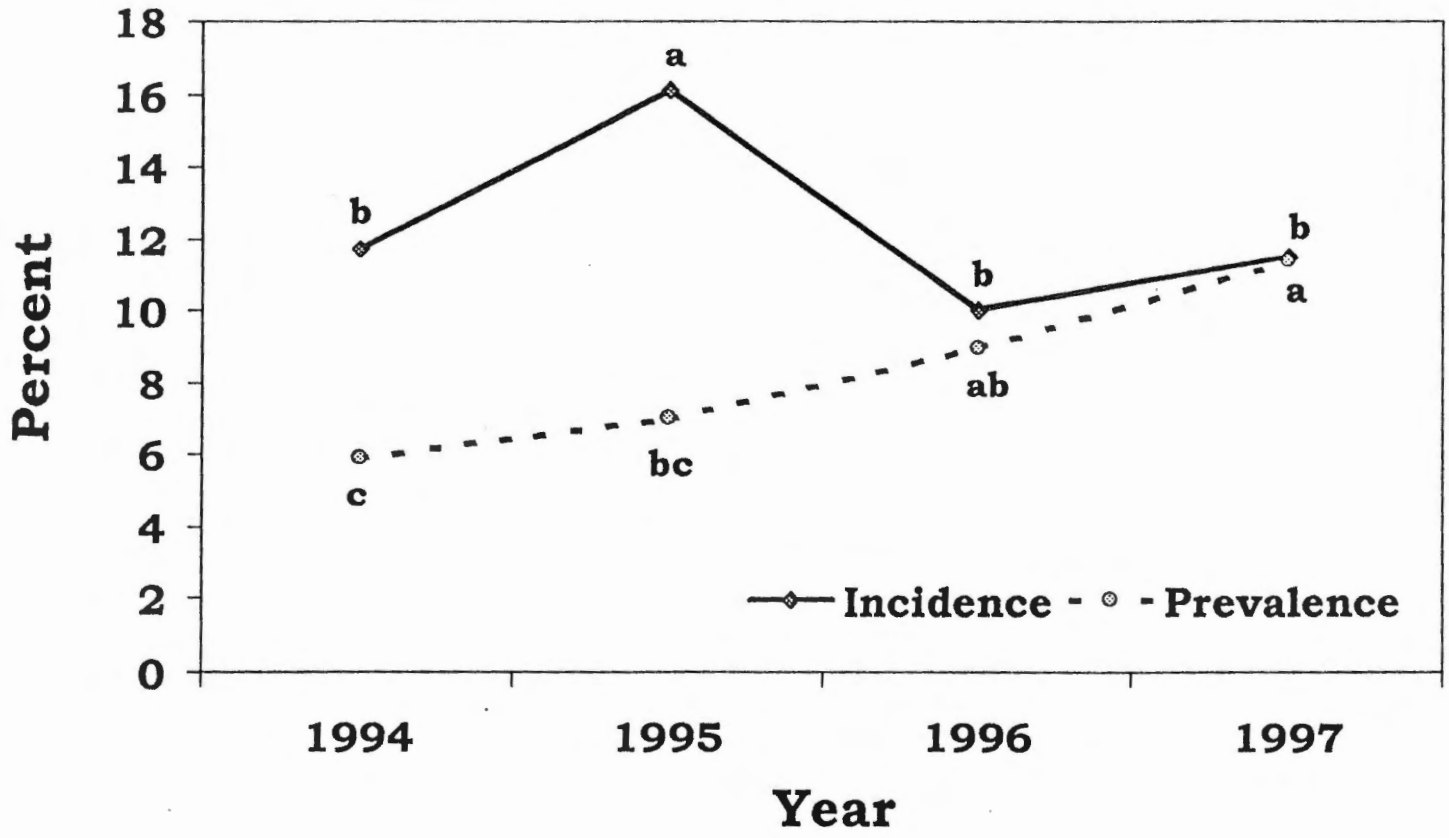


Figure 2-3. Change in mean percent incidence and mean percent prevalence of *Nectria* spp. by year for the period 1994-1997. Points on each line that share a letter do not differ significantly. *Note:* Mean separations by Duncan's Multiple Range Test (P=0.05).

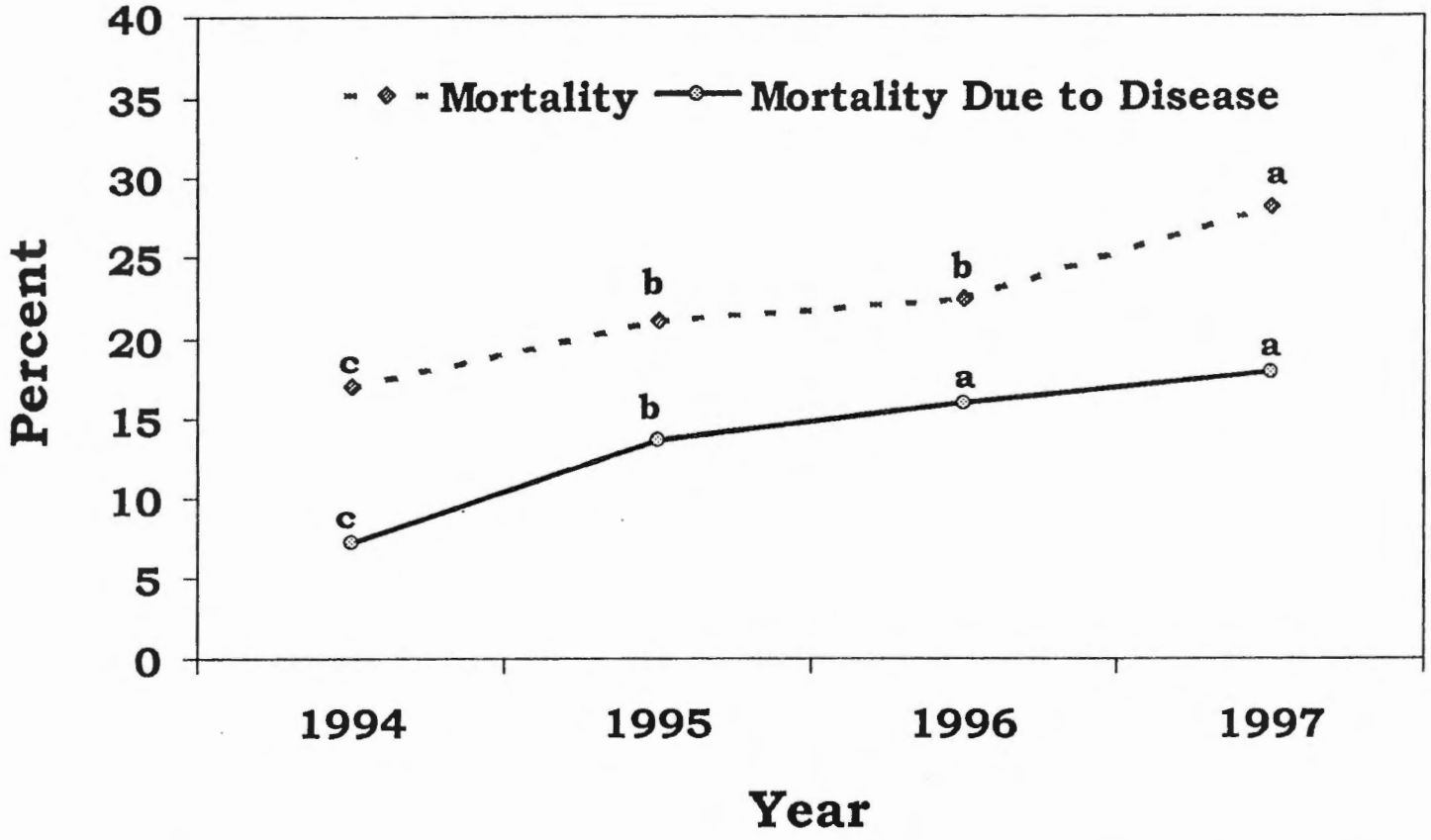


Figure 2-5. Change in mean percent mortality and mean percent mortality due to disease for the period 1994-1997. Points on each line that share a letter do not differ significantly. *Note:* Mean separations by Duncan's Multiple Range Test (P=0.05).

Trees just outside of plot E also exhibited signs of BBD. In general, the trend seems to be an increase in percent incidence both within the plots and among the pooled plots. The obvious exception is the plot on Forney Ridge Trail (C), which is in the latter stages of epidemic development. The significant reduction in percent incidence during this study is indicative of the high (90.9%) mortality within the plot. As trees reach the late stages of BBD, much of the bark which served as habitat for the *Nectria* spp. is missing from the trees or becomes heavily colonized by more competitive saprophytic fungi (Shigo 1964, personal observation).

