EFFECTS OF FUEL REDUCTION TREATMENTS ON SPECIES OF *PHYTOPHTHORA* AND *LEPTOGRAPHIUM* IN FOREST ECOSYSTEMS

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ABSTRACT

The objective of the National Fire and Fire Surrogate Study was to investigate the effects of four fuel reduction treatments (prescribed burning, mechanical fuel reduction, mechanical followed by prescribed burning, and a non-treated control) on a number of ecosystem variables (e.g., soils, vegetation, insects, wildlife, fuels, fire behavior, economics, and tree pathogens) in multiple forest ecosystems across the nation. As part of the national study, the effects of fuel reduction treatments on the incidence levels of two forest tree pathogens, species of *Leptographium* and *Phytophthora*, were investigated over a six-year period. In the Clemson Experimental Forest, near Clemson, South Carolina, the incidence of *Leptographium* spp. in roots of southern pine trees initially was lower after fuel reduction treatments were applied; however, over time (i.e., five years after the initial treatment application), incidence levels were similar to pre-treatment levels, which suggests that these treatments had no long-term effect. *L. procerum* and *L. terebrantis* were found most frequently in roots of southern pine trees, but several other species also were found throughout the study site. Therefore, species of *Leptographium* appear to be a normal component of southern pine forests. In the Green River Game Land Management Area in western North Carolina, fuel reduction treatments did not affect the incidence of *Phytophthora* spp. in soil over the six-year period of this study. Incidence levels of *Phytophthora* spp. in soil samples were similar before treatments were applied, immediately after treatment application, and then three years later. *P. cinnamomi* and *P. heveae* were the only two species recovered; *P. cinnamomi* was found in all treatment plots and *P. heveae* was found in only three of the twelve plots. This study established the

widespread distribution of *Phytophthora* spp. in forest soil in the Green River Game Land Management Area.

To more fully understand the direct effect of prescribed fire on species of *Phytophthora* in soil in forests of the southern Appalachian Mountains, the persistence of *P. cinnamomi* in soil after three low-intensity prescribed fires was investigated. Although persistence of *P. cinnamomi* was significantly reduced at 2 cm beneath the soil surface after one of the three fires, overall, soil temperatures were not elevated for long enough to significantly affect populations of this soilborne plant pathogen either at 2 or 10 cm beneath the soil surface—depths at which *P. cinnamomi* routinely has been detected. Therefore, prescribed fire as a management tool does not appear to be adequate to eliminate *P. cinnamomi* from forest soil.

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CHAPTER ONE

LITERATURE REVIEW AND INTRODUCTION

Role of Species of *Leptographium* in Conifer Forests

Leptographium species, commonly known as blue-stain fungi, have been associated with root diseases of conifers and other trees in several parts of the world (Eckhardt 2003; Hill et al. 2003; Jacobs and Wingfield 2001; Smith 1967; Wingfield and Knox-Davies 1980; Wingfield and Marasas 1983). Over 50 species of *Leptographium* have been described, and these fungi most often are found in roots and boles of conifers and other forest trees (Jacobs and Wingfield 2001). The taxonomic classification of these Ascomycetes has undergone several changes since they first were described in 1851 as species of *Scopularia*. Debate still surrounds the taxonomy of *Leptographium* and closely related genera such as *Pesotum* and *Graphium* (Zipfel et al. 2006). Traditionally, taxonomic relationships were based on morphological characteristics and host relationships, which placed species of *Leptographium* as anamorphs of species of *Ophiostoma* and *Ceratocystis*; however, recent DNA sequence comparisons have shown species of *Leptographium* to be anamorphs of the genus *Grosmannia*, wood-inhabiting fungi closely related to *Ophiostoma* and *Ceratocystis* (Zipfel et al. 2006).

The biology of species of *Leptographium* is complex and important in investigating the role of these fungi in conifer forests. Transmission of *Leptographium* spp. can occur through root to root contact (Landis and Helburg, 1976) and by fungus

growth in soil (Hicks et al. 1980; Lewis et al. 1987; Swai and Hindal 1981); however, long-distance dispersal via bark-infesting insects probably is the most common means of dispersal (Barras and Perry 1971; Goheen and Cobb 1980; Highley and Tatter 1985; Klepzig et al. 1991; Lewis and Alexander 1986; Nevill and Alexander 1992; Otrosina et al. 1997; Wingfield 1983a; Witcosky and Hansen 1985; Witcosky et al. 1986). Although some bark beetles are able to attack apparently healthy trees (Demars and Roettgering 1982), many bark beetles generally are attracted to stressed or dying trees (Amman et al. 1990; Connor and Wilkinson 1983; Kegley et al. 1997). During feeding, the beetles deposit fungus spores in the wood, and, as the fungus grows and colonizes the wood, it produces conidiophores, stalks bearing masses of sticky conidia (Eckhardt 2003; Witcosky et al. 1986; Witcosky and Hansen 1985). As beetles emerge from galleries, spores adhere to the beetles and can be transmitted to other susceptible hosts. Typically, these dematiacious fungi colonize rays or vascular tissue and produce a dark stain in the wood. Infection by root-colonizing fungi such as *Leptographium* spp. predisposes a tree to further attack by bark beetles and root-feeding insects (Eckhardt 2003; Goheen and Cobb 1980; Witcosky and Hansen 1985); although, other abiotic stresses (e.g. drought) also may predispose trees to attack by these insect vectors (Eckhardt et al. 2007).

Attention was drawn to the genus of *Leptographium* in the 1970s when *L. wageneri* was found to cause black-stain root disease (Goheen and Hansen 1978; Landis and Helburg 1976; Smith 1967). This fungus has caused considerable damage to forests in the western United States—affecting mature Douglas-fir (*Pseudotsuga menziesii*), ponderosa pine (*P. ponderosa*), pinyon pine (*P. edulis*), and several other conifer species.

L. wageneri colonizes the vascular system resulting in dark-stained wood, reduced tree height, needle chlorosis, crown thinning, and eventually tree death (Wagener and Mielke 1961). Occurrence of this disease has been associated with site disturbance, such as when roads were built for thinning operations, as well as in high-use recreation areas and timber-producing areas (Goheen and Hansen 1978; Hansen 1978; Hessburg et al. 1995; Hessburg et al. 2001). *L. wageneri* appears to be native to western North America and has not been found outside of the natural range of its hosts (Harrington and Cobb 1988; Hessburg et al. 1995; Jacobs and Wingfield 2001; Walters and Walters 1977).

Other species of *Leptographium* have been associated with various declines of pine trees in North America. The presence of *L. procerum* has been associated with white pine root decline and red pine decline in the eastern and midwestern United States (Dochinger 1967; Horner et al. 1987; Klepzig et al. 1991; Towers 1977). The potential of *L. procerum* to cause mortality in pine trees is debatable; some studies have shown *L. procerum* to cause symptoms and mortality in eastern white pine seedlings (*P. strobus*), eastern white pine Christmas tree plantations in Virginia, and other species of pine (Lackner and Alexander 1983; Lackner and Alexander 1984; Nevill and Alexander 1992). Others believe this fungus has little capacity to incite significant disease in eastern white pine (Wingfield 1983a; Wingfield 1983b; Wingfield 1986) and other pine species (Eckhardt et al. 2004; Nevill et al. 1995; Rane and Tatter 1987). Symptoms of white pine root decline include reduced shoot elongation, needle retention and wilting, and resin-soaked wood at the base of the tree (Jacobs and Wingfield 2001). Symptoms of white pine root decline have been found on sites where *L. procerum* has not been detected,

suggesting site factors such as high soil moisture also may result in symptom expression (Jacobs and Wingfield 2001). Weevils and bark beetles play a role in tree stress and transmission of *L. procerum* (Eckhardt et al. 2007; Nevill and Alexander 1992; Lewis and Alexander 1986; Klepzig et al. 1991; Wingfield 1983a), but spores of this fungus also can persist in soil and may serve as a source of primary inoculum (Swai and Hindal 1981). *L. procerum*, by itself, may be a weak pathogen of pine trees, but this fungus appears to be one of several factors in a complex that leads to pine decline.

In the southeastern United States, *L. terebrantis* is believed to play a role, among other factors, in loblolly pine decline (Eckhardt 2003; Eckhardt et al. 2004; Hess et al. 2000; Nevill et al. 1995; Otrosina et al. 1997) and also has been associated with longleaf pine decline (Otrosina et al. 1999). Loblolly pine decline affects over 400,000 ha of forest in the southeastern United States. Symptoms include sparse crowns, needle chlorosis, and reduced radial growth of the trunk (Hess et al. 2000). *L. terebrantis* first was described in association with the black turpentine beetle, *Dendroctonus terebrans*, attacking loblolly pine (*P. taeda*) (Barras and Perry 1971). Since then, several other insects have been associated with transmission of this fungus (Eckhardt 2003; Otrosina et al. 1997). In pathogenicity studies, *L. terebrantis* appears to be more pathogenic than *L. procerum* on southern pines (Eckhardt et al. 2004; Nevill et al. 1995; Wingfield 1986), but there have been no reports of this organism being a primary pathogen in nature.

Other species of *Leptographium* have been found in forests of the southeastern United States—*L. huntii*, *L. serpens*, and *L. truncatum*. *L. huntii* has been found in pine forests in Arizona, New York, and Alabama—suggesting a wide distribution of this

species (Davidson and Robinson-Jeffrey 1965; Eckhardt, *personal communication*); however, the potential of this fungus to cause disease in pine trees has not been investigated. *L. serpens* and *L. truncatum* recently have been found in pine forests of the southeastern United States. In pathogenicity studies, these species are as aggressive as *L. terebrantis* on loblolly pine and have been associated with decline of this tree species (Eckhardt 2003; Eckhardt et al. 2004). *L. serpens* has been reported to cause disease on *P. pinaster* and *P. radiata* in South Africa (Wingfield and Knox-Davies 1980). *L. truncatum* has been isolated from diseased roots of *P. strobus* in South Africa and New Zealand (Wingfield and Marasas 1983). The role of these fungi in pine forests of the southeastern United States is unclear and warrants further investigation.

Role of Species of *Phytophthora* in Forests

The biology of *Phytophthora* spp. is what makes these fungus-like organisms successful plant pathogens: they can survive long periods of unfavorable conditions, produce an abundance of spores when adequate environmental conditions are present, and can be disseminated through water, soil, and plant material (Ribeiro 1983; Weste 1983). Long-term survival is achieved through oospores and chlamydospores (Weste 1983). These structures can survive long periods of unfavorable conditions because they have a thick cell wall and are resistant to dessication. Oospores are sexual structures formed from the union of an antheridium and oogonium and readily are formed by homothallic species—i.e., ones that can reproduce sexually from a single isolate (Erwin and Ribeiro, 1996). Chlamydospores are asexual spores that can survive extended periods

of unfavorable conditions in some homothallic species and in many heterothallic species (Hwang and Ko, 1978; Kassaby et al. 1977; Erwin and Riberio 1996)—i.e., ones that require two isolates of opposite mating types for sexual reproduction to occur. Sporangia, sac-like structures bearing several to many biflagellate zoospores, are produced under moist conditions (Gisi 1983). Upon release from the sporangium, zoospores are able to swim in water allowing these organisms to utilize waterways as a means of dissemination.

The classification of the genus *Phytophthora* has undergone major changes in recent years (Cooke et al. 2000; Dick 2001). These organisms traditionally have been classified in the Kingdom Fungi because they have features similar to true fungi (e.g., vegetative growth as hyphae (mycelium, collectively) and reproduction through spores) (Alexopoulos et al. 1996). However, they also differ in several ways from true fungi (e.g., asexual reproduction, spore structure, nuclear state of thallus, sexual reproduction, cell wall components, and other biochemical and molecular characteristics) and now are considered to be more closely related to brown algae (Dick 2001). Currently, the classification of the genus *Phytophthora,* according to the CABI Bioscience Database (www.indexfungorum.org), is:

- Kingdom Chromista
- Phylum Oomycota
- Class Oomycetes
- Order Pythiales
- Family Pythiaceae

- Genus *Phytophthora*

Early history of *Phytophthora* spp. was summarized by Bourke (1991) in a symposium volume honoring the centenary of the death of Miles J. Berkeley, an early pioneer in plant pathology. *Phytophthora* spp. first gained recognition when late blight of potato appeared in Europe in 1845. Much debate surrounded the cause of the blight, yet, Berkeley is acknowledged as the first to correctly determine the causal agent to be a fungus. It was not until 1861 to 1863 that de Bary published the life cycle of *Botrytis infestans*, which he later renamed *Phytophthora infestans* in 1876. Since that time, many species of *Phytophthora* have been described and are known for the devastation they can cause in many agriculture production systems—e.g. field and forage, greenhouse and nursery, vegetable and fruit, and nut crops (Erwin and Ribeiro 1996). Furthermore, these straminipilous fungi have had devastating ecological effects in forest ecosystems around the world (Brasier 1999; Crandall et al. 1945; Hansen 1999; Hansen et al. 2000; Hunt 1959; Weste and Taylor 1971).

Phytophthora species have played a role in shaping forested landscapes in Europe. Since the first half of the 1900s, European chestnut (*Castanea sativa*) has suffered severe mortality from root and collar rots caused by *P. cinnamomi* and *P. cambivora*, two introduced species (Brasier 1999). Over 10,000 riparian alder trees (*Alnus* spp.) have succumbed to infection by a new species of *Phytophthora*, *P. alni*, which apparently arose from the hybridization of two other species of *Phytophthora*, probably *P. cambivora* and an unknown species closely related to *P. fragariae* (Brasier et al. 1999; Brasier et al. 2004). Several other species of *Phytophthora* have been associated

with tree decline. European beech (*Fagus sylvatica*) has been reported as declining from several species of *Phytophthora* in both Europe and northeastern North America (Jung et al. 2005). Association between oak decline and the presence of *Phytophthora* spp. in soil and roots also has been reported from several parts of Europe (Balci and Halmschlager 2003a; Balci and Halmschlager 2003b; Brasier 1999; Gallego et al. 1999; Jung et al. 2000; Moreira and Martins 2005). Specifically, *P. cactorum, P. cambivora, P. citricola, P. cinnamomi, P. syringae*, and *P. gonapodyides* have been associated with one or more species of declining oaks. Interestingly, the same suite of species of *Phytophthora* may be found in sites with no apparent disease (Hansen and Delatour 1999), which suggests that other factors may be involved in these decline syndromes.

The potential of *Phytophthora* spp. to have a devastating impact on a forest ecosystem is most evident in the eucalyptus or jarrah forests of Western Australia. The disease, commonly known as jarrah dieback, is caused by *P. cinnamomi* and was identified as the causal agent in 1968 (Podger 1972). Since that time, the pathogen has spread rapidly throughout the region leaving dead eucalyptus (*E. marginata*) and other native plants (e.g., *Banksia* spp. and *Xanthorrhoea australis*) in its wake (Weste and Ashton 1994; Weste and Taylor 1971). Changes in plant communities have occurred since this pathogen was introduced; the dominant tree species, *Eucalyptus marginata*, and the mid-story species, *Banksia grandis*, have been removed or thinned and other species such as sedges and legumes have established or increased in ground cover in their absence (Weste and Law 1973; Weste et al. 1973; Weste and Ashton 1994). *P.*

cinnamomi continues to be a threat to native plant communities in Australia, particularly those that include rare and endangered species (Grant and Barrett 2001).

In the western United States, two species of *Phytophthora*, *P. lateralis* and *P. ramorum,* have been introduced and caused extensive mortality in some tree species. *P. lateralis* was introduced in the 1950s to forests of southwestern Oregon where it has caused extensive mortality of the economically valuable Port-Orford-cedar (*Chamaecyparis lawsoniana*) (Hansen et al. 2000; Roth et al. 1957; Tucker and Milbrath 1942). The only known hosts of this pathogen are Port-Orford-cedar and the less susceptible Pacific yew (*Taxus brevifolia*) (Murray and Hansen 1997). Spread of *P. lateralis* is associated with roadways, where transmission of infested soil to non-infested areas can occur, and the pathogen then can enter and be spread along waterways to infest entire drainages (Hansen et al. 2000). Today, management of this pathogen involves preventing its spread into mature, non-infected stands and breeding for resistance to *P. lateralis* (Hansen et al. 2000; Oh et al. 2006).

In contrast to the narrow host range of *P. lateralis*, the introduction of *P. ramorum* to coastal forests of California and southwestern Oregon has caused lethal girdling cankers on a number of native oaks, including tanoak (Goheen et al. 2002; Rizzo et al. 2002). The disease is known commonly as sudden oak death. This pathogen has a broad host range that includes over 45 plant species (http://www.aphis.usda.gov/plant_health/plant_pest_info/pram/index.shtml). *P. ramorum* also causes ramorum blight, which affects many understory plants in the forest and nursery crops, such as species of *Camellia*, *Rhododendron*, and *Viburnum* (Davidson et

al. 2003; Hansen et al. 2005; Tooley et al. 2004). Currently, this pathogen only has been found in forests of coastal California and southwestern Oregon; however, because of the broad host range of *P. ramorum* and its association with nursery crops, many natural ecosystems are at risk—including forests in the eastern United States (Tooley and Kyde 2007).

The American chestnut (*Castanea dentata*) once was a dominant tree species in many forests of the eastern United States. However, the introduction of *P. cinnamomi* in the 1800s resulted in the near loss of this tree species from forests of the southern Appalachian Mountains (Crandall et al. 1945). Reports of dying *Castanea* spp. date to 1824, but *P. cinnamomi* was not described as the causal agent until 1945. Since that time, American chestnuts and chinkapins have receded from forests of the southeastern United States—even before the chestnut blight fungus, *Cryphonectria parasitica*, was introduced. *Castanea* spp. still can be found in forests of the region; however, they no longer dominate the canopy and seedlings and juvenile trees remain susceptible. Efforts are underway to breed a hybrid chestnut that will be resistant to both blight and root rot (Jeffers et al. 2007).

P. cinnamomi has spread throughout the southeastern and mid-Atlantic regions of the United States (Balci et al. 2007; Campbell 1951; Campbell et al. 1963; Roth 1954; Wood et al. 2001). It is known to cause disease on over 900 hosts, many of them present in forests of the southern Appalachian Mountains (Zentmeyer 1980). Yet, there is little evidence that this organism is causing disease in hardwood forests of this region. A similar situation has been reported from dieback sites in Australia (Old and Dudzinsky

1999) and in oak decline sites in Europe (Hansen and Delatour 1999) where species of *Phytophthora* and known hosts are present and coexisting, without disease being observed. This phenomenon suggests that other factors may be involved in tree declines associated with *Phytophthora* spp.

P. heveae also has been found, consistently but infrequently, in hardwood forest soils and streams of the southern Appalachian Mountains (Campbell and Gallegly 1965; Hwang et al. 2007; Wood et al. 2001; Zwart 2004). This oomycete has been found causing diseases on a range of hosts in other parts of the world—on rubber in Malaysia (Erwin and Ribiero 1996), Brazil nut in Brazil, kauri in New Zealand, avocado in California and Central America (Zentmeyer et al. 1976), and rhododendron in North Carolina (Benson and Jones 1980). In pathogenicity studies on hosts native to the southern Appalachian Mountains, Zwart (2004) reported *P. heveae* to be a weak pathogen of *Rhododendron maximum* and *Kalmia latifolia*—only attacking leaves that had been wounded. The forests of the southeastern United States contain a diversity of trees, shrubs, and herbaceous plants that are hosts or potential hosts to species of *Phytophthora* and, yet, our knowledge on the role of these cosmopolitan organisms in forests of the southern Appalachian Mountains is limited.

Role of Fire in Forests

Fire has played an important role in shaping many North American forests (Kozlowski and Ahlgren 1974; Vale 2002). Before human settlement, lightning was the most common source of ignition (Komarek 1964) and is believed to have played a role in

shaping some fire-adapted plant communities (Allen 2002; Komarek 1964; Weaver 1974). Such communities exist in several different ecosystems—such as conifer forests in Alaska, ponderosa pine forests in Arizona, and southern pine forests in Florida. However, many fire-adapted plant communities appear to have resulted mostly from human influences (Kozlowski and Ahlgren 1974; Vale 2002). Tallgrass prairies of the midwestern states were burned regularly by Native Americans, primarily to attract wildlife for hunting (Vogl 1974). Chaparral in California also has been shaped by fire; although, there has been debate over the role humans played in shaping this plant community (Bendix 2002; Biswell 1974; Keeley 2002; Riggan et al. 1988). In the southern Appalachian Mountains, Native Americans used fire to clear the understory vegetation for easier travel and hunting, reduce the potential of wildfire outbreak, and make gathering of acorns and chestnuts easier (Delcourt and Delcourt 1997; Van Lear and Waldrop 1989).

Since European settlement in North America, vegetation patterns in fire-adapted plant communities have changed as a result of fire suppression (Baker 1994; Barrett 1988; Bonnicksen and Stone 1982; Christensen 1977; Lorimer 1984; Schuler and Gillespie 2000) and the introduction of the chestnut blight fungus (Chapman et al. 2006). In the early 1900s, several severe and fatal wildfires in the western United States prompted the federal government to initiate fire suppression policies throughout forests of the nation (Pyne 2004). At the time, the Forest Service was only a few years old; information on forest management was learned and implemented as the organization developed. Fire suppression policies persisted throughout the past century despite the

efforts of some early fire ecologists who felt that fire exclusion was deleterious in fire-adapted communities (Pyne 2004). As a result, many fire-adapted forests have accumulated unprecedented amounts of fuels, which provide ample opportunity for wildfire outbreak (Barrett 1988; Kilgore and Taylor 1979; Vankat 1977). In addition, some fire-adapted tree species are not regenerating and are being replaced by fire-sensitive species, changing forest structure and composition (Abrams and Nowacki 1992; Lorimer 1984; Schuler and Gillespie 2000; Williams 1998).

Fire has been an important disturbance in shaping forests of the southeastern United States (Abrams 1992; Delcourt and Delcourt 1997; Harmon 1982; Van Lear and Waldrop 1989; Waldrop et al. 1987; Williams 1998). In the Coastal Plain and Piedmont regions of the Carolinas, pine forests—including longleaf (*P. palustris*), slash (*P. elliotii*), and loblolly (*P. taeda*) pine forests—have adapted to fire (Komarek 1974; Pyne 1982; Waldrop et al. 1987). Humans, both Native Americans and European settlers, frequently used fire in these forests to reduce the hardwood understory creating an almost park-like forest setting, which was ideal for travel and hunting. Continued use of fire in these forests is important because regeneration is dependant on disturbance, such as fire and windthrown trees, to provide an appropriate seedbed for seed germination.

Fire also has played a role in shaping mixed pine-oak forests of the southern Appalachian Mountains (Abrams 1992; Barden and Woods 1976; Delcourt and Delcourt 1997; Harmon 1982; Van Lear and Waldrop 1989). Oaks are a dominant tree species in many forests of the southern Appalachian Mountains and have been associated with recurring fire. Several adaptations allow them to survive and regenerate after fire (e.g.,

extensive root system, thick bark, and ability of acorns to sprout on bare mineral soil) (Abrams 1992). Ridgetops in mixed pine-oak forests typically are dominated by several different species of pine such as Table Mountain pine (*P. pungens*) and pitch pine (*P. rigida*), which also require recurring fire for regeneration. Evidence from fire-scars and charcoal and pollen deposits in mountain lakes indicate a shift toward more fire-tolerant species over the last 4000 years (Delcourt and Delcourt 1997; Delcourt et al. 1998; Harmon 1982). However, fire suppression policies initiated at the national level in the early 1900s have resulted in changes to the forest ecosystem (Abrams 1992; Harmon 1982; Lorimer 1984; Williams 1998). The lack of regeneration in oak and pine forests in the southern Appalachian Mountains has instigated more research on the importance of fire in these forests (Barden and Woods 1976; Delcourt and Delcourt 1997; Phillips et al. 2007; Williams 1998).

Today, prescribed fire has become a common forest management practice in forests of the southeastern United States. Haines et al. (2001) reported more than 4.1 million acres of pine forest were prescription burned from 1985 to 1994 in the southern United States. Benefits of prescribed burning in pine forests of the coastal plain and in hardwood forests in the southern Appalachian Mountains include: fuel reduction and reduced risk of wildfire outbreak, forest regeneration, increased plant diversity, and reduction or removal of undesirable tree species (Barden and Woods 1976; Elliot et al. 1999; Phelps et al. 1978; Waldrop et al. 1987).

Fire and Fire Surrogate Study

In an attempt to restore forest ecosystems and to reduce the accumulation of fuels, and, therefore, the risk of wildfire outbreak, forest managers have used thinning and prescribed burning. However, there has been a lack of information to suggest that these practices have similar effects on the ecosystem. Knowledge about these forest management practices is critical and timely as our forests are changing as a result of human practices. In response, the Joint Fire Science Program, a partnership between the United States Department of Agriculture and the United States Department of the Interior, initiated the Fire and Fire Surrogate Study in 2000 at 13 sites located across the country (Weatherspoon and McIver 2000). This long-term study investigates the effects of fuel reduction treatments (i.e., thinning, burning, and their combined effect) on a number of ecosystem variables—including wildlife, soils, insects, fuels, fire behavior, silvicultural economics, and tree pathogens. Eight of these study sites are located in pine forests of the western United States, and five sites are located in the eastern United States—two of which are near Clemson University. The Southern Appalachian study site is one of two sites in hardwood forests, and the Southeastern Piedmont study site is located in a southern pine forest. Researchers from Clemson University have been involved in a number of projects sponsored by the Fire and Fire Surrogate Study, including this one.

Research Objectives

The overall goal of this project was to determine the initial effects of fuel reduction treatments on two forest tree pathogens (*Leptographium* spp. and *Phytophthora* spp.) immediately after treatments were applied and several years thereafter. The initial phase of this study was conducted by Zwart (2004). The study reported here investigated the effects of fuel reduction treatments on these forest pathogens over time. There were three objectives in this study.

