**Some Like it Hot: Fire and Legume Germination
in the Longleaf Pine Ecosystem**

by

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Abstract

Fire is a globally distributed disturbance and is important for structuring and maintaining the diversity of many frequently burned ecosystems. Competitive relationships and vegetation patterns may be affected by fire's influence on survival, reproduction, and recruitment of plants. Each fire is unique and the effects of a given fire are not necessarily uniform throughout the area burned. Within the southeastern United States, the once-vast longleaf pine (*Pinus palustris* Mill.) ecosystem has been reduced to a mere fraction of its former range by overharvesting and land conversion. This fire-dependent system is also home to one of the most diverse plant assemblages known in North America. The effectiveness of restoration and management of this imperiled system may be improved by increased knowledge of fire-related germination and recruitment responses for functionally important groups of plant species.

This project first investigated potential mechanisms of fire-induced germination and then sought to understand how fine-scale fire heterogeneity may influence germination of common native legume species. For the first part of the project, six legume species were used to examine the effects of exposure to various components of fire: 1) elevated temperatures (five levels with dry heat temperatures ranging from presumably non-lethal to lethal) over three durations; 2) duration (three intervals) of moist heat (i.e., steam); 3) mechanical scarification; and 4) smoke. Legume species used represented a range of seed hardness, varying from soft-seeded to extremely hard-seeded. Results indicate that response to both dry and moist heat was species-specific, but moist heat had the greatest potential to stimulate germination. The legumes studied

also exhibited high heat tolerance, which may be a function of their low seed moisture contents. Smoke had no detectable effect on germination of most species, but may have inhibited *Centrosema virginianum*.

Two studies were used for the second part of the project to examine post-fire germination of nine legume species in response to fuel variation in laboratory and field settings. For both studies, fine fuel loads (longleaf pine needles) and woody fuels (longleaf pine cones) were manipulated. Germination and mortality were assessed for six species after experimental laboratory fires. Germination of seven species was assessed for four months in the field following experimental burns of small plots. In both studies, exposure to fire reduced seed germination compared to unburned controls. Furthermore, germination of seeds that were under burned pine cones was reduced more than treatments burned with pine needles alone. Manipulation of biomass of fine fuel loads did not affect germination in either study.

Together, the results of all experiments indicate the potential influence of fire on germination and mortality of legume seeds. Such impacts may, in turn, affect recruitment of these and similar species, thereby influencing post-fire vegetation patterns. Additionally, a hypothetical model is presented that describes the potential influence of pine cones on post-fire seedling recruitment.

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List of Abbreviations

ANOVA   Analysis of Variance

CEVI   *Centrosema virginianum*

DECI   *Desmodium ciliare*

DEFL   *Desmodium floridanum*

LEAN   *Lespedeza angustifolia*

MIQU   *Mimosa quadrivalvis*

PECA   *Pediomelum canescens*

RCB   Randomized Complete Block

SD   Standard Deviation

SE   Standard Error

TEVI   *Tephrosia virginiana*

TZ   2,3,5-Triphenyl Tetrazolium Chloride

CHAPTER 1

INTRODUCTION

PROJECT OVERVIEW

Fire is an integral disturbance agent for many ecosystems around the world and a driver of vegetation distribution and diversity (Bond et al. 2005; Bond and Keeley 2005). Within these systems, fire's effects range from altered survival and reproduction of individuals to altered successional patterns (Bond and van Wilgen 1996; DeBano et al. 1998). Furthermore, each fire is unique and variation in fuel loads, types, and arrangement coupled with species-specific tolerances to fire creates a heterogeneous suite of conditions and possible responses to a given fire (Whelan 1995; Bond and van Wilgen 1996).

Frequent fire has played a critical role in the evolution, structure, and diversity of the longleaf pine (*Pinus palustris* Mill.) ecosystem of the southeastern United States (Frost 2006). Legumes are important components of this diverse ecosystem's groundcover, contributing to the system's overall floristic richness (Drew et al. 1998; Hainds et al. 1999), replenishing nitrogen lost during fires (Hendricks and Boring 1999; Hiers et al. 2003), and serving as valuable forage for a variety of game and non-game species (Yarrow and Yarrow 1999; Miller and Miller 2005). Fires have been demonstrated to stimulate germination of many leguminous species (reviewed in Baskin and Baskin 2004; Keeley and Fotheringham 2000) and, consequently, to influence legume germination and establishment in post-fire environments.

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This project examined post-fire germination responses of a suite of native legumes common to longleaf pine stands in Georgia's Gulf Coastal Plain. Potential mechanisms of fire-related germination cues were investigated, including elevated temperatures, moist vs. dry heat, mechanical scarification, and smoke. Additionally, germination responses to fire heterogeneity were studied by manipulating fuels prior to fires.

The following sections review literature pertinent to the role of fire on reproduction and recruitment of plants, physical seed dormancy in hard-seeded species, and heterogeneity of fuels and fires. Particular attention is paid to legumes within longleaf pine savannas.

LITERATURE REVIEW

Plant Populations

Fires affect plant populations in complex ways (Whelan 1995; Bond and van Wilgen 1996). Availability of resources (e.g., nutrients, water, light), individual survival, and reproduction may all be influenced by fires (Bond and van Wilgen 1996; DeBano et al. 1998); however, both direction (i.e., positive or negative) and magnitude of plant population responses to a given fire depend on numerous factors, such as season of burn, fire intensity, and species identity (Bond and van Wilgen 1996).

Fires may kill certain species, while favoring others. For instance, Mallik and Gimingham (1985) found that several English heathland shrubs differed in their abilities to survive and resprout following experimental fires. Similarly, individuals within each species may exhibit variable responses to fire depending on age or size. Moreno and Oechel (1991b, 1993) found that while resprouting of chamise (*Adenostoma fasciculatum* Hook. & Arn.) shrubs in

California chaparral was reduced following fires, large individuals were better able to survive and resprout following high intensity fires than small individuals.

Seedling recruitment may be influenced by seed availability, suitable microsite availability, or both (Eriksson and Ehrlen 1992; Setterfield 2002). Fires may increase the available seed pool following fires by increasing reproductive output. This phenomenon has been particularly well-documented within longleaf pine stands for numerous species including grasses (van Eerden 1997; Shepherd et al. *In Press*), legumes (Hiers et al. 2000), and other forb species (Platt et al. 1988a; Brewer and Platt 1994; Norden and Kirkman 2004). Seeds within the soil seed bank may also be stimulated to germinate by fires and, as previously noted for survival of mature individuals, seedling recruitment following fires may differ among species and within species (Bond and van Wilgen 1996). For example, Moreno and Oechel (1991a) used increased fuel loads to increase intensities of experimental fires in California chaparral. In that study, *Adenostoma fasciculatum* seedling recruitment decreased with increasing intensities, as did overall herbaceous recruitment; however, recruitment of the hard-seeded leguminous shrub *Lotus strigosus* (Nutt.) Greene increased with increasing intensities. Similarly, seedling recruitment of hard-seeded *Acacia* spp. was greater following high intensity fires than after low intensity fires in jarrah (*Eucalyptus marginata* Sm.) forests of Western Australia (Shea et al. 1979). Additionally, fires may create open sites suitable for seedling recruitment by killing potential competitors or removing litter (Bond and van Wilgen 1996). These open spaces also may have increased nutrients and water available for seedling use (Bond and van Wilgen 1996).

Germination and Physical Dormancy

Several factors associated with fire have been demonstrated to influence germination and survival of seeds. Fire-related germination cues that may operate in combination include, among

others, heat (Cushwa et al. 1968; Martin et al. 1975; Auld and O'Connell 1991), scarification of the seed coat (Teketay 1996), smoke (Clarke and French 2005; Lindon and Menges 2008), chemicals leached from charred plant material (Keeley 1987; Newton et al. 2006), and increased light (McPherson and Muller 1969; Christensen and Muller 1975; Keeley and Keeley 1987).

Hard seed coats occur in at least 15 plant families, including, for example, the Anacardiaceae, Cannaceae, Convolvulaceae, Cucurbitaceae, Geranicaceae, Fabaceae, Malvaceae, Rhamnaceae, and Sapindaceae (Baskin and Baskin 1998; Baskin et al. 2000). While hard-seededness is common in several families, not all species within these families have hard seeds (Keeley and Fotheringham 2000). Hard seed coats impose physical dormancy on embryos in seeds by reducing permeability of the seed coat to the fluids and gases that are required for germination (Ballard 1973; Baskin and Baskin 1998). Often, once physical dormancy has been broken, seeds will readily germinate unless secondary physiological dormancy (e.g., requiring specific temperature or light regimes to germinate) is also present (Baskin and Baskin 1998; Baskin et al. 2000). Such secondary physiological dormancy occurs in at least seven families, including the Fabaceae (Baskin et al. 2000). Dormant seeds allow for the formation of persistent seed banks that can replenish aboveground plant populations following an environmental disturbance, such as fire (Baskin and Baskin 1998). Heat from fires is thought to stimulate germination of many hard-seeded species by scarifying seeds (i.e., disrupting the seed coat and making it permeable to water and gases), thus breaking this physical dormancy (Baskin and Baskin 1998). Both dry heat and moist heat have been found to increase germination rates of hard-seeded species (Martin et al. 1975). Furthermore, duration of exposure to elevated temperatures may influence seed survival and germination (Levitt 1980).

Smoke-induced germination has been found in a wide variety of plant species worldwide (Keeley and Fotheringham 2000; Van Staden et al. 2000). Keeley and Fotheringham (2000) assert that smoke-induced germination may be non-existent in the Fabaceae. Smoke may, however, play a secondary role in germination once the seed coat has been disrupted by fire because compounds in smoke that act as potential cues may persist in the soil or on plant tissues following a fire's passage (Van Staden et al. 2000). Smoke is a complex substance and many attempts to isolate compounds that may influence germination have met with little success (Van Staden et al. 2000). Recently, however, two butenolide compounds have been isolated from smoke and found to have opposing effects on germination (Light et al. 2010). In their study of these butenolides, Light et al. (2010) found that 3-methyl-2*H*-furo[2,3-*c*]pyran-2-one (also called karrikin 1; Soós et al. 2010) promoted germination, while the related 3,4,5-trimethylfuran-2(5*H*)-one inhibited germination. Furthermore, it is likely that a variety of other compounds in smoke may also influence germination (Soós et al. 2010).

Fire Heterogeneity

Variations in fuel loads, types, and arrangement are important determinants of fire intensity (Whelan 1995; Bond and van Wilgen 1996), which in turn influences the ecological effects of a given fire (Kennard and Outcalt 2006). Within longleaf pine stands, pine needles are an abundant fuel and may represent over half of the fuel load (Ottmar and Vihanek 2000; Ottmar et al. 2003). Furthermore, longleaf pine needles have higher energy contents than other potential fuels (e.g., live woody plants, other leaf litter, live and dead herbaceous species [Robertson 2007]) possibly due to higher contents of terpenes, which are highly flammable (Ormeño et al. 2009). Increased fire intensities may result from this combination of high energy content and abundance of pine needle litter under pine canopies. In the Florida sandhills, oaks and juvenile

pines located near large longleaf pine trees suffered greater mortality during fires than those located farther away (Williamson and Black 1981; Rebertus et al. 1989a,b), presumably due to increased fire intensity associated with increased fuel accumulation beneath the pine canopies. Other studies in longleaf pine stands have found similarly increased fire intensities associated with increased fuel loads (Thaxton and Platt 2006; Jack et al. 2010).

In longleaf pine stands, the distribution of fuels is related to the distribution and density of pine trees (Platt et al. 1988b; Robertson 2005; Varner 2005; Mitchell et al. 2009), tending to increase concomitantly with stand basal area and proximity to mature trees. Fine fuels and coarse fuels from pine trees differ in their burning characteristics and may therefore differ in their ecological effects. In contrast to pine needles, which may burn for several minutes (Fonda 2001), pine cones may burn for well over half an hour (Fonda and Varner 2005). Furthermore, pine cones represent a potentially significant amount of fuel, with individual trees capable of producing >100 cones during a mast year (Boyer and White 1989; Mitchell et al. 2009), but the ecological effects of pine cones as fuel remain poorly understood (Fonda and Varner 2005). The impact of longleaf pine cones on fire effects is also likely greater following mast years, when pine cones are particularly abundant.

OBJECTIVES

The objectives of the research presented herein were to: 1) determine the species-specific effects of fire on legume seed germination and mortality using fire surrogates that represent environmental factors associated with exposure to fire (Chapter 2); and 2) examine the effects of variable fine fuel loads and the presence of longer burning fuels (pine cones) on the survivorship and germination of a suite of common legume species (Chapter 3). Specifically, in Chapter 2, six

legume species common to longleaf pine stands in southwestern Georgia were used to determine

the effects of the following treatments: 1) elevated temperatures (five levels with dry heat

temperatures ranging from presumably non-lethal to lethal) over various durations (three time

intervals); 2) duration (three intervals) of moist heat (i.e., steam); 3) physical scarification; and 4)

smoke. Chapter 3 presents two experiments: first, germination and survival of six legume species

following experimental fires were assessed in the laboratory; and second, post-fire germination

responses of seven legume species to fuel manipulations (both pine needles and pine cones) were

determined in the field.