The prevalence variable, by considering the presence of *Nectria* spp. on live trees only, provides a way to counter the problem of decreased incidence due to tree mortality. The advantage it provides is ensuring that incidence calculations do not become 'watered down' with dead trees that could not serve as habitat for the weakly saprophytic species of *Nectria*. In plot C, prevalence provides a more accurate measure of the presence of *Nectria* spp.. The low incidence in that plot is calculated using the full number of rated trees in the plot, though many of those trees are dead and are unlikely to have signs of *Nectria* spp.. In contrast, the prevalence remains high, indicating that nearly 80% of the trees in plot C that could potentially be infected are infected. The weakness of prevalence lies in the danger of underestimating disease by missing trees that have only recently died and still bear signs of the fungi. These features make it a valuable

addition, but not a replacement, to simple percent incidence in monitoring BBD in the southeastern United States.

The general trend in percent prevalence is similar to that of incidence. The significant rise in four of the plots and even the nonsignificant increase in three others seems to indicate that BBD is advancing among the live trees in the plots, and is likely indicative of the progress of the disease throughout much of GSMNP where beech are abundant.

The mean ratings are an indicator of the abundance of fungal signs in the permanent study plots. The general trend towards increasing mean north and overall abundance ratings may reflect an increase in disease severity within the plots. With BBD, greater numbers of perithecia on a single tree are associated with more numerous or larger dead areas on the bark. However, these increases may also reflect the increase in percent prevalence in the plots.

The pattern of greater incidence, prevalence, and north, south, and overall ratings in fall 1996 followed by decreases in spring 1997 was observed in several plots. This pattern can likely be best explained by the life cycle of the *Nectria* spp.. The perithecia, upon which the abundance ratings and incidence figures are solely based, are known to be formed late in the growing season (Ehrlich 1934). Peaks of perithecial abundance might well be expected to occur in the fall. Alternatively, some factor(s) relating

to tree resistance or the environment could be impacting the abundance of *Nectria* spp. in these plots.

A comparison between percent mortality and mortality due to disease offers a reminder of the reality that trees in the plots have died and are dying of natural causes other than BBD. Several plots contain trees that are tagged for inclusion in the study, though they appear to have died some time ago due to natural successional processes. Plot D, for example has nearly 15% mortality, but none of it has been associated with the presence of BBD. Other causes of tree mortality have been high winds and severe winter conditions. However, it is possible that trees weakened by undetected BBD succumbed more readily to other destructive events. The variable mortality due to disease simply adds another dimension to analysis of the impact of BBD in our area.

The common pattern of fluctuation in percent incidence and north, south, and overall ratings from 1994 to 1997 is striking. This pattern is indicative of the large increase in percent mortality due to disease between 1994 and 1995, most of which occurred in plot C. During this period, total disease mortality nearly doubled, from 7.2% to 13.6% (Figure 2-5), and disease mortality in plot C increased from 51% to 77%. This significant rise in disease mortality was accompanied by a significant increase in incidence and abundance of *Nectria* spp. in 1995 and followed by large decreases in percent incidence and mean abundance ratings in 1996. The cycle of

buildup and decline of fungal signs fits well with the idea that as the fungus overwhelms the trees, there is a increase in the formation of fungal fruiting structures. As trees die and begin to degrade, they are quickly colonized by saprophytic organisms, and are no longer suitable hosts for the *Nectria* spp., which explains the observed sharp decline in incidence and abundance.

The rise in percent incidence between 1996 and 1997, though not significant, may be an indication that the cycle of fungal buildup and decline is already beginning again. Significant increases in percent incidence of *Nectria* spp. in plots F, G, I, and J support this idea. The steady rise in percent prevalence and percent mortality due to disease indicate that as the number of beech trees dying from beech bark disease increases, the number of live trees becoming infected is also increasing. It is apparent that BBD is both firmly established and increasing in GSMNP.

Each of the variables discussed thus far, incidence, prevalence, mean abundance ratings, mortality, and mortality due to disease, have been observed to vary significantly in magnitude, and in most cases, changes over time (dynamics). It may be that dispersal of the beech scale and *Nectria* spp. is the major factor limiting the spread and severity of BBD. Other biotic and abiotic variables may also be responsible for some of the observed variations in the advance of BBD in GSMNP.

Chapter 3

THE ROLE OF *ASCODICHAENA RUGOSA* BUTIN IN THE EPIDEMIOLOGY OF BEECH BARK DISEASE IN THE GREAT SMOKY MOUNTAINS NATIONAL PARK

i. Introduction

Beech bark disease (BBD) was first confirmed to be present in Great Smoky Mountains National Park (GSMNP) in 1993 (Houston 1994b). Colonies of beech scale (*Cryptoccus fagisuga* Lindinger) and perithecia of *Nectria* Fries spp. (*Nectria coccinea* var. *faginata* Lohman, Watson, and Ayers or *Nectria galligena* Bresadola) were associated with dead and dying American beech (*Fagus grandifolia* Ehrhart) trees in several high elevation beech stands. In the spring of 1994, ten permanent plots were established to monitor the incidence of BBD and its causal organisms in GSMNP (Rutherford 1996, Vance 1995). At that time, seven of the ten plots had some incidence of beech scale, and perithecia of *Nectria* spp. were found on beech trees in six of the ten plots (Rutherford 1996, Vance 1995). After spring and fall ratings in 1995, eight plots contained beech scale populations and nine of the ten contained *Nectria* spp. (Rutherford 1996, Vance 1995).

Black stroma and fruiting bodies of an unknown fungus were observed growing on the boles of American beech trees in the permanent

plots in 1995. Trees with relatively high abundance of this unknown fungus appeared to have low abundance of the perithecia of *Nectria* spp.. On the basis of host and growth habit, the fungus was tentatively identified as *Ascodichaena rugosa* Butin [=*Dichaena rugosa* (Linnaeus) Fries] Further evidence was provided by a photograph of *A. rugosa* in Cotter (1977). Identification of the fungus was later confirmed by Houston (personal communication).

Ascodichaena rugosa is a member of the phylum Ascomycota, order Helotiales, and is characterized by small (0.3-0.45 mm x 0.25-0.3 mm), blackish-brown apothecia that form in dense groups atop black perennial stroma (Butin 1977). The anamorph is *Polymorphum quercinum* (Persoon) Chevallier [=*Dichaena faginea* (Persoon) Fries), a member of the Coelomycetes (Butin 1977, Hawksworth 1983). The imperfect stage is macroscopically similar in appearance to the perfect stage, forming small, black pycnidia also in dense groups on the perennial stroma (Butin 1977). *Polymorphum quercinum* is the more commonly observed stage on beech (Butin 1977).

Ascodichaena rugosa is a specialized parasite that can commonly be observed as black, elliptical to horizontally elongated patches of stroma and fruiting bodies on boles of both European and American beech (Plate 3-1) (Butin 1977, Butin and Parameswaran 1980). The disease has been named 'Black Bark Scab of Beech', but has little impact on infected trees (Butin