First, in the Southeastern Piedmont study site in the Clemson Experimental Forest, the incidence of *Leptographium* spp. initially was reduced after fuel reduction treatments (i.e., thinning, burning, and thinning followed by burning) were applied (Zwart 2004). However, during the investigation, some study plots were compromised by attacked from southern pine beetles, and, therefore, it is unclear whether the reduction in incidence of *Leptographium* spp. was due to the treatments or to beetle infestation. Therefore, the first objective of this study was to determine the effects of fuel reduction treatments on the incidence of *Leptographium* spp. in roots of southern pine trees over time (i.e., six years after initial treatment application).

In the southern Appalachian Mountain study site in western North Carolina, the initial phase of the study, after the first application of fuel reduction treatments, resulted in no change in the incidences of species of *Phytophthora* in forest soil. However, occurrence of *Phytophthora* spp. in sub-plots from before to after fuel reduction treatments were applied was not highly correlated—i.e, *Phytophthora* spp. were detected in different sub-plots before treatment application and after treatment application in all

treatment plots except the non-treated control plots. Zwart (2004) attributed this, in part, to equipment used for treatments which may have transferred soil infested with *Phytophthora* spp. between plots. Also, the initial treatment application, after over five years of fire suppression, may not have had much effect initially on *Phytophthora* spp. and, therefore, long-term effects need to be evaluated. The second objective of this study was to investigate the long-term effects of fuel reduction treatments on the incidence of *Phytophthora* spp. in a hardwood forest soil.

Several studies have attempted to use fire in an attempt to eradicate species of *Phytophthora* from natural ecosystems; however, in each of these studies, the organism still could be recovered after fire (Betlejewski 2007; Hansen and Sutton 2005; Marks et al. 1975). In the southern Appalachian Mountains, *P. cinnamomi* continues to pose a threat to native vegetation, so, with the increased use of prescribed fire in these forests, it is important to understand how fire may affect survival of *Phytophthora* spp. Therfore, the third objective of this study was to determine the direct effect of prescribed fire on survival of *P. cinnamomi* in a forest soil.

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CHAPTER TWO

EFFECTS OF FUEL REDUCTION TREATMENTS ON THE INCIDENCE OF *LEPTOGRAPHIUM* SPECIES IN A PINE FOREST OF THE SOUTH CAROLINA PIEDMONT

Introduction

Fire as a disturbance plays a role in shaping forest communities (Barbour et al. 1999). Many forests have adapted to fire, whether caused naturally by lightning (Allen 2002; Komarek 1964) or started intentionally by Native Americans (Delcourt and Delcourt 1997; Keeley 2002; Vale 2002; Van Lear and Waldrop 1989). However, in the early 1900s, fire suppression policies were administered in an attempt to protect the forests and the people that manage them (Pyne 1982). As a result, fuels have accumulated in fire-adapted forests, which have increased the risk of wildfire outbreaks (Barrett 1988; Dodge 1972; Keifer et al. 2006; Kilgore and Taylor 1979). Forest managers have attempted to reduce excessive accumulation of fuels by thinning and prescribed burning; however, there is a lack of data on the effects of these practices on many components of forest ecosystems. To address this issue, the Joint Fire Science Program, a partnership between the United States Department of Agriculture and the United States Department of the Interior, initiated the national Fire and Fire Surrogate Study in 2000 at 13 sites located across the country. This study addresses the long-term effects of fuel reduction treatments (i.e., thinning, prescribed burning, and a combination of thinning and burning)

on a number of ecosystem variables—e.g., wildlife, insects, vegetation, economics, fuels, fire behavior, soils, and tree pathogens.

One of the national study sites is located in the Clemson Experimental Forest near Clemson, South Carolina. This site is composed primarily of loblolly pine (*Pinus taeda*) but also has shortleaf pine (*P. echinata*) and Virginia pine (*P. virginiana*). If weakened or stressed, each of these pines is susceptible to attack by bark beetles and root-feeding insects (Connor and Wilkinson 1998; Eckhardt et al. 2007). Some of these insects—such as species of *Hylastes*, *Hylobius,* and *Dendroctonus*—have been associated with the transmission of *Leptographium* spp., a genus of wood-staining fungi commonly found in roots and boles of pine trees (Barras and Perry 1971; Bramble and Holst 1940; Eckhardt et al. 2004b; Eckhardt et al. 2007; Lackner and Alexander 1983; Wingfield 1983a).

At least one species of *Leptographium* is known to cause considerable economic loss to forests. *L. wageneri*, the causal agent of black-stain root disease, infects Douglas-fir (*Pseudotsuga menziesii*), ponderosa pine (*Pinus ponderosa*), and several other pines in the western United States (Goheen and Hansen 1978; Harrington and Cobb 1983; Landis and Helburg 1976; Smith 1967). Colonization by this fungus results in reduced tree growth, dark-stained wood, and eventually tree death (Hessburg et al. 1995). Occurrence of this disease has been associated with site disturbance, such as when roads were built for thinning operations (Goheen and Hansen 1978; Hansen 1978; Hessburg et al. 2001). This pathogen has not been found outside its natural range of hosts (Harrington and Cobb 1988; Jacobs and Wingfield 2001; Walters and Walters 1977).

In contrast to *L. wageneri*, the pathogenicities of other *Leptographium* spp. are not well understood and open to debate. *L. procerum* has been suggested to be the causal agent of white pine root decline (Dochinger 1967; Horner et al. 1987; Towers 1977) and red pine decline (Klepzig et al. 1991) in forests in the northern United States. *L. procerum* has been reported to be an aggressive pathogen on *Pinus strobus* (Lackner and Alexander 1982; Lackner and Alexander 1983; Nevill and Alexander 1992) but, in other studies, investigators reported this fungus to be a weak pathogen associated with wounds—unable to produce significant lesions in white pine and other pines (Eckhardt et al. 2004a; Klepzig et al. 1991; Nevill et al. 1995; Rane and Tatter 1987; Wingfield 1983a; Wingfield 1983b; Wingfield 1986). Although *L. procerum* has been found frequently in pine forests of the southeastern United States (Eckhardt 2003; Nevill et al. 1995; Otrosina et al. 1997; Otrosina et al. 1999), the impact of this fungus in these forests is not clear.

In the southeastern United States, *L. terebrantis* may be a factor in a complex that leads to loblolly pine decline (Eckhardt 2003; Eckhardt et al. 2004b; Eckhardt et al. 2007; Hess et al. 2002) and also has been reported as a factor in longleaf pine decline (Otrosina et al. 1999). This species has caused significant lesion development during pathogenicity studies using loblolly pine (*P. taeda*), white pine (*P. strobus*), and longleaf pine (*P. palustris*) (Eckhardt et al. 2004a; Nevill et al. 1995; Otrosina et al. 2002; Wingfield 1983b), and infection has resulted in tree mortality in one study (Wingfield 1983b). Other species of *Leptographium*—e.g., *L. serpens*, *L. huntii*, and *L. truncatum*—also have been found in southern pine forests but little is known about the potential of these fungi to be pathogens of southern pine trees (Eckhardt 2003; Eckhardt et al. 2004b).

To date, the role *Leptographium* spp. play in southeastern pine forests is not well understood and needs investigation. Likewise, the effect of management practices on the incidences of these fungi in southern pine trees is not known. Therefore, the objective of this study was to investigate the effects of fuel reduction treatments on the incidence of *Leptographium* spp. in roots of southern pine trees five years after the initial treatments were applied. The initial part of this study, comparing incidence levels before and one year after the initial treatment application, was conducted and reported previously (Zwart 2004).

Materials and Methods

Study site. The Clemson Experimental Forest is located near Clemson, South Carolina (primarily within 34°45' to 34°34'N and 82°54' to 82°49'W) and encompasses over 7,000 ha. In the late 1800s and early 1900s, poor farming practices led to erosion, low soil fertility, and farm abandonment (Dunn and Holladay 1977). To prevent further land degradation, the federal government obtained this land in the 1930s and granted it to Clemson College (which later became Clemson University) for the purpose of research and education (Cox et al. 2007; Dunn and Holladay 1977). Today, much of this forest contains second- or third-growth southern pine stands with an understory of mixed hardwood trees (Waldrop 2001). Ultisols of the Cecil-Lloyd-Madison association are the dominant soil type in this forest with elevation ranging from 200 to 300 m above sea level (Waldrop 2001).

Experimental design. The experiment was established in 2000 as a randomized complete block design with tree size as the blocking factor: Block 1 was composed primarily of pulpwood-size trees (diameters at breast height [dbh]=15 to 25 cm); Block 2 was a mixture of pulpwood-size and saw-timber-size trees (dbh >25 cm); and Block 3 was primarily saw-timber-size trees. The treatment design was a 2 × 2 factorial; prescribed burning and thinning were the two factors with presence and absence of each factor as the two levels. Therefore, four treatments were used: prescribed burning (Burn), thinning (Thin), thinning followed by prescribed burning (Thin+burn), and a non-treated control (Control). One replicate of each treatment was assigned randomly to one of the four treatment plots in each block (Fig. 2.1); a total of 12 treatment plots were used in this study. Each treatment plot was approximately 14 ha. Up to 40 gridpoints were placed at 50-m intervals within the innermost 10 ha of each plot; the surrounding 4 ha served as a buffer zone. Ten 1000-m^2 sub-plots (20 m x 50 m) were established from every fourth gridpoint (Fig. 2.2).

Treatment application. Thinning operations were conducted by a commercial contractor between December 2000 and April 2001 in the Thin and Thin+burn plots. A feller buncher was used to remove primarily diseased or weak trees and small trees in the understory or mid-story were removed as needed to produce a basal area of approximately 18 m^2/ha. All cut shrubs and trees were left on-site.

Prescribed burning was conducted by an experienced team from the Clemson Experimental Forest and the USDA Forest Service, Southern Research Station. The goal for all prescription burns was to reduce fuels and eliminate most shrubs as well as some

trees in the mid-story. A combination of strip headfires and flanking fires were set for all burns; spot fires were used in areas where flanking and strip headfires did not burn.

Burn plots were burned one year after plots were established. Block 1 was burned on 10 April 2001; air temperature ranged from 22 to 30°C and relative humidity ranged from 42 to 51%. Wind speed ranged from 8 to 13 mph from the southwest. Block 2 was burned on 12 April 2001; air temperature ranged from 23 to 29°C and relative humidity ranged from 45 to 56%. Wind speed ranged from 8 to 16 mph from the south. Block 3 was burned on 11 April 2001; air temperature ranged from 24 to 29°C and relative humidity was 46%. Wind speed ranged from 6 to 14 mph from the south. Flame heights in all three blocks were generally 1 to 2 m. Some hot spots did occur where an accumulation of fuels from beetle-killed trees occurred and in areas where gullies created a chimney effect and carried the flames into the tree canopy.

After the first burn, extensive mortality due to a heavy infestation by southern pine beetles (*Dendroctonus frontalis*) was noticed in one Control plot and all three Burn plots. Consequently, these plots were replaced in late 2002 and early 2003 to sites with similar stand conditions within the Clemson Experimental Forest. Replaced Burn plots were burned in 2004. Block 1 was burned on 24 March 2004; air temperature at the start of the fire was 16°C and relative humidity ranged from 26 to 30%. Wind speed ranged from 7 to 10 mph from the southeast. Block 2 was burned on 10 March 2004; air temperature at the start of the fire was 12°C and relative humidity ranged from 33 to 37%. Wind speed ranged from 4 to 8 mph from the south. Block 3 was burned on 19 April 2001; air temperature ranged from 29 to 32°C and relative humidity ranged from 24

to 29%. Wind speed ranged from 0 to 5 mph from the southwest. Flame heights in all three blocks were generally less than 1 m.

Thin+burn plots were burned one year after the thinning operation, in March 2002—which allowed the cut shrubs and trees to partially desiccate and decompose prior to burning. Block 1 was burned on 7 March 2002; air temperature at the start of the fire was 20°C and relative humidity ranged from 22 to 45%. Wind speed ranged from 6 to 11 mph from the southwest. Block 2 was burned on 25 March 2002; air temperature at the start of the fire was 18°C and relative humidity ranged from 41 to 42%. Wind speed ranged from 6 to 11 mph from the southwest. Block 3 was burned on 28 March 2002; air temperature at the start of the fire was 18°C and relative humidity was 56%. Wind speed ranged from 6 to 11 mph from the southeast. Flame heights in all three blocks were generally less than 1 m.

Thin+burn plots were burned for a second time three years later, in March through May 2005. Block 1 was burned on 6 April 2005; air temperature ranged from 23 to 24°C and relative humidity ranged from 46 to 47%. Wind speed ranged from 5 to 10 mph from the southwest. Block 2 was burned on 10 March 2005; air temperature ranged from 10 to 13°C and relative humidity ranged from 40 to 42%. Wind speed ranged from 3 to 11 mph from the southwest. Block 3 was burned on 4 May 2005; air temperature ranged from 18 to 23°C and relative humidity ranged from 38 to 49%. Wind speed ranged from 5 to 8 mph from the northeast. Flame heights in all three blocks were generally less than 1 m.

Sampling procedure. To determine the effect of fuel reduction treatments on the incidence of *Leptographium* spp. in roots of southern pine trees, three live trees (dbh >15

cm) were sampled in each of the 10 sub-plots per treatment plot; where there were fewer than three live trees, zero, one, or two were sampled. In 2000, four trees were sampled in a few of the sub-plots. From each tree, two lateral roots (diameter >5 cm) on opposite sides of the tree were excavated up to 1 m from the tree base. From each root, three root cores (5 mm in diameter and approximately 30 mm in length) were collected using an increment hammer (Suunto USA, Inc., Ogden, UT). The hammer head was rinsed in 95% ethanol after each tree was sampled and allowed to air-dry. Samples were placed in plastic bags, transported back to the laboratory in a cool ice chest, and then kept at 4°C until processed.

All plots were sampled three times over a 6-yr period from 2000 to 2006. The first sample period occurred in fall 2000, before treatments were applied, to determine the baseline incidence of *Leptographium* spp. in treatment plots. The second sample period occurred in fall 2002, after plots were thinned and burned. The third sample period occurred in summer 2006, after Thin+burn plots were burned for a second time and the new Burn plots were burned for the first time. In 2000 and 2002, trees were sampled after beetles known to vector *Leptographium* spp., such as the southern pine beetle, had emerged to prevent attraction of beetles to trees wounded by sampling. Trees sampled in a previous sample period were avoided whenever possible to keep from using potentially compromised trees; however, some trees were sampled a second time in 2002 and 2006 if there were fewer than three live trees that had not been sampled previously.

Isolation of *Leptographium* spp. In the laboratory, each root core was sliced aseptically into 10 disks approximately 2 mm thick. Disks were placed on 1.25% malt

extract agar (MEA) amended with cycloheximide to encourage the growth of Ophiostomatoid fungi while inhibiting the growth of most other fungi and bacteria (Jacobs and Wingfield 2001); MEA contained per liter: 15 g of Bacto agar (Becton, Dickinson, and Company, Sparks, MD), 12.5 g of malt extract (Becton, Dickinson, and Company), and 200 mg of cycloheximide (Sigma-Aldrich, Inc., St. Louis, MO). Two isolation plates were used for each tree, and five disks from each root were placed on each plate. Plates were held at room temperature (22 to 25°C) for 3 weeks and observed regularly for the presence of dematiaceous fungi. Species of *Leptographium* were sub-cultured on MEA and representative isolates were retained for identification.

Identification of *Leptographium* spp. In 2000 and 2002, isolates were confirmed as species of *Leptographium*, but in 2006 isolates were identified to species. Isolates of *Leptographium* spp. were grown on 1.25% MEA in the dark at 25°C for 7 to 10 days to encourage production of conidiophores and conidia. Isolates were identified using morphological features (examined at 100 to 400×) such as the presence or absence of rhizoids, primary branching pattern of conidiophores, presence or absence of serpentine hyphae, presence or absence of aerial hyphae, and color of conidium droplets (Jacobs and Wingfield 2001). Identifications of representative isolates were confirmed by an expert (L.G. Eckhardt, School of Forestry and Wildlife Sciences, Auburn University, Auburn, AL). Representative isolates (Appendix 1) were stored on 2% malt extract agar in 8-ml glass vials at 4°C in the dark.

Data analysis. Proportions of pine trees and tree roots infected by *Leptographium* spp. were used to determine incidence levels in treatment plots in all analyses; however,

data are reported as percentages in tables and figures. There were inherent differences in incidence levels among the treatment plots prior to treatment application based on data collected in 2000; specifically, the incidence level was significantly lower in the Control plots than in the other treatment plots. To account for this difference in treatment comparisons, a linear contrast was used to estimate the initial difference and this estimate was used to adjust incidence levels in Control plots in all sample periods.

A model for the experimental design was created with factors for sample period (2000, 2002, and 2006), treatment plots (Control, Thin, Burn, and Thin+burn), and blocks (three). A two-way analysis of variance (ANOVA) was conducted to analyze the model factors (i.e. sample period and treatment plot) while adjusting for block effects. Fisher's protected least significant difference (LSD) was used to separate specific means of any model factor determined to be significant. Within each sample period, a one-way ANOVA also was used to identify differences among treatment plots, again, adjusting for block effects, and LSD was used to separate the treatment plot means. All analyses were conducted using the software program SAS, ver. 9.1 for Windows (SAS Institute Inc., Cary, NC) and statistical tests were performed with $\alpha = 0.05$.

Results

Incidence levels of *Leptographium* spp. in 2006. In 2006, 656 roots from 328 trees in the Clemson Experimental Forest were sampled from May through July. *Leptographium* spp. were isolated from a total of 79 trees (24%) and 96 roots (15%) (Tables 2.1, 2.2, and 2.3). Six species were recovered with *L. terebrantis* (20% of trees

and 12% of roots) and *L. procerum* (18% of trees and 13% of roots) isolated most frequently (Table 2.1). *L. huntii*, *L. serpens*, *L. truncatum*, and *Leptographium sp*., a species that has not been described yet (L.G. Eckhardt, *personal communication*), were recovered only occasionally—each from ≤5% of trees and ≤4% of roots (Table 2.1). Frequently, two or more species were isolated from the same tree and from the same root. Isolates of *Leptographium* spp. were found in all but one treatment plot (i.e., one Control plot in Block 1). Each of the species of *Leptographium* isolated was found in all three blocks except for *L. truncatum,* which was found only in Block 3, and *Leptographium* sp., which was found one time in each of Blocks 1 and 2 (Table 2.1). There were no differences in incidence levels of *Leptographium* spp. among the treatments in 2006 (Fig. 2.3). Mean percentages of infected trees and roots, respectively, were: Control, 28% and 27%; Burn, 36% and 27%; Thin, 20% and 16%; and Thin+burn, 25% and 16% (Tables 2.2, 2.3, and Fig. 2.3).

Changes in incidence levels over time. Other co-workers collected data on the incidence levels of *Leptographium* spp. in trees and roots in 2000 and 2002, before and immediately after treatments were applied the first time (Zwart 2004). These data are reported here so effects of treatments over time could be evaluated. However, because one Control plot and all three Burn plots were replaced in 2002, it is important to note that no pre-treatment data were collected on the new plots, and, therefore, we must use caution in determining if any changes occurred over time in these plots.

The numbers of trees (Table 2.2) and roots (Table 2.3) sampled and infected in individual treatment plots at different sample periods varied. Before treatment application

in 2000, 21 to 35 trees were sampled per plot (313 trees total) and incidence of *Leptographium* spp. ranged from 12 to 43% (25% overall) (Table 2.2). In 2002, after the first application of treatments and during a severe of southern pine beetle infestation, fewer trees were sampled: 11 to 30 trees were sampled per plot (255 trees total) and incidence ranged from 0 to 44% (9% overall) (Table 2.2). In 2006, six years after the first samples were collected, 18 to 30 trees were sampled per plot (328 trees total) and incidence ranged from 0 to 47% (24% overall) (Table 2.2). Incidence of *Leptographium* spp. in roots ranged from 6 to 29% in 2000 (17% overall), 0 to 34% in 2002 (6% overall), and 0 to 37% in 2006 (15% overall) (Table 2.3).

Results from the two-way ANOVA indicated the treatment plot × sample period interaction was not significant (Table 2.4); therefore, main effects of treatment plot and sample period could be examined. There were no treatment plot effects for the three sample periods combined; however, sample period was a significant factor in the incidence of *Leptographium* spp. in both trees and tree roots (P=0.0070 and P=0.0151, respectively). A pairwise comparison of sample periods for the treatments combined indicated a significant change in *Leptographium* incidence in both trees and roots from 2000 to 2002 and from 2002 to 2006; incidence was lower in 2002. However, there was no difference in incidence levels between 2000 and 2006 (Fig. 2.3). Results from one-way ANOVAs for each of the three sample periods indicated no block effect and no treatment plot effect except in 2002 (Table 2.4). Results of pairwise comparisons among all treatment plots in 2002 showed that incidence levels of *Leptographium* spp. in trees

and roots in the Control plots were significantly higher than those in the other three treatments (Fig. 2.3).

Discussion

Leptographium species appear to be widespread in the Clemson Experimental Forest and, probably, in other southern pine forests as well. Results from this study are consistent with those from other studies, which have reported a widespread distribution of these wood-staining fungi in pine forests elsewhere in the southeastern United States (Eckhardt et al. 2004b; Hess et al. 1999, Otrosina et al. 1999, Otrosina et al. 2002). For example, *L. terebrantis* and *L. procerum* were the two species most frequently encountered in trees in the Clemson Experimental Forest study site, and these two species also were predominant in trees in other forests studied in Alabama, South Carolina, and Florida (Eckhardt 2003; Otrosina et al. 1997, Otrosina et al. 1999, Otrosina et al. 2002). No species of *Leptographium* was found in this study that has not been found before in southern pine trees—including the one unknown species, which was collected previously by L.G. Eckhardt (*personal communication*).

There was an inherent difference in incidence levels between the Control plots and the other treatment plots before treatments were applied. It was assumed that this difference was similar throughout the six-year period of the study, so incidence levels in the Control plots were adjusted to account for this initial difference in all analyses. The incidence levels of *Leptographium* spp. in the Thin, Burn, and Thin+burn plots in 2002 were lower than those in 2000 and 2006. This may be due, in part, to the severe beetle infestation that occurred during the 2002 sample period. The presence of *Leptographium*

spp. in southern pine trees predisposes them to attack by southern pine beetles and other wood-inhabiting beetles—such as species of *Hylastes* (Eckhardt et al. 2004b; Eckhardt et al. 2007; Otrosina 1997). Therefore, it is possible that trees initially infected with *Leptographium* spp. were colonized selectively by southern pine beetles and killed, as were many trees in one Control plot. In addition, stressed trees may have been targeted during the thinning operations and also may have been debilitated or killed during the prescribed burn.

Over time, the fuel reduction treatments used in this study (i.e., prescribed burning and thinning) did not appear to have a significant effect on the incidence of *Leptographium* spp. in the roots of southern pine trees. More importantly, these treatments did not stress trees enough to cause the incidence of these opportunistic and potentially pathogenic fungi to increase. In the Thin and Thin+burn plots, the lower incidence levels observed in 2002, if not from beetle attack, may be due to the thinning operation removing infected trees that were obviously less fit and weak. However, over time, incidence levels returned to pre-treatment levels, and, ultimately, thinning had no effect on the incidence of *Leptographium* spp. in southern pine trees after five years. To date, there is no evidence in the literature of an association between thinning and the incidence of *Leptographium* spp. in southern pine trees; however, thinning does reduce the risk of attack by the southern pine beetle—a known vector of these potential pathogens—by eliminating competition and increasing vigor of the trees (Belanger et al. 1993; Burkhart et al. 1986). Furthermore, the presence of *Leptographium* spp. in southern pine tree roots has been associated with high stand basal area (Otrosina et al. 1997),

which further illustrates the importance of thinning to promote tree health in southern pine forests.

In this study, prescribed burning also had no long-term effect on the incidence of *Leptographium* spp. in southern pine trees. To date, there has been only a limited amount of research on the effects of prescribed fire on *Leptographium* spp. in southern pine forests. Fire directly can affect a tree by heating and killing the cambium, which can lead to immediate mortality; however, development of decline symptoms after prescribed burning followed by mortality has been observed (Otrosina et al. 2002)—suggesting disease agents such as species of *Leptographium* might be involved in tree death. Certainly, fire characteristics (e.g., fire intensity and duration) may influence how fires affect tree health. Otrosina et al. (2002) found that decline symptoms in longleaf pine forests increased with increasing burn intensity; however, *Leptographium* spp. were not associated with symptom class or burn intensity. In another study (Otrosina et al. 2007), the incidence of *Leptographium* spp. was high one year after a wildfire outbreak, but, over time, severely affected trees succumbed and *Leptographium* spp. were not detected in remaining trees eight years after the fire. The effects of prescribed fire on the susceptibility of southern pine trees to *Leptographium* spp. is not conclusive and warrants further research.

Because prescribed fire and thinning operations are common forest management practices, it is essential to know the impact these practices have on incidence levels of diseases within the forest and on the overall health of trees. However, very little research has been conducted on the effects of these practices on the many diseases affecting forest

trees. Because forests are such dynamic ecosystems that constantly change over time, it would be wise to continue to monitor the treatment plots at the study sites in the Clemson Experimental Forest.

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Table 2.1. Numbers and percentages of southern pine trees, tree roots, treatment plots, and blocks in the Clemson Experimental Forest in which *Leptographium* species were isolated in 2006 after applications of fuel reduction treatments

	Trees (*n*=328)		**Roots (*n*=656)**		**Plots (*n*=12)**		**Blocks (*n*=3)**	
Species	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**
L. terebrantis	65	20	76	12	11	92	3	100
L. procerum	60	18	85	13	11	92	3	100
L. huntii	15	5	26	4	9	75	3	100
L. serpens	4	1	5	1	3	25	3	100
L. truncatum	2	<1	2	<1	2	17	1	33
Leptographium sp.[a]	2	<1	2	<1	2	17	2	67
All species	79	24	96	15	11	92	3	100

[a]Two isolates were identified as a species that has not yet been described.