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CHAPTER 2

MECHANISMS OF FIRE-INDUCED GERMINATION AND SURVIVAL OF SIX LEGUME

SPECIES IN THE LONGLEAF PINE ECOSYSTEM

ABSTRACT

Fire is an important driver of community diversity and structure for ecosystems worldwide. Fires affect competitive relationships between and within species by influencing individual survival, growth, reproduction, and recruitment. Physical seed dormancy via an impermeable seed coat is a characteristic of many species, especially those found within fire-maintained ecosystems. In such fire prone sites, fire may enhance germination of these species. Understanding how fire-related factors affect germination can help predict fire regimes and predict how fire heterogeneity influences patterns of diversity within frequently burned systems. Six leguminous species common to the frequently burned longleaf pine ecosystem of southwestern Georgia, USA were used to examine the effects of: 1) elevated temperatures (five levels with dry heat temperatures ranging from presumably non-lethal to lethal) over three durations; 2) duration (three intervals) of moist heat (i.e., steam); 3) physical scarification; and 4) smoke. Legume species chosen represented a range of seed hardness, varying from soft-seeded to extremely hard-seeded. Response to both dry and moist heat was species-specific, but moist heat had the greatest potential to stimulate germination. The legumes studied also exhibited high heat tolerance, which may be a function of their low seed moisture contents. Smoke had no detectable effect on germination of most species, but may have inhibited germination of *Centrosema virginianum*. Potential ecological and managerial implications of these results are discussed.

KEYWORDS: germination; fire; fire surrogates; longleaf pine ecosystem; legumes; Fabaceae

INTRODUCTION

Fire is an important driver of plant diversity within many ecosystems worldwide (Whelan 1995; Bond and van Wilgen 1996). However, each fire is unique and the effects of a given fire are not necessarily uniform throughout the area burned. The behavior, intensity, and severity of a given fire are strongly affected by a variety of factors, including, time since last burn, season of burn, fuel characteristics (e.g., type, arrangement, amount, and moisture), weather (e.g., temperature, humidity, and wind speed), and landscape position (Whelan 1995; DeBano et al. 1998). Furthermore, within a given fire, fire intensities may exhibit marked heterogeneity at a variety of scales (Kennard and Outcalt 2006) resulting from patchy distribution of fuels (Hiers et al. 2009).

Fires affect plant populations in complex ways (Whelan 1995). Availability of resources (e.g., nutrients, water, light), competitive relationships, survival, and reproduction may all be influenced by fires (Whelan 1995; DeBano et al. 1998). In particular, fire's effects on plant reproduction and recruitment have been widely studied. Fires may promote or reduce post-fire vegetative reproduction (Hoffmann 1998; Liu and Menges 2005), fruit and seed production (Hiers et al. 2000; Norden and Kirkman 2004), and seedling recruitment (Setterfield 2002; Liu and Menges 2005). The magnitude and direction (i.e., positive or negative) of these effects, however, depends on species identity, season of burn, fire behavior, and location within the burned area (Knapp et al. 2009; Stephan et al. 2010).

Leguminous species are an important component of many frequently burned systems, including, for example, North American prairies (Woodmansee et al. 1981; Leach and Givnish 1996) and longleaf pine savannas (Drew et al. 1998; Hainds et al. 1999) as well as Mediterranean-type ecosystems worldwide (Arianoutsou and Thanos 1996). Because of their

ability to fix atmospheric nitrogen, legumes are important for replenishing nitrogen lost to volatilization during fires (Hendricks and Boring 1999; Hiers et al. 2003). Additionally, legumes provide forage for numerous game and non-game animal species (Yarrow and Yarrow 1999; Miller and Miller 2005).

Legume abundance is often enhanced in frequently burned systems (e.g., Stoddard 1931; Chapman 1932; Lemon 1949; Towne and Knapp 1996). Increased light availability following fires may promote nitrogen-fixing species on nitrogen poor sites (Leach and Givnish 1996). In the absence of fire, these species may have a competitive disadvantage during seedling establishment because of inadequate light levels required for the energetically expensive symbiotic nitrogen fixation (Cathey 2005). Legume abundance may also be enhanced following fires if their soil-stored seeds require fire to germinate. Many members of the Fabaceae are hard-seeded (Ballard 1973; Morrison et al. 1998). These hard seed coats impose physical dormancy on embryos of these species by reducing permeability of the seed coat to fluids and gases, thereby limiting hydration and subsequent germination (Ballard 1973; Baskin and Baskin 1998). Once the seed coat is scarified, however, these seeds often readily germinate (Baskin et al. 2000).

Heat from fires is thought to stimulate germination of many hard-seeded species by scarifying seeds (i.e., disrupting the seed coat and making it permeable to water and gases), thus breaking this physical dormancy (Baskin and Baskin 1998). Heat may also directly stimulate embryos to germinate—as has been noted for several species in South Africa (Blommaert 1972; Musil and De Witt 1991; Van de Venter and Esterhuizen 1988). Both dry heat and moist heat have been found to increase germination rates of hard-seeded species (Martin et al. 1975). Furthermore, duration of exposure to elevated temperatures may influence seed survival and germination (Levitt 1980). In addition to heat, chemicals in smoke (Clarke and French 2005;

Lindon and Menges 2008), ash, and charate (Keeley 1987; Newton et al. 2006) have also been found to stimulate germination.

Understanding how fire-related factors affect seed germination, vegetative recruitment, and seed production is a preliminary step for predicting how fire regimes and fire heterogeneity may influence patterns of diversity within frequently burned systems (Driscoll et al. 2010), such as the longleaf pine (*Pinus palustris* Mill.) ecosystem of the southeastern United States. The necessity of process-based restoration of the longleaf pine community is becoming increasingly recognized (e.g., Van Lear et al. 2005; Mitchell et al. 2005, 2006; Kirkman et al. 2006). Thus, a better understanding of germination and recruitment strategies of important groundcover species may improve the development of ecologically sustainable management tools.

Longleaf pine stands once covered more than 37 million hectares (Ware et al. 1993), but have been reduced to less than 5% of their original extent (Noss 1989). Fire has played a critical role in the evolution, structure, and diversity of the longleaf pine ecosystem (Frost 2006). Natural longleaf pine communities have highly diverse groundcover layers, which may have >40 species per square meter (Peet and Allard 1993; Kirkman et al. 2001) and are home to numerous endemic and threatened species (Hardin and White 1989). Groundcover species account for the high plant species richness found within natural longleaf pine stands that are frequently burned (Drew et al. 1998; Kirkman et al. 2004). Knowledge of specific fire-related germination cues within the longleaf pine system is limited to only a small fraction of the system's numerous species (e.g., Cushwa et al. 1968; Martin et al. 1975; Young et al. 2007; Bolin 2009).

The goal of this study was to determine the species-specific effects of fire on legume seed germination and mortality, using fire surrogates that represent components of environmental factors associated with fire. Specifically, for six legume species common to longleaf pine stands

in southwestern Georgia, the effects of the following treatments were examined: 1) elevated temperatures (five levels with dry heat temperatures ranging from presumably non-lethal to lethal) over various durations (three); 2) duration (three) of moist heat (i.e., steam); 3) physical scarification; and 4) smoke. Additionally, the legume seeds chosen for this study represent a range of seed hardness, varying from relatively soft-seeded to extremely hard-seeded.

MATERIALS AND METHODS

Study Species

Six legume species native to the southeastern United States were selected for this study: *Centrosema virginianum* (L.) Benth., *Crotalaria rotundifolia* Walter ex J.F. Gmel., *Desmodium floridanum* Chapm., *Lespedeza angustifolia* (Pursh) Elliot, *Mimosa quadrivalvis* L., and *Rhynchosia reniformis* DC; nomenclature follows Wunderlin and Hansen (2003). These species were selected based on their perennial life history, common occurrence throughout natural longleaf pine stands within the study site, and because they represent a range of seed hardness (based on scarification tests). Similar to other *Desmodium* species (Martin et al. 1975; Coffey and Kirkman 2006), *D. floridanum* is soft-seeded and does not typically require scarification for germination. As determined by earlier germination trials (L.K. Kirkman, unpublished data), *Ce. virginianum* is moderately hard-seeded, requiring some scarification to germinate (2 s in a Forsberg® tumble scarifier lined with 150 grit sandpaper; Forsberg, Inc., Thief River Falls, Minnesota, USA), while *L. angustifolia, R. reniformis*, *M. quadrivalvis*, and *Cr. rotundifolia* are extremely hard-seeded, requiring more intensive scarification (L.K. Kirkman, unpublished data: 9, 9, 15, and 17 s, respectively) for maximum germination to occur.

All seeds, except those of *R. reniformis*, were harvested in 2005 or 2006 from a native seed production garden of the Joseph W. Jones Ecological Research Center at Ichauway (Ichauway) in Baker County, Georgia, USA. *Rhynchosia reniformis* seeds were harvested from natural longleaf pine stands at Ichauway in 2007. Seeds were stored in burlap or paper bags in a climate-controlled laboratory at 21°C prior to experimentation.

Fire Effect Surrogate Treatments

All species were subjected to heat treatments representing a range of fire conditions: 1) untreated control; 2) factorial combinations of dry heat at five temperatures (40, 60, 80, 100, and 150ºC) and three durations (1, 4, and 10 min); 3) moist heat (i.e., steam) at four durations (0.16, 1, 4, and 10 min); 4) manual scarification (seed coat abrasion); and 5) exposure to pine needle smoke. Heat treatment durations were chosen to represent a range of potential exposure times experienced during actual fires (Heyward 1938; Beadle 1940; Robertson 2007). Four replicates of 20 seeds species^{-1} for each treatment and control (20 seeds × 22 treatments × 4 replicates = 1,760 seeds species^{-1}) were used.

DRY HEAT – Dry heat treatments were applied by placing seeds of all species in Pyrex® glass petri dishes and heating each replicate dish in one of four ovens. Timing of each dry heat treatment period began when appropriate treatment temperatures (± 3ºC) were reached, as indicated by a chromel-alumel (K-type) thermocouple suspended in the center of each oven. Exposure to 60ºC for short periods of time has been found to be lethal to plant tissue, although seeds often have higher lethal temperature thresholds than other plant tissues (Levitt 1980; DeBano et al. 1998).

For the purposes of this study, it was assumed that 40ºC for the durations used would not be lethal or induce germination; therefore, 40ºC was used in lieu of untreated controls for the

statistical analyses described below. Temperatures near the soil surface may reach or exceed 40ºC on a hot summer day within longleaf pine stands in southwest Georgia (G. Iacona, unpublished data). Furthermore, after examining the effects of heat on 35 legume species, Auld and O'Connell (1991) concluded that temperatures of 40ºC were not sufficient to induce germination of these species.

MOIST HEAT – Moist heat treatments were applied by placing seeds into a nylon mesh bag and suspending the bag 2.5 cm from the nozzle of an active commercial steam cleaner (Vapor Clean TR 4, Commital-Sami, Marostica, Italy) for the appropriate durations. A chromel-alumel thermocouple was placed immediately behind each mesh bag to record initial and final temperatures of steam for each replicate.

SCARIFICATION – Seeds were manually scarified by nicking the seed coat opposite to each seed's hilum with a razor.

SMOKE – A custom-built "smoker" was used to expose seeds to smoke derived from pine needles. The smoker consisted of one upright and one inverted steel bucket (30 cm diameter by 35 cm deep) attached 30 cm above the upright bucket's base using a 6 cm × 15 cm steel pipe. Air-dried longleaf pine needles were burned in the upright bucket and smoke was passively transferred to the inverted bucket via the connecting pipe after sealing the upright bucket with a steel lid. Seeds were then placed in Pyrex® glass petri dishes under the smoker and exposed to smoke for 10 min. To minimize heat transfer into the smoke chamber (i.e., the inverted bucket), water mist was sprayed on the exterior of the smoker to cool it. Temperatures associated with smoke treatments were monitored with a K-type thermocouple placed in the center of the smoke chamber and level with the seeds. Maximum temperatures were recorded for each replicate treatment.

Data Collection

GERMINATION – Immediately following the treatments, seeds of each species were placed in covered plastic petri dishes (20 seeds per dish) on filter paper (Whatman No. 1) moistened with deionized water. Seeds were then allowed to germinate for three weeks (January-February 2008) in a heated (10ºC minimum) greenhouse at Ichauway under ambient winter light conditions. Baskin and Baskin (1998) note that 2-4 weeks are typically adequate durations for germination experiments. Furthermore, previous experience with these and related species indicated that fungal attacks on seeds increase with the time seeds are exposed to damp filter paper (pers. obs.). Based on these personal observations and similar ones by Baskin and Baskin (1998), three weeks were considered adequate to observe most germination while minimizing fungal infestation. Seeds were monitored for germination (defined as protrusion of the radicle through the seed coat) three times per week. Rotten seeds (identified by extensive visible fungal infestation and/or being easily crushed under light pressure) were considered dead (Baskin and Baskin 1998). Following each round of data collection, germinated and rotten seeds were removed from petri dishes and discarded. Germination response for each replicate was determined as the proportion of seeds germinated.