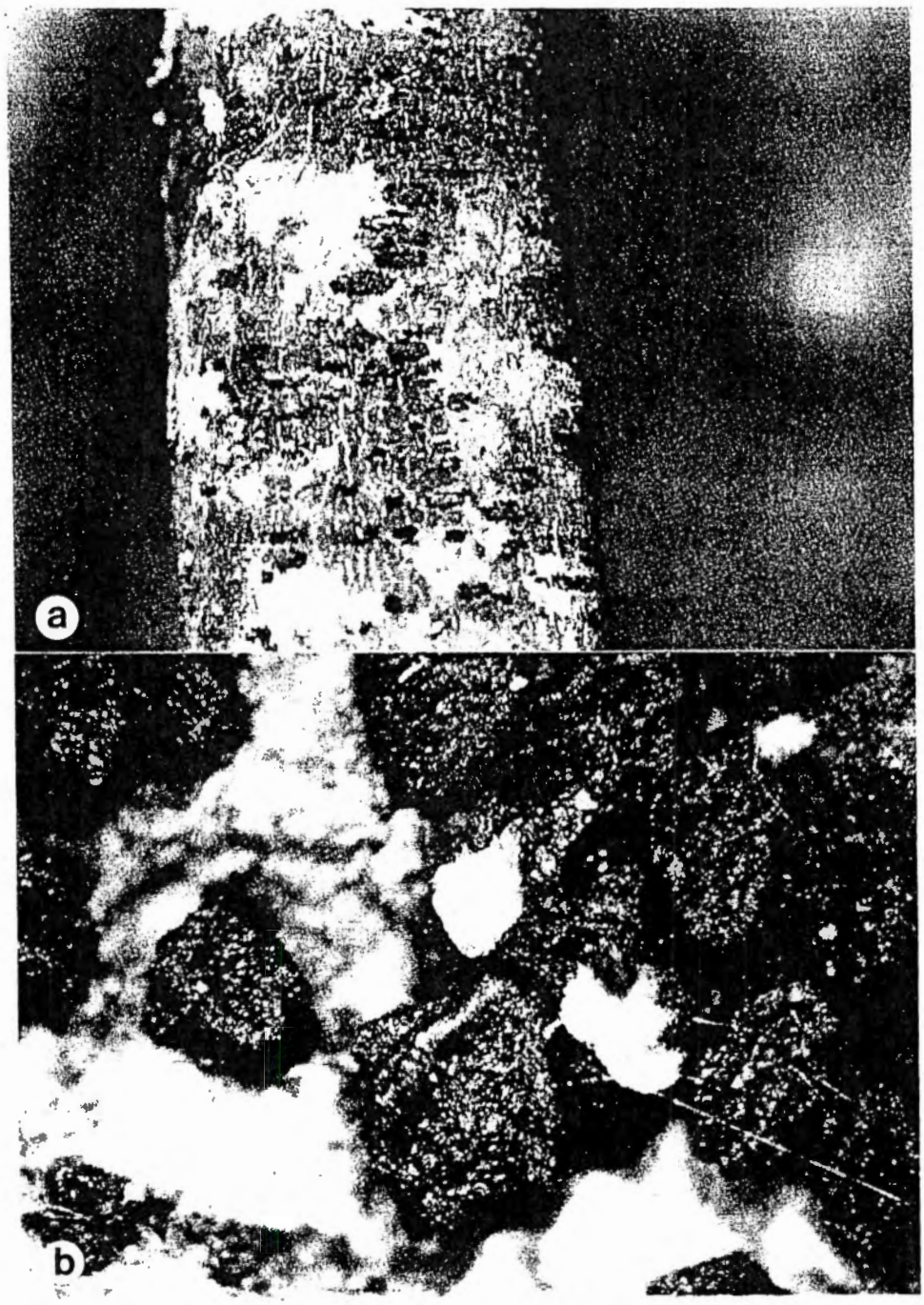


Plate 3-1. Stroma and fruiting bodies of *Ascodichaena rugosa* on the bark of American beech: (a) horizontal and elliptical patches of stroma and fruiting bodies (pycnidia) on the bole, and (b) a magnified view of sporulating pycnidia atop black stromata.

1995). Symptoms include a two- to three-fold increase in the thickness of the periderm resulting from increased production of cork cells and enlargement of infected cells (Butin and Parameswaran 1980).

A negative association has been found between the relative frequencies of *A. rugosa* and the beech scale on European beech *(Fagus sylvatica* Linnaeus) trees in England (Houston 1976). The heavy colonization of European beech bark by *A. rugosa* may have reduced the negative effects of beech scale and the development of beech bark disease (Houston 1976). Speer and Butin (1980) concluded that the presence of *A. rugosa* generally promoted the distribution of the beech scale, and only when beech trees formed secondary periderm were attacks of the insect limited by the fungus. It is currently believed that the more sparse colonization of American beech by this fungus offers no protection against the beech scale in North American forests, but may, in fact, provide refuge for the insects (D. R. Houston, personal communication). Neither hypothesis has yet been tested on American beech trees.

The purpose of this research was to determine what, if any, effects *A. rugosa* populations have on the spread of beech bark disease in GSMNP. The objectives of this study were to: 1) monitor the incidence and abundance of *A. rugosa* on American beech trees within ten permanent study plots, 2) test the hypothesis that heavy colonization by *A. rugosa* may reduce the abundance of beech scale on American beech trees, and 3)

document the relationship between *A. rugosa* population size with the incidence of beech bark disease.

ii. Materials and Methods

Incidence and Abundance of Ascodichaena rugosa

Boles of beech trees in ten long term study plots were sampled and rated on the north side, south side, and overall for relative abundance of the stroma and fruiting bodies of *A. rugosa* . The same rating scale (Table 2-2) and rating techniques that were used to rate beech trees for abundance of *Nectria* spp. were used to collect relative abundance data for *A. rugosa.* Permanent plot descriptions, rating techniques, and rating scale are described in Chapter 2 of this thesis. Each plot was rated twice for *A. rugosa*, once during the fall of 1996 and again in the spring of 1997.

The percent incidence of *A. rugosa* was calculated for each plot as the number of beech trees, live or dead, with signs of the fungus, divided by the total number of sampled beech trees in that plot. Percent prevalence was calculated as the number of live trees with signs of *A. rugosa* divided by the total number of live trees in the plot at that time. Means were also calculated for north side, south side, and overall tree abundance ratings. Plot means were separated by year and season using LSD. Significance for all analyses were determined at the 95% (P=0.05) confidence level.

The Association Between Abundances of Ascodichaena rugosa and Cryptococcus fagisuga.

Relative abundance ratings for *C. fagisuga* were taken in fall of 1996 and again in the spring of 1997 by Wiggins (1997). Ratings were taken in the same manner as Vance (1995) with the same rating scale used throughout this thesis (Table 2-2). North side, south side, and overall abundance ratings for *A. rugosa* and *C. fagisuga* were each placed into one of three classes: 0 = none, 1-2 = low, and 3-6 = moderate to high. Data were arranged by north, south, and overall ratings from fall 1996 and spring 1997 into 3 x 3 contingency tables and analyzed using Fisher's Exact Test for Independence (Schlottzhauer and Littell 1987). The MEASURES option was added to the TABLES statement in the SAS program (version 6.12) and the Kendall's Tau-b statistic was analyzed to determine the positive or negative nature of associations, when they occurred (Schlotzhauer and Littell 1987). Significance was determined at the P=0.05 level.

The Association Between Ascodichaena rugosa Abundance Ratings and Incidence of Beech Bark Disease

Beech trees in the ten plots were rated for relative abundance of *Nectria* spp. as described in Chapter 2 of this thesis. BBD was deemed present when the rating for *Nectria* spp. was greater than zero. North side,

south side, and overall abundance ratings for *Nectria* spp. were placed into one of two classes: 0 = not present or 1-6 = present.

Relative north, south, and overall abundance ratings for *A. rugosa* were also placed into two classes. Ratings between 0 and 2 were counted as "none to low" and ratings between 3 and 6 were counted as "moderate to high". *Ascodichaena rugosa* abundance classes and incidence of BBD were arranged by north, south, and overall abundance ratings into 2 x 2 contingency tables and analyzed for independence using Fisher's Exact Test (Schlotzhauer and Littell 1987). The MEASURES option was added to the TABLES statement in the SAS program (version 6.12) and the Kendall's Tau-b statistic was analyzed to determine the positive or negative nature of associations, when they occurred (Schlotzhauer and Littell 1987). Significance was determined at the P=0.05 level.

Comparison of Nectria spp. and Ascodichaena rugosa Abundance on North and South Tree Sides

As a further exploration into the relationship between *A. rugosa* and *Nectria* spp., the mean north and south tree side abundance ratings for each fungus were compared. The means of 3,039 north side and 3,034 south side observations for abundance of *Nectria* spp. perithecia from seven sampling periods (1994-1997) were calculated and tested for differences using Duncan's Multiple Range Test. Likewise, means from a total of 849 north side and 849 south side observations for abundance of *A. rugosa* from

two sampling periods (1996-1997) were calculated and tested for differences. Significance was determined at the P=0.05 level.

iii. Results

Incidence and Abundance of Ascodichaena rugosa

Ascodichaena rugosa was found in all ten plots during this study (Figure 3-1a). In general, incidence of *A. rugosa* on beech trees within the plots was common, with 65% and 66% of the trees showing signs of the fungus in fall 1996 and spring 1997, respectively. Significant differences in percent incidence of *A. rugosa* were observed among plots in both 1996 and 1997 (Figure 3-1a). Three plots (A, B, and F) had greater than 90% incidence in 1996, and incidence of greater than 90% was observed in four plots (A, B, D, and F) in 1997. In seven of the plots (A, B, D, E, F, G, and H) *A. rugosa* was observed on more than 50% of all beech trees. Two plots (D and J) experienced significant changes in percent incidence of the fungus between the fall 1996 and spring 1997 sampling dates (Figure 3-1a).