Table 2.2. Incidence levels of *Leptographium* spp. in southern pine trees at the Clemson Experimental Forest before (2000) and after (2002 and 2006) applications of fuel reduction treatments to plots in three replicate blocks [a]

		2000			2002			2006		
		Sampled	Infected		Sampled	Infected		Sampled	Infected	
Treatment[b]	Block	(no.)	No.	%	(no.)	No.	%	(no.)	No.	%
Control	1	28	4	14	11	0	0	30	0	0
	2	25	4	16	15	3	20	18	2	11
	3	26	3	12	25	11	44	30	8	27
Burn	1	22	5	23	12	0	0	30	12	40
	2	28	9	32	21	1	5	30	6	20
	3	26	6	23	22	0	0	30	14	47
Thin	1	26	11	42	30	0	0	24	11	46
	2	25	3	12	23	0	0	30	3	10
	3	25	10	40	15	0	0	18	1	6
Thin+burn	1	21	9	43	26	1	4	28	9	32
	2	26	7	27	26	7	27	30	5	17
	3	35	8	23	29	1	3	30	8	27
All treatments	1-3	313	79	25	255	24	9	328	79	24

[a] Samples were collected during the fall of 2000, fall of 2002, and summer of 2006.

[b] Fuel reduction treatments were not applied (Control) or were two applications (April 2001, March through April 2004) of prescribed burning (Burn), one application (December 2000 through April 2001) of thinning (Thin), or one application (December 2000 through April 2001) of thinning followed by two applications (March 2002, March through May 2005) of prescribed burning (Thin+burn).

Table 2.3. Incidence levels of *Leptographium* spp. in southern pine tree roots at the Clemson Experimental Forest before (2000) and after (2002 and 2006) applications of fuel reduction treatments to plots in three replicate blocks [a]

		2000			2002			2006		
		Sampled	Infected		Sampled	Infected		Sampled	Infected	
Treatment[b]	Block	(no.)	No.	%	(no.)	No.	%	(no.)	No.	%
Control	1	56	6	11	22	0	0	60	0	0
	2	50	5	10	30	4	13	36	3	8
	3	52	4	8	50	17	34	60	11	18
Burn	1	44	6	14	24	0	0	60	22	37
	2	56	10	18	42	1	2	60	6	10
	3	52	9	17	44	0	0	60	21	35
Thin	1	52	15	29	60	0	0	48	3	6
	2	50	3	6	46	0	0	60	2	3
	3	50	14	28	30	0	0	36	2	3
Thin+burn	1	42	12	29	52	1	2	56	13	23
	2	52	8	15	52	7	13	60	5	8
	3	70	12	17	58	2	3	60	10	17
All treatments	1-3	626	104	17	510	32	6	656	96	15

[a] Samples were collected during the fall of 2000, fall of 2002, and summer of 2006.

[b] Fuel reduction treatments were not applied (Control) or were two applications (April 2001, March through April 2004) of prescribed burning (Burn), one application (December 2000 through April 2001) of thinning (Thin), or one application (December 2000 through April 2001) of thinning followed by two applications (March 2002, March through May 2005) of prescribed burning (Thin+burn).

Table 2.4. Analyses of variance (ANOVA) of proportions of southern pine trees and tree roots in the Clemson Experimental Forest infected with *Leptographium* spp. at three sample periods: before (2000) and after (2002 and 2006) applications of four fuel reduction treatments[a]

		Trees			Roots		
ANOVA	**Source**	**df**	***F***	***P*>*F*** [b]	**df**	***F***	***P*>*F*** [b]
2-way	Treatment plot	3	2.20	0.1279	3	1.44	0.2693
	Sample period	2	6.88	**0.0070**	2	5.51	**0.0151**
	Block	2	0.48	0.6247	2	1.58	0.2358
	Treatment plot × block	6	1.70	0.1863	6	1.46	0.2545
	Treatment plot × sample period	6	2.29	0.0872	6	2.15	0.1035
1-way 2000	Treatment plot	3	0.16	0.9227	3	0.25	0.8616
	Block	2	0.70	0.5332	2	1.28	0.3438
1-way 2002	Treatment plot	3	5.25	**0.0408**	3	5.14	**0.0428**
	Block	2	1.06	0.4047	2	1.04	0.4083
1-way 2006	Treatment plot	3	0.54	0.6726	3	0.59	0.6642
	Block	2	1.13	0.3825	2	1.96	0.2216

[a] Fuel reduction treatments were applied to treatment plots that were assigned to three replicate blocks; tree age was used as the blocking factor.

[b] *P*-values in bold are those that were significant (α=0.05).

Fig. 2.1. Map of the study site included in the Fire and Fire Surrogate Study at the Clemson Experimental Forest. Treatment plots are highlighted in green and are labeled with treatment application (Control, Thin, Burn, and Thin+burn) and block number (1 to 3).

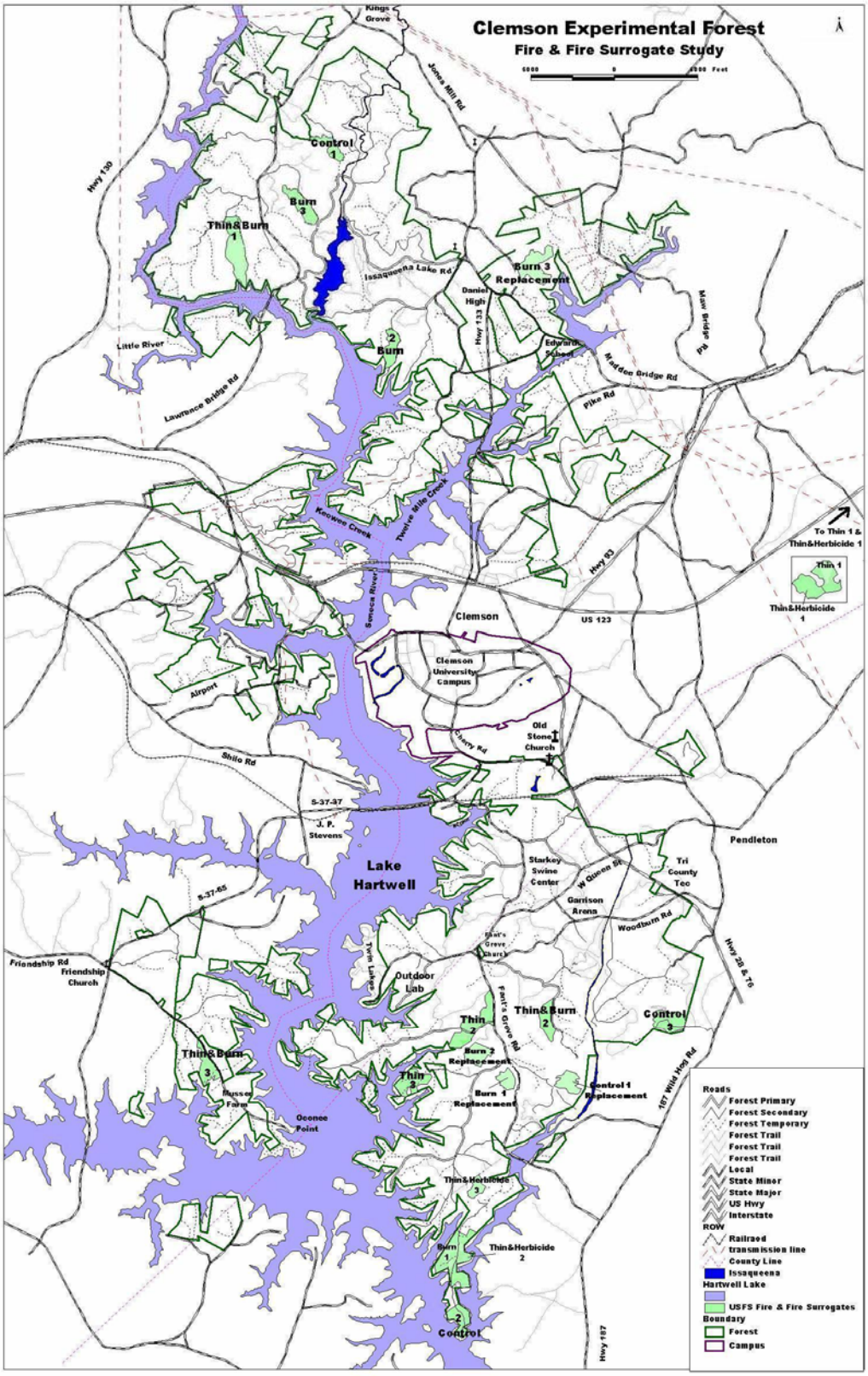

Clemson Experimental Forest
Fire & Fire Surrogate Study
Kings Grove
James Mill Rd
Hwy 130
Control 1
Burn 3
Thin&Burn 1
Issaqueena Lake Rd
Burn 3 Replacement
Daniel High
Hwy 133
Edwards School
Mauldin Bridge Rd
Madden Bridge Rd
Pike Rd
Little River
Lawrence Bridge Rd
Burn 2
Keowee Creek
Twelve Mile Creek
Seneca River
Hwy 93
US 123
To Thin 1 & Thin&Herbicide 1
Thin 1
Thin&Herbicide 1
Clemson
Clemson University Campus
Airport
Cherry Rd
Old Stone Church
Shiloh Rd
S-37-37
J. P. Stevens
Lake Hartwell
S-37-65
Pendleton
Starkey Swine Center
W Queen St
Tri County Tec
Garrison Arena
Woodburn Rd
Hwy 28 & 76
Friendship Rd
Friendship Church
Twin Lakes
Outdoor Lab
Fant's Grove Church
Fant's Grove Rd
Thin 2
Thin&Burn 2
Control 3
Thin&Burn 3
Burn 2 Replacement
Thin 3
Burn 1 Replacement
Control 1 Replacement
187 Wild Hog Rd
Russell Farm
Oconee Point
Thin&Herbicide 3
Burn 1
Thin&Herbicide 2
Control 2
Hwy 187
Roads
Forest Primary
Forest Secondary
Forest Temporary
Forest Trail
Forest Trail
Forest Trail
Local
State Minor
State Major
US Hwy
Interstate
ROW
Railraod
transmission line
County Line
Issaqueena
Hartwell Lake
USFS Fire & Fire Surrogates
Boundary
Forest
Campus

Fig. 2.2. Diagram of one treatment plot from the study site at the Clemson Experimental Forest. Gridpoints were located at 50-m intervals and 10 sub-plots (20 m × 50 m) were established—one from every fourth gridpoint.

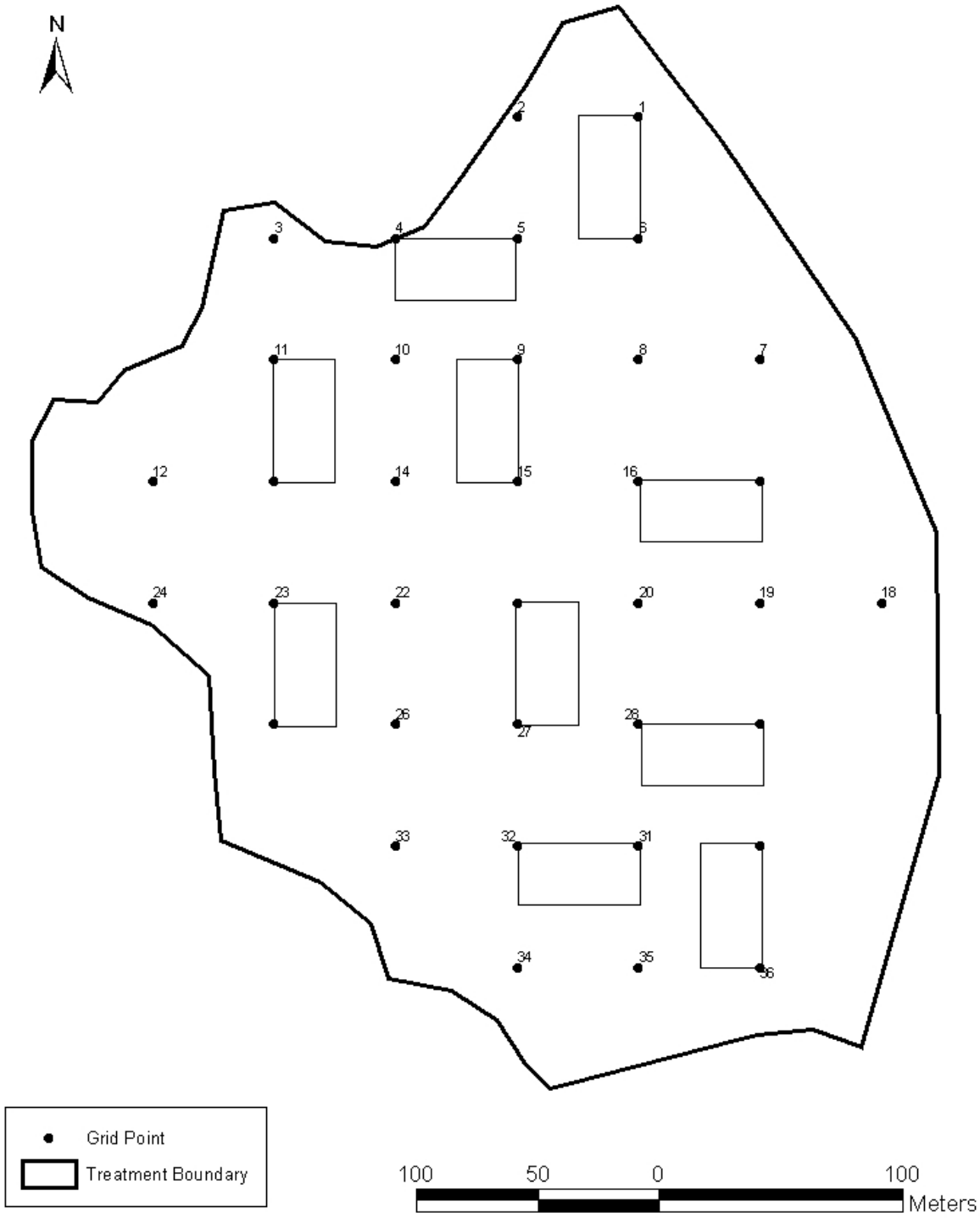
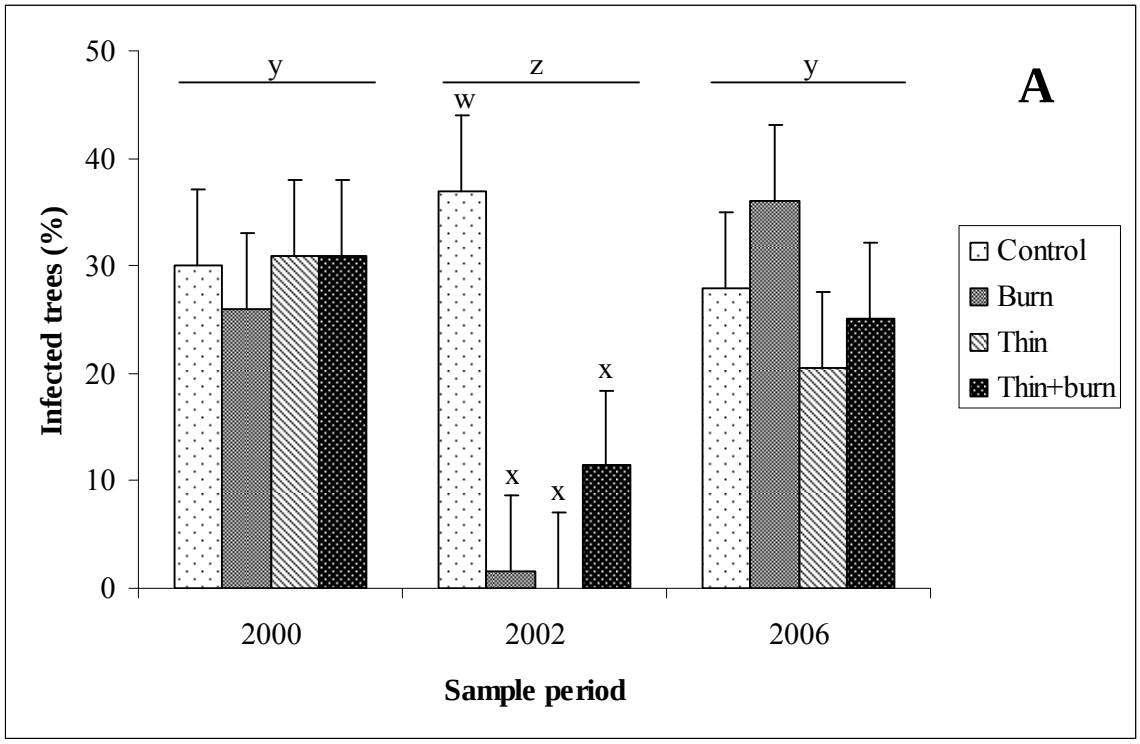
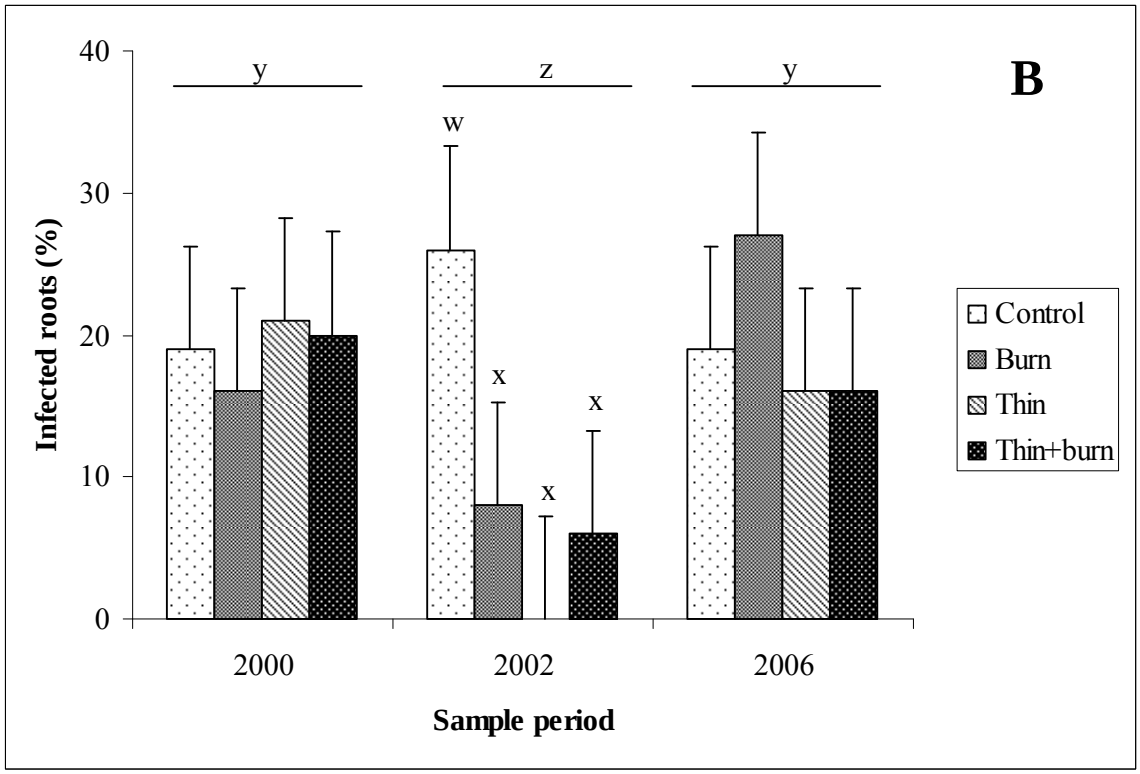

N
1
2
3
4
5
6
7
8
9
10
11
12
14
15
16
18
19
20
22
23
24
26
27
28
31
32
33
34
35
36
Grid Point
Treatment Boundary
100
50
0
100
Meters

Fig. 2.3. Percentages of **A**, southern pine trees and **B**, tree roots in the Clemson Experimental Forest from which *Leptographium* spp. were recovered before (2000) and after (2002 and 2006) applications of fuel reduction treatments. Data are means of three replicate plots with 11 to 35 trees and two roots per tree sampled in each plot; error bars represent standard errors. There were significant differences ($P \leq 0.05$) among sample periods (y vs. z) and among treatments in the 2002 sample period (w vs. x) based on analyses of variance and pairwise comparisons.


A
Infected trees (%)
50
40
30
20
10
0
y
z
y
w
x
x
x
2000
2002
2006
Sample period
Control
Burn
Thin
Thin+burn


B
Infected roots (%)
40
30
20
10
0
y
z
y
w
x
x
x
2000
2002
2006
Sample period
Control
Burn
Thin
Thin+burn

CHAPTER THREE

EFFECTS OF FUEL REDUCTION TREATMENTS ON THE INCIDENCE OF *PHYTOPHTHORA* SPECIES IN SOIL IN A HARDWOOD FOREST OF THE SOUTHERN APPALACHIAN MOUNTAINS

Introduction

Natural and human-related disturbances, particularly fire, have influenced forest communities of the southern Appalachian Mountains (Barden and Woods 1976; Delcourt and Delcourt 1997; Harmon 1982; Van Lear and Waldrop 1989). Before human settlement, lightning was the most prominent source of ignition (Komarek 1964); however, shifts in forest communities of the southern Appalachian Mountains have occurred within the last 4,000 years primarily due to the use of fire by Native Americans (Delcourt and Delcourt 1997). Throughout the past century, fire usually has been excluded from forests in the United States due to national fire suppression policies administered by the federal government (Pyne 2004). As a result, many fire-adapted forests have accumulated an excessive amount of fuels and, therefore, are at risk of wildfire outbreak (Barrett 1988; Kilgore and Taylor 1979; Vankat 1977). Furthermore, in some forests in the eastern United States, oaks are being replaced by less fire-tolerant species such as red maple (*Acer rubrum*), which had been suppressed when fire was recurring (Christensen 1977; Lorimer 1984; Schuler and Gillespie 2000).

Mechanical fuel reduction practices (e.g., cutting, thinning, brush removal, mastication, etc.), have been used in forests to reduce the accumulation of fuels and to remove undesirable tree and shrub species; however, there is a lack of information confirming that these practices have the same effect as fire in hardwood forests of the eastern United States. Furthermore, the effects of these practices on forest ecosystems are not well understood. The national Fire and Fire Surrogate Study (Weatherspoon and McIvers 2000) was established in 2000 by the United States Department of the Interior and the United States Department of Agriculture (USDI-USDA) Joint Fire Science Program to address the long-term effects of fuel reduction treatments (i.e., mechanical fuel reduction and prescribed burning) in fire-adapted forests on ecosystem variables—i.e., wildlife, insects, vegetation, economics, fuels, fire behavior, soils, and tree pathogens. One of the study sites is located in a hardwood forest in the southern Appalachian Mountains. *Phytophthora* spp. are widespread in forest soils in this region and have the potential to cause disease on many trees and shrubs (Campbell et al. 1963; Campbell and Gallegly 1965; Wood et al. 2001; Zentmeyer 1980).

Species of *Phytophthora* are important pathogens of many agricultural crops (Erwin and Ribiero 1996); however, several *Phytophthora* spp. also are known to cause severe mortality in forests where they have been introduced and become established (Crandall et al. 1945; Goheen et al. 2002; Hansen et al. 2000; Rizzo et al. 2002; Tainter et al. 2000; Weste and Taylor 1971). In recent years, species of *Phytophthora* also have been associated with decline of several tree species in the forests of Europe (Balci and Halmschlager 2003a, 2003b; Brasier et al. 2004; Cerny et al. 2007; Jung et al. 1999,

2000, 2002) and in the northeastern United States (Jung et al. 2005). Declines of European beech (*Fagus sylvatica*) in the USA and Europe and decline of several oak species (*Quercus* spp.) in Europe have been associated with the presence of multiple species of *Phytophthora* in soil, fine roots, or root and collar lesions (Brasier 1999; Gallego et al. 1999; Jung et al. 2000, 2002, 2005; Moreira and Martins 2005). However, these organisms also have been found in forest soils where known susceptible hosts are present but with no evidence of disease, suggesting that other factors may be involved in disease development (Hansen and Delatour 1999; Marks et al. 1975; Wood et al. 2001).

In the southeastern United States, *P. cinnamomi* is believed to have been introduced over 150 years ago and since that time has spread throughout forests of this region (Campbell 1951; Campbell et al. 1963; Crandall et al. 1945; Roth 1954; Wood et al. 2001; Zentmeyer 1980). This organism is responsible for almost eliminating American chestnut (*Castanea dentata*) from southeastern forests before chestnut blight was introduced (Campbell et al. 1963; Crandall et al. 1945; Zentmeyer 1980). Many species of hardwood trees and shrubs native to forests of the southern Appalachian Mountains—e.g., *Rhododendron* spp., *Pinus strobus*, and *Quercus* spp.—are susceptible to *P. cinnamomi* in plant nurseries or inoculation studies (Coyier and Roane 1988; Jordan and Tainter 1996; Spainhour et al. 2001; Zentmeyer 1980); however, there are no reports of *P. cinnamomi* causing disease on these hosts in the forest.

A number of other species of *Phytophthora* have been found in forest soils and streams of the southeastern United States. Recent surveys of forest streams in the region have revealed a large diversity of species of *Phytophthora*—some of which have not been

described (Hwang et al. 2007). The overall role of *Phytophthora* spp. in forests of the southern Appalachian Mountains is not known. The potential for these organisms to cause disease continues to pose a threat to native vegetation (Jordan and Tainter 1996; Wood et al. 2001).

As forest management practices such as prescribed burning and mechanical fuel reduction become increasingly common in hardwood forests of the southeastern United States, it is important to understand their effects on tree pathogens, such as species of *Phytophthora*. Therefore, the objective of this study was to evaluate the effects of fuel reduction treatments (i.e., mechanical fuel reduction and prescribed burning) on the incidence of *Phytophthora* spp. in a forest soil of the southern Appalachian Mountains five years after the initial treatments were applied. A comparison of incidence levels of *Phytophthora* spp. before and one year after treatment application was reported previously (Zwart 2004). This project is part of the ongoing Fire and Fire Surrogate (FFS) Study funded by the USDI-USDA Joint Fire Science Program.