VIABILITY AND MORTALITY ASSESSMENTS – Following the three week greenhouse germination period, viability of all remaining seeds was determined using a 2,3,5-triphenyl tetrazolium chloride (TZ) test (Peters 2000). To perform the TZ test, the seed coats of all remaining seeds were cut with a razor opposite the hilum and the seeds were allowed to imbibe water on filter paper moistened with deionized water in petri dishes for 12 to 24 h. Following imbibition of water, excess moisture was blotted from the filter paper, the paper was remoistened with 1.0% TZ, and then the covered petri dishes were wrapped in aluminum foil to eliminate

light contamination and stored in a laboratory at 21ºC for 48 h. Seeds were considered viable if the radicle and cotyledons were both stained or if a radicle had emerged from a seed coat. Unstained seeds and seeds with only cotyledons or radicle stained were considered non-viable (Peters 2000). Seed mortality was calculated as the total percentage of dead (rotten and non-viable) seeds for each replicate.

SEED WEIGHT AND MOISTURE CONTENT – Mean seed weights and percent moisture content by weight were determined for each species. Six non-scarified seeds of each species were weighed individually and then oven-dried at 80ºC. Seed weights were recorded every other day for six days, until seed weights stabilized. The difference in seed weight before and after drying was then used to calculate percent moisture content.

Statistical Analyses

Data from each species were analyzed separately. All analyses were performed using the SAS software package (v9.1, SAS Institute, Cary, North Carolina). Germination and mortality percentages were normalized prior to analyses using arcsine square root transformations. A two-way ANOVA (Littell et al. 2002) was used to analyze seed germination and mortality responses to dry heat exposure time and temperature. A one-way ANOVA (Littell et al. 2002) was used to determine if germination or mortality varied in response to steam duration. For this analysis, we included the control treatment as a zero minute duration. Where ANOVAs indicated significant differences between treatments, a Tukey's test ($\alpha = 0.05$; Littell et al. 2002) was used for post-hoc pairwise means comparisons. Separate two-sample *t*-tests (Littell et al. 2002) were used to compare germination and mortality responses of untreated controls with smoke and manually scarified treatments. For the *t*-tests, Satterthwaite corrections were used to adjust the degrees of freedom when *F*-tests indicated that variances were unequal (Littell et al. 2002). Species

experiencing significantly enhanced germination following heat treatments were considered to have heat-induced germination, while those that merely survived were considered heat tolerant.

RESULTS

UNTREATED CONTROLS – Germination of untreated seeds tended to follow patterns of relative seed hardness, with hard-seeded species typically exhibiting lower germination rates than soft-seeded species. Low mortality of controls (Table 2.1) indicated that initial viability of all species was high. Despite high viability, germination of the untreated hard-seeded species (*Cr. rotundifolia*, *L. angustifolia*, *M. quadrivalvis*, and *R. reniformis*) was low (i.e., <25%). In contrast, over half of the untreated seeds from the soft-seeded *D. floridanum* and the moderately hard-seeded *Ce. virginianum* germinated over the course of the study.

DRY HEAT – All species, regardless of relative seed hardness, exhibited the capacity to survive exposure to high temperatures. With the exception of the most extreme treatment (10 min exposure to 150ºC), which killed most or all seeds of all species, mortality did not differ among the combinations of temperature and durations tested for any species (Fig. 2.1, Table 2.2).

Germination responses were more variable than mortality responses. Increasing temperatures above 40ºC did not affect germination of *M. quadrivalvis* and *R. reniformis* (Fig. 2.1, Table 2.3). In contrast, increasing temperatures did significantly affect germination of *Ce. virginianum*, *Cr. rotundifolia*, and *D. floridanum*. Duration of elevated temperatures also affected germination of *Ce. virginianum, Cr. rotundifolia*, and *D. floridanum* (Table 2.3). Post-hoc analyses did not indicate any germination differences between durations for *Cr. rotundifolia* or *D. floridanum* and only indicated significant differences between 1 and 4 min durations for *Ce. virginianum*. Furthermore, the interaction between duration and temperature significantly

affected germination of all species except *M. quadrivalvis* and *R. reniformis* (Table 2.3).

Germination of *Ce. virginianum*, *Cr. rotundifolia*, and *D. floridanum* was significantly reduced

after exposure to 10 min of 150ºC dry heat (Table 2.3; Fig. 2.1). *Crotalaria rotundifolia* tolerated

high temperatures, but exhibited a more complex response than the other species studied (Table

2.3; Fig. 2.1). Specifically, germination was enhanced following 4 and 10 min exposures to 80ºC

and 100ºC and seeds survived brief (1 and 4 min) exposures to 150ºC without significantly

increased mortality. Germination of *L. angustifolia* also was increased by high temperatures, but

only following exposure to 100ºC for 10 min (Table 2.3; Fig 2.1).

MOIST HEAT – Steam influenced both germination and mortality of most species (Table

2.4, Fig. 2.2). Only germination of *R. reniformis*, which was low regardless of treatment, did not

significantly respond to variation in steam duration. Furthermore, all species exhibited similar

response patterns to increased duration of steam, with brief exposure to steam (0.16 min) tending

to increase germination without significantly affecting mortality of any species (Table 2.4; Fig.

2.2). Similarly, although their germination remained relatively low, the hard-seeded species *L.

angustifolia* and *R. reniformis* experienced 5.8- and 2.6-fold increased germination (respectively)

following 0.16 min exposure to steam. Species with the hardest seed coats (*M. quadrivalvis* and

Cr. rotundifolia) experienced greatest germination rates following 1 min exposure to steam.

Exposure to 4 and 10 min of steam significantly increased mortality of seeds from all species

regardless of seed hardness.

SCARIFICATION – Manual scarification increased germination rates of all species and had

no effect on mortality (Table 2.1). Hard-seeded species experienced the greatest increases in

germination, increasing by 3.8× to 19.5× that of untreated controls (*Cr. rotundifolia* and *L.

angustifolia*, respectively). Scarification also significantly increased germination of the soft-

seeded *D. floridanum* and moderately hard-seeded *Ce. virginianum* by 1.3× and 1.8×, respectively.

SMOKE – Exposure to pine needle smoke typically did not affect germination or seed mortality (Table 2.1). The only exception was *Ce. virginianum*, which experienced a 65% reduction in germination compared with controls ($t = 3.10_6$, $P = 0.039$). However, this reduced germination of *Ce. virginianum* was not accompanied by significantly increased mortality ($t = -1.00_3$, $P = 0.391$).

TREATMENT TEMPERATURES – Temperatures of dry heat, steam, and smoke treatments are listed in Table 2.5. Actual temperatures of dry heat treatments averaged to within <1.5ºC of their prescribed temperatures. Final temperatures of steam treatments typically were between about 80-90ºC. Final smoke temperatures tended to be 20-25ºC, but occasionally briefly rose to 30-50ºC during treatments; however, spraying the exterior of the smoke chamber with water mist rapidly cooled interior temperatures and limited these increases to less than 30 s.

SEED WEIGHT AND MOISTURE CONTENT – Of seeds used in the study, those of *L. angustifolia* were the smallest, weighing 1.99 ± 0.55 mg (mean ± SD dry weight), followed by *Cr. rotundifolia* (2.65 ± 0.47 mg), *D. floridanum* (3.97 ± 0.69 mg), *M. quadrivalvis* (8.18 ± 1.90 mg), *R. reniformis* (11.77 ± 1.34 mg), and *Ce. virginianum* (12.33 ± 3.02 mg). Seed moisture content of species used in this study was relatively low, typically less than 10%. *Mimosa quadrivalvis* and *R. reniformis* had the lowest seed moisture contents (4.3 ± 1.5% and 5.3 ± 1.1%, respectively). Highest moisture contents were found in seeds of *Ce. virginianum* (9.9 ± 0.8%) and *Cr. rotundifolia* (9.0 ± 0.6%). *Desmodium floridanum* and *L. angustifolia* had moisture contents between these extremes (7.4 ± 0.6% and 6.7 ± 1.5%, respectively).

DISCUSSION

Overall, heat treatments enhanced germination of five of the six species examined in the current study. The finding that heat exposure enhanced germination of nearly all of the legume species examined is consistent with fire-related germination cues reported for other legume species of the longleaf pine system in the southeastern United States (e.g., Cushwa et al. 1968; Martin et al. 1975; Young et al. 2007). Similar to these previous studies, the degree of germination responses to individual temperature, duration, and moisture conditions of the heat treatments varied by species.

The observed increases in germination following mechanical scarification of the seed coat suggest that disruption of the seed coat is the likely mechanism by which heat-stimulated germination occurs within the species studied. This is consistent with observations of other authors (reviewed in Murdoch and Ellis 2000). Weak spots on the seed coat such as the lens (lens-shaped area of raised tissue near the hilum that is also called the strophiole by some authors) or hilum (scar where the seed was attached to the funiculus during development) may be cracked during heat (Murdoch and Ellis 2000; Baskin et al. 2000). Heat may increase pressure within seeds due to water vaporization (Hanna 1984) or cause differential swelling and shrinking of cells (Serrato-Valenti et al. 1995), thereby cracking weak spots or other areas of the seed coat. During the present study, cracked seed coats of individual seeds were occasionally noticed following application of the 100ºC and 150ºC dry heat treatments. Similarly, seeds subjected to moist heat treatments, particularly for durations of ≥1 min, were often noticeably larger and softer post-treatment than pre-treatment. Steam appeared to have penetrated the seed coat and subsequently hydrated the seeds.

Only *R. reniformis* germination was not enhanced by either dry or moist heat treatments. One explanation may be the length of storage since seed harvest. Unlike the one- and two-year-old seeds of other species obtained for this study, seeds of *R. reniformis* were harvested only a few months prior to the experiment. Baskin and Baskin (1998) noted that germination results of seeds requiring a period of after-ripening may differ if tested prior to or following after-ripening of the seeds. After-ripening may increase the ambient temperatures required for germination. It is not known if after-ripening of *R. reniformis* occurs; however, nearly 90% of manually scarified *R. reniformis* seeds germinated, so it is unlikely that after-ripening, if required, influenced the observed results. Another fire-related cue—such as ash, leachates, higher temperatures, longer durations of increased heat, or some combination thereof, may influence germination of this species. Alternatively, it is possible that fire simply does not enhance germination of *R. reniformis*.

Differential germination responses to moist *versus* dry heat have also been reported previously (Cushwa et al. 1968; Martin et al. 1975; Kauffman and Martin 1991; Herranz et al. 1998, 1999; Le Fer and Parker 2005). Of the species studied herein, dry heat enhanced germination of two species, whereas moist heat enhanced germination of five species. Of the two species enhanced by exposure to dry heat (*Cr. rotundifolia* and *L. angustifolia*), *Cr. rotundifolia* appears to require the most intense heat environment to elicit germination. Germination of the moderately hard-seeded *Ce. virginianum* and soft-seeded *D. floridanum* remained high at all but the most extreme combination of temperature and duration (i.e., 150ºC for 10 min). Reduced germination following the most intense heat shock is similar to results of Rivas et al. (2006), who found that germination of six Australian legume species was reduced after exposures to 5 and 10 min of 150ºC. Interestingly, under similar temperatures and durations, the soft-seeded *D.*

floridanum and moderately hard-seeded *Ce. virginianum* had similar or higher germination rates than the hard-seeded *Cr. rotundifolia* and *L. angustifolia*.

Brief exposure to moist heat (i.e., ≥0.16 min) increased germination of all species except *R. reniformis*. Germination of *D. floridanum* and *Ce. virginianum* appears to be most sensitive to increasing duration of moist heat, while germination of *Cr. rotundifolia* and *M. quadrivalvis* appears to be most tolerant of increasing duration of moist heat. These observations generally follow patterns of relative seed hardness, where germination of the softer seeded species is reduced by shorter durations of moist heat than germination of the harder seeded species. In addition, the data indicate that some soft-seeded species, such as *Desmodium* spp., may benefit from scarification.

Consistent with earlier studies of legumes within the longleaf pine ecosystem (e.g., Cushwa et al. 1968; Martin et al. 1975; Young et al. 2007), the legume species studied herein exhibited the capacity to survive exposures to temperatures that are generally considered fatal for other plant tissues (i.e., ≥60ºC). Furthermore, this study demonstrated that herbaceous legume seeds have the capacity to survive temperatures 40-50ºC higher than previously noted (i.e., 150 ºC) for this system (Cushwa et al. 1968; Martin et al. 1975); however, seeds of both leguminous and non-leguminous species have been reported to survive brief (i.e., 1-5 min) exposures to 200ºC in Africa (Gashaw and Michelsen 2002). Indeed, with the exception of *D. floridanum*, a small proportion of seeds (up to 40%) of all species examined herein survived exposure to 150ºC dry heat for 10 min, although mortality increased significantly at this extreme exposure.