Percent prevalence among beech trees was 68% in 1996, and rose slightly to 69% in 1997. Significant differences among plots were observed during both 1996 and 1997 when prevalence was analyzed by plot (Figure 3-1b). Four plots (A, B, D, and F) had greater than 90% prevalence of *A. rugosa* during both 1996 and 1997, and a single plot (C) had zero percent

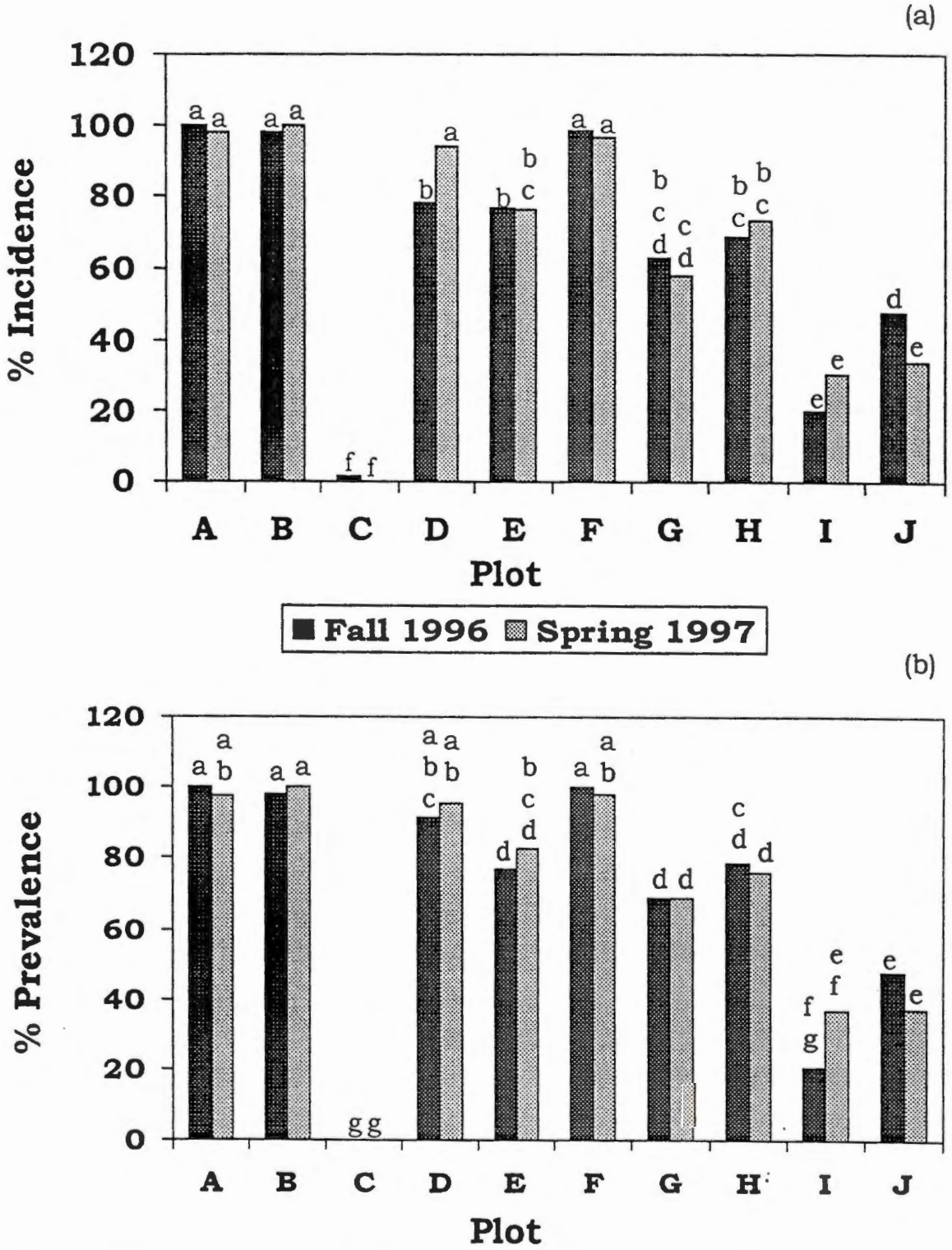


Figure 3-1. Frequency of *Ascodichaena rugosa* in ten permanent plots; (a) percent incidence, and (b) percent prevalence. Columns that share a letter do not differ significantly.
Note: LSD (P=0.05).

prevalence (Figure 3-1b). None of the plots experienced a significant change in percent prevalence of *A. rugosa* between 1996 and 1997 (Figure 3-1b).

Significant differences between mean overall abundance ratings of *A. rugosa* were also observed among plots (Figure 3-2). Five plots (A, B, D, E, and F) had overall means of greater than 2 in fall 1996, but this number fell to three plots (A, B, and D) by spring 1997 (Figure 3-2). Significant changes in mean overall abundance were observed in six of the plots (B, D, E, F, G, and H) between fall 1996 and spring 1997 sampling dates, with reductions in five of those (B, E, F, G, and H) and an increase in only one (D) (Figure 3-2).

The Association Between Abundances of Ascodichaena rugosa and Cryptococcus fagisuga

Analysis of the Fisher's Exact Test probability statistic provided evidence of significant associations (=dependence) between the abundance of *A. rugosa* and abundance of *C. fagisuga* on the north side, south side, and overall tree for both fall 1996 and spring 1997 (Tables 3-1, 3-2, and 3-3). All but two (Tables 3-2a and 3-3a) of the Fisher's Test statistics exceed a 99.9% probability for significant association. The negative values for the Kendall's Tau-b statistic and the associated 95% confidence intervals indicated that all of the tables but one (Table 3-2a) represent negative associations between the abundance of *A. rugosa* and abundance of beech scale. Table 3-2a did have a negative value for the Kendall's tau-b statistic,

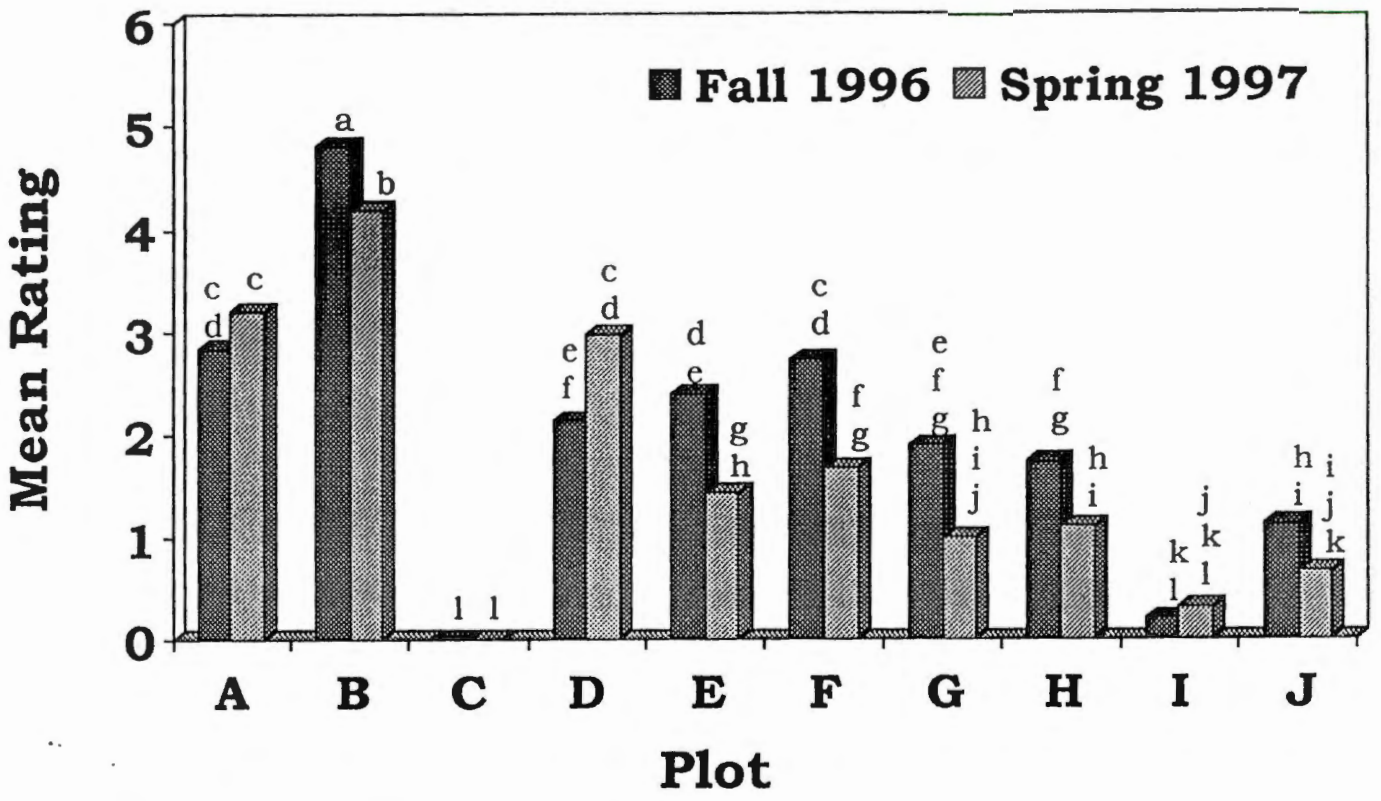


Figure 3-2. Mean overall abundance ratings of *Ascodichaena rugosa* on *Fagus grandifolia* from ten permanent plots.
Note: LSD (P=0.05).