Materials and Methods

Study site. The FFS Southern Appalachian Mountains study site was located in the Green River Game Land Management Area in Polk County, North Carolina (35°22' to 35°15'N and 82°22' to 82°10'W) (Fig. 3.1). The forest is a mixture of xeric and mesic oak species with several other hardwood tree species found throughout the study site—e.g., hickory (*Carya* spp.), sourwood (*Oxydendrum arboretum*), red maple (*Acer rubrum*), and yellow poplar (*Liriodendron tulipifera*) (Phillips et al. 2007). Yellow pines

(*Pinus* spp.) were common on ridges while white pine (*P. strobus*) was found in moist coves. The shrub layer primarily consisted of rhododendron (*Rhododendron* spp.), mountain laurel (*Kalmia latifolia*), and blueberry (*Vaccinium* spp.). The study site had not been cut or thinned within the previous decade, and it had not been burned within the previous five years (Waldrop 2001). Due to excessive fuel accumulation, the study site was considered to be at risk of severe wildfire outbreak and, therefore, was chosen for the national FFS Study.

Experimental design. The FFS study site was established in 2000 as a randomized complete block design with one replicate of each treatment in each of three blocks (Fig. 3.1). Location was used as the blocking factor, and all plots were selected based on similar stand conditions—including tree age and size, dominant tree species, and management history. Four fuel reduction treatments were applied: prescription burning (Burn), mechanical fuel reduction (Mechanical), mechanical followed by burning (Mech+burn), and a non-treated control (Control). Each treatment plot was approximately 14 ha with 36 to 40 gridpoints installed at 50-m intervals within the innermost 10 ha; the other 4 ha served as a buffer between plots. Ten 1000-m^2 sub-plots (20 m × 50 m) were established within each treatment plot—one sub-plot at every fourth gridpoint. A number of experiments involving different ecosystem variables were conducted at this study site. The effects of the four fuel reduction treatments on the incidence of *Phytophthora* spp. in soil was evaluated in this study.

Treatment application. Treatment applications followed those outlined by the FFS protocol for the Southern Appalachian Mountains study site (Waldrop 2001).

Mechanical fuel reduction operations were conducted by a chainsaw crew in December 2001 through March 2002. The goal of the fuel reduction operation was to cut most rhododendron and mountain laurel shrubs and small trees (<10 cm diameter at breast height [dbh] and greater than 2 m in height). All cut vegetation was left on-site to avoid excessive costs associated with removal. Mechanical fuel reduction was conducted one time throughout this study in both Mechanical and Mech+burn treatment plots.

Prescription burns were conducted by the North Carolina Wildlife Resources Commission and the USDA Forest Service. In the Mech+burn plots, the burn occurred one year after mechanical fuel reduction operations were conducted to allow the cut vegetation to desiccate and partially decompose. Prescribed burns were conducted in the Burn and Mech+burn plots in Block 3 on 12 March 2003; air temperature ranged from 17 to 27°C and relative humidity ranged from 30 to 61%. To set the fires, strip fire ignition was used in the Burn plot and spot ignition was used in the Mech+burn plot. Flame height ranged from 0.25 to 1 m. The Burn and Mech+burn plots in Blocks 1 and 2 were burned on 13 March 2003; air temperature ranged from 20 to 26°C and relative humidity ranged from 39 to 49%. Fires were set by aerial ignition and flame heights again ranged from 0.25 to 1 m in length.

Prescribed burning was conducted a second time in both the Burn and Mech+burn plots. Plots in Block 3 were burned on 16 February 2006; air temperature ranged from 16 to 21°C and relative humidity ranged from 24 to 39%. Plots in Blocks 1 and 2 were burned on 1 March 2006; air temperature ranged from 23 to 25°C and relative humidity ranged from 29 to 36%. Strip fire ignition was used to set fires in all three blocks. The

objective of all burns was to reduce the woody shrub component in treatment plots (Waldrop 2001).

Sampling procedure. Samples initially were collected in December 2001 through February 2002 before treatments were applied to determine the natural distribution of *Phytophthora* spp. in forest soil in the treatment plots. Post-treatment samples were collected twice—in November 2003 through February 2004, one year after both prescribed fire applications and approximately two years after pre-treatment samples were collected, and again in January through April 2007, approximately five years after pre-treatment samples were collected. These sample periods will be referred to by the primary year in which they occurred: 2002, 2004, and 2007. In each treatment plot, 20 soil cores (2 cm in diameter and 20 cm in length) were removed systematically from each of the 10 sub-plots using a soil sampling tube (Oakfield Apparatus, Inc., Oakfield, WI) and combined into one plastic bag. Therefore, 120 soil samples were collected from the 12 treatment plots. Composite soil samples contained 1 to 2 liters and were transported in a cool ice chest to the laboratory and maintained at 10°C until processing.

Detection of *Phytophthora* spp. Detection of *Phytophthora* spp. from soil samples was conducted using a baiting bioassay (Ferguson and Jeffers 1999; Wood et al. 2001; Zwart 2004). All soil samples were sieved through a 2-mm-mesh screen to remove rocks and large organic matter and mixed thoroughly. For each composite soil sample, three 100-ml aliquots of soil were baited. Each aliquot was placed in a separate 450-ml plastic box and flooded with 200 ml of distilled water; six camellia leaf disks (5 mm in diameter) were placed in each box as baits for *Phytophthora* spp., and boxes were kept at

room temperature (approx. 22 to 25°C) for 3 days. Baits then were removed, blotted dry, and embedded in PARPH-V8, a medium selective for *Phytophthora* spp. (Ferguson and Jeffers 1999)—which contained per liter: 950 ml of distilled water, 50 ml of buffered and clarified V8 juice (Campbell Soup Company, Camden, NJ), 15 g of Bacto agar (Becton, Dickinson, and Company, Sparks, MD), 10 mg of pimaricin as Delvocid Instant (DSM Food Specialties, Delft, The Netherlands), 250 mg of ampicillin sodium salt (Shelton Scientific, Inc., Shelton, CT), 10 mg of rifamycin SV sodium salt (Sigma-Aldrich, St. Louis, MO), 50 mg of penta-chloro-nitro-benzene as Terraclor (Chemtura USA Corp., Middlebury, CT), and 50 mg of hymexazol as Tachigaren 70WP (Sankyo Co., Ltd., Tokyo, Japan). Buffered and clarified V8 juice was prepared by mixing 1g of $CaCO_3$ with each 100 ml of V8 juice; the suspension was centrifuged at 7970×g for 10 min and the clarified supernatant was frozen (-20°C) until used.

Isolation plates were held at 20°C in the dark for up to 7 days and were observed regularly for colonies of *Phytophthora* spp. Any soil sub-sample from which *Phytophthora* spp. were not detected was assayed a second time. In addition, aliquots of soil from sub-plots in which *P. heveae* had been detected previously (Zwart 2004) were allowed to air-dry for 3 days and then were re-moistened, held at room temperature for 3 days, and assayed using the same protocol as described above. Air drying and re-moistening soil samples has been shown to enhance detection of *Phytophthora* spp. that survive as oospores (Ferguson and Jeffers 1999). The numbers of sub-plots and 100-ml soil sub-samples from which *Phytophthora* spp. were recovered were counted, and

proportions and percentages were calculated. Colonies of *Phytophthora* spp. recovered from soil samples were sub-cultured and retained for identification.

Identification of *Phytophthora* spp. Isolates of *Phytophthora* spp. usually were identified directly on PARPH-V8 after incubation at 20°C in the dark for 7 days. Identification was based on unique morphological features—such as colony pattern and production and shape of oospores, chlamydospores, and sporangia (Erwin and Riberio 1996). Representative isolates were placed on corn meal agar (Becton, Dickinson, and Company) in 8-ml glass vials, and placed in a permanent collection at 15°C in the dark.

Data analysis. Proportions of sub-plots and soil sub-samples from which *Phytophthora* spp. were detected were used in an analysis of variance (ANOVA) but are reported in tables as numbers and percentages. A model for the experimental design was created with factors for sample period (2001, 2004, and 2007), treatment plots (Control, Mechanical, Burn, and Mech+burn), and blocks (1 to 3). ANOVA was used to analyze the model factors sample period and treatment plot while adjusting for block effects. Orthogonal linear contrasts were used to determine differences among the levels within significant model factors in the ANOVA ($P \leq 0.05$).

Presence or absence of *Phytophthora* spp. in each of the 120 sub-plots was determined during each sampling period. Comparisons of the consistency of occurrence of *Phytophthora* spp. in sub-plots between pairs of sample periods were conducted by using two-way contingency tables and testing for agreement with Cohen's Kappa Coefficient. All analyses were conducted using SAS, ver. 9.1 for Windows (SAS Institute Inc., Cary, NC), and all statistical tests were performed with $\alpha = 0.05$.

Results

2007 sample period. In 2007, *Phytophthora* spp. were detected in 71 of 120 sub-plots (58%) and in 181 of 360 soil sub-samples (50%) (Table 3.1). *Phytophthora* spp. were detected in all treatment plots in all blocks, but incidence levels varied among the treatment plots: Mechanical (17/30 sub-plots); Burn, 77% (23/30 sub-plots); Mech, 53% (16/30 sub-plots); and Control, 50% (15/30 sub-plots) (Table 3.1 and Fig. 3.3). Percentages of soil sub-samples in which *Phytophthora* spp. were detected were: Mechanical, 48% (43/90); Burn, 68% (61/90); Mech+burn, 43% (39/90); and Control, 42% (38/90) (Table 3.1). Incidence levels of *Phytophthora* spp. in Blocks 1 and 2 were 45% (18/40) and 48% (19/40), respectively, but incidence was greatest in Block 3 where *Phytophthora* spp. were detected in 85% (34/40) of the sub-plots.

P. cinnamomi and *P. heveae* were the only two species detected in soil at the Green River Game Land Management Area study site. *P. cinnamomi* was found in all treatment plots, with incidence levels ranging from 47% to 73% sub-plots, and in all three blocks, where incidence levels were 43% or 85% of sub-plots (Tables 3.1 and 3.2). This species was detected in over half (68) of the 120 sub-plots and was recovered from nearly half (175) of the 360 sub-samples assayed (Table 3.1). In contrast, *P. heveae* was detected in just three sub-plots—one in each of three treatment plots—and was recovered from only six soil sub-samples (Table 3.1 and Fig. 3.3). *P. cinnamomi* and *P. heveae* were not detected in the same sub-plots (Fig. 3.3).

Changes in incidence of *Phytophthora* spp. over time. Other co-workers collected samples in 2001 and 2004, before and immediately after fuel reduction

treatments were applied (Zwart 2004); data are included here so changes over time could be determined. Percentages of sub-plots in which *Phytophthora* spp. were detected were similar in 2001, 35% (42/120), and 2004, 34% (41/120), but increased to 59% (71/120) in 2007 (Table 3.1 and Fig. 3.3). This pattern was similar for detection of *Phytophthora* spp. in soil sub-samples: 30% (109/360) in 2002, 28% (99/360) in 2004, and 50% (181/360) in 2007. In all sample periods, incidence of *Phytophthora* spp. was greatest in Block 3 where it ranged from 20 to 34 sub-plots. In Blocks 1 and 2, incidence of *Phytophthora* spp. ranged from 10 to 18 and 9 to 19 sub-plots, respectively (Table 3.1 and Fig. 3.3).

Results from the two-way ANOVA of proportions of sub-plots and soil sub-samples that were positive for *Phytophthora* spp. revealed no treatment plot × sample period or treatment plot × block interactions (Table 3.2); therefore, effects of the individual model factors could be evaluated. There was no significant effect of treatment plot on the incidence of *Phytophthora* spp.; however, sample period and block effects were significant in sub-plots (P=0.0008 and P=0.0002, respectively) and soil sub-samples (P=0.0021 and P=0.0010, respectively).

Orthogonal linear contrasts of incidence levels were used to identify where differences among sample periods occurred. In comparing incidence levels of *Phytophthora* spp. before (2002) and after treatment application (2004+2007), incidence levels were significantly greater in sub-plots (P=0.0461) after treatments were applied, and incidence levels were significantly greater in both sub-plots and soil sub-samples (P=0.006 and P =0.0012, respectively) in 2007 than in 2004 (Tables 3.1 and 3.2). In comparing incidence levels of *Phytophthora* spp. between 2002 and 2004 combined (i.e.,

before treatment application and after mechanical and burn treatments had been applied once—data collected previously) (Zwart 2004) and 2007 (i.e., after plots were burned the second time—data collected during the current study), incidence levels were significantly greater in both sub-plots and soil sub-samples (P=0.0002 and P=0.0006, respectively) in 2007 (Tables 3.1 and 3.2). Incidence levels in 2002 and 2004 were not significantly different (Table 3.2).

Occurrence in sub-plots. The Kappa Test for Agreement was conducted to determine the consistency of detection of *Phyophthora* spp. in soil from sub-plots within treatment plots between pairs of sample periods: 2002 and 2004, 2004 and 2007, and 2002 and 2007 (Table 3.3). There was no significant difference in the numbers of sub-plots from which *Phytophthora* spp. were detected for any of the treatment plots except those from Mechanical plots and Mech+burn plots between 2002 and 2004—indicating that most of the sub-plots in which *Phytophthora* spp. were detected before application of treatments in 2002 were the same sub-plots in which *Phytophthora* spp. were detected after treatments were applied in 2004 and 2007. Consistency in detection varied from 17 to 26 sub-plots between sample periods 2002 and 2004, from 18 to 23 sub-plots between 2004 and 2007, and from 19 to 25 sub-plots between 2001 and 2007.

Discussion

In this study, *Phytophthora* species were recovered from forest soils in all treatment plots in the FFS Southern Appalachian Mountains study site, which is consistent with results from the initial phase of this study (Zwart 2004) and with results

from other studies that investigated the distribution of *Phytophthora* spp. in forest soils of the southeastern USA (Campbell 1951; Wood et al. 2001). Results from all these studies combined indicate a widespread distribution of *Phytophthora* spp. in hardwood forest soils in this region of the southern Appalachian Mountains. Recently, *Phytophthora* spp. also have been found in forest soils in the mid-Atlantic and north central states (Balci et al. 2007) and previously have been found in natural ecosystems in western New York (Jeffers and Aldwinckle 1988).

P. cinnamomi was detected most frequently in the soils in the Green River Game Land Management Area. In the southeastern USA, this oomycete is known to cause ink disease of *Castanea* spp. as well as littleleaf disease on shortleaf pine (*P. echinata*) and has been recovered frequently from sites where these diseases occurred (Campbell 1951; Campbell et al. 1963; Crandall et al. 1945; Zentmeyer 1980). *P. cinnamomi* is regarded as a notorious plant pathogen worldwide (Erwin and Ribeiro 1996; Zentmeyer 1980); however, this organism has been shown to persist in soils in the presence of known susceptible hosts without causing disease (Hansen and Delatour 1999; Marks et al. 1975). Although hosts known to be susceptible to *P. cinnamomi* (Coyier and Roane 1988; Jordan and Tainter 1996; Zwart 2004) were present throughout the FFS Southern Appalachian Mountains study site, no obvious symptoms of Phytophthora root rot or leaf blight were observed during the course of this study.

P. heveae was detected much less frequently than *P. cinnamomi* in soils from the study site, which is consistent with results from Zwart (2004) and Wood et al. (2001). *P. heveae* was detected in three sub-plots in each of the three sample periods (2002, 2004,

and 2007), and it was detected in two sub-plots consistently. Although, *P. heveae* is not found commonly in the United States (Erwin and Ribeiro 1996), it does appear to be established in soils of hardwood forests in the southern Appalachian Mountains. Previously, it has been reported in forest soils of western South Carolina, western North Carolina, and eastern Tennessee (Campbell and Gallegly 1965; Wood et al. 2001); in forest streams in western North Carolina (Hwang et al. 2007); as well as on rhododendron in a forest site (S.N. Jeffers, *personal communication*) and in ornamental plant nurseries in North Carolina (Benson and Jones 1980). Zwart (2004) tested the susceptibility of two plant species native to the southern Appalachian Mountains, mountain laurel (*Kalmia latifolia*) and rhododendron (*Rhododendron maximum*), to *P. heveae* but found this species to be only a weak pathogen.

Over the five-year course of the FFS study in western North Carolina, fuel reduction treatments had no effect on the incidence of *Phytophthora* spp. in a hardwood forest soil. Few studies have investigated the effects of similar treatments. Prescribed burning has been used in attempts to eliminate introduced species of *Phytophthora* in Oregon forests (DeNitto 1993; Hansen and Sutton 2005); however, in both cases, these species (i.e., *P. lateralis* and *P. ramorum*) still were detectable after fire—suggesting that heat from the fire was not adequate to kill oomycete propagules present in soil. The effects of prescribed fire also were tested in the jarrah forests of Western Australia; again there was no change in the population density of *P. cinnamomi* in soils after fire (Marks et al. 1975). Results from previous studies and these results suggest that prescribed fire does not affect the survival of *Phytophthora* spp. in soil; however, more studies need to

be conducted to determine the direct effect of soil heating from prescribed fire on soilborne species of *Phytophthora*.

Mechanical fuel reduction also had no effect on the incidence of *Phytophthora* spp. in soil. In contrast with our study, populations of *P. cinnamomi* in soil increased with decreasing canopy cover from young eucalyptus trees in Australia (Marks et al. 1975)—suggesting that selective logging may increase root rot. The authors attributed this result to changes in soil moisture; a thinner canopy resulted in higher soil moisture, and, therefore, mortality from root rot increased. There are several factors associated with mechanical fuel reduction that may influence the incidence and distribution of soilborne species of *Phytophthora*—changes in soil moisture and temperature as a result of the removal of vegetation cover, presence and abundance of host plants, and movement of infested soil and debris to non-infested areas.

Although there was no direct effect of fuel reduction treatments on the incidence of *Phytophthora* spp. in soil, there was an increase in incidence over time, particularly from 2004 to 2007. The source of this difference could be due to several factors. Although the same protocol and equipment were used, the data in 2002 and 2004 were collected by a different individual (Zwart 2004) than samples collected in 2007 (this study). Therefore, the greater incidence in 2007 may be due to a human factor. Another possible explanation is that the region had been experiencing drought conditions from 1998 to 2002, which may have affected recovery of *Phytophthora* spp. in the first two sample periods. Seasonal fluctuations in recovery of *P. cinnamomi* due to changes in precipitation have been reported (Shearer and Shea 1987) and may be a factor in the

differences observed in our study. Also, because numerous research projects are involved with the Fire and Fire Surrogate Study, there is an unusually high amount of traffic, both foot and vehicular, at the study site; therefore, infested soil on shoes and equipment may have been transferred among sub-plots. Overall, there was consistent recovery of *Phytophthora* spp. in sub-plots between sample periods. Therefore, presence or absence of *Phytophthora* spp. in the sub-plots remained relatively unchanged throughout the project duration.

The effects of fuel reduction treatments in this study did not affect the incidence of *Phytophthora* spp. in soil of a hardwood forest in western North Carolina over a 5-year period. Therefore, additional research into the effect of fuel reduction treatments on *Phytophthora* spp. and on other soilborne organisms should be conducted before management decisions are made. Also, further investigations into the role of species of *Phytophthora* in forest ecosystems of the southern Appalachian Mountains are needed.

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Table 3.1. Numbers and percentages of sub-plots and soil sub-samples from which *Phytophthora cinnamomi* and *P. heveae* were detected before (2002) and after (2004 and 2007) fuel reduction treatments were applied to plots in a hardwood forest in western North Carolina

	Sub-plots[a]											
	2002				**2004**				**2007**			
	P. cinnamomi		***P. heveae***		***P. cinnamomi***		***P. heveae***		***P. cinnamomi***		***P. heveae***	
Treatment[b]	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**
Mechanical	12	40	1	3	11	37	1	3	16	53	1	3
Burn	12	40	1	3	11	40	1	3	22	73	1	3
Mech+burn	7	23	0	0	6	20	0	0	16	53	0	0
Control	9	30	1	3	9	30	1	3	14	47	1	3
Block 1	12	30	1	<1	9	23	1	<1	17	43	1	<1
Block 2	9	23	1	<1	8	20	1	<1	17	43	2	1
Block 3	19	48	1	<1	20	50	1	<1	34	85	0	0
All treatments	40	33	3	<1	37	31	3	<1	68	57	3	<1

	Soil sub-samples[c]											
	2002				**2004**				**2007**			
	P. cinnamomi		***P. heveae***		***P. cinnamomi***		***P. heveae***		***P. cinnamomi***		***P. heveae***	
Treatment[b]	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**
Mechanical	33	14	0	0	28	31	3	3	42	47	1	1
Burn	32	36	3	3	30	33	1	1	58	64	3	3
Mech+burn	13	14	0	0	13	14	0	0	39	43	0	0
Control	25	28	3	3	21	23	3	3	36	40	2	2
All treatments	103	29	6	7	92	26	7	8	175	49	6	7

[a] At each sample period, soil samples were collected from 10 sub-plots per treatment plot in each of three blocks; therefore, 30 sub-plots were sampled for each treatment, 40 sub-plots were sampled in each block, and 120 sub-plots were sampled overall.

[b] Four fuel reduction treatments were assigned to three replicate blocks in different locations: mechanical fuel reduction (Mechanical) was conducted in December 2001 through March 2002, prescribed burning (Burn) was done in March 2003 and again in February through March 2006, mechanical followed by burning (Mech+burn), and a non-treated control (Control).

[c] From each sub-plot, three soil sub-samples were assayed; therefore, 90 soil sub-samples were assayed per treatment and 360 sub-samples were assayed overall.

Table 3.2. Two-way analysis of variance (ANOVA) of proportions of sub-plots and soil sub-samples in which *Phytophthora* spp. were detected before (2002) and after (2004 and 2007) fuel reduction treatments were applied to plots in a hardwood forest in western North Carolina[a]

	Sub-plots			Soil sub-samples[b]		
Source	**df**	***F***	***P>F*[c]**	**df**	***F***	***P>F*[c]**
Treatment plot	3	1.77	0.2533	3	2.90	0.1234
Sample period	2	11.58	**0.0008**	2	9.24	**0.0021**
Block	2	15.35	**0.0002**	2	11.05	**0.0010**
Treatment plot × block	6	1.81	0.1609	6	1.47	0.2517
Treatment plot × sample period	6	0.55	0.7621	6	0.52	0.7883
Orthogonal contrasts[d]						
2002 vs. (2004 + 2007)	1	2.16	**0.0461**	1	1.72	0.1047
2004 vs. 2007	1	4.30	**0.0006**	1	3.94	**0.0012**
(2002 + 2004) vs. 2007	1	4.80	**0.0002**	1	4.27	**0.0006**
2002 vs. 2004	1	0.28	0.7851	1	0.48	0.6378

[a] Four fuel reduction treatments were assigned to three replicate blocks in different locations: mechanical fuel reduction was conducted in December 2001 through March 2002, prescribed burning was done in March 2003 and again in February through March 2006, mechanical followed by burning, and a non-treated control.

[b] At each sample period, soil was collected from ten sub-plots in each of the 12 treatment plots; three soil sub-samples then were assayed from each sub-plot.

[c] *P*-values were judged significant at $\alpha \leq 0.05$; those that were significant are in bold.

[d] Two sets of single degree-of-freedom orthogonal linear contrasts comparing sample periods: before versus after treatment plots had mechanical fuel reduction once and burned twice (2002 vs. [2004 + 2007]) and before and after plots had mechanical fuel reduction and burned once versus after plots were burned a second time ([2002 + 2004] vs. 2007).

Table 3.3. Comparisons between sample periods of the numbers of sub-plots in treatment plots with consistent results from a baiting bioassay for detection of *Phytophthora* species[a]

Treatment[b]	2002 and 2004[c]	2004 and 2007	2002 and 2007
Mechanical	17*	21	20
Burn	20	19	19
Mech+burn	21*	18	19
Control	26	23	25
Total (*n*=120)	84	81	83

[a] Soil samples were collected in three different years from 30 sub-plots within in each treatment plot in a hardwood forest in western North Carolina: 2002—before treatments were applied, 2004—after plots had mechanical fuel reduction and were prescription burned one time, and 2007—after the second burn.

[b] Four fuel reduction treatments were assigned to three replicate blocks in different locations: mechanical fuel reduction (Mechanical) was conducted in December 2001 through March 2002, prescribed burning (Burn) was done in March 2003 and again in February through March 2006, mechanical followed by burning (Mech+burn), and a non-treated control (Control).

[c] * = A significant change in the number of sub-plots in which *Phytophthora* spp. were detected; differences between sample periods were determined using 2×2 contingency tables and the Kappa Test for Agreement (α=0.05).

Fig. 3.1. A map of the Fire and Fire Surrogate study site in western North Carolina: location of the Green River Game Land Management Area (inset) and the locations of the three replicate blocks in the Green River Game Land Management Area. One replicate of each of four fuel reduction treatments were applied to plots in each block; treatments were: prescribed burning (B), mechanical fuel reduction (M), mechanical followed by burning (MB), and a non-treated control (C).