Various properties of seeds have been shown to be associated with the ability to survive high temperatures, including seed coat thickness (DeBano et al. 1998), internal moisture content (Levitt 1980), and size (Williams et al. 2003; Overbeck et al. 2006). Little is known about the

physical properties (e.g., thickness) of the seed coats of the species studied herein. As noted above, relatively hard-seeded species tolerated greater exposure to moist heat; however, no similar pattern was evident for dry heat treatments. Species with small seeds may survive exposures to high temperatures better than larger seeded species (Williams et al. 2003; Overbeck et al. 2006), but no such pattern was apparent in the current study. My observations, however, are qualitative because statistical comparisons were not made between species. The relatively low seed moisture contents of the species in this study are similar to values reported for hard-seeded species (Morrison et al. 1998) and this characteristic is typically associated with increased ability to tolerate elevated temperatures (Levitt 1980; Le Fer and Parker 2005).

Heat-induced germination of hard seeds requires adequate heat to scarify seeds, but not so much heat that seeds are killed (Banda et al. 2006), suggesting that both temperature and duration are important. This study provides some evidence regarding upper threshold temperatures for seed survival or induced germination when exposed to dry heat. Additionally, the interaction of temperature and soil moisture was not examined in this study, although other studies indicate that this may be important because of moist soil's ability to buffer elevated temperatures of brief fires (Cushwa et al. 1968; Martin et al. 1975; Le Fer and Parker 2005). As described here and elsewhere (e.g., Levitt 1980; Herranz et al. 1999; Banda et al. 2006), duration of elevated temperatures (dry or moist heat) appears to be critical for seed survival and germination of these species. Tolerance of high temperatures may allow seeds to persist within the soil seed bank until environmental conditions favor germination. Alternatively, species with heat-stimulated germination may find the post-fire environment favorable for seedlings (Bond and van Wilgen 1996) because of increased availability of nutrients, light, and open space.

In particular, hard-seeded species requiring scarification to break dormancy may be favored in fire-maintained systems (Whelan 1995; Towne and Knapp 1996). Dormant seeds may survive in the soil seed bank for years or even many decades (Baskin and Baskin 1998; Fenner and Thompson 2005) and, thus, provide a buffer against localized extirpation of their species (Thompson et al. 1998; Stocklin and Fisher 1999). Evidence from a buried seed bag study indicates that hard-seeded legumes may have the capacity to form a long-term persistent seed bank within the longleaf pine system (L.K. Kirkman, unpublished data). Persistent seed banks may maintain genetic diversity within frequently burned systems (Dolan et al. 2008) or promote species spread by colonizing open gaps following fires.

Although smoke-induced germination has been found in a wide variety of plant species worldwide (Keeley and Fotheringham 2000; Van Staden et al. 2000), the present study found no evidence that longleaf pine needle smoke induces germination of the observed legume species. While it is possible that plant-derived smoke from sources other than longleaf pine needles may induce germination in these species, the results of this study are consistent with Keeley and Fotheringham's (2000) assertion that smoke-induced germination may be non-existent in the Fabaceae. In addition, Van Staden et al. (2000) note that smoke may play a secondary role in germination once the seed coat has been disrupted by fire because compounds in smoke that act as potential cues may persist in the soil or on plant tissues following a fire's passage.

The only species apparently influenced by smoke was *Ce. virginianum*, which experienced reduced germination following smoke exposure. The observed reduction in germination was not accompanied by increased mortality and, therefore, may represent a potential inhibition response to a chemical (or chemicals) in longleaf pine needle smoke. Such an inhibitory effect of smoke has been reported for various species around the world (Keeley and

Fotheringham 2000), while Lindon and Menges (2008) found similarly decreased germination of the Florida native legume *Crotalaria pallida* (Aiton) following 10 and 30 min smoke exposures. The butenolide 3,4,5-trimethylfuran-2(*5H*)-one has recently been isolated from smoke and identified as a germination inhibitor (Light et al. 2010), but its presence in longleaf pine-derived smoke is presently unknown. Further investigation is required to determine if germination of *Ce. virginianum* is in fact inhibited by longleaf pine needle-derived smoke.

Restoration efforts within the longleaf pine ecosystem may be enhanced by knowledge of germination requirements of a suite of functionally important species as well as their responses to particular fire regimes (Driscoll et al. 2010). Results presented herein provide additional information regarding potential mechanisms of germination and survival of legume seeds within this system. However, caution must be used when attempting to apply these results to responses of legume seeds in the field. First, seeds of these species may respond differently to fire-related cues in soil compared to cues established in the lab. For example, Christensen and Muller (1975) found higher germination of a variety of herbaceous chaparral species when seeds were heated in chaparral soils compared to seeds exposed to heat without soil in the lab. The authors attributed those differences to the likely presence of chaparral leachates in the soils. Second, the behavior and impact of each fire is unique (Whelan 1995). Variation in fuel features (load, type, distribution, and moisture), weather, and local topography all interact to create fires that are spatially and temporally diverse. Within longleaf pine stands distribution of fuel loads and types has been found to vary at fine scales (i.e., 0.1-10 m^2; Hiers et al. 2009) which may in turn influence local intensities and residence times of potentially lethal temperatures (Thaxton and Platt 2006; Hiers et al. 2009). Companion studies (presented in Chapter 3) were performed to investigate how fuel-mediated variation in fire effects may alter recruitment of plant species via

differential germination and survival of seeds following fires. In particular, these companion studies indicate that fuels that increase durations of lethal temperatures (such as pine cones) may create a mosaic of open spaces available for recruitment of species from the soil seed bank that are able tolerate the increased intensity of the fine-scale fire environment. Thus, those species that survive and germinate within these open spaces may have an initial competitive advantage over neighboring individuals that are unable to take advantage of the increased resource availability within these open spaces.

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Species	Treatment	Germination (%)			Mortality (%)		
Ce. virginianum	Control	50.6 ± 1.9	B	a	0.0 ± 0.0	A	a
	Scarified	93.6 ± 3.9	A		6.4 ± 3.9	A	
	Smoke	33.0 ± 5.1		b	1.3 ± 1.3		a
Cr. rotundifolia	Control	21.8 ± 2.5	B	a	21.9 ± 3.5	A	a
	Scarified	83.8 ± 2.4	A		16.3 ± 2.4	A	
	Smoke	21.4 ± 5.1		a	12.8 ± 4.5		a
D. floridanum	Control	63.8 ± 3.1	B	a	15.0 ± 3.5	A	a
	Scarified	84.3 ± 3.2	A		15.7 ± 3.2	A	
	Smoke	61.9 ± 3.7		a	6.3 ± 2.4		a
L. angustifolia	Control	5.0 ± 3.5	B	a	5.0 ± 2.0	A	a
	Scarified	97.5 ± 1.4	A		2.5 ± 1.4	A	
	Smoke	7.3 ± 4.2		a	3.8 ± 3.8		a
M. quadrivalvis	Control	11.3 ± 3.8	B	a	4.8 ± 3.4	A	a
	Scarified	98.8 ± 1.3	A		0.0 ± 0.0	A	
	Smoke	6.3 ± 1.3		a	1.3 ± 1.3		a
R. reniformis	Control	5.1 ± 3.0	B	a	8.8 ± 1.2	A	a
	Scarified	87.4 ± 2.5	A		12.6 ± 2.5	A	
	Smoke	3.8 ± 1.3		a	11.3 ± 2.4		a

Table 2.1. Legume seed germination and mortality responses to untreated (control), scarification, and smoke treatments. Within each response, different uppercase letters indicate significant differences between control and scarified seeds of each species, while different lowercase letters indicate significant differences between controls and smoke treatments as determined by separate two-sample t-tests ($\alpha = 0.05$).

Species	Treatment	df	F	P
Ce. virginianum	Temperature	2,45	4.97	<0.0001
	Time	4,45	13.37	0.011
	Temp. × Time	8,45	6.62	<0.0001
Cr. rotundifolia	Temperature	2,45	14.53	<0.0001
	Time	4,45	10.08	<0.0001
	Temp. × Time	8,45	8.54	<0.0001
D. floridanum	Temperature	2,45	6.20	0.004
	Time	4,45	21.72	<0.0001
	Temp. × Time	8,45	8.75	<0.0001
L. angustifolia	Temperature	2,45	11.27	<0.001
	Time	4,45	25.22	<0.0001
	Temp. × Time	8,45	13.37	<0.0001
M. quadrivalvis	Temperature	2,45	3.36	0.043
	Time	4,45	2.40	0.064
	Temp. × Time	8,45	3.65	0.002
R. reniformis	Temperature	2,45	2.61	0.084
	Time	4,45	11.63	<0.0001
	Temp. × Time	8,45	5.19	<0.001

Table 2.2. Mortality responses of legume seeds to dry heat treatments. Where ANOVAs were significant, Tukey's mean separation tests ($\alpha = 0.05$) always indicated that mortality at 150ºC, 10 min, and their combination (i.e., 150ºC for 10 min) were significantly greater than all others. Post-hoc analyses did not indicate any other significant differences. Means are illustrated in Fig. 2.1.

Treatment	*Ce. virginianum*			*Cr. rotundifolia*			*D. floridanum*		
	df	*F*	*P*	df	*F*	*P*	df	*F*	*P*
Temperature (°C)	2,45	5.44	0.007	2,45	0.29	0.747	2,45	2	0.146
40			a						
60			a						
80			a						
100			a						
150			b						
Time (min)	4,45	9.92	<0.0001	4,45	5.21	0.001	4,45	16.08	<0.0001
1			a			a			a
4			ab			a			a
10			b			a			a
Temp. × Time	8,45	6.65	<0.0001	8,45	9.57	<0.0001	8,45	9.34	<0.0001
40,1			a			bcd			a
40,4			a			cd			a
40,10			a			de			a
60,1			a			bcd			a
60,4			a			de			a
60,10			a			cd			a
80,1			a			cd			a
80,4			a			cd			a
80,10			a			ab			a
100,1			a			cd			a
100,4			a			abce			a
100,10			a			a			a
150,1			a			abce			a
150,4			a			abc			a
150,10			b			d			b

Table 2.3. (Continued on next page.) Germination of legume seeds following dry heat treatments. Different *letters* under *P*-values indicate significant differences between response variables within a species using Tukey's mean separation tests ($\alpha = 0.05$). Means are illustrated in Fig. 2.1.

Treatment	L. angustifolia			M. quadrivalvis			R. reniformis		
	df	F	P	df	F	P	df	F	P
Temperature (°C)	2,41	0.32	0.731	2,45	2.3	0.112	2,45	1.21	0.307
40									
60									
80									
100									
150									
Time (min)	4,41	1.68	0.173	4,45	0.29	0.882	4,45	2.4	0.063
1									
4									
10									
Temp. × Time	8,41	4.78	<0.001	8,45	1.7	0.125	8,45	0.41	0.906
40,1			ab						
40,4			b						
40,10			b						
60,1			ab						
60,4			ab						
60,10			b						
80,1			ab						
80,4			ab						
80,10			ab						
100,1			b						
100,4			b						
100,10			a						
150,1			ab						
150,4			ab						
150,10			b						

Table 2.3. (Continued from previous page.)

Species	Response	df	F	P	Duration (min)				
					0	0.16	1	4	10
Ce. virginianum	Germination	4,15	24.23	<0.0001	ab	a	ab	b	c
	Mortality	4,15	38.19	<0.0001	c	c	c	b	a
Cr. rotundifolia	Germination	4,15	7.49	0.001	b	a	a	a	ab
	Mortality	4,15	5.24	0.007	b	b	b	ab	a
D. floridanum	Germination	4,15	35.53	<0.0001	ab	a	bc	c	d
	Mortality	4,15	26.23	<0.0001	cd	d	cb	b	a
L. angustifolia	Germination	4,15	5.88	0.004	b	a	ab	ab	b
	Mortality	4,15	7.41	0.001	b	b	b	ab	a
M. quadrivalvis	Germination	4,15	7.17	0.002	b	ab	a	a	a
	Mortality	4,15	7.19	0.001	b	b	b	ab	a
R. reniformis	Germination	4,15	2.43	0.093					
	Mortality	4,15	19.57	<0.0001	c	c	bc	b	a

Table 2.4. Germination and mortality of legume seeds following moist heat treatments. Different *letters* under duration indicate significant differences between moist heat treatment times as determined by Tukey's mean separation tests ($\alpha = 0.05$). Means are illustrated in Fig. 2.2.