Table 3-1. Contingency tables testing for association between abundance of *Ascodichaena rugosa* and abundance of *Cryptococcus fagisuga* on the north-facing tree side for (a) fall 1996, and (b) spring 1997.
Note: O=observed values and E=expected values.
Note: C.I.=95% confidence interval.
Note: P=0.05.

(a)

	Abundance of *A. rugosa*			
Abundance of *C. fagisuga*	None Present	Low	Moderate to High	**Total**
None Present	O_{11}=18 E_{11}=27	O_{12}=23 E_{12}=23	O_{13}=41 E_{13}=32	82
Low	O_{21}=75 E_{21}=65	O_{22}=44 E_{22}=56	O_{23}=78 E_{23}=77	197
Moderate to High	O_{31}=22 E_{31}=23	O_{32}=32 E_{32}=20	O_{33}=17 E_{33}=28	71
Total	115	99	136	**350**

Fisher's Exact Test: $P=3.73\times10^{-4}$
Kendall's tau-b: -0.126, C.I.=(-0.04,-0.212)

(b)

	Abundance of *A. rugosa*			
Abundance of *C. fagisuga*	None Present	Low	Moderate to High	**Total**
None Present	O_{11}=16 E_{11}=25	O_{12}=19 E_{12}=22	O_{13}=35 E_{13}=23	70
Low	O_{21}=70 E_{21}=65	O_{22}=45 E_{22}=57	O_{23}=68 E_{23}=61	183
Moderate to High	O_{31}=29 E_{31}=25	O_{32}=37 E_{32}=22	O_{33}= 4 E_{33}=23	70
Total	115	101	107	**323**

Fisher's Exact Test: $P=2.41\times10^{-9}$
Kendall's tau-b: -0.219, C.I.=(-0.133, -0.305)

Table 3-2. Contingency tables testing for association between abundance of *Ascodichaena rugosa* and abundance of *Cryptococcus fagisuga* on the south-facing tree side for (a) fall 1996, and (b) spring 1997.
Note: O=observed values and E=expected values.
Note: C.I.=95% confidence interval.
Note: P=0.05.

(a)

	Abundance of *A. rugosa*			
Abundance of *C. fagisuga*	None Present	Low	Moderate to High	**Total**
None Present	O_{11}=13 E_{11}=22	O_{12}=29 E_{12}=22	O_{13}=40 E_{13}=38	82
Low	O_{21}=62 E_{21}=53	O_{22}=44 E_{22}=53	O_{23}=94 E_{23}=94	200
Moderate to High	O_{31}=18 E_{31}=18	O_{32}=20 E_{32}=18	O_{33}=30 E_{33}=32	68
Total	93	93	164	**350**

Fisher's Exact Test: P=0.046
Kendall's tau-b: -0.053, C.I.=(.037,-0.143)

(b)

	Abundance of *A. rugosa*			
Abundance of *C. fagisuga*	None Present	Low	Moderate to High	**Total**
None Present	O_{11}=14 E_{11}=21	O_{12}=18 E_{12}=28	O_{13}=45 E_{13}=28	77
Low	O_{21}=53 E_{21}=50	O_{22}=60 E_{22}=65	O_{23}=67 E_{23}=65	180
Moderate to High	O_{31}=23 E_{31}=18	O_{32}=39 E_{32}=24	O_{33}= 4 E_{33}=24	66
Total	90	117	116	**323**

Fisher's Exact Test: $P=3.44\times10^{-10}$
Kendall's tau-b: -0.264, C.I.=(-0.178, -0.35)

Table 3-3. Contingency tables testing for association between abundance of *Ascodichaena rugosa* and abundance of *Cryptococcus fagisuga* on the overall tree for (a) fall 1996, and (b) spring 1997.
Note: O=observed values and E=expected values.
Note: C.I.=95% confidence interval.
Note: P=0.05.

(a)

	Abundance of *A. rugosa*			
Abundance of *C. fagisuga*	None Present	Low	Moderate to High	**Total**
None Present	O_{11}=2 E_{11}=11	O_{12}=13 E_{12}=11	O_{13}=35 E_{13}=28	50
Low	O_{21}=54 E_{21}=47	O_{22}=43 E_{22}=48	O_{23}=115 E_{23}=117	212
Moderate to High	O_{31}=22 E_{31}=19	O_{32}=23 E_{32}=20	O_{33}=43 E_{33}=49	88
Total	78	79	193	**350**

Fisher's Exact Test: $P=3.85\times10^{-3}$
Kendall's tau-b: -0.119, C.I.=(-0.029,-0.209)

(b)

	Abundance of *A. rugosa*			
Abundance of *C. fagisuga*	None Present	Low	Moderate to High	**Total**
None Present	O_{11}=3 E_{11}=8	O_{12}=10 E_{12}=14	O_{13}=28 E_{13}=19	41
Low	O_{21}=42 E_{21}=38	O_{22}=48 E_{22}=64	O_{23}=101 E_{23}= 89	191
Moderate to High	O_{31}=20 E_{31}=18	O_{32}=50 E_{32}=31	O_{33}=22 E_{33}=43	92
Total	65	108	151	**324**

Fisher's Exact Test: $P=1.37\times10^{-7}$
Kendall's tau-b: -0.230, C.I.=(-0.186, -0.274)

however the confidence interval included a slight area of positive value, indicating that there was some uncertainty as to the positive/negative nature of this association.

Analysis of the observed data versus expected values (Tables 3-1, 3-2, and 3-3) revealed the patterns of inconsistency from which the significant negative associations are formed. For column 1 (*A. rugosa*--none present), each table had fewer observations than expected for *C.* fagisuga--none present, and a greater number of observations than expected for low abundance of the insect. In four of the six tables (3-1b, 3-2b, 3-3a, and 3-3b) cells in row 3 column 1 have more trees with moderate to high abundance of *C. fagisuga* than expected.

Columns 2 and 3 of Tables 3-1, 3-2, and 3-3 provided two distinct patterns. First, when abundance of *A. rugosa* was low (column 2), there were consistently fewer than expected trees with low abundance of beech scale and more than expected with moderate to high abundance of the insect. In contrast, when abundance of the fungus was moderate to high (column 3), there were consistently more trees than expected with no incidence of beech scale and less than expected with moderate to high abundance of the insect.

The Association Between Ascodichaena rugosa Abundance Ratings and Incidence of Beech Bark Disease

The Fisher's Exact Test probability statistic provided evidence of

significant associations between the abundance of *A. rugosa* and incidence of *Nectria* spp. on the north side and overall tree for fall 1996 and spring 1997 (Tables 3-4 and 3-5). Negative values for Kendall's tau-b statistics and the associated confidence intervals indicated that these tables represent negative associations. There were no significant associations between the two variables on the south side, either in fall 1996 or in spring 1997 (Table 3-6).

The pattern of inconsistency between the observed and expected values was the same for every table (Tables 3-4, 3-5, and 3-6). When abundance of *A. rugosa* was none to low, there were fewer trees than expected without signs of *Nectria* spp. and more trees than expected with these signs. When abundance of *A. rugosa* was moderate to high, there were consistently more trees than expected without incidence of *Nectria* spp. and fewer than expected with incidence of *Nectria* species.

Comparison of Nectria spp. and Ascodichaena rugosa Abundance on North and South Tree Sides

Mean overall abundance ratings for both *Nectria* spp. and *A. rugosa* differed significantly from north side to south side (P=0.05). The perithecia of *Nectria* spp. were found in greater abundance on the north side (n=3,034), with a mean rating of 0.14, than on the south side (n=3,039), where the mean abundance rating was 0.09. In contrast, signs of *A. rugosa*

Table 3-4. Contingency tables testing for association between abundance of *Ascodichaena rugosa* and incidence of *Nectria* spp. on the north-facing tree side for (a) fall 1996, and (b) spring 1997.
Note: O=observed values and E=expected values.
Note: C.I.=95% confidence interval.
Note: P=0.05.

(a)

	Abundance of *A. rugosa*		
Presence of *Nectria spp.*	None to Low	Moderate to High	**Total**
Not Present	O_{11}=286 E_{11}=293	O_{12}=137 E_{12}=130	423
Present	O_{21}=24 E_{21}=17	O_{22}=1 E_{22}=8	25
Total	310	138	**448**

Fisher's Exact Test: $P=1.40\times10^{-3}$
Kendall's tau-b: -0.141, C.I.=(-0.091, -0.191)

(b)

	Abundance of *A. rugosa*		
Presence of *Nectria spp.*	None to Low	Moderate to High	**Total**
Not Present	O_{11}=271 E_{11}=276	O_{12}=110 E_{12}=105	381
Present	O_{21}=20 E_{21}=15	O_{22}=0 E_{22}=5	20
Total	291	110	**401**

Fisher's Exact Test: $P=1.71\times10^{-3}$
Kendall's tau-b: -0.141, C.I.=(-0.107, -0.175)

Table 3-5. Contingency tables testing for association between abundance of *Ascodichaena rugosa* and incidence of *Nectria* spp. on the overall tree for (a) fall 1996, and (b) spring 1997.
Note: O=observed values and E=expected values.
Note: C.I.=95% confidence interval.
Note: P=0.05.