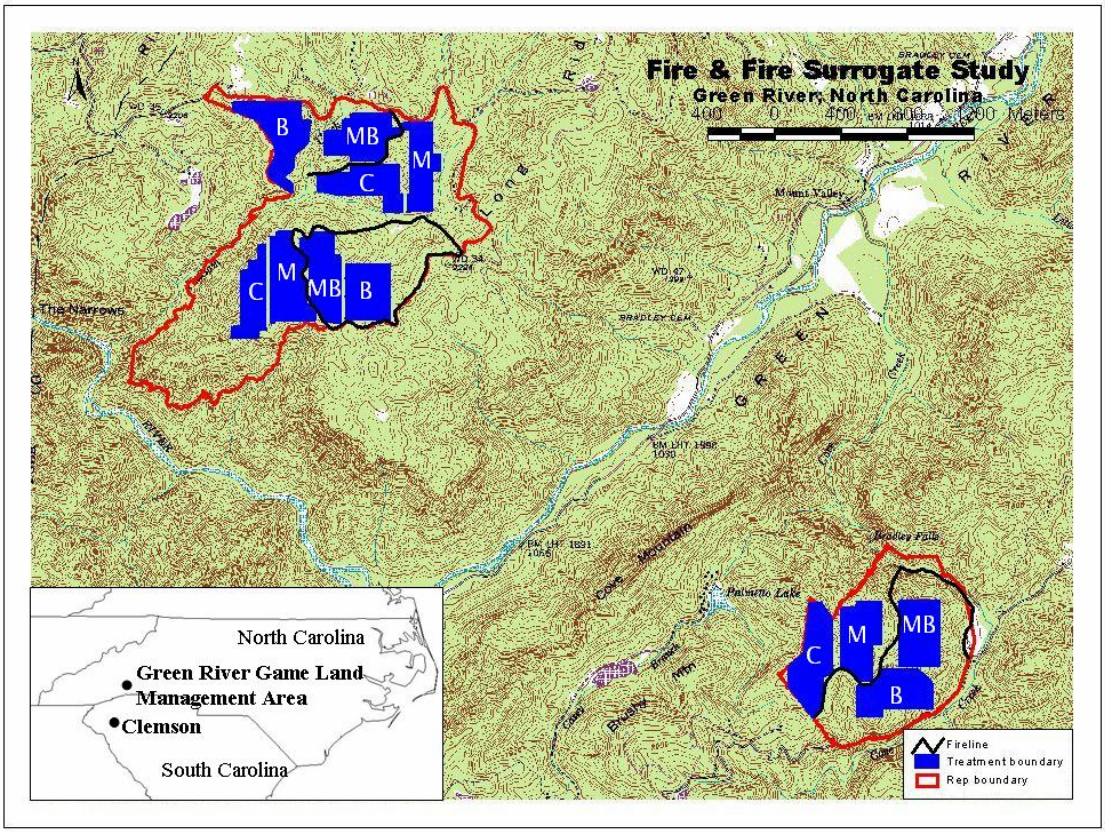

Fire & Fire Surrogate Study
Green River, North Carolina
400 0 400 800 1200 Meters
B
MB
M
C
C
M
MB
B
The Narrows
Lone Rd
Green River
Miami Valley
Bradley Creek
Palmetto Lake
Bradley Falls
C
M
MB
B
North Carolina
Green River Game Land Management Area
Clemson
South Carolina
Fireline
Treatment boundary
Rep boundary

Fig. 3.2. Map of one replicate block in the Fire and Fire Surrogate Study at the Green River Game Land Management Area study site in western North Carolina. One replicate of each of four treatments was applied to plots in each block: prescribed burning (Burn), mechanical fuel reduction (Mechanical), mechanical followed by burning (Mech+burn), and a non-treated control (Control). Within each treatment plot, 36 to 40 gridpoints were located at 50-m intervals and ten sub-plots (20 m × 50 m) were established—one from every fourth gridpoint.

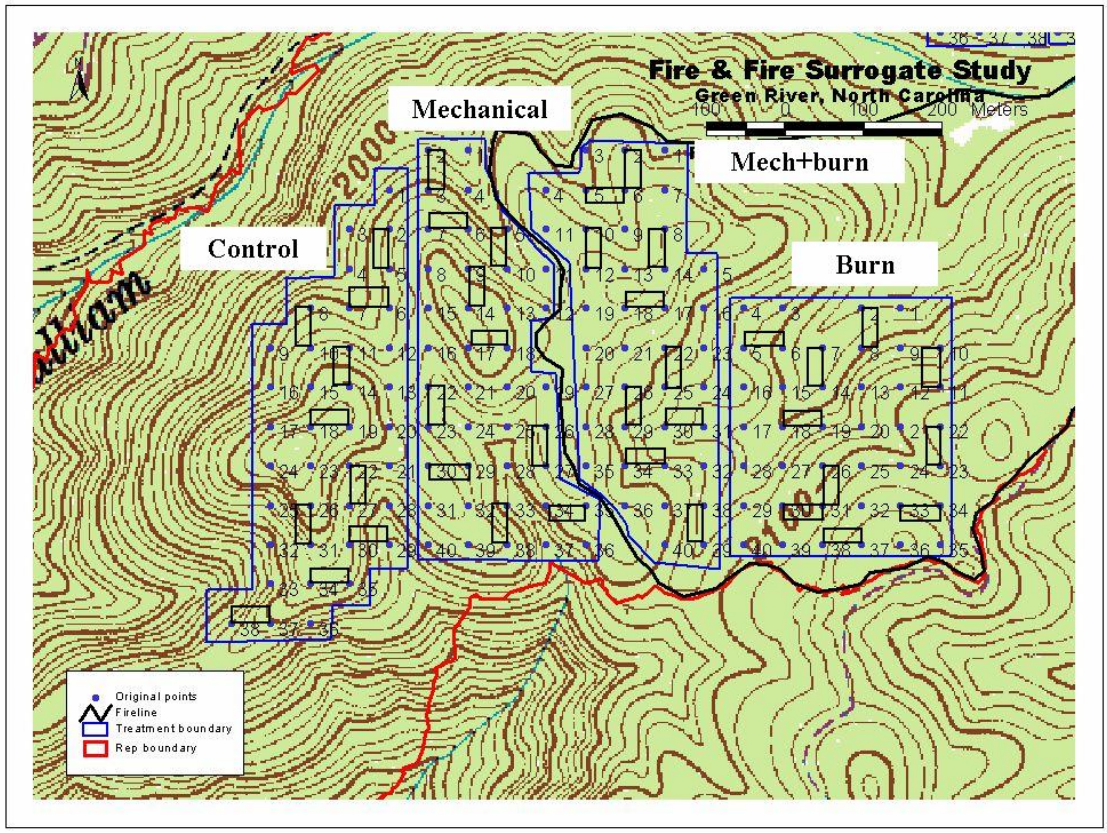
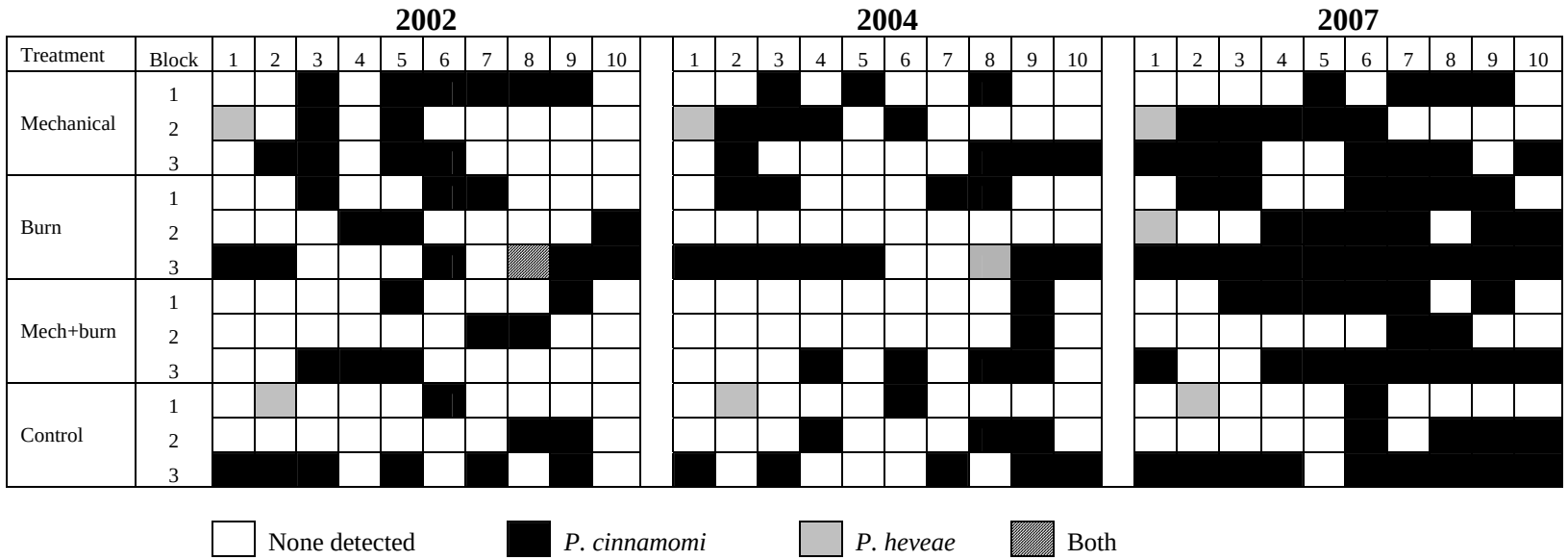

Fire & Fire Surrogate Study
Green River, North Carolina
180 0 180 200 Meters
Mechanical
Mech+burn
Control
Burn
2000
2100
Original points
Fireline
Treatment boundary
Rep boundary

Fig. 3.3. Diagramatic representation of the sub-plots in which *Phytophthora cinnamomi* and *P. heveae* were detected before (2002) and after (2004 and 2007) fuel reduction treatments were applied to plots in a hardwood forest in western North Carolina. Four treatments were assigned to plots in three replicate blocks: mechanical fuel reduction (Mechanical) was conducted in December 2001 through March 2002, prescribed burning (Burn) was done in March 2003 and again in February through March 2006, mechanical fuel reduction followed by prescribed burning (Mech+burn), and a non-treated control (Control). Soil samples were collected from ten sub-plots in each of the 12 treatment plots, and *Phytophthora* spp. were detected with a baiting bioassay.


2002
2004
2007
Treatment
Block
1 2 3 4 5 6 7 8 9 10
Mechanical
Burn
Mech+burn
Control
None detected
P. cinnamomi
P. heveae
Both

CHAPTER FOUR

EFFECTS OF PRESCRIBED FIRE ON THE PERSISTENCE OF *PHYTOPHTHORA CINNAMOMI* IN SOIL IN A HARDWOOD FOREST

Introduction

The use of prescribed fire in forests of the southern Appalachian Mountains can be a useful management tool to decrease the risk of wildfire outbreak, restore declining forest ecosystems, increase plant diversity, and improve habitat for wildlife (Barden and Woods 1976; Brose and Waldrop 2000; Elliot et al. 1999; Phelps et al. 1997; Waldrop et al. 1987). The impact of this forest management practice on soil microorganisms, particularly soilborne plant pathogens, is of interest and has been evaluated in some forest ecosystems (DeNitto 1993; Dickman and Cook 1988; Phelps et al. 1997; Whitney and Irwin 2005; Zwart et al. 2004). Prescribed fire has been shown to reduce the incidence of some plant diseases in forest settings (Dickman and Cook 1988; Phelps et al. 1997; Whitney and Irwin 2005), but results have not been consistent and suggest that additional factors may be involved. Heating soil is a well established practice for managing soilborne plant pathogens (Baker and Roistacher 1957; Katan and DeVay 1991). Therefore, the direct effect of soil heating from prescribed fire in forest ecosystems could be used to manage soilborne plant pathogens, such as species of *Phytophthora*, if soil temperatures get hot enough for a sustained duration.

Since it was introduced approximately 150 years ago, *P. cinnamomi* has become widespread in forests of the southeastern United States (Campbell 1951; Crandall et al. 1945; Roth 1954; Wood et al. 2001; Zentmeyer 1980; Zwart 2004). It has the potential to cause disease in over 900 plant species—many of which are native to this region (Jordan and Tainter 1996; Spainhour et al. 2001; Zentmeyer 1980; Zwart 2004). For example, *P. cinnamomi* is responsible for causing ink disease on American chestnut (*Castanea dentata*) and, in the early 1900s, nearly eliminated this dominant tree species from hardwood forests of the southern Appalachian Mountains before chestnut blight was introduced (Crandall et al. 1945; Zentmeyer 1980). Recently, *P. cinnamomi* has been found in hardwood forest soils of the mid-Atlantic and north central states (Balci et al. 2007).

Investigations on the use of prescribed fire to eliminate or reduce populations of species of *Phytophthora* in soil have been limited (DeNitto 1993; Hansen and Sutton 2005; Marks et al. 1975; Zwart 2004). In all cases, the target species still could be recovered after fire treatment. In a previous study (Chapter 3), the use of prescribed fire as a fuel reduction treatment had no effect on the incidence of *Phytophthora* spp., particularly *P. cinnamomi*, in soil in a hardwood forest in western North Carolina. However, the direct effect of fire on the persistence of *P. cinnamomi* has not been studied. Therefore, the objectives of this study were to determine the direct effect of prescribed fires on persistence of *P. cinnamomi* in forest soil and to measure temperature in forest soil during prescribed fires.

Materials and Methods

Study sites. Two hardwood forests in the southern Appalachian Mountains where *P. cinnamomi* is known to occur naturally were chosen as study locations (Wood et al. 2001; Zwart 2004). One location was in the Green River Game Land Management Area in western North Carolina (GR) and two locations were in the Jocassee Gorges Natural Area in northwestern South Carolina (JG 1 and JG 2). All forests had a mixed oak (*Quercus* spp.) and hickory (*Carya* spp.) forest community with some yellow pines (*Pinus* spp.) on ridgetops and white pine (*P. strobus*) in moist coves. Rhododendron (*Rhododendron* spp.) and mountain laurel (*Kalmia latifolia*) were predominant understory species.

Experimental design. At the GR study site, two plots were selected in the area to be burned and two plots were selected in an adjacent area outside the burn area to serve as non-treated controls. Within each plot, seven aluminum-mesh packets (10 cm × 15 cm; made from 2-mm screen) containing approximately 100 ml of soil naturally infested with *P. cinnamomi* were buried at 2 and 10 cm beneath the soil surface—depths at which *P. cinnamomi* is known to occur (*unpublished data*). In each plot, two temperature probes were placed at each depth in close proximity to the soil packets; therefore, four measurements were taken at each depth. Probes were attached to HOBO U12 dataloggers (Onset Computer Corporation, Pocasset, MA), which was set to record temperature every 30 s during the burn. Dataloggers were placed in waterproof plastic electrical boxes and buried at least 10 cm beneath the soil surface to protect them from heat from the fire. Plots were established the day before the burn.

At the JG 1 site, six plots were established in the area to be burned and two plots were established outside the burn area to serve as the non-treated controls. At the JG 2 site, four plots were placed in the area to be burned and one plot was placed in the adjacent non-burned area. Within each plot, seven aluminum-mesh packets containing infested soil were buried at 2 cm and at 10 cm beneath the soil surface. Temperature probes were placed in each soil packet; each probe was connected to a HOBO Type K datalogger that was programmed to record temperature every 1.5 s during the fire. At the JG 2 location, a temperature sensor also was placed in each of the four plots to measure temperature at 30 cm above the soil surface to determine the temperature of the fire as it passed through the plot; these dataloggers also recorded temperature every 1.5 s during the fire.

Prescribed fires. A prescribed fire was set on 1 March 2006 at the GR site. The objective of the fire was to reduce the understory shrub component (i.e., rhododendron and mountain laurel) and accumulated fuels. Air temperature ranged from 23 to 25°C and relative humidity ranged from 29 to 36%. A backing fire was used along the perimeter and strip fire ignition was used in the interior of the burn area; flame heights were approximately 1 m.

The objective of the prescribed burns at the JG 1 and JG 2 sites was to reduce the understory component (i.e., mountain laurel and rhododendron) in an effort to restore the forest to pre-European settlement conditions. Backing fires were set around the perimeter of the area to be burned and strip fire ignition was used within the burn area. The JG 1 site was burned on 12 February 2007; air temperature at the start of the fire was 55°C and

relative humidity ranged from 22 to 35% during the fire. Flame heights were generally below 1 m; however, intense fires occurred near two plots in JG 1 where flame heights reached into the tree canopy. The JG 2 site was burned on 27 February 2007; air temperature at the start of the fire was approximately 56°C and relative humidity ranged from 22 to 38%. Flame heights generally were less than 0.5 m.

Persistence of *P. cinnamomi*. Persistence of *P. cinnamomi* was determined with a baiting bioassay, which was developed to detect multiple species of *Phytophthora* in soil (Ferguson and Jeffers 1999). Soil packets were retrieved the day after each fire and transported to the laboratory in a cool ice chest. Soil from each packet was placed into a plastic freezer box (450 ml) and flooded with approximately 200 ml of distilled water. Six camellia leaf pieces were placed in each box as baits for *P. cinnamomi,* and boxes were kept at room temperature (approx. 22 to 25°C) for 3 days. Baits then were removed, blotted dry, and embedded in PARPH-V8—a medium selective for *Phytophthora* spp. (Ferguson and Jeffers 1999)—which contained per liter: 950 ml of distilled water, 50 ml of buffered and clarified V8 juice (Campbell Soup Company, Camden, NJ), 15 g of Bacto agar (Becton, Dickinson, and Company, Sparks, MD), 5 mg of pimaricin as Delvocid Instant (DSM Food Specialties, Delft, The Netherlands), 250 mg of ampicillin sodium salt (Shelton Scientific, Inc., Shelton, CT), 10 mg of rifamycin SV sodium salt (Sigma-Aldrich, St. Louis, MO), 50 mg of penta-chloro-nitro-benzene as Terraclor (Chemtura USA Corp., Middlebury, CT), and 50 mg of hymexazol as Tachigaren 70WP (Sankyo Co., Ltd., Tokyo, Japan). Buffered and clarified V8 juice was prepared by mixing 1g of $CaCO_3$ with each 100 ml of V8 juice; the suspension was centrifuged at

7970×g for 10 min and the clarified supernatant was frozen (-20°C) until used. Isolation plates were placed at 20°C in the dark for 3 to 7 days and examined regularly for colonies of *P. cinnamomi*. The number of baits out of five (GR) or six (JG 1 and JG 2) from which *P. cinnamomi* grew was recorded, and proportions and percentages of baits colonized were calculated. The numbers and percentages of soil packets positive for *P. cinnamomi* were determined for each plot and for each treatment × depth at each location.

Data analysis. All analyses were conducted using SAS, ver. 9.1 for Windows (SAS Institute Inc., Cary, NC). Mean proportions of leaf baits with *P. cinnamomi* were used to conduct all analyses. A model for the experimental design was created with factors for location (GR, JG 1, and JG 2), treatment (Burn and Control), and depth (2 and 10 cm). A three-way ANOVA using the GLIMMIX procedure was conducted to analyze model factors, and Fisher's protected least significant difference (α=0.05) was used to separate means.

Results

Soil temperature. Mean maximum soil temperatures varied among locations, and were hotter in the Burn plots at the GR site than in the Burn plots at the JG 1 and JG 2 sites (Table 4.1). Soil temperatures at the 2-cm depth during the burn at GR were 21°C and 25°C hotter than those at JG 1 and JG 2, respectively. Soil temperatures at 10 cm in the Burn plots at GR were 7 and 5°C hotter than those at JG 1 and JG 2, respectively. Maximum soil temperature at 2- and 10-cm depths in Burn plots at all locations ranged from 9 to 51°C and 9 to 18°C, respectively. Soil temperatures at both depths in the

Control plots remained relatively constant at all locations, and ranged from 9 to 13°C at 2 cm and from 9 to 15°C at 10 cm.

In general, soil temperatures at 2 cm beneath the soil surface in the Burn plots at all locations were greater than those at 10 cm and were greater than those at both depths in the Control plots. At the GR site, mean maximum soil temperature at 2 cm was 21°C hotter than at 10 cm and at least 25°C hotter than at 2- and 10- cm depths in the Control plots (Table 4.1). Mean maximum soil temperature at the 10-cm depth in Burn plots was only 4 to 6°C warmer than those in Control plots at both depths. The average amount of time for soil temperatures at 2 cm in Burn plots to return to pre-burn levels was 5.0 h (Fig. 4.1). A temperature spike was recorded by one of the four probes placed in the Burn plots at the 2-cm depth; soil temperature reached 51°C for 30 min.

At the JG 1 site, mean maximum soil temperature at 2 cm in the Burn plots was 7°C hotter than at 10 cm and 6 to 7°C hotter than at both depths in the Control plots (Table 4.1). Mean maximum soil temperature at 10 cm in Burn plots was similar to the temperatures in Control plots at both depths. The average amount of time for elevated soil temperatures at 2 cm beneath the soil surface to return to pre-burn levels was 5.1 h. A soil temperature spike was recorded in one plot where soil temperature reached 42°C for 42 min.

At the JG 2 site, mean maximum soil temperature at 2 cm was only 1°C hotter than at 10 cm and only 4°C hotter than at both depths in the Control plots (Table 4.1). The average amount of time for elevated soil temperatures to return to pre-burn levels was 7.2 h. No soil temperature spikes were recorded at this location. During the fires,

temperatures above ground also varied among plots. As the fire passed through the JG 2 site, maximum temperatures 30 cm above the soil surface in each plot were: 47, 62, 91, and 111°C; these elevated temperatures lasted 8, 21, 12, and 10 min, respectively (Fig. 4.2).

Persistence of *P. cinnamomi*. *P. cinnamomi* was recovered from both depths in all treatment plots at all three locations; however, persistence was somewhat less at 2 cm beneath the soil surface at two locations (Table 4.1). At the GR site, *P. cinnamomi* was not detected in three soil packets out of 14 at the 2-cm depth in the Burn plots, where soil temperatures reached 51°C for 30 min. At the JG 1 site, *P. cinnamomi* was eliminated from one of the 42 soil packets buried at 2 cm in Burn plots. This packet was in the plot where soil temperature reached 42°C for 42 min. *P. cinnamomi* was detected from 100% of the soil packets buried at 10 cm in the Burn plots and at both depths in the Control plots at all three locations.

When proportions of baits were analyzed by 3-way ANOVA, all interactions among the treatment, depth, and location model factors were significant as were all three model factors (Table 4.1). However, upon closer examination of the individual means, the only significant difference was observed in packets buried at 2 cm in the Burn plots at the GR site, where recovery of *P. cinnamomi* was significantly less than in any other treatment×depth×location combination (Table 4.1). The reduced survival in soil packets buried at 2 cm deep in the Burn plots at the JG 1 site (0.952) was not significantly different. *P. cinnamomi* was recovered from 99 to 100% of baits used to assay packets buried at 10 cm in Burn plots and at both depths in Control plots (Table 4.1).

Discussion

During all fires, soil temperatures were noticeably elevated only at 2 cm beneath the soil surface. Soil temperatures at 10 cm beneath the soil surface were only slightly higher than those in non-burned control plots. These results were expected because soil temperature decreases exponentially with increasing depth during fire (Bradstock and Auld 1995). Soil is a poor conductor of heat and very little heat produced from a fire travels downward (Boerner 2006). In previous studies, temperatures measured in soil 1 to 2 cm beneath the soil surface during fires have varied (Heyward 1938; Iverson and Hutchinson 2002; Swift et al. 1993) but were similar to those found in this study.

In this study, soil temperatures of 40°C for over 40 min were adequate to inactivate or eliminate naturally occurring propagules of *P. cinnamomi* in soil, which is consistent with other reports. Survival of soil microorganisms is known to be a function of temperature and duration (Baker and Roistacher 1957; Neary et al. 1999). Several laboratory studies have reported that propagules of *P. cinnamomi* are inactivated at temperatures as low as 38°C for 30 to 60 min or 44 to 45°C for 10 to 20 min (Barbercheck and von Broembson 1986; Benson 1978; Gallo et al. 2007; Jaurez-Palacios et al. 1991). In South Africa, *P. cinnamomi* did not survive in soil after 3 to 6 weeks of solarization where maximum temperatures reached 35 to 45°C for sustained periods. DeNitto (1993) observed no *Phytophthora* spp. in soil that had reached as high as 54°C during a prescribed fire, but it is not known how long the soil remained at this temperature. In general, soil microorganisms are killed at temperatures between 50 to 121°C, and a temperature of 70°C for 10 min is reported to kill most soilborne fungi and

bacteria (Lawrence 1956). Baker and Roistacher (1957) recommend exposure to moist heat at 65°C for 30 min to destroy most important plant pathogens. However, this study and others (Baker and Roistacher 1957; Barbercheck and Broembsen 1986; Benson 1978; Gallo et al. 2007; Jaurez-Palacios et al. 1991; Lopez-Herrera 1997; Pinkas 1984; Pinkerton et al. 2000) indicate that *P. cinnamomi* might be more sensitive if exposed to lower temperatures for a longer period of time, which can occur during low-intensity prescribed fires.

The prescribed fires in this study had little effect on the persistence of *P. cinnamomi* in the top 2 cm of soil and no effect on persistence at 10 cm deep. Prescribed burning has been used in attempts to eliminate or reduce populations of *Phytophthora* spp. from forest soil in only a few studies, and our results are consistent with the results from these studies. Since the introduction of *P. ramorum*, the causal agent of sudden oak death, to coastal forests of southwestern Oregon, forest managers have been using prescribed fire in an attempt to eradicate this pathogen from plants and soil; however, two years after the fire, the organism still could be detected (Hansen and Sutton 2005). Prescribed fire also was used in an attempt to eradicate or reduce populations of *P. lateralis* in soil in a Port-orford cedar forest, but results also were unsuccessful (DeNitto 1993). In Australia, populations of *P. cinnamomi* in soil were only reduced for a short time after a prescribed burn (Marks et al. 1975).