Treatment	Duration (min)			
	0.16	1	4	10
Dry Heat (ºC)				
40		39.0 ± 0.4	38.7 ± 0.7	39.9 ± 0.6
60		59.4 ± 0.6	59.7 ± 0.6	59.7 ± 0.6
80		78.7 ± 0.6	79.0 ± 0.5	79.5 ± 0.7
100		99.1 ± 0.7	99.3 ± 0.7	99.2 ± 0.6
150		148.8 ± 0.8	148.8 ± 0.8	149.2 ± 0.8
Steam	91.0 ± 0.7	89.2 ± 0.7	79.1 ± 3.9	82.5 ± 1.6
Smoke				22.1 ± 1.1

Table 2.5. Temperatures (mean ± SE) of dry heat, steam, and smoke treatments. Temperatures of dry heat treatments represent means of initial and final temperatures ($n = 8$). Values for steam and smoke represent mean final temperatures ($n = 4$).
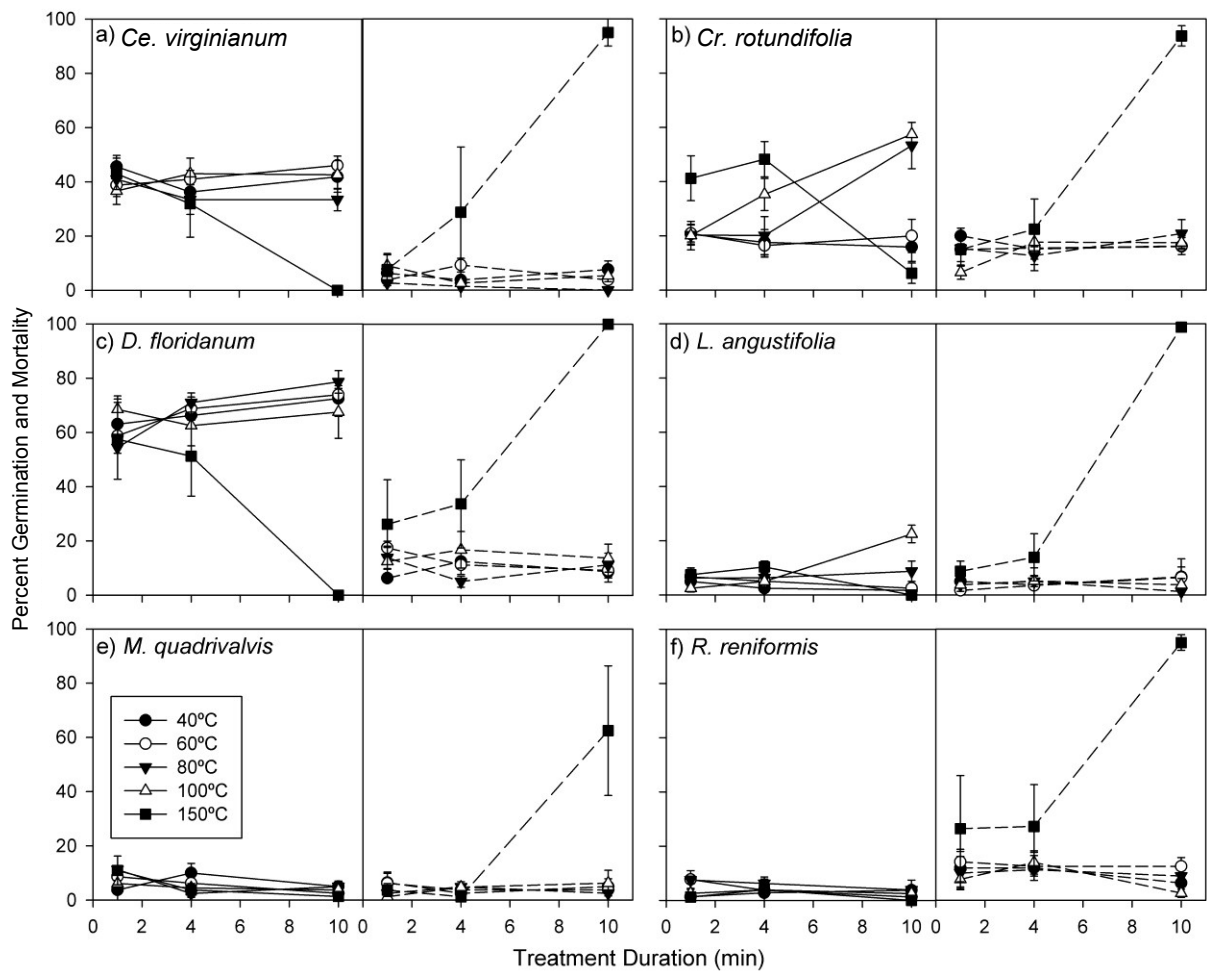
Figure 2.1. Mortality and germination responses of legume seeds to dry heat treatments. Values represent mean germination (solid lines) or mortality (dashed lines). Error bars represent ± one SE. Results of statistical analyses for mortality and germination can be found in Tables 2.2 and 2.3, respectively.
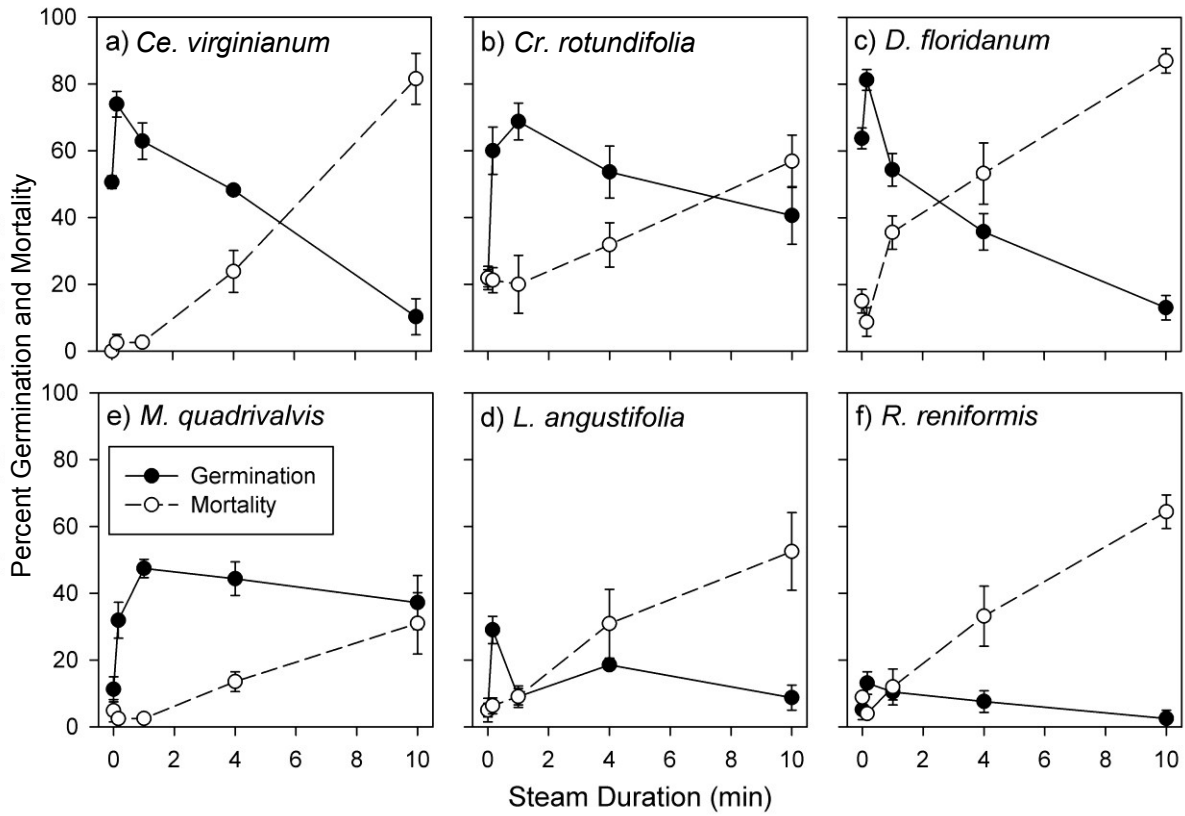
Figure 2.2. Mortality and germination responses of legume seeds to steam. Values represent mean germination (solid lines) or mortality (dashed lines). Error bars represent ± one SE. ANOVA results and post-hoc Tukey's means separations test results are presented in Table 2.4.

CHAPTER 3

IMPACTS OF FINE-SCALE VARIATION IN SURFACE FIRE ENVIRONMENT ON

LEGUME GERMINATION IN THE LONGLEAF PINE ECOSYSTEM

ABSTRACT

By influencing fire behavior, fine-scale fuel heterogeneity may influence patterns of plant recruitment and regeneration from soil seed banks. In particular, hard-seeded species, such as many members of the Fabaceae, may experience differential germination and survival as a result of heterogeneous fire intensities mediated by variable distribution of fine fuel. Post-fire germination was examined for nine legume species native to longleaf pine stands (*Pinus palustris*) of the southeastern United States in response to fuel variation using laboratory and field studies. For both studies, fine fuel loads (longleaf pine needles) and woody fuels (presence or absence of longleaf pine cones) were manipulated. Germination and mortality were assessed for six species after exposure to experimental laboratory fires. Germination of seven species was assessed for four months in the field following experimental burns of small plots. Burning reduced germination compared to unburned controls in both studies. Furthermore, legumes exposed to burning pine cones experienced greater reductions in germination than when exposed to burning pine needles alone. Manipulation of fine fuel loads did not affect germination in either study. Additionally, a hypothetical model of pine cone effects on recruitment from the soil seed bank following fires is presented.

KEYWORDS: fuel heterogeneity; fine-scale fire heterogeneity; legumes; germination; seed survival.

INTRODUCTION

Recurrent fires maintain the structure and diversity of numerous ecosystems worldwide (Bond and Keeley 2005; Bond et al. 2005). Within these systems, variations in fuel loads, types, and arrangement are important determinants of fire behavior and intensity (Whelan 1995; Bond and van Wilgen 1996), which in turn influence the ecological effects of a given fire (Kennard and Outcalt 2006). Even at fine scales (i.e., <10 m^2) and in frequently burned ecosystems, woodland fuels may exhibit markedly heterogeneous distribution (Hiers et al. 2009), which may influence fire's impacts at similarly small scales. The importance of fine-scale fire heterogeneity as a potential driver of plant diversity within fire-maintained systems is becoming increasingly recognized (e.g., Rebertus et al. 1989; Mitchell et al. 2006; Thaxton and Platt 2006).

An example of an exceptionally species rich and fire-dependent ecosystem is the longleaf pine (*Pinus palustris* Mill.) savanna of the southeastern United States (Peet and Allard 1993). This system's diverse groundcover and abundant pine needle litter provide fine fuels necessary for carrying fires across the forest floor (O'Brien et al. 2008). Bunch grasses common to this system—*Aristida* spp. and *Andropogon* spp.—are highly flammable and serve as perches for other fine fuels, such as pine needles (Hendricks et al. 2002; Hermann 2007). Together, the bunch grasses and perched fine fuels provide a continuous fuel layer and greatly enhance the pyrogenicity of longleaf pine stands. Groundcover species alone, however, may not sustain fires if pine litter, particularly pine needles, is absent or occurs in insufficient abundance (O'Brien et al. 2008).

In longleaf pine savannas, accumulation of needles and woody litter under pine trees may increase fine-scale fire intensities (Mitchell et al. 2009; Jack et al. 2010). Indeed, increased pine litter and associated increases in fire temperatures and intensities have been found to reduce the

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survival and growth of oaks (Williamson and Black 1981; Rebertus et al. 1989). Elevated pine

fuel loads have also been found to reduce the survival and resprouting of shrubs in longleaf pine

stands (Thaxton and Platt 2006) and increase mortality of longleaf pine seedlings (Jack et al.

2010).

Fine fuels and coarse fuels from pine trees differ in their burning characteristics and may

therefore differ in their ecological effects. In contrast to pine needles, which may burn for only a

few minutes (Fonda 2001), pine cones may burn for well over half an hour (Fonda and Varner

2005). Although pine cones represent a potentially significant source of fuel (Mitchell et al.

2009), the ecological effects of pine cones as a fuel source remain poorly understood (Fonda and

Varner 2005). Because distribution of cones is influenced by the distribution and density of pine

trees (Platt et al. 1988; Varner 2005; Mitchell et al. 2009), the impact of longleaf pine cones on

fire effects is likely greater following mast seeding years when pine cones are particularly

abundant.

Legume species are an important component of the groundcover flora in some longleaf

pine forests; in some cases, accounting for nearly 10% of species present (Drew et al. 1998) and

up to 120,000 stems per ha (Hainds et al. 1999). Within this ecosystem, legumes provide forage

for wildlife and are important sources of nitrogen replenishment to the soil following loss due to

volatilization in fires (Hendricks and Boring 1999; Hiers et al. 2003). Many leguminous species

are hard-seeded, possessing an impermeable seed coat (Ballard 1973; Morrison et al. 1998). Hard

seed coats impose a physical dormancy on seeds of these species by limiting uptake of water by

the embryo (Ballard 1973) and thus allow for the formation of persistent seed banks (Baskin and

Baskin 1998). Exposure to fire enhances germination of some hard-seeded species by breaking

the moisture barrier that imparts dormancy (Morrison et al. 1998); however, little is known about

how small-scale fire variation may affect species distribution by influencing seed germination or mortality of individuals of a species.