(a)

	Abundance of *A. rugosa*		
Presence of *Nectria spp.*	None to Low	Moderate to High	**Total**
Not Present	O_{11}=210 E_{11}=221	O_{12}=182 E_{12}=172	392
Present	O_{21}=42 E_{21}=32	O_{22}=14 E_{22}=25	56
Total	252	196	**448**

Fisher's Exact Test: $P=2.42\times10^{-3}$
Kendall's tau-b: -0.143, C.I.=(-0.057, -0.229)

(b)

	Abundance of *A. rugosa*		
Presence of *Nectria spp.*	None to Low	Moderate to High	**Total**
Not Present	O_{11}=208 E_{11}=218	O_{12}=147 E_{12}=137	355
Present	O_{21}=38 E_{21}=28	O_{22}= 8 E_{22}=18	46
Total	246	155	**401**

Fisher's Exact Test: $P=1.26\times10^{-3}$
Kendall's tau-b: -0.157, C.I.=(-0.075, -0.239)

Table 3-6. Contingency tables testing for association between abundance of *Ascodichaena rugosa* and incidence of *Nectria* spp. on the south-facing tree side for (a) fall 1996, and (b) spring 1997.
Note: O=observed values and E=expected values.
Note: C.I.=95% confidence interval.
Note: P=0.05.

(a)

	Abundance of *A. rugosa*		
Presence of *Nectria spp.*	None to Low	Moderate to High	**Total**
Not Present	O_{11}=270 E_{11}=273	O_{12}=162 E_{11}=159	432
Present	O_{21}=13 E_{21}=10	O_{22}=3 E_{22}=6	16
Total	283	165	**448**

Fisher's Exact Test: P=0.186

(b)

	Abundance of *A. rugosa*		
Presence of *Nectria spp.*	None to Low	Moderate to High	**Total**
Not Present	O_{11}=277 E_{11}=278	O_{12}=118 E_{12}=117	395
Present	O_{21}=5 E_{21}=4	O_{22}=1 E_{22}=2	6
Total	282	119	**401**

Fisher's Exact Test: P=0.674

were found in greater abundance on the south side (n=849), with a mean rating of 1.62, than the north side, where the mean overall rating was 1.47.

iv. Discussion

Incidence and prevalence data indicate that *A. rugosa* is a common inhabitant on the bark of *F. grandifolia* within the ten study plots. The high frequency of the fungus on beech trees in many of the plots indicates that the fungus spreads easily from tree to tree, at least over a small area. The low incidence of *A. rugosa* in plot C may be due to the high beech tree mortality in that plot (90.9%); however, two considerations may suggest otherwise. First, the percent prevalence in plot C was zero both in fall 1996 and spring 1997, indicating that the fungus was not observed on any of the live trees in the plot. Second, the stroma and, to some extent the pycnidia, of *A. rugosa* are perennial in nature and would be expected to remain observable on trees even after death of the bark. Factors relating to host susceptibility or the environment may play roles in regulating the establishment and subsequent spread of *A. rugosa* in beech stands.

The differences in mean overall abundance ratings between plots somewhat reflect the differences in incidence/prevalence, but not entirely. For example, plots A, B, and F show no significant differences in incidence or prevalence but contain four statistically different mean rating values. The variations in abundance of *A. rugosa* may be further evidence that

populations of the fungus are favored by particular host genotypes or certain environmental conditions.

The significant changes, particularly the reductions, in mean abundance rating within plots between fall 1996 and spring 1997 is puzzling. The maturation of the pycnidia occurs in June and July (Butin 1977), and the spring 1997 sampling period ended by early June. Therefore, it might be logical to reason that a peak in abundance might be expected during the fall, followed by reduced abundance in the spring, as was the pattern with the abundance of *Nectria* spp. in Chapter 2 of this thesis. However, as mentioned earlier, the stroma of *A. rugosa* are perennial on the tree bark. As the sampling method for abundance of *A. rugosa* included stroma and pycnidia, no significant reductions were expected to occur. The fact that these reductions did occur in 50% of the plots suggests either that many pycnidia were developing on young stroma that were unrecognizable in the spring, or that inconsistencies existed within the sampling technique.

The significant negative associations between the abundances of *A. rugosa* and *C. fagisuga* support the findings of Houston (1976). The important difference, of course, is that Houston's study was conducted in England on European beech. This study provides the first evidence that populations of beech scale on American beech are negatively associated with *A. rugosa* in North America.

Fisher's Exact Test and Kendall's tau-b statistics do not imply anything about cause-and-effect in this relationship. Of particular interest in forming cause-and-effect hypotheses are the differences between observed and expected values in the contingency tables. Two consistent patterns exist: 1) increased numbers of trees with moderate to high scale populations where *A. rugosa* populations are low, and 2) decreased numbers of trees with moderate to high populations of scale where *A. rugosa* populations are moderate to high. These patterns provide important clues as to the likely cause and effect nature of the association. If one makes the reasonable assumption that *A. rugosa* was present on the bark prior to infestation with the beech scale, it follows that as abundance of *A. rugosa* increases from low to moderate or high, the abundance of the beech scale decreases. The fact that observed values for low *A. rugosa* and moderate to high beech scale are usually substantially greater than the expected values indicates that low abundances of the fungus may actually favor infestation of the trees by the insect, as suggested by Houston (personal communication).

The significant negative associations of *A. rugosa* abundance with the incidence of BBD on the north side and overall tree provide additional support for the idea that high *A. rugosa* equals none or low beech scale. In the current model of beech bark disease progression, infestation of beech trees by the scale is prerequisite for the development of the disease. If

populations of the scale are excluded or reduced by high abundance of *A. rugosa*, it follows that the appearance of *Nectria* spp. and subsequent disease will be excluded or, more likely, delayed and reduced. The lack of a statistical association between *A. rugosa* and incidence of BBD on the south side poses a dilemma. However, upon examination of the differences in observed and expected values, the exact same pattern is seen in the south side contingency tables as that in north side and overall tree. The low total values for row 2 of the south side contingency tables indicate that few trees had incidence of *Nectria* spp. on their south facing side. The cell values in row 2 of the south side tables simply resulted in differences that were too small for the statistical analysis to detect.

Mean north and south side abundance ratings for *Nectria* spp. and *A. rugosa* provide another look at how distribution of the two fungi relate. The two fungi had significantly greater mean abundance ratings on opposite tree sides, with *Nectria* spp. more abundant on the north side and *A. rugosa* more abundant on the south side. Taken separately, these differing abundances may indicate that different environmental conditions favor the growth and fruiting of the two fungi. Taken together, these results may lend further support to the concept that where abundance of *A. rugosa* is high, the occurrence or abundance of BBD is lower than in areas where *A. rugosa* is not present or is in low abundance.

The results of this research provide evidence that the abundance of *A. rugosa* on the boles of American beech may be an important influence on the spread and severity of BBD in the southern Appalachians. Because the development of BBD is entirely dependent on infestation and weakening of beech trees by the beech scale, the spread of BBD is governed by the dispersal and buildup of these insects (Houston et al. 1979b). The consistently negative associations between abundance of *A. rugosa* and abundance of beecn scale indicate that *A. rugosa* can act to limit the spread and development of infestation of the insects, in effect also reducing the occurrence and severity of BBD.

Knowledge of this association could prove useful in predicting the spread and severity of beech scale infestations and subsequent BBD epidemics. Further research should be conducted with a larger and more spatially diverse sample size, to determine if the negative association between *A. rugosa* and beech scale abundance is conserved over large areas in the southern Appalachians. Future research could also focus on the interactions between *A. rugosa* and *C. fagisuga* at the histological level, attempting to gain a better understanding of the precise mode of action of this fungus-host-insect interaction.

Chapter 4

INFLUENCE OF SITE AND STAND FACTORS ON INCIDENCE AND PREVALENCE OF INFECTION, PERITHECIAL ABUNDANCE, AND *NECTRIA*-RELATED MORTALITY

i. Introduction

Plant disease epidemics occurring in a natural setting result primarily from interactions among the pathogen, host, and environment (Agrios 1997). When sufficient numbers of a virulent pathogen are present at a given time, the development and spread of disease are largely controlled by characteristics of the host and environmental conditions. Host factors may include individual plant qualities such as size, age, and resistance, while environmental factors include liquid and vapor moisture, temperature, light, and wind (Agrios 1997). Environmental factors are highly interdependent and may also be affected by characteristics of a particular site, such as elevation, aspect, degree of slope, or soils.