Prescribed fire in this study had a very limited effect on the persistence of *P. cinnamomi* in soil and, therefore, does not appear to be a practical management tool to reduce populations of this soilborne plant pathogen. Only temperatures in the top 2 cm of

soil were elevated in some plots during the prescribed fires used in this study; soil temperatures at 10 cm beneath the soil surface were relatively unaffected. *P. cinnamomi* has been found consistently in soil cores from 0 to 10 cm deep and from 10 to 20 cm deep (*unpublished data*), so some propagules were never exposed to elevated, deleterious temperatures. Furthermore, soil temperatures necessary to inactivate propagules of *P. cinnamomi* during the prescribed fires were not reached consistently in the study plots—even at the 2-cm depth. Temperatures hot enough to inactivate *P. cinnamomi* may be reached only in areas where fuels have accumulated and fire burns longer; therefore, soil temperatures in these places may become elevated for an extended period of time. Consequently, soil temperatures during a typical prescribed fire usually are not uniformly hot enough throughout a prescribed burn site and the heat generated does not travel deep enough in the soil profile to adversely affect persistence of *P. cinnamomi*. This may help explain why incidence levels of *Phytophthora* spp. were not affected by prescribed fire in the previous study (Chapter 3).

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Table 4.1. Persistence of *Phytophthora cinnamomi* in soil after prescribed fires at three locations in the southern Appalachian Mountains: numbers and percentages of soil packets and leaf baits from which *P. cinnamomi* was detected (*Pcin*), maximum soil temperatures, and three-way analysis of variance (ANOVA)[a]

Location	Treatment	Depth (cm)	No. plots	Packets			Baits				Maximum temp. (°C)[c]	
				No.	*Pcin*	%*Pcin*	No.	*Pcin*	%*Pcin*	Mean[b]	Mean	Range
GR	Burn	2	2	14	11	79	70	50	71	0.714*	38	23-51
		10	2	14	14	100	70	70	100	1.000	17	17-18
	Control	2	2	14	14	100	70	70	100	1.000	13	12-13
		10	2	14	14	100	70	70	100	1.000	11	10-11
JG 1	Burn	2	6	42	41	97	252	240	95	0.952	17	13-42
		10	6	42	42	100	252	250	99	0.992	10	9-18
	Control	2	2	14	14	100	84	84	100	1.000	11	9-13
		10	2	14	14	100	84	83	99	0.988	10	7-15
JG 2	Burn	2	4	28	28	100	168	168	100	1.000	13	9-16
		10	4	28	28	100	168	168	100	1.000	12	11-13
	Control	2	1	7	7	100	42	42	100	1.000	9	9
		10	1	7	7	100	42	42	100	1.000	9	9

3-way ANOVA Model Factors	df	*F*	*P>F*
Location	2	3.55	0.057
Treatment	1	6.12	0.031
Depth	1	7.23	0.008
Location × treatment	2	3.88	0.053
Location × depth	2	5.39	0.005
Treatment × depth	1	8.37	0.004
Location × treatment × depth	2	4.90	0.008

[a] Prescribed fires occurred on: 1 March 2006 at the Green River Game Land in western North Carolina (GR) and 12 and 27 February 2007 at Jocassee Gorges (JG 1 and JG 2, respectively). Different numbers of treatment plots were established at each location and were placed in the area to be burned (Burn) and in an adjacent area not burned (Control). Within each plot, seven aluminum-mesh packets of soil (100 ml) naturally infested with *P. cinnamomi* were placed at 2 cm and 10 cm beneath the soil surface. A baiting bioassay with camellia leaf pieces as baits was used to detect *P. cinnamomi* in the soil from each packet after the fires.

[b] Mean proportions of baits positive for *P. cinnamomi* per packet, averaged across treatment plots, were used in the ANOVA; *****=significant difference based on Fisher's protected least significant difference (*P*=0.05).

[c] HOBO dataloggers with temperature probes were used to record soil temperatures at both depths near the soil packets at GR and in each soil packet at JG 1 and JG 2.

Fig. 4.1. Representative temperature profiles at 2 and 10 cm beneath the soil surface from a burned plot (Burn) and a non-burned control plot (Con) during a prescribed fire in a hardwood forest at the Green River Game Land Management Area in western North Carolina.

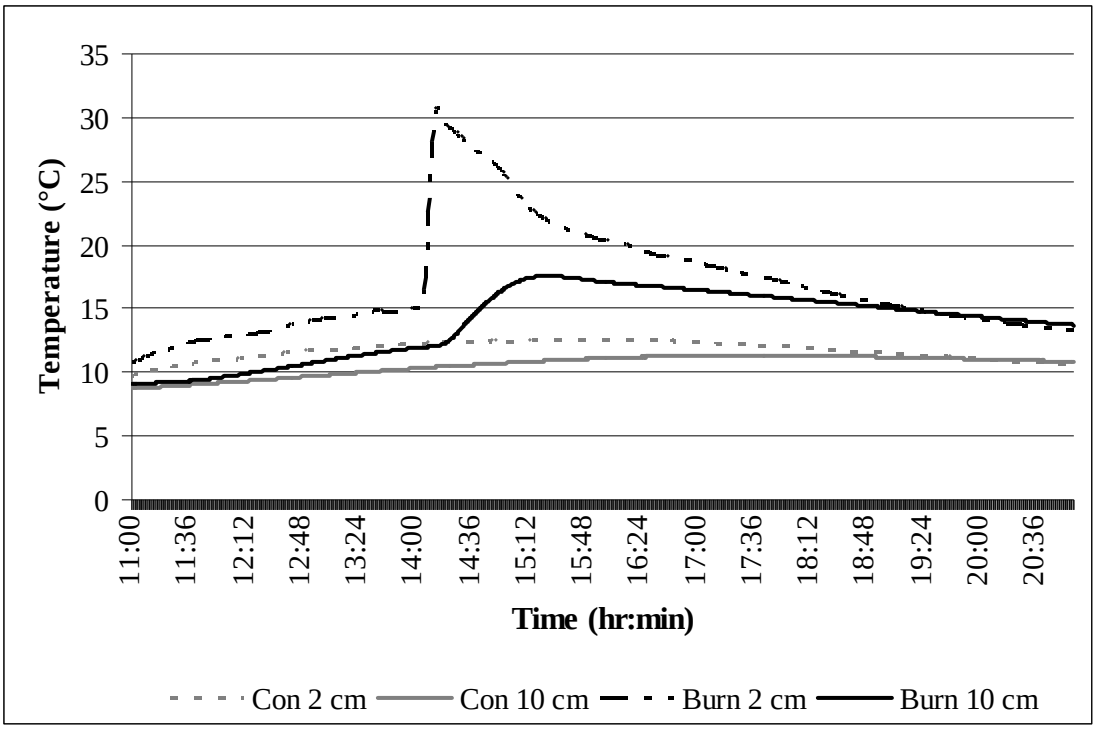
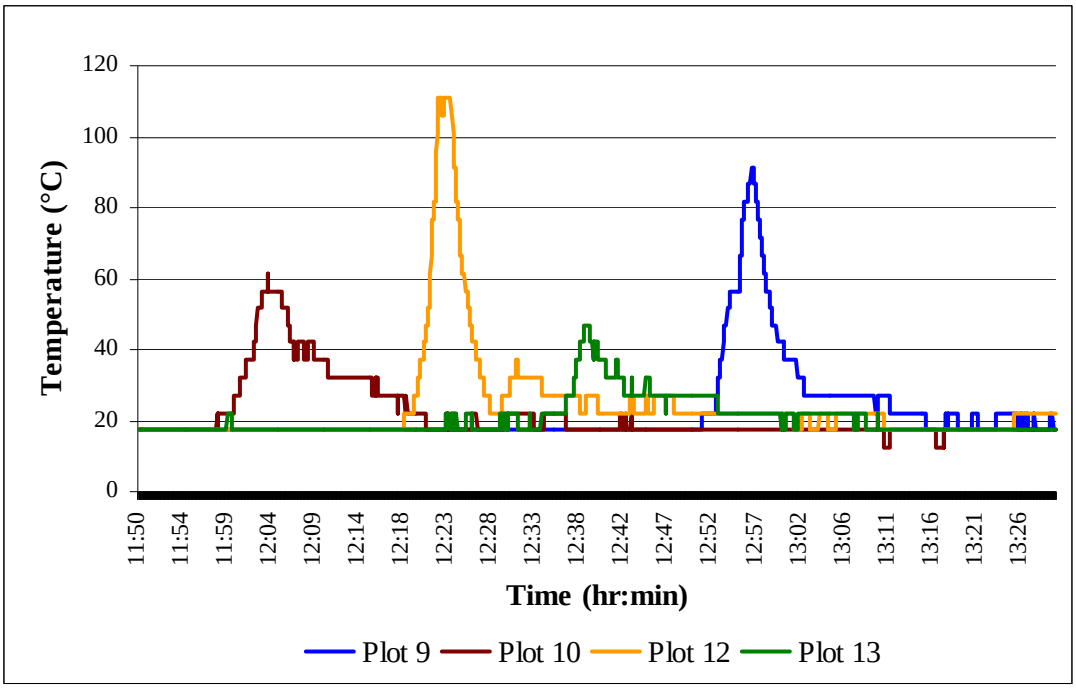

35
30
25
20
15
10
5
0
Temperature (°C)
11:00
11:36
12:12
12:48
13:24
14:00
14:36
15:12
15:48
16:24
17:00
17:36
18:12
18:48
19:24
20:00
20:36
Time (hr:min)
Con 2 cm
Con 10 cm
Burn 2 cm
Burn 10 cm

Fig. 4.2. Temperature profiles approximately 30 cm above the soil surface in four plots during a prescribed burn in a hardwood forest at the Jocassee Gorges Natural Area (site JG 2) in northwestern South Carolina.


120
100
80
60
40
20
0
Temperature (°C)
11:50
11:54
11:59
12:04
12:09
12:14
12:18
12:23
12:28
12:33
12:38
12:42
12:47
12:52
12:57
13:02
13:06
13:11
13:16
13:21
13:26
Time (hr:min)
Plot 9
Plot 10
Plot 12
Plot 13

CHAPTER 5

STUDY CONCLUSIONS

The overall objective of the USDI-USDA Joint Fire Science Program Fire and Fire Surrogate Study was to determine the effects of fuel reduction treatments (i.e., mechanical fuel reduction and prescribed burning) on several economic and ecological variables in fire-adapted forest ecosystems across the nation. This study is the first of its kind to attempt to compare effects of fuel reduction treatments over several years and across multiple study sites. Information collected from this study will be compiled and available to forest managers and scientists. Although this study has created a large amount of information, it is only the beginning in understanding the impacts of these forest management practices on the ecosystem as a whole.

The first two objectives of this project were to determine the effects of fuel reduction treatments on the incidence levels of two common root pathogens of forest trees in the southeastern United States: *Leptographium* spp. in a southern pine forest in the Piedmont of South Carolina and *Phytophthora* spp. in a hardwood forest in western North Carolina. Initial results were reported previously (Zwart 2004), and long-term effects (i.e., after 5 to 6 years) are reported here (Chapters 2 and 3). The third objective was to determine the direct effect of soil heating during prescribed fires on the persistence of *Phytophthora cinnamomi* in a hardwood forest soil.

Leptographium spp. appear to be widespread in roots of southern pine trees in the Clemson Experimental Forest, and, therefore, these fungi appear to be a normal component of southern pine forests. However, very little is known about the role of these wood-staining fungi in southern pine forests and their involvement in decline syndromes. After the initial application of fuel reduction, the incidence of *Leptographium* spp. in roots of southern pine trees in the Clemson Experimental Forest was less than the incidence before treatment application (Zwart 2004); however, these results should be viewed with caution because widespread tree mortality occurred in some treatment plots due to southern pine beetle infestation during the investigation. Over time, fuel reduction treatments did not affect the incidence of *Leptographium* spp.; more importantly, these treatments did not increase the incidence of these potentially damaging wood-staining fungi. This information could help forest managers to make decisions about the impact of fuel reduction treatments on tree health. Furthermore, there is a need to more fully understand how these treatments affect overall tree health and susceptibility to attack by insects and pathogens. Although this study investigated the effects of fuel reduction treatments over time, it would be valuable to continue to monitor this study site to determine effects over a longer period of time.

In this study, fuel reduction treatments did not affect the incidence of *Phytophthora* spp. in soil, initially and over the long-term. This study established the widespread distribution of *Phytophthora* spp. in the forests in the Green River Game Land Management Area. Little is known about the role of *Phytophthora* spp. in forests of the southern Appalachian Mountains; yet, these organisms—especially *P. cinnamomi*—

are widespread in forest soils of this region and continue to pose a threat to native vegetation. Therefore, it is important to continue to investigate the role of these oomycetes in the forests of this region. Results from this study will be useful for future research on the management of *Phytophthora* spp. in forest soils and can be of use to land-use managers in estimating the impacts of fuel reduction treatments on soilborne plant pathogens. Furthermore, it provides information on the natural distribution of *Phytophthora* spp. in the region.

The use of prescribed fire had little effect on the persistence of *P. cinnamomi* in soil in a hardwood forest in the southern Appalachian Mountains. Soil heating from low-intensity prescribed fire, like those used in this study, may reduce inoculum of this organism in the top layer of soil; however, this management practice is not sufficient to eradicate *Phytophthora* spp. from soil, and hotter fires would likely not meet burn objectives. Also, this study confirms that low-intensity prescribed fire in a hardwood forest has little effect on soil temperatures and, therefore, does not pose much threat to the survival of soilborne microorganisms and probably roots below the surface layer. Future research on soilborne *Phytophthora* spp. in forest soils should involve where these organisms survive in the soil profile and how indirect effects, such as changes in soil moisture or vegetation after multiple prescribed fires, may affect their distribution and persistence in soil.

APPENDICES

APPENDIX 1

Isolation of *Leptographium* Species from Roots of Southern Pine Trees in the Clemson Experimental Forest

Results from all attempts to isolate species of *Leptographium* from roots of southern pine trees in the Clemson Experimental Forest in 2006 are reported here. Four fuel reduction treatments were applied to plots in three replicate blocks (1 to 3): prescribed burning (Burn), thinning (Thin), thinning followed by prescribed burning (Thin+burn), and a non-treated control (Control). There were ten sub-plots in each treatment plot; two roots (A and B) on each of one to three live pine trees were sampled per sub-plot with an increment hammer. Ten disks from each root were placed on 1.25% malt extract agar amended with 200 mg/liter of cycloheximide. These data are summarized and discussed in Chapter 2.

Appendix 1. (continued)

Block	Treatment	Sub-plot	Tree no.	*Pinus* spp.	No. root disks with *Leptographium* spp.	
					Root A	Root B
1	Burn	1	962	*P. taeda*	0	0
			944	*P. taeda*	0	0
			934	*P. taeda*	0	0
		5	969	*P. taeda*	0	0
			970	*P. taeda*	0	0
			979	*P. taeda*	0	0
		9	835	*P. taeda*	0	0
			843	*P. taeda*	0	0
			857	*P. taeda*	7	10
		13	882	*P. taeda*	4	8
			884	*P. taeda*	5	2
			891	*P. taeda*	0	1
		17	750	*P. taeda*	0	0
			745	*P. taeda*	4	7
			735	*P. taeda*	0	0
		21	784	*P. taeda*	7	7
			788	*P. taeda*	3	1
			795	*P. taeda*	0	0
		25	462	*P. taeda*	2	4
			465	*P. taeda*	3	3
			469	*P. taeda*	0	0
		29	68	*P. taeda*	1	0
			59	*P. taeda*	0	0
			53	*P. taeda*	0	0
		32	47	*P. taeda*	3	1
			492	*P. taeda*	0	0
			496	*P. taeda*	0	0
		36	76	*P. taeda*	0	0
			83	*P. taeda*	0	0
			85	*P. taeda*	6	2
	Control	2	1642	*P. taeda*	0	0
			1639	*P. virginiana*	0	0
			1635	*P. virginiana*	0	0
		6	1678	*P. taeda*	0	0
			1684	*P. virginiana*	0	0
			1682	*P. virginiana*	0	0
		10	65	*P. taeda*	0	0
			63	*P. taeda*	0	0
			51	*P. taeda*	0	0
		14	1152	*P. taeda*	0	0
			1150	*P. taeda*	0	0
			1149	*P. virginiana*	0	0
		18	1164	*P. virginiana*	0	0

Appendix 1. (continued)

Block	Treatment	Sub-plot	Tree no.	*Pinus* spp.	No. root disks with *Leptographium* spp.	
					Root A	Root B
			1166	*P. taeda*	0	0
			1168	*P. taeda*	0	0
		22	1257	*P. taeda*	0	0
			1248	*P. taeda*	0	0
			1246	*P. taeda*	0	0
		26	1889	*P. taeda*	0	0
			1899	*P. taeda*	0	0
			1890	*P. taeda*	0	0
		30	1820	*P. taeda*	0	0
			1824	*P. taeda*	0	0
			1833	*P. taeda*	0	0
		34	1787	*P. virginiana*	0	0
			1766	*P. taeda*	0	0
			1767	*P. taeda*	0	0
		38	1230	*P. taeda*	0	0
			1223	*P. taeda*	0	0
			1210	*P. taeda*	0	0
	Thin	6	102	*P. taeda*	0	0
			111	*P. taeda*	0	0
			130	*P. taeda*	0	0
		10	971	*P. taeda*	3	4
			186	*P. taeda*	0	0
		14	57	*P. taeda*	0	0
			59	*P. taeda*	0	0
			60	*P. taeda*	1	0
		18	21	*P. taeda*	8	4
			16	*P. taeda*	1	1
			17	*P. taeda*	0	0
		22	83	*P. taeda*	0	0
			84	*P. taeda*	0	0
			34	*P. taeda*	1	2
		26	565	*P. taeda*	7	4
			569	*P. taeda*	7	3
			571	*P. taeda*	3	0
		30	94	*P. taeda*	8	0
			96	*P. taeda*	2	8
			299	*P. taeda*	0	0
		34	1056	*P. taeda*	0	2
		38	1077	*P. taeda*	0	0
			1078	*P. taeda*	0	0
			1080	*P. taeda*	0	0
	Thin+burn	2	278	*P. taeda*	0	0
			480	*P. taeda*	0	0
			492	*P. taeda*	0	0

Appendix 1. (continued)

Block	Treatment	Sub-plot	Tree no.	*Pinus* spp.	No. root disks with *Leptographium* spp.	
					Root A	Root B
		6	716	*P. taeda*	0	0
			713	*P. taeda*	0	0
			734	*P. taeda*	0	0
		10	53	*P. taeda*	0	3
			55	*P. taeda*	0	0
			61	*P. taeda*	0	10
		12	56	*P. taeda*	0	0
			1255	*P. taeda*	0	0
			62	*P. taeda*	0	0
		16	803	*P. taeda*	0	0
		20	956	*P. taeda*	0	10
			957	*P. taeda*	0	0
			955	*P. taeda*	4	10
		24	995	*P. taeda*	0	0
			993	*P. taeda*	0	0
			978	*P. taeda*	0	0
		28	866	*P. taeda*	0	0
			864	*P. taeda*	0	0
			865	*P. taeda*	0	0
		32	699	*P. taeda*	0	0
			698	*P. taeda*	0	1
			694	*P. taeda*	9	10
		36	76	*P. taeda*	7	2
			74	*P. taeda*	10	8
			839	*P. taeda*	0	7
2	Burn	2	98	*P. virginiana*	0	1
			87	*P. virginiana*	0	2
			603	*P. taeda*	0	0
		6	629	*P. taeda*	0	0
			650	*P. taeda*	0	0
			656	*P. taeda*	0	0
		10	671	*P. virginiana*	0	7
			677	*P. taeda*	0	0
			683	*P. taeda*	0	0
		14	1441	*P. taeda*	3	0
			48	*P. taeda*	0	0
			56	*P. echinata*	1	0
		18	71	*P. taeda*	0	0
			76	*P. taeda*	0	0
			85	*P. taeda*	0	0
		22	55	*P. taeda*	0	0
			51	*P. taeda*	0	0
			53	*P. taeda*	0	0
		26	58	*P. taeda*	0	0

Appendix 1. (continued)

Block	Treatment	Sub-plot	Tree no.	*Pinus* spp.	No. root disks with *Leptographium* spp.	
					Root A	Root B
			57	*P. taeda*	0	0
			50	*P. taeda*	0	0
		30	68	*P. taeda*	0	0
			69	*P. taeda*	0	0
			66	*P. taeda*	0	0
		34	67	*P. echinata*	0	0
			65	*P. taeda*	1	0
			3453	*P. virginiana*	0	0
		38	100	*P. taeda*	0	0
			39	*P. taeda*	0	0
			41	*P. taeda*	0	0
	Control	2	531	*P. taeda*	0	0
		14	582	*P. virginiana*	0	0
			585	*P. virginiana*	0	0
		18	839	*P. taeda*	0	0
			822	*P. taeda*	0	0
			824	*P. taeda*	0	0
		22	891	*P. taeda*	0	0
		26	937	*P. taeda*	4	3
			922	*P. taeda*	0	4
		30	946	*P. taeda*	0	0
			952	*P. taeda*	0	0
			953	*P. taeda*	0	0
		34	701	*P. taeda*	0	0
			704	*P. taeda*	0	0
			717	*P. taeda*	0	0
		38	755	*P. taeda*	0	0
			756	*P. taeda*	0	0
			758	*P. taeda*	0	0
	Thin	2	214	*P. taeda*	0	0
			204	*P. virginiana*	0	1
			210	*P. virginiana*	1	0
		6	254	*P. echinata*	0	0
			249	*P. virginiana*	0	0
			1796	*P. echinata*	0	0
		10	278	*P. echinata*	0	0
			285	*P. echinata*	0	0
			296	*P. virginiana*	0	0
		14	142	*P. echinata*	0	0
			152	*P. echinata*	0	0
			153	*P. virginiana*	0	0
		18	194	*P. echinata*	0	0
			191	*P. echinata*	0	0
			199	*P. taeda*	0	0

Appendix 1. (continued)

Block	Treatment	Sub-plot	Tree no.	*Pinus* spp.	No. root disks with *Leptographium* spp.	
					Root A	Root B
		22	338	*P. virginiana*	0	0
			340	*P. virginiana*	0	0
			334	*P. echinata*	0	0
		26	381	*P. virginiana*	0	0
			374	*P. echinata*	0	0
			372	*P. echinata*	0	0
		30	440	*P. virginiana*	0	0
			439	*P. virginiana*	0	0
			433	*P. virginiana*	7	0
		34	484	*P. virginiana*	0	0
			482	*P. virginiana*	0	0
			481	*P. virginiana*	0	0
		38	501	*P. virginiana*	0	0
			502	*P. virginiana*	0	0
			505	*P. virginiana*	0	0
	Thin+burn	2	64	*P. echinata*	0	0
			50	*P. echinata*	0	0
			5	*P. virginiana*	0	0
		6	113	*P. echinata*	0	0
			132	*P. echinata*	0	0
			142	*P. echinata*	0	4
		10	161	*P. virginiana*	0	0
			175	*P. virginiana*	0	0
			179	*P. echinata*	1	0
		14	283	*P. virginiana*	0	0
			282	*P. virginiana*	0	0
			284	*P. virginiana*	0	0
		18	102	*P. virginiana*	0	0
			123	*P. virginiana*	0	0
			121	*P. virginiana*	0	1
		22	414	*P. echinata*	0	0
			455	*P. echinata*	0	0
			459	*P. virginiana*	0	0
		26	497	*P. virginiana*	0	0
			499	*P. virginiana*	0	0
			341	*P. taeda*	2	0
		30	281	*P. echinata*	0	0
			269	*P. virginiana*	0	0
			274	*P. echinata*	0	0
		34	407	*P. virginiana*	0	0
			558	*P. virginiana*	0	0
			549	*P. virginiana*	0	0
		38	435	*P. virginiana*	0	0
			547	*P. virginiana*	0	0

Appendix 1. (continued)

Block	Treatment	Sub-plot	Tree no.	*Pinus* spp.	No. root disks with *Leptographium* spp.	
					Root A	Root B
			373	*P. virginiana*	3	0
3	Burn	2	61	*P. taeda*	0	1
			54	*P. taeda*	0	0
			47	*P. taeda*	1	0
		6	70	*P. taeda*	0	0
			76	*P. taeda*	5	0
			83	*P. taeda*	0	0
		10	77	*P. taeda*	0	0
			82	*P. taeda*	1	0
			90	*P. taeda*	0	0
		14	76	*P. taeda*	0	0
			70	*P. taeda*	0	0
			95	*P. taeda*	0	0
		18	42	*P. taeda*	0	2
			43	*P. taeda*	10	6
			58	*P. taeda*	0	0
		22	97	*P. taeda*	1	7
			94	*P. taeda*	10	10
			92	*P. taeda*	8	10
		26	287	*P. taeda*	0	0
			284	*P. taeda*	0	0
			81	*P. taeda*	0	0
		30	392	*P. taeda*	8	0
			393	*P. taeda*	0	0
			1289	*P. taeda*	9	4
		34	298	*P. taeda*	0	0
			299	*P. taeda*	0	6
			384	*P. taeda*	1	0
		38	100	*P. taeda*	7	10
			98	*P. taeda*	1	6
			93	*P. taeda*	0	0
	Control	2	900	*P. taeda*	0	0
			898	*P. virginiana*	6	5
			895	*P. virginiana*	0	0
		6	497	*P. taeda*	0	0
			995	*P. taeda*	0	0
			992	*P. taeda*	0	0
		10	117	*P. taeda*	0	0
			114	*P. taeda*	0	0
			113	*P. taeda*	0	0
		14	128	*P. taeda*	4	0
			129	*P. taeda*	0	0
			132	*P. taeda*	0	2
		18	155	*P. taeda*	0	0

Appendix 1. (continued)

Block	Treatment	Sub-plot	Tree no.	*Pinus* spp.	No. root disks with *Leptographium* spp.	
					Root A	Root B
			158	*P. taeda*	0	0
			165	*P. taeda*	0	0
		22	210	*P. taeda*	0	0
			211	*P. taeda*	0	0
			217	*P. taeda*	0	0
		26	264	*P. taeda*	1	0
			256	*P. taeda*	1	0
			252	*P. taeda*	0	0
		30	289	*P. taeda*	0	0
			287	*P. taeda*	0	0
			284	*P. taeda*	0	0
		34	1898	*P. taeda*	0	0
			793	*P. taeda*	5	9
			697	*P. taeda*	10	1
		38	533	*P. taeda*	0	2
			529	*P. taeda*	0	0
			524	*P. taeda*	0	0
	Thin	2	565	*P. taeda*	0	0
			555	*P. taeda*	2	9
			546	*P. taeda*	0	0
		6	573	*P. taeda*	0	0
			575	*P. taeda*	0	0
			578	*P. taeda*	0	0
		14	364	*P. taeda*	0	0
			368	*P. taeda*	0	0
		18	375	*P. taeda*	0	0
			370	*P. taeda*	0	0
		26	969	*P. taeda*	0	0
			963	*P. taeda*	0	0
			957	*P. taeda*	0	0
		30	999	*P. taeda*	0	0
			993	*P. taeda*	0	0
			406	*P. taeda*	0	0
		38	301	*P. taeda*	0	0
	Thin+burn	2	705	*P. taeda*	0	5
			712	*P. taeda*	0	0
			724	*P. taeda*	0	0
		6	400	*P. taeda*	0	0
			789	*P. taeda*	0	1
			781	*P. taeda*	0	0
		10	842	*P. taeda*	0	0
			835	*P. taeda*	0	0
			824	*P. taeda*	0	0
		14	878	*P. echinata*	0	0

Appendix 1. (continued)

Block	Treatment	Sub-plot	Tree no.	*Pinus* spp.	No. root disks with *Leptographium* spp.	
					Root A	Root B
			880	*P. echinata*	0	0
			885	*P. echinata*	0	0
		18	9	*P. virginiana*	0	0
			15	*P. virginiana*	0	0
			13	*P. echinata*	4	0
		22	67	*P. virginiana*	0	1
			64	*P. virginiana*	0	0
			70	*P. virginiana*	10	5
		26	904	*P. taeda*	0	0
			908	*P. taeda*	0	0
			911	*P. taeda*	0	0
		30	974	*P. taeda*	0	0
			975	*P. taeda*	0	0
			973	*P. taeda*	0	0
		34	607	*P. virginiana*	0	0
			615	*P. virginiana*	0	2
			616	*P. virginiana*	0	0
		38	649	*P. virginiana*	1	1
			657	*P. virginiana*	2	0
			658	*P. virginiana*	0	0

APPENDIX 2

Isolates of *Leptographium* Species Recovered from Roots of Southern Pine Trees in the Clemson Experimental Forest

The following table is a list of representative isolates of species of *Leptographium* that were recovered from two lateral roots (A and B) of southern pine trees in the Clemson Experimental Forest in 2006 after fuel reduction treatments had been applied. Four treatments were applied to plots in three replicate blocks (1 to 3): prescribed burning (Burn), thinning (Thin), thinning followed by prescribed burning (Thin+burn), and a non-treated control (Control). There were ten sub-plots in each treatment plot; two roots on each of one to three live pine trees were sample per sub-plot. Identifications of representative isolates were confirmed by an expert (L.G. Eckhardt, School of Forestry and Wildlife Sciences, Auburn University, Auburn, AL). Some isolates were found to contain a mixture of species. All isolates are stored on 2% malt extract agar in 8-ml glass vials at 4°C in the dark. These data are summarized and discussed in Chapter 2.