Seeds within the soil seed bank are uniquely positioned to take advantage of new gaps created by fires as recruitment sites, provided they can survive the fires. Within ecosystems that are regularly burned, the capacity for seeds to survive fires or germinate in response to these fires differs among species (Keeley et al. 1985; Clark and Wilson 1994; Danthu et al. 2003). Thus, species-specific responses to fine-scale fuel and fire variation may contribute to patterns of species recruitment and fine-scale vegetation diversity.

The objective of this study was to examine effects of variable fine fuel loads and presence of longer burning fuels on germination and mortality of a suite of common legume species. First, germination and mortality of six legume species following experimental fires using a gradient of fuel loads were assessed in the laboratory. Second, post-fire germination responses of seven legume species to fuel (i.e., pine needle and pine cones) manipulations were determined in the field.

MATERIALS AND METHODS

Study Site

Seed germination responses to fuel manipulation treatments were examined in the laboratory and in field plots at the Joseph W. Jones Ecological Research Center at Ichauway (Ichauway). Ichauway is a 12,000 ha research property located in Baker County, Georgia, USA and occurs within the Lower Gulf Coastal Plain ecoregion and within the historic range of longleaf pine (Ware et al. 1993). The local climate is characterized by short, mild winters and

long, hot summers (Lynch et al. 1986) with average annual temperatures ranging from lows of 5ºC in winter to highs of 34ºC in the summer (Goebel et al. 1997).

Study Designs and Data Collection

EXPERIMENT 1: LABORATORY GERMINATION

Seed mortality and germination were examined in response to ignition of variable fuel loads under laboratory conditions. The laboratory setting allowed for ready retrieval of the seeds for further testing. Post-treatment retrieval of seeds permitted me to quantify seed mortality as a factor in the absence of germination response.

In this study, seeds of six legume species were used (nomenclature follows Wunderlin and Hansen [2003]): *Centrosema virginianum* (L.) Benth., *Crotalaria rotundifolia* Walter ex J.F. Gmel., *Desmodium floridanum* Chapm., *Lespedeza angustifolia* (Pursh) Elliot, *Mimosa quadrivalvis* L., and *Rhynchosia reniformis* DC. These species were selected based on seed availability, common occurrence within Ichauway's longleaf pine stands, their perennial life histories, and characteristics that permitted ready identification of seeds of each species during post-treatment retrieval. Additionally, the species represent a range of seed coat hardness as determined by their scarification requirements using a Forsberg® tumble scarifier lined with 150 grit sandpaper (Forsberg, Inc., Thief River Falls, Minnesota). Germination trials following scarification indicate that *D. floridanum* is soft-seeded and does not typically require scarification for germination; *Ce. virginianum* is moderately hard-seeded, requiring some scarification (2 s); and *Cr. rotundifolia*, *L. angustifolia*, *M. quadrivalvis*, and *R. reniformis* are extremely hard-seeded, requiring more extensive scarification to elicit germination (9, 9, 15, and 17 s, respectively; L.K. Kirkman, unpublished data). *Rhynchosia reniformis* seeds were harvested by hand from natural longleaf pine stands at Ichauway in October 2007. Seeds of all

other species used were harvested in 2005 and 2006 from a native seed production garden at Ichauway. Seeds were stored in burlap or paper bags in a climate-controlled laboratory at approximately 21°C prior to use.

For this study, four replicate sets of 20 seeds species^{-1} were used for untreated control and four fire treatments (20 seeds × 5 treatments × 4 replicates = 400 seeds species^{-1}). Fuel load treatment rates were selected to approximate typical accumulation of longleaf pine needle litter between 1-2 and 3-4 years since the last fire in natural stands (Ottmar and Vihanek 2000; Ottmar et al. 2003). The low fuel treatment was equivalent to 2.8 Mg/ha of longleaf pine needles, while the high fuel treatment was equivalent to 5.7 Mg/ha of pine needles. Longleaf pine needles were dried for 24 h in an oven at 70ºC prior to use. Two longleaf pine cones (65 to 85 g per cone [150 g total] after oven-drying for 24 h at 70ºC) were used for pine cone treatments. Prior to applying fire treatments, a 61 × 61 × 7 cm wooden box was filled with approximately 5 cm of sand (125-500 μm grain size). The commercially prepared (i.e., washed and sieved) sand was oven-dried for 4 days at 70ºC and allowed to cool for 12 h prior to use. Prior to each treatment, a 6 cm diameter hemispherical steel sieve (approx. 1 mm) was buried level with the sand surface in the center of the box. Next, seeds of all six species were uniformly sowed across the sand within the sieve, lightly pressed into the sand, and then covered with 1-2 mm of sand to minimize direct contact with burning material. Following this, pine needles were spread uniformly over a 707 cm^2 circular area (centered over the sieve). For pine cone fuel treatments, pine cones were placed side-by-side, lengthwise, in opposing directions on top of the pine needles directly above the sieve.

A portable furnace was used to ignite fuels uniformly for the fire treatments in early March 2008. Similar to that described by van Eerden (1997), the circular propane-fueled portable

aluminum furnace (31 cm diameter by 40 cm high) was mounted on a tripod and could be raised and lowered as needed using a hand-crank cable mechanism. To ignite the fuel, the furnace was lowered to within 2.5 cm of the sand's surface, was allowed to stand for 10 s, and then raised. Four chromel-constantan (E-type) thermocouples were placed on the sand surface in a 15 cm × 15 cm square around the sieve and then lightly covered with 1-2 mm of sand (i.e., approximate seed burial depth). Thermocouple data were recorded at 1 s intervals using a CR3000 data logger (Campbell Scientific, North Logan, Utah, USA). Results from the companion study presented in Chapter 2 indicated that brief exposure (i.e., ≤10 min) to temperatures ≤60°C were not lethal to these legume seeds; therefore, when temperatures recorded by all thermocouples fell below 60°C, seeds were separated from the sand by sifting through a 1 mm wire-mesh sieve. Maximum temperature (from any one of the four thermocouples) and duration of temperatures ≥60ºC (i.e., potentially lethal elevated temperatures [Hare 1961] averaged among the four thermocouples) were recorded for each treatment.

Following treatments, seeds were separated by species, counted, and placed on filter paper (Whatman No. 1) moistened with deionized water in plastic petri dishes. Seeds were then allowed to germinate in the laboratory under ambient light at 21ºC for three weeks in March 2008. Baskin and Baskin (1998) note that 2-4 weeks are typically adequate durations for germination experiments. Furthermore, previous experience with these and related species indicated that fungal attacks of seeds increase with the time seeds are exposed to damp filter paper (pers. obs.). Based on these personal observations and similar to those of Baskin and Baskin (1998), three weeks were considered adequate to observe most germination while minimizing fungal infestation. Germination (defined as protrusion of the radicle through the seed coat) was assessed three times per week. Germinated seeds were removed and discarded after

each biweekly assessment. Additionally, rotten seeds (identified by extensive visible fungal infestation and/or by being easily crushed using minimal pressure) were counted and discarded following each assessment. Rotten seeds were counted as dead (Baskin and Baskin 1998).

After the three-week germination period, viability of remaining seeds was assessed using a 2,3,5-triphenyl tetrazolium chloride (TZ) test (Peters 2000). Following manual scarification of the seed coat with a razor, seeds were placed on filter paper moistened with deionized water in petri dishes for 12 to 24 h. Following imbibition of water, excess moisture was blotted from the filter paper and then the paper was remoistened with a 1.0% TZ solution. The covered petri dishes were then wrapped in aluminum foil and stored in a laboratory at 21ºC for 48 h, after which time seeds were dissected to assess the embryo for staining. Seeds were considered viable if the radicle and cotyledons were both stained or if a radicle had emerged from the seed coat. Unstained seeds and seeds with only cotyledons or radicle stained were considered non-viable (Peters 2000).

EXPERIMENT 2: FIELD FIRE INTENSITY MEASURES AND GERMINATION

To examine germination response of legumes to variable fire intensities caused by pre-fire fuel variation, seeds of seven common perennial species were used: four that were also used in the laboratory experiment (*Ce. virginianum*, *D. floridanum*, *L. angustifolia*, and *M. quadrivalvis*) plus three additional species, *Desmodium ciliare* (Muhl. ex Willd.) DC., *Pediomelum canescens* (Michx.) Rydb., and *Tephrosia virginiana* (L.) Pers. Similar to the laboratory study described above, these species were selected because they represent a range of seed hardness, they are relatively common within longleaf pine stands of Ichauway, and because adequate seed quantities were available. Based on scarification requirements for germination as described above, *D. ciliare* and *D. floridanum* are soft-seeded; *Ce. virginianum* is moderately

hard-seeded (2 s scarification); and *L. angustifolia*, *M. quadrivalvis*, *P. canescens*, and *T. virginiana* are extremely hard-seeded, requiring greater scarification (9, 15, 7, and 7 s, respectively). Seeds were collected from Ichauway's native seed production garden in 2005 or 2006.

This study was performed in three blocks of planted wiregrass (*Aristida stricta* Michx.) beds located within a native plant garden on Ichauway. Soils of the garden plot consisted of loamy sand (Orangeburg series, Typic Kandiudult). Planted wiregrass clumps occurred at a density of 5 m^{-2} to approximate natural densities (Clewell 1989).

A randomized complete block split-plot design of two fine fuel loads and two pine cone (i.e., presence or absence) treatments was used for burning treatments. Within the three wiregrass blocks, fine fuel loads were used as whole plots and pine cone treatments were the split-plots. Within each of the three wiregrass blocks, six plots (2 m × 3.5 m) separated by 1 m wide mowed buffer strips were established in February 2007. Two replicates within each block were randomly assigned to one of the main treatments: 1) unburned control treatment; 2) burned with low fine fuel load; or 3) burned with elevated fine fuel load. Additionally, two adjacent split-plots (0.5 m × 2 m) were established within the center of each plot and then randomly assigned to a pine cone treatment: either with or without pine cones. An example of the plot layout (post-fire) is depicted in Fig. 3.1. Within each split-plot, four subsample sites were established by inserting steel rings (15 cm diameter with 5 cm wall height) until the top of each ring was level with the soil surface. Thirty seeds of each legume species were sown into each subsample ring, lightly pressed into the soil, and then covered with 1-2 mm of soil to minimize direct contact with burning fuels. To prevent seed predation by vertebrates, subsample plots were covered with metal screens (~1.5 mm mesh size) affixed to the ground with aluminum nails. To avoid potential increased

conduction of heat to the seeds via the metal screens, the screens were removed immediately prior to and then replaced following application of the burn treatments. A total of 3,600 seeds species^{-1} were used for this study (5 treatments × 6 replicates × 4 subsamples replicate^{-1} × 30 seeds subsample^{-1}).

Experimental fuel loads used for this study were similar to the treatment loads (per unit area) used in the laboratory study described above. In March 2007, air-dried longleaf pine needles were uniformly spread throughout each burn plot. Immediately prior to igniting the prescribed burns, 20 oven-dried (24 h at 70ºC) longleaf pine cones (75.1 ± 29.6 g per cone; mean ± SD, $n = 30$) were placed uniformly throughout one split-plot in each fuel treatment plot. In July 2007, the edges of each plot were ignited using a drip-torch (2:1 diesel:gasoline mixture) and the fire permitted to burn into the center of each plot.

During the treatment applications, infrared thermal imagery (one digital image recorded every 5.5 s) was acquired for six plots using a ThermaCAM™ S60 (FLIR Systems, Boston, Massachusetts) to measure temperatures of the fires. Thermal images were individually analyzed using the ThermaCAM™ Researcher Pro software package (v2.7, FLIR Systems, Boston, Massachusetts). To assess maximum temperatures, two temperature sensitivity ranges of the thermal camera were used during the fires: 0-500°C and 300-1500°C. The lower limit of the highest temperature range (i.e., 300ºC) was used to assess duration of potentially lethal temperatures. Maximum temperatures and duration were derived from the thermal images by using three uniformly spaced digital transects arranged parallel to the long edge of each split-plot. Example thermal images obtained during an experimental fire are depicted in Fig. 3.2.

Beginning approximately two weeks after application of the treatments (July 2007), seed germination within the plots was assessed biweekly for one growing season, ending in November

2007 prior to the first frost. Biweekly assessments were made by counting seedlings within each

sub-plot in the field. Seedlings were readily identified in the field by the distinctive

characteristics of their cotyledons and first true leaves (if present). Emergent seedlings were

removed after each germination assessment to avoid repeated counts of the same individuals.

Statistical Analysis

Data were analyzed using the SAS software package (v9.1, SAS Institute, Cary, North

Carolina). Data from each species were analyzed separately for each experiment. Data were

normalized prior to analyses using arcsine square root transformations for proportions and log

transformations for counts.