Certain site, stand, and tree characteristics were thought by Ehrlich (1934) to influence both the incidence and severity of beech bark disease (BBD) in the Northeastern United States. He observed that the "activities" of the *Nectria* Fries sp. were inhibited by reduced levels of moisture on the beech (*Fagus grandifolia* Ehrhart) tree bark and in the surrounding atmosphere. Reductions in moisture appeared to result from openings in

the forest canopy. Ironically, these areas that appeared to subdue the fungus were often the result of crown dieback associated with the latter stages of the disease (Ehrlich 1934).

Ehrlich (1934) further observed that growth of fungal lesions and fruiting bodies was more abundant and rapid in beech stands with dense crown canopies. Stand variations in sunlight and drying winds were recognized as the basis for the variations in moisture and hence, disease progression (Ehrlich 1934). Statistical analyses, however, showed inconsistent or lacking correlations between crown density and disease incidence, mortality, or severity (Ehrlich 1934). These results are unclear and may have resulted from errors or inconsistencies in estimating "past" crown density in stands that had already lost significant canopy due to BBD.

Small but significant correlations between certain site, stand, or tree factors and disease incidence, severity, and tree mortality have been noted (Ehrlich 1934). Incidence of infection by *Nectria* sp. was positively correlated with percentage of beech in the stand, position on slope, and degree of slope. Severity of disease was positively correlated with diameter at breast height (DBH) of affected trees. Finally, mortality was correlated with DBH, crown class, and position of trees on slope.

Since the initial work by Ehrlich, the role that environmental factors play in the progression of BBD has been limited. Work done by Mize and

Lea (1979) examined the relationship of tree and environmental characteristics to growth and mortality of 417 American beech trees in the Adirondack Mountains of New York. They found that DBH, tree height, and crown width of survivor trees were significantly smaller than for trees that had died during a nine year BBD episode. A risk classification for beech trees based on these and other factors relating to pre-BBD vigor and health was developed (Mize and Lea 1979).

The effects of site factors on the severity of BBD in forests of Massachusetts and New Hampshire were examined by Twery and Patterson (1983). Severity was measured as visible defect due to infection. In agreement with studies by Ehrlich (1934) and Mize and Lea (1979), they found larger beech trees (>23 cm DBH) to have significantly greater defect than saplings (5-12 cm) and poles (12-23 cm). They stated that crown class had a significant effect on disease severity, with mean defect progressively less for dominant, codominant, intermediate, and suppressed trees.

Beech stands with either greater proportion of eastern hemlock [*Tsuga canadensis* (L.) Carr.] basal area or a low slope position have been observed to have significantly more defects due to BBD than beech trees in other stands (Twery and Patterson 1983). The authors suggested these factors may act to provide buffered microsites that offer conditions conducive for survival and pathogenicity of the beech scale and *Nectria* spp.. Twery and Patterson (1984) also suggested that red spruce (*Picea rubens*

Sargent) could potentially play a role similar to that of eastern hemlock in BBD epidemics.

In Great Smoky Mountains National Park (GSMNP) Rutherford (1996) and Vance (1995) looked at a limited number of tree and site variables in relationship to incidence and abundance of the beech scale and *Nectria* spp. in ten permanent plots. Vance (1995) found slight negative correlations between beech scale abundance ratings and average aspect of the plot as well as significant correlations between *Nectria* spp. abundance ratings and elevation. Rutherford (1996) was unable to correlate incidence of *Nectria* spp. with slope, aspect, or elevation after an additional season of data collection. She did find the occurrence of *Nectria* spp. to be negatively correlated to incidence of *Xylococculus betulae* (Pergande) Morrison, another scale insect on beech. These findings indicate that environmental and tree factors may play a role in the epidemiology of BBD in the Smokies, but represent only a short period of observation.

The current research was undertaken to determine if environmental or tree factors impact the incidence/prevalence of beech bark infection, abundance of sexual fruiting bodies of *Nectria* spp., or mortality related to infection by *Nectria* species. The objectives of this study were to: 1) collect or measure a number of biotic and abiotic variables and statistically analyze them for relationship to disease-related variables, and 2) determine if beech trees in particular forest types or localities may be at higher risk for

development of severe beech bark disease outbreaks. These data will provide information about the potential risk to American beech trees in the diverse forests of GSMNP.

ii. Materials and Methods

Biotic and Abiotic Variables

Research was conducted in ten permanent plots established by Rutherford (1996), Vance (1995), and staff of the National Park Service. A number of site and tree variables were obtained from data taken at the time of plot establishment in spring 1994 (Rutherford and Vance, unpublished data sheets). These variables were as follows: plot aspect, percent slope of plot, plot elevation, position of stand on slope, number of beech stems greater than 3.5 cm DBH, and crown class of all beech trees. Plot aspect, position of stand on slope, and crown class were all placed in numerical categories. These categories are defined in Table 4-1.

Additional site, stand, and environmental variables were collected during the summer and winter of 1996. These variables were as follows: fractional interception of photosynthetically active radiation (PAR) during summer and winter, canopy cover during summer and winter, total evergreen basal area in plots, evergreen basal area by species in plots, and number of evergreens in plots.

Figure 4-1. Category values and definitions for independent plot variables.

Variable Name	Category	Definition
Plot Aspect	1 2	NE 315°-134° SW 135°-314°
Slope Position	1 2 3 4 5 6	Summit Shoulder Back slope Foot slope Terrace Bottom
Crown class	1 2 3 4	Suppressed Intermediate Codominant Dominant

Disease-related variables were as follows: incidence, prevalence, perithecial abundance of *Nectria* spp., and *Nectria*-related mortality. Detailed information regarding the collection and interpretation of these data are provided in Chapter 2 of this work. Disease data from the most current ratings, spring 1997 (n=401 trees), were used in the statistical analysis.

Measurement of Variables: Fractional Interception of PAR and Canopy Cover

Transect lines were established on both diagonals in each plot. The lines were constructed from yellow nylon twine with red flagging secured at every two meters with tape. Values for PAR and canopy cover were recorded at 2 m intervals along the transects, for a total of 16 to 31 data points per plot. Readings were recorded at 3 m intervals in plot B. Adjustments in the methodology were also necessary in plot J. There are many trees in that plot and the nearby area that were blown down by remnants of Hurricane Opal in October 1995, and establishment of transects was infeasible. Three points near each of the plot's corner posts and four points near the center post were sampled instead. At the plot corners, points were located approximately 3 m towards each of the other three corners and in the center, samples were taken from the post while facing each of the plot corners. Techniques were consistent within plots for summer and winter data collections.

Photosynthetically active radiation (400-700 nm) was measured with a Sunfleck Ceptometer (Decagon Devices, Inc., Pullman, Washington 99163). At each observation point, the microprocessor/keypad was balanced at breast height directly above the red flagging with the wand of the ceptometer facing due south. Three measurements of PAR were taken sequentially at each point. The values were then averaged by the ceptometer and recorded onto a data sheet.

To standardize values, PAR was measured at open (no canopy) locations before and after sampling each plot. These values were averaged to estimate an ambient light standard for that day. The standard was used to calculate fractional interception at each sample point within the plots. The following formula was used to calculate fractional interception, i_f:

$$i_f = 1 - (\text{plot PAR}/\text{standard PAR})$$

The fractional interception value theoretically provides a standard sample of light intensity below the plot canopies, which is where all observations of fungal incidence and abundance were made.

Canopy cover was measured with a sighting tube constructed of a 15 cm length of 2.5 cm internal diameter PVC pipe fitted on one end with cross hairs of piano wire. Cover was determined by looking straight up through the tube at each position along the transect. A value of "1" was assigned to positions where foliage at the canopy level was observed at the intersection of the cross hairs. Because *F. grandifolia* typically retains its leaves

throughout much of the winter, both live and dead foliage was counted as a "1". A value of "0" was assigned to positions where no foliage was observed at the intersection of crosshairs. The advantages to this method for measurement of canopy closure are discussed by Ganey and Block (1994).

Measurements of PAR and canopy cover were made twice in 1996, once during the growing season and once during the winter months, after all deciduous trees had undergone seasonal senescence. The assumption was made that the standardized values of fractional interception would serve as an accurate representation for light conditions in each plot during these two predominant growth phases.

Measurement of Variables: Evergreen Basal Area

Plot perimeters were demarcated by running a 100-m tape from corner post to corner post. Each evergreen tree or shrub species that was within the plot or had foliage overhanging the plot was considered. Those over 137 cm in height were identified and the DBH was recorded. Basal areas for total evergreens by plot and evergreen species by plot were calculated using the measured diameters.