Appendix 2. (continued)

Isolate no.	*Leptographium* spp.	Block	Treatment	Sub-plot	Tree no.	Host (*Pinus* spp.)	Date collected
1B-857-1A	*L. terebrantis*	1	Burn	9	857	*P. taeda*	May-06
1B-857-1B	*L. terebrantis*	1	Burn	9	857	*P. taeda*	May-06
1B-857-2A	*L. terebrantis, L. huntii*	1	Burn	9	857	*P. taeda*	May-06
1B-857-2B	*L. procerum*	1	Burn	9	857	*P. taeda*	May-06
1B-882-1B	*L. procerum*	1	Burn	13	882	*P. taeda*	May-06
1B-882-2A	*L. procerum*	1	Burn	13	882	*P. taeda*	May-06
1B-882-2B	*L. procerum*	1	Burn	13	882	*P. taeda*	May-06
1B-884-1A	*L. terebrantis*	1	Burn	13	884	*P. taeda*	May-06
1B-884-1B	*L. huntii*	1	Burn	13	884	*P. taeda*	May-06
1B-884-2A	*L. terebrantis*	1	Burn	13	884	*P. taeda*	May-06
1B-884-2B	*L. terebrantis*	1	Burn	13	884	*P. taeda*	May-06
1B-891-1B	*L. terebrantis*	1	Burn	13	891	*P. taeda*	May-06
1B-745-1A	*L. procerum*	1	Burn	17	745	*P. taeda*	May-06
1B-745-2A	*L. procerum*	1	Burn	17	745	*P. taeda*	May-06
1B-745-2B	*L. procerum*	1	Burn	17	745	*P. taeda*	May-06
1B-784-1A	*L. terebrantis*	1	Burn	21	784	*P. taeda*	May-06
1B-784-1B	*L. procerum, L. terebrantis*	1	Burn	21	784	*P. taeda*	May-06
1B-784-2A	*L. procerum, L. terebrantis*	1	Burn	21	784	*P. taeda*	May-06
1B-784-2B	*L. procerum*	1	Burn	21	784	*P. taeda*	May-06
1B-788-1B	*L. procerum*	1	Burn	21	788	*P. taeda*	May-06
1B-788-2A	*L. procerum, L. terebrantis*	1	Burn	21	788	*P. taeda*	May-06
1B-462-1B	*L. procerum*	1	Burn	25	462	*P. taeda*	May-06
1B-462-2A	*L. procerum*	1	Burn	25	462	*P. taeda*	May-06
1B-465-1A	*L. procerum*	1	Burn	25	465	*P. taeda*	May-06
1B-465-1B	*L. procerum, L. terebrantis*	1	Burn	25	465	*P. taeda*	May-06
1B-465-2A	*L. terebrantis*	1	Burn	25	465	*P. taeda*	May-06
1B-465-2B	*L. procerum, L. terebrantis*	1	Burn	25	465	*P. taeda*	May-06
1B-68-1A	*L. procerum, L. huntii*	1	Burn	29	68	*P. taeda*	May-06
1B-47-1A	*L. terebrantis*	1	Burn	32	47	*P. taeda*	May-06
1B-47-1B	*L. terebrantis*	1	Burn	32	47	*P. taeda*	May-06
1B-85-1A	*L. procerum, L. terebrantis*	1	Burn	36	85	*P. taeda*	May-06
1B-85-1B	*L. procerum*	1	Burn	36	85	*P. taeda*	May-06
1B-85-2A	*L. terebrantis*	1	Burn	36	85	*P. taeda*	May-06
1B-85-2B	*L. procerum, L. terebrantis, L. huntii*	1	Burn	36	85	*P. taeda*	May-06
1TB-53-1B	*L. terebrantis*	1	Thin+burn	10	53	*P. taeda*	Jun-06
1TB-53-2B	*L. terebranits*	1	Thin+burn	10	53	*P. taeda*	Jun-06
1TB-61-1B	*L. serpens*	1	Thin+burn	10	61	*P. taeda*	Jun-06
1TB-956-1B	*L. huntii*	1	Thin+burn	20	956	*P. taeda*	Jun-06
1TB-956-2B	*L. huntii*	1	Thin+burn	20	956	*P. taeda*	Jun-06
1TB-955-1A	*L. procerum*	1	Thin+burn	20	955	*P. taeda*	Jun-06
1TB-955-1B-a	*L. procerum, L. terebrantis*	1	Thin+burn	20	955	*P. taeda*	Jun-06
1TB-955-1B-b	*L. procerum, L. terebrantis*	1	Thin+burn	20	955	*P. taeda*	Jun-06
1TB-698-1B	*L. procerum*	1	Thin+burn	32	698	*P. taeda*	Jun-06
1TB-694-1A	*L. procerm, L. terebrantis*	1	Thin+burn	32	698	*P. taeda*	Jun-06

Appendix 2. (continued)

Isolate no.	*Leptographium* spp.	Block	Treatment	Sub-plot	Tree no.	Host (*Pinus* spp.)	Date collected
1TB-694-1B	*L. huntii*	1	Thin+burn	32	698	*P. taeda*	Jun-06
1TB-76-1B	*Leptographium* sp.	1	Thin+burn	36	76	*P. taeda*	Jun-06
1TB-76-2A	*L. terebrantis*	1	Thin+burn	36	76	*P. taeda*	Jun-06
1TB-74-1A	*L. terebrantis, L. procerum*	1	Thin+burn	36	74	*P. taeda*	Jun-06
1TB-74-1B	*L. terebrantis*	1	Thin+burn	36	74	*P. taeda*	Jun-06
1TB-839-1B	*L. terebrantis*	1	Thin+burn	36	839	*P. taeda*	Jun-06
1TB-839-2B	*L. terebrantis*	1	Thin+burn	36	839	*P. taeda*	Jun-06
1T-971-1A	*L. terebrantis, L. procerum*	1	Thin	10	971	*P. taeda*	Jun-06
1T-971-1B	*L. procerum, L. huntii*	1	Thin	10	971	*P. taeda*	Jun-06
1T-971-2B	*L. procerum*	1	Thin	10	971	*P. taeda*	Jun-06
1T-60-1A	*L. terebrantis, L. procerum*	1	Thin	14	60	*P. taeda*	Jun-06
1T-21-1A	*L. procerum, L. terebrantis*	1	Thin	18	21	*P. taeda*	Jun-06
1T-21-1B	*L. terebrantis*	1	Thin	18	21	*P. taeda*	Jun-06
1T-21-2A	*L. terebranits, L. procerum*	1	Thin	18	21	*P. taeda*	Jun-06
1T-21-2B	*L. procerum*	1	Thin	18	21	*P. taeda*	Jun-06
1T-16-1A	*L. procerum, L. terebrantis*	1	Thin	18	16	*P. taeda*	Jun-06
1T-16-2B	*L. terebrantis*	1	Thin	18	16	*P. taeda*	Jun-06
1T-34-1B	*L. terebrantis*	1	Thin	22	34	*P. taeda*	Jun-06
1T-34-2A	*L. procerum*	1	Thin	22	34	*P. taeda*	Jun-06
1T-34-2B	*L. terebrantis*	1	Thin	22	34	*P. taeda*	Jun-06
1T-565-1A	*L. procerum, L. huntii*	1	Thin	26	565	*P. taeda*	Jun-06
1T-565-1B	*L. terebrantis*	1	Thin	26	565	*P. taeda*	Jun-06
1T-565-2A	*L. procerum*	1	Thin	26	565	*P. taeda*	Jun-06
1T-565-2B	*L. procerum*	1	Thin	26	565	*P. taeda*	Jun-06
1T-569-1A	*L. terebrantis, L. huntii*	1	Thin	26	569	*P. taeda*	Jun-06
1T-569-1B	*L. terebrantis, L. procerum*	1	Thin	26	569	*P. taeda*	Jun-06
1T-569-2B	*L. terebrantis, L. huntii*	1	Thin	26	569	*P. taeda*	Jun-06
1T-571-1A	*L. procerum, L. huntii*	1	Thin	26	571	*P. taeda*	Jun-06
1T-571-2A	*L. terebrantis*	1	Thin	26	571	*P. taeda*	Jun-06
1T-94-1A	*L. procerum, L. terebranits*	1	Thin	30	94	*P. taeda*	Jun-06
1T-94-2A	*L. procerum, L. terebranits*	1	Thin	30	94	*P. taeda*	Jun-06
1T-96-1A	*L. terebrantis*	1	Thin	30	96	*P. taeda*	Jun-06
1T-96-1B	*L. terebrantis*	1	Thin	30	96	*P. taeda*	Jun-06
1T-96-2A	*L. terebrantis*	1	Thin	30	96	*P. taeda*	Jun-06
1T-96-2B	*L. terebranits, L. huntii*	1	Thin	30	96	*P. taeda*	Jun-06
1T-1056-1B	*L. terebrantis*	1	Thin	34	1056	*P. taeda*	Jun-06
2B-87-1B	*L. serpens*	2	Burn	2	87	*P. virginiana*	May-06
2B-671-1B	*L. terebrantis*	2	Burn	10	671	*P. virginiana*	May-06
2B-14-41-1A	*Leptographium* sp.	2	Burn	14	14 41	*P. taeda*	May-06
2B-56-1A	*L. terebrantis*	2	Burn	14	56	*P. echinata*	May-06
2TB-142-1B	*L. procerum*	2	Thin+burn	6	142	*P. echinata*	May-06
2TB-179-1A	*L. terebrantis*	2	Thin+burn	10	179	*P. echinata*	May-06
2TB-121-1B	*L. terebrantis, L. procerum*	2	Thin+burn	18	121	*P. virginiana*	May-06
2TB-341-1A	*L. procerum, L. terebrantis*	2	Thin+burn	26	341	*P. virginiana*	May-06
2TB-373-1A	*L. procerum, L. terebrantis*	2	Thin+burn	38	373	*P. virginiana*	May-06

Appendix 2. (continued)

Isolate no.	*Leptographium* spp.	Block	Treatment	Sub-plot	Tree no.	Host (*Pinus* spp.)	Date collected
2T-204-1B	*L. terebrantis, L. procerum*	2	Thin	2	204	*P. virginiana*	Jun-06
2T-210-1A	*L. procerum*	2	Thin	2	210	*P. virginiana*	Jun-06
2T-433-1A	*L. procerum, L. terebrantis*	2	Thin	30	433	*P. virginiana*	Jun-06
2C-937-1A	*L. terebrantis*	2	Control	26	937	*P. taeda*	May-06
2C-937-1B	*L. huntii, L. terebrantis*	2	Control	26	937	*P. taeda*	May-06
2C-937-2A	*L. procerum*	2	Control	26	937	*P. taeda*	May-06
2C-922-1B	*L. terebrantis, L. huntii*	2	Control	26	922	*P. taeda*	May-06
3B-61-1B	*L. terebrantis, L. huntii*	3	Burn	2	61	*P. taeda*	May-06
3B-47-1A	*L. procerum, L. huntii*	3	Burn	2	47	*P. taeda*	May-06
3B-6-76-1A	*L. procerum*	3	Burn	6	6 76	*P. taeda*	May-06
3B-6-76-2A	*L. procerum*	3	Burn	6	6 76	*P. taeda*	May-06
3B-82-1A	*L. procerum*	3	Burn	10	82	*P. taeda*	May-06
3B-42-1B	*L. procerum*	3	Burn	18	42	*P. taeda*	May-06
3B-43-1A	*L. procerum, L. huntii*	3	Burn	18	42	*P. taeda*	May-06
3B-43-1B	*L. procerum, L. huntii*	3	Burn	18	43	*P. taeda*	May-06
3B-43-2A	*L. procerum*	3	Burn	18	43	*P. taeda*	May-06
3B-43-2B	*L. procerum, L. terebrantis*	3	Burn	18	43	*P. taeda*	May-06
3B-97-1A	*L. terebrantis*	3	Burn	22	97	*P. taeda*	May-06
3B-97-1B	*L. procerum*	3	Burn	22	97	*P. taeda*	May-06
3B-97-2B-a	*L. procerum*	3	Burn	22	97	*P. taeda*	May-06
3B-97-2B-b	*L. huntii, L. terebrantis*	3	Burn	22	97	*P. taeda*	May-06
3B-94-1A	*L. terebrantis*	3	Burn	22	94	*P. taeda*	May-06
3B-94-1B	*L. procerum*	3	Burn	22	94	*P. taeda*	May-06
3B-94-2A	*L. terebrantis*	3	Burn	22	94	*P. taeda*	May-06
3B-94-2B	*L. procerum, L. terebrantis*	3	Burn	22	94	*P. taeda*	May-06
3B-92-1A	*L. procerum*	3	Burn	22	92	*P. taeda*	May-06
3B-92-1B	*L. terebrantis, L. procerum*	3	Burn	22	92	*P. taeda*	May-06
3B-92-2A	*L. procerum*	3	Burn	22	92	*P. taeda*	May-06
3B-392-1A	*L. terebrantis, L. procerum*	3	Burn	30	392	*P. taeda*	May-06
3B-392-2A	*L. procerum*	3	Burn	30	392	*P. taeda*	May-06
3B-1289-1A	*L. procerum, L. terebrantis*	3	Burn	30	1289	*P. taeda*	May-06
3B-1289-2A	*L. procerum, L. terebrantis*	3	Burn	30	1289	*P. taeda*	May-06
3B-1289-2B-a	*L. terebrantis*	3	Burn	30	1289	*P. taeda*	May-06
3B-1289-2B-b	*L. procerum*	3	Burn	30	1289	*P. taeda*	May-06
3B-299-1B	*L. procerum, L. terebrantis*	3	Burn	34	299	*P. taeda*	May-06
3B-299-2B	*L. procerum, L. terebrantis*	3	Burn	34	299	*P. taeda*	May-06
3B-384-1A	*L. procerum*	3	Burn	34	384	*P. taeda*	May-06
3B-100-1A	*L. procerum*	3	Burn	38	100	*P. taeda*	May-06
3B-100-1B	*L. procerum, L. terebrantis*	3	Burn	38	100	*P. taeda*	May-06
3B-100-2A	*L. terebrantis, L. huntii*	3	Burn	38	100	*P. taeda*	May-06
3B-100-2B	*L. terebrantis*	3	Burn	38	100	*P. taeda*	May-06
3B-98-1A	*L. procerum*	3	Burn	38	98	*P. taeda*	May-06
3B-98-1B	*L. procerum, L. terebrantis*	3	Burn	38	98	*P. taeda*	May-06
3B-98-2B	*L. terebrantis, L. procerum*	3	Burn	38	98	*P. taeda*	May-06
3TB-705-1B	*L. procerum*	3	Thin+burn	2	705	*P. taeda*	Jun-06

Appendix 2. (continued)

Isolate no.	*Leptographium* spp.	Block	Treatment	Sub-plot	Tree no.	Host (*Pinus* spp.)	Date collected
3TB-789-1B	*L. huntii*	3	Thin+burn	6	789	*P. taeda*	Jun-06
3TB-13-2A	*L. procerum, L. terebrantis*	3	Thin+burn	22	13	*P. virginiana*	Jun-06
3TB-67-1B	*L. terebrantis, L. truncatum*	3	Thin+burn	22	67	*P. virginiana*	Jun-06
3TB-70-2A	*L. procerum, L. terebrantis*	3	Thin+burn	22	70	*P. virginiana*	Jun-06
3TB-615-1B	*L. procerum, L. terebrantis*	3	Thin+burn	34	615	*P. virginiana*	Jun-06
3TB-657-1A	*L. procerum*	3	Thin+burn	38	657	*P. virginiana*	Jun-06
3TB-649-1A	*L. procerum*	3	Thin+burn	38	649	*P. virginiana*	Jun-06
3TB-649-1B	*L. huntii*	3	Thin+burn	38	649	*P. taeda*	Jun-06
3T-555-1B	*L. terebrantis, L. huntii*	3	Thin	2	555	*P. taeda*	May-06
3T-555-2A	*L. procerum*	3	Thin	2	555	*P. taeda*	May-06
3T-555-2B	*L. procerum, L. terebrantis*	3	Thin	2	555	*P. taeda*	May-06
3C-898-1A	*L. terebrantis*	3	Control	2	898	*P. taeda*	May-06
3C-898-1B	*L. procerum*	3	Control	2	898	*P. taeda*	May-06
3C-898-2A-a	*L. terebrantis, L. procerum, L. huntii*	3	Control	2	898	*P. taeda*	May-06
3C-898-2A-b	*L. terebrantis*	3	Control	2	898	*P. taeda*	May-06
3C-898-2B	*L. huntii, L. procerum*	3	Control	2	898	*P. taeda*	May-06
3C-128-1A	*L. terebrantis, L. huntii*	3	Control	14	128	*P. taeda*	May-06
3C-128-2A	*L. procerum, L. terebrantis*	3	Control	14	128	*P. taeda*	May-06
3C-132-1B-a	*L. procerum, L. terebrantis*	3	Control	14	132	*P. taeda*	May-06
3C-132-1B-b	*L. terebrantis*	3	Control	14	132	*P. taeda*	May-06
3C-264-1A	*L. procerum*	3	Control	26	264	*P. taeda*	May-06
3C-256-1A	*L. procerum, L. terebrantis*	3	Control	26	256	*P. taeda*	May-06
3C-793-1A	*L. terebrantis*	3	Control	34	793	*P. virginiana*	May-06
3C-793-1B-a	*L. terebrantis, L. procerum*	3	Control	34	79	*P. virginiana*	May-06
3C-793-1B-b	*L. terebrantis, L. procerum, L. truncatum*	3	Control	34	793	*P. virginiana*	May-06
3C-793-2A	*L. terebrantis*	3	Control	34	793	*P. virginiana*	May-06
3C-793-2B-a	*L. procerum, L. terebrantis*	3	Control	34	793	*P. virginiana*	May-06
3C-793-2B-b	*L. procerum*	3	Control	34	793	*P. virginiana*	May-06
3C-697-1A	*L. huntii, L. procerum*	3	Control	34	697	*P. taeda*	May-06
3C-697-1B	*L. terebrantis*	3	Control	34	697	*P. taeda*	May-06
3C-697-2A	*L. procerum, L. terebrantis*	3	Control	34	697	*P. taeda*	May-06
3C-533-1B	*L. procerum*	3	Control	38	533	*P. taeda*	May-06

APPENDIX 3

Isolation of *Phytophthora* Species from Forest Soil in the Green River Game Land Management Area in Western North Carolina

Results from all attempts to isolate *Phytophthora* spp. from soil in a hardwood forest in western North Carolina after fuel reduction treatments had been applied are reported here. Four treatments were applied to plots in three replicate blocks (1 to 3): prescribed burning (Burn), mechanical fuel reduction (Mech), mechanical fuel reduction followed by prescribed burning (Mech+burn), and a non-treated control (Control). A composite soil sample was collected in 10 sub-plots in each treatment plot, and three 100-ml soil sub-samples were assayed from each sub-plot. Each composite soil sub-sample was placed in a plastic freezer box, flooded, and baited with six camellia leaf disks (i.e., baits). Data in the table are reported as the number of baits from which *Phytophthora* spp. were recovered (No. + baits) out of six baits used per box, the species recovered, and isolate numbers of representative isolates (i.e., one from each Block/Treatment/Sub-plot combination) that were kept for future use. Isolates are stored on corn meal agar in 8-ml glass vials at 15° in the dark. These data are summarized and discussed in Chapter 3.