Addition of pine cones killed all seeds in the laboratory germination study. A one-way

ANOVA was therefore used to compare germination and mortality responses between untreated

controls and those burned under low and high pine needle fuel loads (Littell et al. 2002).

Differences in mean maximum temperature and durations due to treatments (i.e., low and high

pine needle fuel loads and presence/absence of pine cones) from the laboratory study were

examined using a fully factorial ANOVA. Significant ANOVA results were then further

investigated using post-hoc analyses (Tukey's means comparison tests; $\alpha = 0.05$; Littell et al.

1996 and 2002) to identify differences among treatment responses (i.e., germination and

mortality).

Prior to analyzing germination data for the field study, total germination of each species

was first calculated for each sub-plot. Germination data for this study were first analyzed for

differences between control and fire treatments (fuel variation pooled at the plot level) and then

analyzed for differences between burned treatments. To compare controls with fire treatments,

germination totals for all sub-plots within each whole plot (i.e., controls and fuel loads) were

used to calculate mean germination at the whole plot level. These whole plot mean germination values (regardless of fuel load) were then analyzed using a randomized complete block (RCB) ANOVA (Littell et al. 2002) with wiregrass beds used as blocks. To compare germination between fire treatments, sub-plot totals were used to calculate mean germination for each split-plot (i.e., pine cone treatments). These split-plot mean germination values were then analyzed using an RCB split-plot mixed model ANOVA (Littell et al. 1996) to determine responses to fuel treatments. Wiregrass beds were used as blocks, fuel load treatments were used as whole plots, and presence of pine cones was assigned as the split-plot for this analysis. Data for temperatures and durations of temperatures ≥300ºC were analyzed using a nested mixed model ANOVA by nesting pine cone treatments within fuel loads (Littell et al. 1996). Where ANOVAs indicated differences between treatments, post-hoc Tukey's means comparison tests were performed (α = 0.05; Littell et al. 1996, 2002).

RESULTS

EXPERIMENT 1: LABORATORY GERMINATION

Temperature and duration data are presented in Fig. 3.3. Presence of pine cones increased both maximum temperatures ($F = 12.40_{1,12}$, $P = 0.0042$; Fig. 3.3a) and durations ($F = 127.72_{1,12}$, $P < 0.0001$; Fig. 3.3b). Variation in pine needle fuel loads did not affect maximum temperatures ($F = 2.45_{1,12}$, $P = 0.143$; Fig. 3.3a) or duration of temperatures ≥60°C ($F = 0.17_{1,12}$, $P = 0.688$; Fig. 3.3b). No interaction occurred between fuel load and pine cone treatments for either variable (maximum temperature: $F = 3.05_{1,12}$, $P = 0.106$; duration: $F = 0.29_{1,12}$, $P = 0.598$).

For all species, germination coupled with TZ tests indicated high initial viability of untreated seeds (i.e., >85%); however, germination levels of untreated seeds were highly variable

(Table 3.1). Germination levels generally reflected patterns of relative seed hardness (i.e., hard-seeded species experienced the least germination). The relatively soft-seeded *D. floridanum* and moderately hard-seeded *Ce. virginianum* experienced the highest germination levels (about 70% and 50%, respectively).

Overall, burning treatments reduced the germination rate of soft-seeded *D. floridanum* and moderately hard-seeded *Ce. virginianum*, while germination of hard-seeded species—*Cr. rotundifolia*, *L. angustifolia*, *M. quadrivalvis*, and *R. reniformis*—was unaffected by burning (Table 3.1). Regardless of relative seed hardness, mortality of all species increased following experimental burns. As noted in the methods, presence of pine cones killed all seeds, regardless of species or fuel load. Pine needle load did not affect germination or mortality of any species (Table 3.1).

EXPERIMENT 2: FIELD FIRE INTENSITY MEASURES AND GERMINATION

Maximum fire temperatures did not differ among fuel treatments (fuel load: $F = 0.33_{1,1}$, $P = 0.667$; pine cones: $F = 0.61_{1,3}$, $P = 0.490$) (Fig. 3.3c). Maximum temperatures typically ranged from about 725 to 875°C and occurred during flaming combustion. These temperatures are similar to those reported in longleaf pine stands in southern Georgia (Robertson 2007; Jack et al. 2010) and are within the range of flaming combustion temperatures reported elsewhere (Neary et al. 1999). Contrary to expectations, altering pine needle loads did not affect mean duration of temperatures ≥300°C ($F = 0.14_{1,1}$, $P = 0.771$); however, presence of pine cones significantly increased duration of temperatures ≥300°C ($F = 10.20_{1,3}$, $P = 0.049$) (Table 3.3d). No interaction occurred between fuel load and pine cone treatments for the documented temperature variables (maximum temperature: $F = 1.21_{1,3}$, $P = 0.352$; duration: $F = 0.06_{1,3}$, $P = 0.823$).

Although the soft-seeded *D. floridanum* had more than 50% germination on average, germination of the remaining species was generally low (<20%) regardless of treatment (Table 3.2, Fig. 3.4). Relative to unburned controls, burning significantly reduced germination of six of the seven legume species, although the magnitude of response to burning treatments varied by species and with fuel treatment (Fig. 3.4). Only germination of *M. quadrivalvis*, which had the hardest seed coat of the species used, did not differ from unburned controls. Among burning treatments, pine needle fuel load did not influence germination of any species (Table 3.3). The presence of pine cones reduced germination of all species except *D. ciliare* and *Ce. virginianum*, although the former species was marginally reduced (Table 3.3, Fig. 3.5). No interaction between fuel load and presence of pine cones occurred (Table 3.3) and no significant block effects were found.

DISCUSSION

By providing a source for regeneration of populations and recruitment of new individuals, persistent soil seed banks buffer against localized extirpation of some species (Thompson et al. 1998; Stocklin and Fisher 1999) and help maintain genetic diversity within these species (Dolan et al. 2008). Fires influence seedling recruitment from the soil seed bank both directly and indirectly (Bond and van Wilgen 1996). The present study demonstrated that fires have the potential to directly influence post-fire recruitment by killing seeds present in the soil seed bank. Although not observed under the conditions studied herein, fires may also enhance germination of some species (such as hard-seeded legumes) within longleaf pine stands (e.g., Chapter 2; Cushwa et al. 1968; Martin et al. 1975). Fires may also act as indirect filters for seedling establishment. Open spaces may be increased after fires via litter consumption and

reduced plant cover (Laughlin and Fulé 2008; Zimmermann et al. 2008; Myers and Harms *In Press*), thus potentially increasing the availability of suitable microsites for seedling recruitment (Eriksson and Ehrlen 1992; Setterfield 2002).

Results of the present studies indicate that fire has the potential to significantly affect post-fire regeneration of legumes from the soil seed bank by influencing seed survival and germination. Fires did not appear to stimulate germination under the conditions studied, as has been reported in other studies of post-fire regeneration from the seed bank (e.g., Shea et al. 1979; Moreno and Oechel 1991). On the contrary, fires reduced germination for eight of the nine legume species studied. Only germination of *M. quadrivalvis* was not significantly reduced following fires in either the laboratory or field study. Additionally, germination responses differed between studies. Of the four species common to both studies (*Ce. virginianum, D. floridanum, L. angustifolia*, and *M. quadrivalvis*), only *Ce. virginianum* and *D. floridanum* experienced reduced germination following experimental fires in both cases; *Ce. virginianum* and *D. floridanum* have the softest seed coats of these four species and, as indicated in Chapter 1, may be less tolerant of elevated temperatures than those with harder seed coats. As indicated by the laboratory study, post-fire reductions in germination may be the result of increased seed mortality.

Variation in fine fuel loads did not influence germination in either study, while presence of pine cones significantly reduced germination in the field study and eliminated it altogether in the laboratory study. These observations are likely linked to increased fire intensities under burning pine cones compared with pine needles alone. Indeed, a variety of studies have linked increased fine fuel loads with increased maximum temperatures during fires in longleaf pine systems (e.g., Mejeur 1998; Thaxton and Platt 2006; Jack et al. 2010). Unlike those studies,

however, the fine fuel load treatments used in the present studies did not significantly influence

temperatures (Figs. 3.3a and c). Thaxton and Platt (2006) found that addition of woody fuels

further increased temperatures compared to fine fuel addition alone. Similarly, pine cones

increased temperatures in the current laboratory study. No such trend was found for the field

study, however. These observations may be due, in part, to the methods used to measure

temperatures in each study: thermocouples were used in the laboratory to measure temperatures

occurring just below the substrate's surface directly under the combusting material, whereas the

field study used a thermal camera that measured temperatures using emitted radiation from the

burning material. Residence times of temperatures ≥60°C and ≥300°C were similarly influenced

by fuel types and loads, increasing with addition of pine cones, but not with additional fine fuel.

Soils buffer elevated temperatures during fires and attenuation of temperatures with

increasing soil depth is a well-documented phenomenon (e.g., Heyward 1938; Beadle 1940;

Massman and Frank 2004); therefore, location within the soil profile will influence seed fate.

The temperature range for maximum germination is typically within 70-100°C for hard-seeded,

heat-stimulated legumes (Cushwa et al. 1968, Martin et al. 1975, Auld and O'Connell 1991,

Williams et al. 2003). Temperatures reached near the soil surface in the current studies were well

above this range of typical heat-stimulated germination. Studies of soil seed banks within

longleaf pine stands that were encountered during the literature search sampled soils to depths of

5 or 10 cm, but did not describe the vertical distribution of seeds within these samples (e.g.,

Cohen et al. 2004; Ruth et al. 2008). Seed bank studies from other fire-prone ecosystems (e.g.,

South African fynbos [Pieterse and Cairns 1986; Fourie 2008]; Australian jarrah forest [Shea et

al. 1979]; Argentinian grassland [Laterra et al. 2006]) indicate that most seeds likely occur

within the upper 5 cm. Further studies within longleaf pine stands are needed in order to quantify the vertical distribution of seeds within the soil seed bank.

Conceptual models have been developed to explain and predict post-fire germination responses. Martin and Cushwa (1966) note that, below a given threshold, temperatures have no effect on germination of heat-stimulated seeds. Above this temperature threshold, germination increases linearly with temperature until maximum germination is reached. Auld (1986) developed this model further by coupling soil temperature profiles with experimentally derived germination curves of the hard-seeded legume *Acacia suaveolens* (Sm.) Willd. to predict post-fire germination responses within the soil profile under high- and low-intensity fires. Auld's (1986) models predict that temperatures sufficient to stimulate germination of *A. suaveolens* would occur nearer to the soil's surface under low-intensity fires than high-intensity fires. Under high-intensity fires, lethal temperatures will penetrate deeper into the soil profile, thereby increasing the depth at which germination will be stimulated during these fires.

Burning woody fuels have the potential to elevate soil temperatures both vertically and laterally. Monsanto and Agee (2008) found that potentially lethal temperatures (i.e., 60°C) readily penetrated to 10 cm beneath and beside burned logs. One can visualize these elevated temperatures as a roughly bowl- or cone-shaped area of soil centered on the burning logs. Other long-burning fuels like pine cones may have similar effects. For example, a companion study to the current studies (Appendix 1) found that burning longleaf pine cones could increase temperatures 2.5 cm below the soil surface roughly 20-80°C higher than fires without pine cones.

Based on the above models and observations of the influence of pine cone fuels, a conceptual model was developed to illustrate the potential impact of pine cones on seeds within

the soil seed bank (Fig. 3.6). This model assumes that: 1) temperatures decrease with increasing distance (both laterally and vertically within the soil) from the pine cone; 2) direct seed mortality of all species decreases with increasing distance from the pine cone; and 3) seeds of some species will be stimulated to germinate by elevated temperatures from the burning pine cone. This model predicts that most or all seeds located under and immediately adjacent to a pine cone (or other fuel source that prolongs fire) will be killed by the higher intensity fire (Zone of Mortality). As depth and lateral distance from a pine cone increase, temperatures will descend into the favorable range for germination (Zone of Germination). Each species, particularly those with heat-stimulated germination, will have a particular optimum temperature range and thus an optimum distance/depth from a pine cone for germination. Eventually, distance and/or depth from a pine cone will be sufficiently great to have no effect on seeds in the soil seed bank because soil temperature will not rise into the range that stimulates germination (Zone of No Effect).

By killing mature plants and seeds, pine cones create open spaces that are available for colonization. Within the soil seed bank, seeds that are able to survive and germinate closer to burned pine cones may be able to take advantage of the open space created, thus providing them with an initial competitive advantage over species that are less tolerant of high intensity fires. Following germination in the open spaces created by pine cones, other fire-mediated factors, such as water or light availability, may be important for seedling survival (Bond and van Wilgen 1996). In particular, within longleaf pine stands, moisture availability may be the most important resource for seedling establishment (Iacona et al. 2010). Because pine cones and other long-burning fuels are not distributed uniformly throughout longleaf pine stands (Hiers et al. 2009),

these open spaces will exhibit similarly heterogeneous distribution, thus influencing fine-scale distribution patterns of species.