Statistical Analysis

Data were analyzed using Proc GLM and Proc Corr of SAS Version 6.12. Backward selection was used to test each of the environmental or stand variables for significant relationship to disease/*Nectria*-related

variables. Factors showing significant effects on the dependent variables of disease were then grouped into linear models. These independent variables were then removed one by one until the models contained only factors with a significant relationship to the dependent variables. Proc Corr was used to determine the positive or negative correlation when significant relationships were found. Significance for all statistics was determined at P=0.05.

These statistical methods were conducted twice; the first set of analyses included data from plot C (Forney Ridge), and the second set were conducted without data from plot C. Inclusion of the plot was necessary to capture relationships between the dependent variables and most of the independent variables. The plot had to be excluded for determination of relationships between fractional interception, canopy cover, and dependent variables, due to the high percentage of dead trees (90.9%) and resultant openings in the canopy.

Additional analyses of the effect of tree size on disease variables were conducted. DBH was recorded for all beech trees in the study plots during the spring of the years 1994, 1995, and 1996 by staff of the National Park Service (Glenn Taylor, personal communication). Diameter measurements for each tree were included as the 1994 data when it was available. When no measurement was taken for an individual tree in 1994, the earliest available measurement from the following two years was used as the DBH.

It was assumed that the trees had not increased significantly in diameter over the three year period. Means were calculated for trees having an overall *Nectria* spp. rating greater than zero at any point during the period 1994-1997 and for trees that had died due to the disease. These means were compared with DBH of trees having no incidence of *Nectria* spp. and otherwise dead or live trees, respectively. Means were separated using Duncan's Multiple Range Test (P=0.05).

iii. Results

Linear models accounting for significant portions of the variation in incidence and overall abundance ratings of *Nectria* spp. were developed. Probability and R-square values for each model from analysis of variance, and probability values for each of the linear model components are given in Table 4-2a and b, along with correlation coefficients and probabilities for each of the components. The linear models relate variations in both incidence and overall abundance of *Nectria* spp. with variations in crown class of trees, plot slope, and number of beech stems in the plots. Since neither fractional interception nor canopy cover accounted for significant amounts of variation in these dependent variables, data from all ten of the permanent plots (n=401 trees), including plot C, were used to develop the models.

Table 4-2. Linear models accounting for significant portions of variability in (a) incidence of *Nectria* spp., and (b) overall abundance ratings for *Nectria* species.
Note: Statistics for correlation between the model components and the dependent variables are provided.
Note: P=0.05.

(a)

Dependent Variable: Incidence of Nectria species			
ANOVA Pr > F	0.0001		
Model R-square	0.0680		
Model Components:	Pr > F	**Correlation Coefficient**	**Correlation P Value**
Crown class	0.0032	0.12	0.018
Slope	0.0001	0.14	0.004
Number of beech stems	0.0018	-0.12	0.020

(b)

Dependent Variable: Overall Abundance for Nectria species			
ANOVA Pr > F	0.0001		
Model R-square	0.0798		
Model Components:	Pr > F	**Correlation Coefficient**	**Correlation P Value**
Crown class	0.0043	0.11	0.027
Slope	0.0001	0.15	0.002
Number of beech stems	0.0001	-0.14	0.004

A significant portion of the variability in mortality due to disease was explained by a linear model consisting of crown class, elevation, total evergreen basal area, and number of beech stems. Probability and R-square values for the model from analysis of variance, and probability values for linear model components are given in Table 4-3, along with correlation coefficients and probabilities for each of the components. Again, neither fractional interception nor canopy cover accounted for significant portions of variation in this variable, so data from all ten plots was used to develop the model.

Tree size (DBH) had no effect on incidence of *Nectria* spp.. *Nectria* spp. were found on 213 trees (n=453) during the period of spring 1994 to spring 1997. These trees averaged 13.2 cm in diameter, only slightly less than the 13.4 cm mean diameter for trees with no incidence of *Nectria* spp..

Tree size did have an effect on mortality due to disease. A total of 83 beech trees (n=453) have died and had some incidence of *Nectria* spp.. Those trees averaged 10.2 cm, significantly less than the 14.0 cm for trees that remained alive or had died in the absence of *Nectria* spp..

iv. Discussion

With R-square values of 0.07 and 0.08 respectively, the models explaining variation in incidence and overall abundance of *Nectria* spp. are quite weak. Correlation coefficients for each of the model components,

Table 4-3. Linear model accounting for a significant portion of the variability in mortality due to disease.
Note: Statistics for correlation between the model components and the dependent variable are provided.
Note: P=0.05.

Dependent Variable: Mortality Due to Disease			
ANOVA Pr > F	0.0001		
Model R-square	0.3662		
Model Components:	Pr > F	**Correlation Coefficient**	**Correlation P Value**
Crown class	0.0027	-0.15	0.0025
Elevation	0.0001	0.36	0.0001
Number of beech stems	0.0137	0.58	0.0001
Total evergreen basal area	0.0001	-0.20	0.0001

though statistically significant, are only slight. The large sample size likely accounts for the statistical significance; however, two of the variables, number of beech stems and plot slope, were also found by Ehrlich (1934) to relate to variations in incidence of disease in Canada and the northeastern United States.

The positive correlation of incidence of *Nectria* spp. with slope in GSMNP matches the positive correlation that Ehrlich (1934) found, but the negative correlation of incidence with number of beech stems in GSMNP is just the opposite of the positive correlation that existed in Canada and the northeastern United States. This opposite response could possibly be explained by the high mortality and subsequent low current incidence of *Nectria* spp. in plot C, which has the greatest number of tagged beech trees (77) of all the plots. Such a large number of trees no longer suitable for infection by *Nectria* spp. could certainly skew the results of the analysis. It seems advisable to disregard the role that number of stems plays in disease incidence on the basis of these results.

The positive correlations between incidence and abundance of *Nectria* spp. and crown class of beech trees parallel the findings from the northeastern United States (Ehrlich 1934, Twery and Patterson 1983). The current findings indicate that dominant and codominant trees may be at a slightly higher risk of becoming infected with BBD. This intuitively seems contrary to the findings of the analysis of tree size and disease variables,

where DBH of infected trees did not differ significantly from that of uninfected trees. It must be emphasized, however, that no correlation between crown class and DBH has been established for trees in the permanent study plots.

The R-square of the model relating to variations in mortality due to disease (=0.37) is relatively more substantial. The negative correlation between tree crown class and disease mortality, however, is opposite of the relationship established by Ehrlich (1934) and later by Mize and Lea (1979). This negative correlation also appears to be in opposition to the earlier conclusion of this analysis that dominant and codominant trees may be at a higher risk of becoming infected by *Nectria* spp.. It may be that dominant/codominant beech trees have a higher risk of becoming infected but are also more likely to survive than intermediate and suppressed trees in GSMNP. The significantly smaller DBH of trees that have died due to disease may lend some support to this idea. An alternative and more likely hypothesis is that the sample size was insufficient to provide an effective account of the variation in mortality among beech crown classes. While the number of trees examined for this statistic was relatively large (n=421), the data set of Ehrlich (1934) included observations of 4,483 beech trees.

The negative correlation of total evergreen basal area in plots to disease mortality is also the opposite of what might have been expected based on the earlier works of Twery and Patterson (1983). This finding is

most certainly a result of the small number of plots sampled in the present study. Sufficient variability in evergreen basal area may not have been included in this study and thus conclusions concerning the effect of plot evergreens on disease mortality in GSMNP may not be reliable.

Elevation and number of beech stems in the plots were both strongly positively correlated with disease mortality. Again, due to the small number of elevation observations, it is uncertain whether sufficient variability in elevation was included in this study. However, the variability of elevation alone accounts for nearly 13% of the variation in disease mortality. In this model, number of beech stems in the plot accounts for almost 34% of the variability in mortality due to disease. Additionally, these data indicate that as the number of beech stems in a plot increases, the mortality due to disease increases. Again, this variable represents a sample size of just ten plots, and this analysis of the results should be viewed conservatively until they can be verified by a study with a larger number of plots.

The inconsistencies between the model relating site and stand factors with disease mortality and previous BBD research may indicate that the disease is governed by different factors in the southeastern United States than in the northeastern United States. It is much more likely that sufficient variation in the independent variables was not represented by the relatively low number of plots. The low model R-square values and low correlation coefficients indicate that the current study falls substantially

short of explaining the site variations in disease incidence and abundance of fruiting structures. However, the fact that the relationships of slope and cover class to incidence of disease concur with earlier findings may indicate that these factors are playing some role in the epidemiology of the disease in GSMNP. In addition, Ehrlich (1934) himself felt that in the face of so many known and unknown factors likely affecting the local variations in BBD, even a "small definite tendency" for incidence to vary with one of them should be considered.

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