Appendix 3. (continued)

Block	Treatment	Sub-plot	Isolation box no.	No. + baits (*n*=6)	Species	Isolate no.	Date collected
1	Burn	2	1	0			
1	Burn	2	2	0			
1	Burn	2	3	0			
1	Burn	6	1	6	*P. cinnamomi*	IM.1B-6-1	Mar 2007
1	Burn	6	2	6	*P. cinnamomi*		
1	Burn	6	3	6	*P. cinnamomi*		
1	Burn	10	1	5	*P. cinnamomi*	IM.1B-10-1	Mar 2007
1	Burn	10	2	6	*P. cinnamomi*		
1	Burn	10	3	6	*P. cinnamomi*		
1	Burn	14	1	0			
1	Burn	14	2	0			
1	Burn	14	3	0			
1	Burn	18	1	0			
1	Burn	18	2	0			
1	Burn	18	3	0			
1	Burn	22	1	4	*P. cinnamomi*	IM.1B-22-1	Mar 2007
1	Burn	22	2	6	*P. cinnamomi*		
1	Burn	22	3	6	*P. cinnamomi*		
1	Burn	26	1	6	*P. cinnamomi*	IM.1B-26-1	Mar 2007
1	Burn	26	2	6	*P. cinnamomi*		
1	Burn	26	3	6	*P. cinnamomi*		
1	Burn	30	1	6	*P. cinnamomi*	IM.1B-30-1	Mar 2007
1	Burn	30	2	6	*P. cinnamomi*		
1	Burn	30	3	6	*P. cinnamomi*		
1	Burn	34	1	6	*P. cinnamomi*	IM.1B-34-1	Mar 2007
1	Burn	34	2	6	*P. cinnamomi*		
1	Burn	34	3	6	*P. cinnamomi*		
1	Burn	38	1	0			
1	Burn	38	2	0			
1	Burn	38	3	0			
1	Control	1	1	0			
1	Control	1	2	0			
1	Control	1	3	0			
1	Control	5	1	0			
1	Control	5	2	1	*P. heveae*	IM.1C-5-2	Mar 2007
1	Control	5	3	1	*P. heveae*		
1	Control	9	1	0			
1	Control	9	2	0			
1	Control	9	3	0			
1	Control	13	1	0			
1	Control	13	2	0			
1	Control	13	3	0			
1	Control	17	1	6	*P. cinnamomi*	IM.1C-17-1	Mar 2007

Appendix 3. (continued)

Block	Treatment	Sub-plot	Isolation box no.	No. + baits (*n*=6)	Species	Isolate no.	Date collected
1	Control	17	2	5	*P. cinnamomi*		
1	Control	17	3	6	*P. cinnamomi*		
1	Control	21	1	0			
1	Control	21	2	0			
1	Control	21	3	0			
1	Control	25	1	0			
1	Control	25	2	0			
1	Control	25	3	0			
1	Control	29	1	0			
1	Control	29	2	0			
1	Control	29	3	0			
1	Control	33	1	0			
1	Control	33	2	0			
1	Control	33	3	0			
1	Control	37	1	0			
1	Control	37	2	0			
1	Control	37	3	0			
1	Mech	2	1	0			
1	Mech	2	2	0			
1	Mech	2	3	0			
1	Mech	6	1	0			
1	Mech	6	2	0			
1	Mech	6	3	0			
1	Mech	10	1	0			
1	Mech	10	2	0			
1	Mech	10	3	0			
1	Mech	14	1	0			
1	Mech	14	2	0			
1	Mech	14	3	0			
1	Mech	18	1	6	*P. cinnamomi*	IM.1M-18-1	Mar 2007
1	Mech	18	2	0			
1	Mech	18	3	5	*P. cinnamomi*		
1	Mech	22	1	0			
1	Mech	22	2	0			
1	Mech	22	3	0			
1	Mech	26	1	5	*P. cinnamomi*	IM.1M-26-4	Mar 2007
1	Mech	26	2	0			
1	Mech	26	3	0			
1	Mech	30	1	6	*P. cinnamomi*	IM.1M-30-1	Mar 2007
1	Mech	30	2	6	*P. cinnamomi*		
1	Mech	30	3	6	*P. cinnamomi*		
1	Mech	34	1	4	*P. cinnamomi*	IM.1M-34-1	Mar 2007
1	Mech	34	2	3	*P. cinnamomi*		
1	Mech	34	3	0			

Appendix 3. (continued)

Block	Treatment	Sub-plot	Isolation box no.	No. + baits (*n*=6)	Species	Isolate no.	Date collected
1	Mech	38	1	0			
1	Mech	38	2	0			
1	Mech	38	3	0			
1	Mech+burn	2	1	0			
1	Mech+burn	2	2	0			
1	Mech+burn	2	3	0			
1	Mech+burn	6	1	0			
1	Mech+burn	6	2	0			
1	Mech+burn	6	3	0			
1	Mech+burn	10	1	0			
1	Mech+burn	10	2	0			
1	Mech+burn	10	3	4	*P. cinnamomi*	IM.1MB-10-3	Mar 2007
1	Mech+burn	14	1	6	*P. cinnamomi*	IM.1MB-14-1	Mar 2007
1	Mech+burn	14	2	6	*P. cinnamomi*		
1	Mech+burn	14	3	6	*P. cinnamomi*		
1	Mech+burn	18	1	6	*P. cinnamomi*	IM.1MB-18-1	Mar 2007
1	Mech+burn	18	2	6	*P. cinnamomi*		
1	Mech+burn	18	3	6	*P. cinnamomi*		
1	Mech+burn	22	1	6	*P. cinnamomi*	IM.1MB-22-1	Mar 2007
1	Mech+burn	22	2	0			
1	Mech+burn	22	3	2	*P. cinnamomi*		
1	Mech+burn	26	1	6	*P. cinnamomi*	IM.1MB-26-1	Mar 2007
1	Mech+burn	26	2	6	*P. cinnamomi*		
1	Mech+burn	26	3	6	*P. cinnamomi*		
1	Mech+burn	30	1	0			
1	Mech+burn	30	2	0			
1	Mech+burn	30	3	0			
1	Mech+burn	34	1	6	*P. cinnamomi*	IM.1MB-34-1	Mar 2007
1	Mech+burn	34	2	6	*P. cinnamomi*		
1	Mech+burn	34	3	6	*P. cinnamomi*		
1	Mech+burn	38	1	0			
1	Mech+burn	38	2	0			
1	Mech+burn	38	3	0			
2	Burn	2	1	1	*P. heveae*	IM.2B-2-3	Mar 2007
2	Burn	2	2	1	*P. heveae*		
2	Burn	2	3	3	*P. heveae*		
2	Burn	6	1	0			
2	Burn	6	2	0			
2	Burn	6	3	0			
2	Burn	10	1	0			
2	Burn	10	2	0			
2	Burn	10	3	0			
2	Burn	14	1	4	*P. cinnamomi*	IM.2B-14-1	Mar 2007
2	Burn	14	2	4	*P. cinnamomi*		

Appendix 3. (continued)

Block	Treatment	Sub-plot	Isolation box no.	No. + baits (*n*=6)	Species	Isolate no.	Date collected
2	Burn	14	3	6	*P. cinnamomi*		
2	Burn	18	1	4	*P. cinnamomi*	IM.2B-18-1	Mar 2007
2	Burn	18	2	0			
2	Burn	18	3	0			
2	Burn	22	1	6	*P. cinnamomi*	IM.2B-22-1	Mar 2007
2	Burn	22	2	6	*P. cinnamomi*		
2	Burn	22	3	6	*P. cinnamomi*		
2	Burn	26	1	3	*P. cinnamomi*	IM.2B-26-1	Mar 2007
2	Burn	26	2	6	*P. cinnamomi*		
2	Burn	26	3	2	*P. cinnamomi*		
2	Burn	30	1	0			
2	Burn	30	2	0			
2	Burn	30	3	0			
2	Burn	34	1	6	*P. cinnamomi*	IM.2B-34-1	Mar 2007
2	Burn	34	2	6	*P. cinnamomi*		
2	Burn	34	3	6	*P. cinnamomi*		
2	Burn	38	1	6	*P. cinnamomi*	IM.2B-38-1	Mar 2007
2	Burn	38	2	6	*P. cinnamomi*		
2	Burn	38	3	3	*P. cinnamomi*		
2	Control	2	1	0			
2	Control	2	2	0			
2	Control	2	3	0			
2	Control	6	1	0			
2	Control	6	2	0			
2	Control	6	3	0			
2	Control	10	1	0			
2	Control	10	2	0			
2	Control	10	3	0			
2	Control	14	1	0			
2	Control	14	2	0			
2	Control	14	3	0			
2	Control	18	1	0			
2	Control	18	2	0			
2	Control	18	3	0			
2	Control	22	1	0			
2	Control	22	2	4	*P. cinnamomi*	IM.2C-22-2	Apr 2007
2	Control	22	3	0			
2	Control	26	1	0			
2	Control	26	2	0			
2	Control	26	3	0			
2	Control	30	1	6	*P. cinnamomi*		
2	Control	30	2	6	*P. cinnamomi*	IM.2C-30-2	Apr 2007
2	Control	30	3	6	*P. cinnamomi*		
2	Control	34	1	6	*P. cinnamomi*	IM.2C-34-3	Apr 2007

Appendix 3. (continued)

Block	Treatment	Sub-plot	Isolation box no.	No. + baits (*n*=6)	Species	Isolate no.	Date collected
2	Control	34	2	6	*P. cinnamomi*		
2	Control	34	3	6	*P. cinnamomi*		
2	Control	38	1	6	*P. cinnamomi*	IM.2C-38-1	Apr 2007
2	Control	38	2	6	*P. cinnamomi*		
2	Control	38	3	4	*P. cinnamomi*		
2	Mech	2	1	2	*P. cinnamomi*	IM.2M-2-1	Mar 2007
2	Mech	2	2	0			
2	Mech	2	3	0			
2	Mech	6	1	6	*P. cinnamomi*	IM.2M-6-1	Mar 2007
2	Mech	6	2	6	*P. cinnamomi*		
2	Mech	6	3	6	*P. cinnamomi*		
2	Mech	10	1	6	*P. cinnamomi*	IM.2M-10-1	Mar 2007
2	Mech	10	2	6	*P. cinnamomi*		
2	Mech	10	3	6	*P. cinnamomi*		
2	Mech	14	1	6	*P. cinnamomi*	IM.2M-14-1	Mar 2007
2	Mech	14	2	6	*P. cinnamomi*		
2	Mech	14	3	6	*P. cinnamomi*		
2	Mech	18	1	6	*P. cinnamomi*	IM.2M-18-1	Mar 2007
2	Mech	18	2	6	*P. cinnamomi*		
2	Mech	18	3	6	*P. cinnamomi*		
2	Mech	22	1	6	*P. cinnamomi*	IM.2M-22-1	Apr 2007
2	Mech	22	2	6	*P. cinnamomi*		
2	Mech	22	3	6	*P. cinnamomi*		
2	Mech	26	1	6	*P. cinnamomi*	IM.2M-26-1	Apr 2007
2	Mech	26	2	6	*P. cinnamomi*		
2	Mech	26	3	6	*P. cinnamomi*		
2	Mech	30	1	0			
2	Mech	30	2	0			
2	Mech	30	3	0			
2	Mech	34	1	0			
2	Mech	34	2	0			
2	Mech	34	3	0			
2	Mech	38	1	0			
2	Mech	38	2	0			
2	Mech	38	3	0			
2	Mech+burn	2	1	0			
2	Mech+burn	2	2	0			
2	Mech+burn	2	3	0			
2	Mech+burn	6	1	0			
2	Mech+burn	6	2	0			
2	Mech+burn	6	3	0			
2	Mech+burn	10	1	0			
2	Mech+burn	10	2	0			
2	Mech+burn	10	3	0			

Appendix 3. (continued)

Block	Treatment	Sub-plot	Isolation box no.	No. + baits (*n*=6)	Species	Isolate no.	Date collected
2	Mech+burn	14	1	0			
2	Mech+burn	14	2	0			
2	Mech+burn	14	3	0			
2	Mech+burn	18	1	0			
2	Mech+burn	18	2	0			
2	Mech+burn	18	3	0			
2	Mech+burn	22	1	0			
2	Mech+burn	22	2	0			
2	Mech+burn	22	3	0			
2	Mech+burn	26	1	0			
2	Mech+burn	26	2	0			
2	Mech+burn	26	3	2	*P. cinnamomi*	IM.2MB-26-6	Mar 2007
2	Mech+burn	30	1	6	*P. cinnamomi*	IM.2MB-30-1	Mar 2007
2	Mech+burn	30	2	6	*P. cinnamomi*		
2	Mech+burn	30	3	6	*P. cinnamomi*		
2	Mech+burn	34	1	0			
2	Mech+burn	34	2	0			
2	Mech+burn	34	3	0			
2	Mech+burn	38	1	0			
2	Mech+burn	38	2	0			
2	Mech+burn	38	3	0			
3	Burn	2	1	6	*P. cinnamomi*	IM.3B-2-1	Mar 2007
3	Burn	2	2	6	*P. cinnamomi*		
3	Burn	2	3	6	*P. cinnamomi*		
3	Burn	6	1	6	*P. cinnamomi*	IM.3B-6-1	Mar 2007
3	Burn	6	2	6	*P. cinnamomi*		
3	Burn	6	3	6	*P. cinnamomi*		
3	Burn	10	1	3	*P. cinnamomi*	IM.3B-10-4	Mar 2007
3	Burn	10	2	0			
3	Burn	10	3	0			
3	Burn	14	1	6	*P. cinnamomi*	IM.3B-14-1	Mar 2007
3	Burn	14	2	4	*P. cinnamomi*		
3	Burn	14	3	6	*P. cinnamomi*		
3	Burn	18	1	6	*P. cinnamomi*	IM.3B-18-1	Mar 2007
3	Burn	18	2	6	*P. cinnamomi*		
3	Burn	18	3	6	*P. cinnamomi*		
3	Burn	22	1	6	*P. cinnamomi*	IM.3B-22-1	Mar 2007
3	Burn	22	2	6	*P. cinnamomi*		
3	Burn	22	3	6	*P. cinnamomi*		
3	Burn	26	1	6	*P. cinnamomi*	IM.3B-26-1	Mar 2007
3	Burn	26	2	0			
3	Burn	26	3	0			
3	Burn	30	1	6	*P. cinnamomi*	IM.3B-30-1	Mar 2007
3	Burn	30	2	6	*P. cinnamomi*		

Appendix 3. (continued)

Block	Treatment	Sub-plot	Isolation box no.	No. + baits (*n*=6)	Species	Isolate no.	Date collected
3	Burn	30	3	6	*P. cinnamomi*		
3	Burn	34	1	6	*P. cinnamomi*	IM.3B-34-1	Mar 2007
3	Burn	34	2	6	*P. cinnamomi*		
3	Burn	34	3	6	*P. cinnamomi*		
3	Burn	38	1	0			
3	Burn	38	2	6	*P. cinnamomi*	IM.3B-38-2	Mar 2007
3	Burn	38	3	6	*P. cinnamomi*		
3	Control	2	1	6	*P. cinnamomi*	IM.3C-2-1	Feb 2007
3	Control	2	2	5	*P. cinnamomi*		
3	Control	2	3	6	*P. cinnamomi*		
3	Control	6	1	6	*P. cinnamomi*	IM.3C-6-1	Feb 2007
3	Control	6	2	0			
3	Control	6	3	0			
3	Control	10	1	6	*P. cinnamomi*	IM.3C-10-1	Feb 2007
3	Control	10	2	6	*P. cinnamomi*		
3	Control	10	3	6	*P. cinnamomi*		
3	Control	14	1	6	*P. cinnamomi*	IM.3C-14-1	Feb 2007
3	Control	14	2	6	*P. cinnamomi*		
3	Control	14	3	6	*P. cinnamomi*		
3	Control	18	1	6	*P. cinnamomi*	IM.3C-18-1	Feb 2007
3	Control	18	2	6	*P. cinnamomi*		
3	Control	18	3	2	*P. cinnamomi*		
3	Control	22	1	0			
3	Control	22	2	0			
3	Control	22	3	0			
3	Control	26	1	6	*P. cinnamomi*	IM.3C-26-1	Feb 2007
3	Control	26	2	6	*P. cinnamomi*		
3	Control	26	3	6	*P. cinnamomi*		
3	Control	30	1	5	*P. cinnamomi*	IM.3C-30-1	Feb 2007
3	Control	30	2	0			
3	Control	30	3	4	*P. cinnamomi*		
3	Control	34	1	6	*P. cinnamomi*	IM.3C-34-1	Feb 2007
3	Control	34	2	6	*P. cinnamomi*		
3	Control	34	3	6	*P. cinnamomi*		
3	Control	38	1	4	*P. cinnamomi*	IM.3C-38-1	Feb 2007
3	Control	38	2	4	*P. cinnamomi*		
3	Control	38	3	0			
3	Mech	2	1	0			
3	Mech	2	2	0			
3	Mech	2	3	2	*P. cinnamomi*	IM.3M-2-3	Jan 2007
3	Mech	6	1	5	*P. cinnamomi*	IM.3M-6-1	Jan 2007
3	Mech	6	2	6	*P. cinnamomi*		
3	Mech	6	3	6	*P. cinnamomi*		
3	Mech	10	1	6	*P. cinnamomi*	IM.3M-10-1	Jan 2007

Appendix 3. (continued)

Block	Treatment	Sub-plot	Isolation box no.	No. + baits (*n*=6)	Species	Isolate no.	Date collected
3	Mech	10	2	6	*P. cinnamomi*		
3	Mech	10	3	6	*P. cinnamomi*		
3	Mech	14	1	0			
3	Mech	14	2	0			
3	Mech	14	3	0			
3	Mech	18	1	0			
3	Mech	18	2	0			
3	Mech	18	3	0			
3	Mech	22	1	6	*P. cinnamomi*	IM.3M-22-1	Jan 2007
3	Mech	22	2	6	*P. cinnamomi*		
3	Mech	22	3	6	*P. cinnamomi*		
3	Mech	26	1	6	*P. cinnamomi*	IM.3M-26-1	Jan 2007
3	Mech	26	2	6	*P. cinnamomi*		
3	Mech	26	3	6	*P. cinnamomi*		
3	Mech	30	1	6	*P. cinnamomi*	IM.3M-30-1	Jan 2007
3	Mech	30	2	6	*P. cinnamomi*		
3	Mech	30	3	6	*P. cinnamomi*		
3	Mech	34	1	0			
3	Mech	34	2	0			
3	Mech	34	3	0			
3	Mech	38	1	3	*P. cinnamomi*	IM.3M-38-1	Jan 2007
3	Mech	38	2	6	*P. cinnamomi*		
3	Mech	38	3	6	*P. cinnamomi*		
3	Mech+burn	2	1	6	*P. cinnamomi*	IM.3MB-2-1	Jan 2007
3	Mech+burn	2	2	6	*P. cinnamomi*		
3	Mech+burn	2	3	6	*P. cinnamomi*		
3	Mech+burn	6	1	0			
3	Mech+burn	6	2	0			
3	Mech+burn	6	3	0			
3	Mech+burn	10	1	0			
3	Mech+burn	10	2	0			
3	Mech+burn	10	3	0			
3	Mech+burn	14	1	6	*P. cinnamomi*	IM.3MB-14-1	Jan 2007
3	Mech+burn	14	2	4	*P. cinnamomi*		
3	Mech+burn	14	3	0			
3	Mech+burn	18	1	6	*P. cinnamomi*	IM.3MB-18-1	Jan 2007
3	Mech+burn	18	2	6	*P. cinnamomi*		
3	Mech+burn	18	3	4	*P. cinnamomi*		
3	Mech+burn	22	1	6	*P. cinnamomi*	IM.3MB-22-1	Jan 2007
3	Mech+burn	22	2	0			
3	Mech+burn	22	3	6	*P. cinnamomi*		
3	Mech+burn	26	1	6	*P. cinnamomi*	IM.3MB-26-1	Jan 2007
3	Mech+burn	26	2	6	*P. cinnamomi*		
3	Mech+burn	26	3	6	*P. cinnamomi*		

Appendix 3. (continued)

Block	Treatment	Sub-plot	Isolation box no.	No. + baits (*n*=6)	Species	Isolate no.	Date collected
3	Mech+burn	30	1	6	*P. cinnamomi*	IM.3MB-30-1	Jan 2007
3	Mech+burn	30	2	6	*P. cinnamomi*		
3	Mech+burn	30	3	0			
3	Mech+burn	34	1	5	*P. cinnamomi*	IM.3MB-34-1	Jan 2007
3	Mech+burn	34	2	1	*P. cinnamomi*		
3	Mech+burn	34	3	0			
3	Mech+burn	38	1	6	*P. cinnamomi*	IM.3MB-38-1	Jan 2007
3	Mech+burn	38	2	6	*P. cinnamomi*		
3	Mech+burn	38	3	6	*P. cinnamomi*		

APPENDIX 4

Persistence of *Phytophthora cinnamomi* in Soil at Two Depths After Prescribed Fire in a Hardwood Forest

Three fires were used to determine the direct effect of prescribed fire on the persistence of *P. cinnamomi* in soil. The first fire occurred in the Green River Game Land Management Area in western North Carolina (GR) on 1 March 2006, and the second and third burns occurred in the Jocassee Gorges Natural Area in western South Carolina (JG 1 and JG 2) on 12 and 27 February 2007, respectively. Plots were placed in the area to be burned (Burn) or in an adjacent area not burned (Control). In each plot, seven packets of soil naturally infested with *P. cinnamomi* were placed at 2 cm and 10 cm beneath the soil surface. After the burns, soil from each bag was placed in a 450-ml plastic freezer box, flooded with 200 ml of distilled water, and baited with camellia leaf disks (five disks per box for GR samples and six disks per box for JG 1 and JG 2 samples). Soil temperatures also were recorded during the fires using dataloggers with temperature probes. In GR, one datalogger was placed in each plot and two probes were placed at each depth in close proximity to the soil packets. At JG 1 and JG 2, a temperature probe was placed in each soil packet and attached to a datalogger. Maximum temperature is reported in this table; some temperature data are missing (-). Data are reported as the number of baits from which *P. cinnamomi* was recovered (Positive) out of the numbers of baits used (Total) per box. These data are summarized and discussed in Chapter 4.

Appendix 4. (continued)

Location	Treatment	Site	Depth (cm)	Packet	Number baits		Maximum temp. (°C)
					Positive	Total	
GR	Burn	A	2	1	5	5	
				2	0	5	50.8
				3	0	5	
				4	1	5	30.8
				5	4	5	
				6	5	5	
				7	5	5	
			10	1	5	5	
				2	5	5	17.6
				3	5	5	
				4	5	5	17.5
				5	5	5	
				6	5	5	
				7	5	5	
	Burn	B	2	1	5	5	
				2	0	5	
				3	5	5	36.7
				4	5	5	23.7
				5	5	5	
				6	5	5	
				7	5	5	
			10	1	5	5	
				2	5	5	
				3	5	5	16.9
				4	5	5	18.1
				5	5	5	
				6	5	5	
				7	5	5	
	Control	C	2	1	5	5	
				2	5	5	
				3	5	5	12.6
				4	5	5	-
				5	5	5	
				6	5	5	
				7	5	5	
			10	1	5	5	
				2	5	5	10.1
				3	5	5	
				4	5	5	-
				5	5	5	
				6	5	5	

Appendix 4. (continued)

Location	Treatment	Site	Depth (cm)	Packet	Number baits		Maximum temp. (°C)
					Positive	Total	
				7	5	5	
	Control	D	2	1	5	5	
				2	5	5	
				3	5	5	11.3
				4	5	5	
				5	5	5	12.1
				6	5	5	
				7	5	5	
			10	1	5	5	
				2	5	5	
				3	5	5	
				4	5	5	11.0
				5	5	5	10.3
				6	5	5	
				7	5	5	
JG1	Burn	1	2	1	6	6	14.8
				2	6	6	8.8
				3	6	6	12.8
				4	6	6	12.8
				5	6	6	8.8
				6	6	6	8.8
				7	6	6	8.8
			10	1	6	6	8.8
				2	6	6	8.8
				3	6	6	8.8
				4	6	6	8.8
				5	6	6	8.8
				6	6	6	10.8
				7	6	6	8.8
	Control	2	2	1	6	6	8.8
				2	6	6	-
				3	6	6	8.8
				4	6	6	8.8
				5	6	6	8.8
				6	6	6	6.6
				7	6	6	8.8
			10	1	5	6	6.8
				2	6	6	6.8
				3	6	6	8.8
				4	6	6	-
				5	6	6	6.8
				6	6	6	6.8
				7	6	6	6.8

Appendix 4. (continued)

Location	Treatment	Site	Depth (cm)	Packet	Number baits		Maximum temp. (°C)
					Positive	Total	
	Burn	3	2	1	5	6	16.6
				2	6	6	16.6
				3	6	6	14.8
				4	6	6	10.8
				5	6	6	-
				6	6	6	12.8
				7	6	6	16.6
			10	1	6	6	8.8
				2	5	6	8.8
				3	6	6	8.8
				4	6	6	8.8
				5	6	6	8.8
				6	6	6	8.8
				7	6	6	8.8
		4	2	1	6	6	22
				2	6	6	17.5
				3	6	6	17.5
				4	5	6	22
				5	6	6	16.6
				6	6	6	22
				7	0	6	42
			10	1	5	6	17.5
				2	6	6	17.5
				3	6	6	12.5
				4	6	6	17.8
				5	6	6	17.8
				6	6	6	16.6
				7	6	6	17.5
		5	2	1	6	6	12.8
			2	2	6	6	10.8
			2	3	6	6	10.8
			2	4	6	6	10.8
			2	5	6	6	10.8
			2	6	6	6	10.8
			2	7	5	6	8.8
			10	1	6	6	8.8
				2	6	6	8.8
				3	6	6	8.8
				4	6	6	8.8
				5	6	6	8.8
				6	6	6	8.8
				7	6	6	8.8
		6	2	1	5	6	14.8
				2	6	6	18.6

Appendix 4. (continued)

Location	Treatment	Site	Depth (cm)	Packet	Number baits		Maximum temp. (°C)
					Positive	Total	
				3	6	6	14.8
				4	6	6	0.8
				5	6	6	10.8
				6	6	6	10.8
				7	4	6	-
			10	1	6	6	8.8
				2	6	6	8.8
				3	6	6	10.8
				4	6	6	8.8
				5	6	6	10.8
				6	6	6	10.8
				7	6	6	10.8
		7	2	1	6	6	10.8
				2	6	6	8.8
				3	6	6	8.8
				4	6	6	12.8
				5	6	6	10.8
				6	6	6	10.8
				7	6	6	10.8
			10	1	6	6	8.8
				2	6	6	8.8
				3	6	6	8.8
				4	6	6	8.8
				5	6	6	8.8
				6	6	6	8.8
				7	6	6	8.8
	Control	8	2	1	6	6	8.8
				2	6	6	10.8
				3	6	6	10.8
				4	6	6	12.8
				5	6	6	8.8
				6	6	6	8.8
				7	6	6	10.8
			10	1	6	6	10.8
				2	6	6	12.8
				3	6	6	-
				4	6	6	14.8
				5	6	6	12.8
				6	6	6	12.8
				7	6	6	10.8
JG2	Burn	9	2	1	6	6	13.3
				2	6	6	13.0
				3	6	6	12.5
				4	6	6	12.5

Appendix 4. (continued)

Location	Treatment	Site	Depth (cm)	Packet	Number baits		Maximum temp. (°C)
					Positive	Total	
				5	6	6	12.6
				6	6	6	12.6
				7	6	6	13.2
			10	1	6	6	12.6
				2	6	6	13.3
				3	6	6	12.6
				4	6	6	11.4
				5	6	6	12.2
				6	6	6	14.1
				7	6	6	12.9
		10	2	1	6	6	19.4
				2	6	6	-
				3	6	6	15.6
				4	6	6	12.6
				5	6	6	14.5
				6	6	6	16.4
				7	6	6	12.6
			10	1	6	6	12.9
				2	6	6	12.9
				3	6	6	12.2
				4	6	6	12.9
				5	6	6	11.4
				6	6	6	11.4
				7	6	6	11.4
	Control	11	2	1	6	6	6.6
				2	6	6	8.6
				3	6	6	7.4
				4	6	6	-
				5	6	6	8.6
				6	6	6	8.6
				7	6	6	9.0
			10	1	6	6	8.2
				2	6	6	8.6
				3	6	6	8.2
				4	6	6	8.6
				5	6	6	8.2
				6	6	6	9.0
				7	6	6	9.0
	Burn	12	2	1	6	6	10.2
				2	6	6	10.2
				3	6	6	10.2
				4	6	6	10.2
				5	6	6	9.0
				6	6	6	9.0

Appendix 4. (continued)

Location	Treatment	Site	Depth (cm)	Packet	Number baits		Maximum temp. (°C)
					Positive	Total	
				7	6	6	11
			10	1	6	6	10.2
				2	6	6	9.4
				3	6	6	10.2
				4	6	6	10.2
				5	6	6	9.4
				6	6	6	9.0
				7	6	6	11.0
		13	2	1	6	6	10.6
				2	6	6	11.4
				3	6	6	11.4
				4	6	6	12.9
				5	6	6	-
				6	6	6	11.0
				7	6	6	12.16
			10	1	6	6	12.6
				2	6	6	13.3
				3	6	6	11.4
				4	6	6	12.6
				5	6	6	12.6
				6	6	6	12.2
				7	6	6	12.2