The potential influence of pine cones on fine-scale vegetation dynamics within the longleaf pine ecosystem and other frequently burned pine-dominated systems, such as ponderosa pine (*Pinus ponderosa* [Dougl.]) ecosystems of the western United States, needs to be tested in the field. To test this hypothetical model, lateral and vertical soil temperature profiles need to be characterized for burning pine cones, as well as the influence of soil properties such as moisture content, compaction, porosity, and texture. Season of fire may also influence the ecological effects of pine cones, as soil and fuel moistures as well as ambient temperatures will likely vary with season. Additionally, each species likely has a maximum survivable germination depth. Seeds stimulated to germinate below this maximum depth will be indirectly killed by the fire because of their inability to reach the surface before exhausting their energy stores. No studies were encountered that have investigated maximum survivable germination depth for species native to the longleaf pine ecosystem. Finally, the applicability of this model to other long-burning fuels needs to be assessed by experiments that compare burning characteristics of pine cones to other plant materials, such logs and tree limbs.

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Species	Treatment	Germination (%)	Mortality (%)
Ce. virginianum	Control	48.8 ± 4.3 a	7.5 ± 2.5 b
	Low Fuel	10.0 ± 5.0 b	71.3 ± 15.3 a
	High Fuel	8.8 ± 5.2 b	90.0 ± 6.1 a
Cr. rotundifolia	Control	16.3 ± 7.2 a	15.0 ± 2.0 b
	Low Fuel	21.3 ± 8.8 a	68.8 ± 12.0 a
	High Fuel	11.3 ± 8.0 a	77.4 ± 9.6 a
D. floridanum	Control	70.0 ± 4.6 a	13.8 ± 1.3 b
	Low Fuel	13.3 ± 13.3 b	87.5 ± 12.6 a
	High Fuel	0.0 ± 0.0 b	97.5 ± 1.4 a
L. angustifolia	Control	1.3 ± 1.3 a	1.3 ± 1.3 b
	Low Fuel	3.8 ± 3.8 a	87.4 ± 9.2 a
	High Fuel	2.5 ± 2.5 a	83.6 ± 8.5 a
M. quadrivalvis	Control	7.5 ± 1.4 a	3.8 ± 1.3 b
	Low Fuel	2.5 ± 2.5 a	56.8 ± 18.3 a
	High Fuel	2.5 ± 1.4 a	74.2 ± 9.5 a
R. reniformis	Control	2.5 ± 1.4 a	2.5 ± 1.4 b
	Low Fuel	1.8 ± 1.8 a	64.5 ± 14.3 a
	High Fuel	0.0 ± 0.0 a	92.5 ± 4.8 a

Table 3.1. Influence of fine fuel load variation on post-burn germination and mortality of legume seeds (mean ± SE) for the laboratory germination study. Different letters indicate significant differences as indicated by post-hoc Tukey's means separation tests ($P < 0.05$).

Species	Model Component	df	F	P
Ce. virginianum	Bed	2,14	1.57	0.243
	Burned	1,14	12.91	0.003
D. ciliare	Bed	2,14	3.34	0.065
	Burned	1,14	22.39	<0.001
D. floridanum	Bed	2,14	0.06	0.945
	Burned	1,14	75.01	<0.0001
L. angustifolia	Bed	2,14	0.81	0.466
	Burned	1,14	38.28	<0.0001
M. quadrivalvis	Bed	2,14	0.57	0.579
	Burned	1,14	0.59	0.456
P. canescens	Bed	2,14	3.50	0.058
	Burned	1,14	66.50	<0.0001
T. virginiana	Bed	2,14	0.53	0.602
	Burned	1,14	13.33	0.003

Table 3.2. Effect of burning on germination of legume seeds (July – November 2007; $n = 30$) in the field germination study. Bed was used as the blocking component of the RCB split-plot ANOVA. Mean germination values from burned treatments and untreated controls are depicted in Fig. 3.3.

Species	Model Component	df	F	P
Ce. virginianum	Bed	2,2	2.63	0.276
	Fuel Load	1,2	0.46	0.567
	Pine Cones	1,16	3.08	0.099
	Fuel × Cones	1,16	0.64	0.435
D. ciliare	Bed	2,2	5.00	0.167
	Fuel Load	1,2	3.71	0.194
	Pine Cones	1,16	3.71	0.072
	Fuel × Cones	1,16	4.30	0.055
D. floridanum	Bed	2,2	3.14	0.242
	Fuel Load	1,2	2.58	0.249
	Pine Cones	1,16	10.89	0.005
	Fuel × Cones	1,16	2.21	0.157
L. angustifolia	Bed	2,2	1.31	0.433
	Fuel Load	1,2	0.27	0.656
	Pine Cones	1,16	7.11	0.017
	Fuel × Cones	1,16	0.01	0.910
M. quadrivalvis	Bed	2,2	4.95	0.168
	Fuel Load	1,2	0.18	0.711
	Pine Cones	1,16	6.01	0.026
	Fuel × Cones	1,16	0.20	0.663
P. canescens	Bed	2,2	5.17	0.162
	Fuel Load	1,2	0.05	0.838
	Pine Cones	1,16	10.49	0.005
	Fuel × Cones	1,16	0.00	1.000
T. virginiana	Bed	2,2	2.12	0.321
	Fuel Load	1,2	0.66	0.502
	Pine Cones	1,16	12.90	0.002
	Fuel × Cones	1,16	0.88	0.361

Table 3.3. Effect of fuel variation on post-burn germination of legume seeds for the field germination study. Bed was used as the blocking component of the RCB split-plot ANOVA. Mean germination values from burned treatments with and without pine cones are depicted in Fig. 3.4.
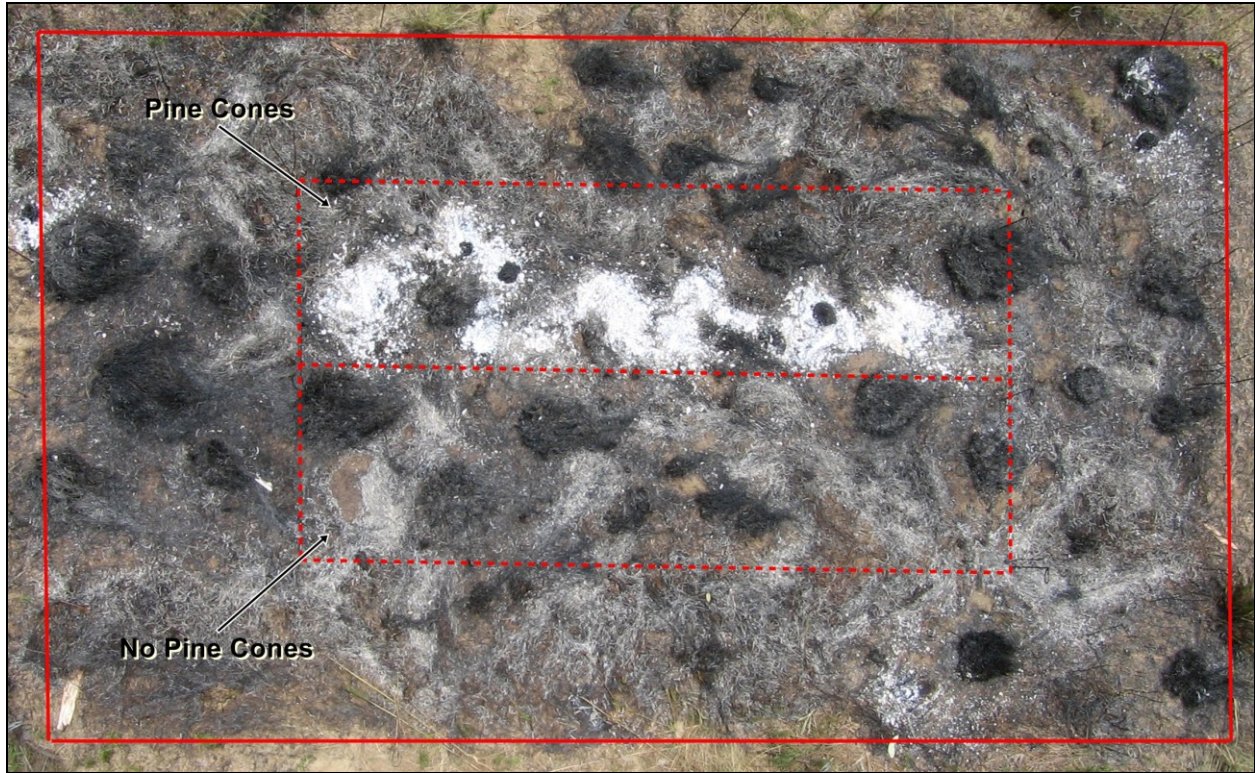
Figure 3.1. Example of plot layout (post-fire). Solid line is plot boundary (pine needle treatments) and dashed line is split-plot boundary (pine cones treatments). White ash within the pine cone treatment split-plot is remains of longleaf pine cones and round, black areas are wiregrass clumps.
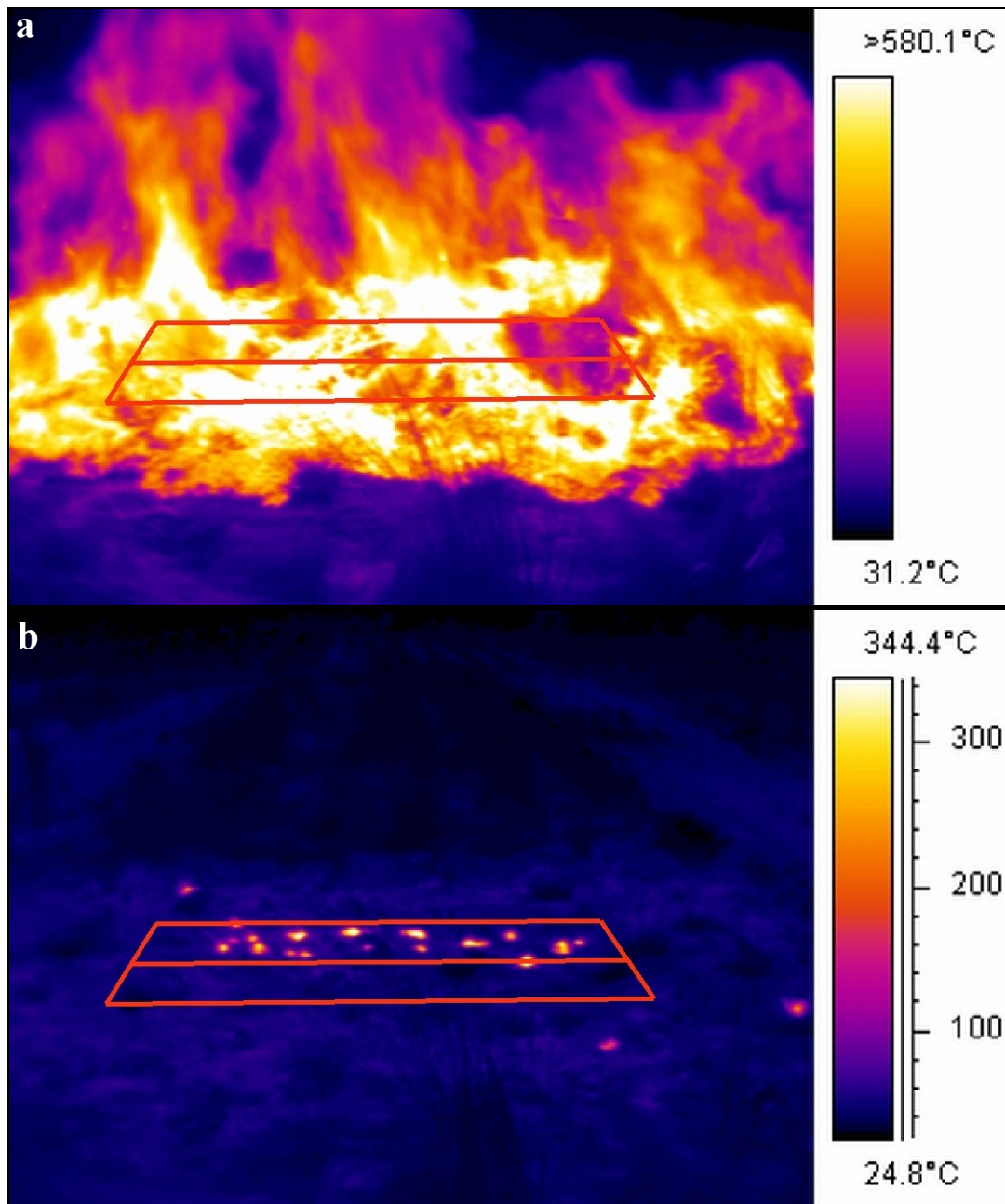
Figure 3.2. Thermal imagery of a typical burn (a) during flaming combustion (at approx. 0.5 min after ignition of fire) and (b) during smoldering/glowing combustion with smoldering pine cones visible (at approx. 30 min after ignition). Red outlines in the centers of the images indicate approximate split-plot boundaries.
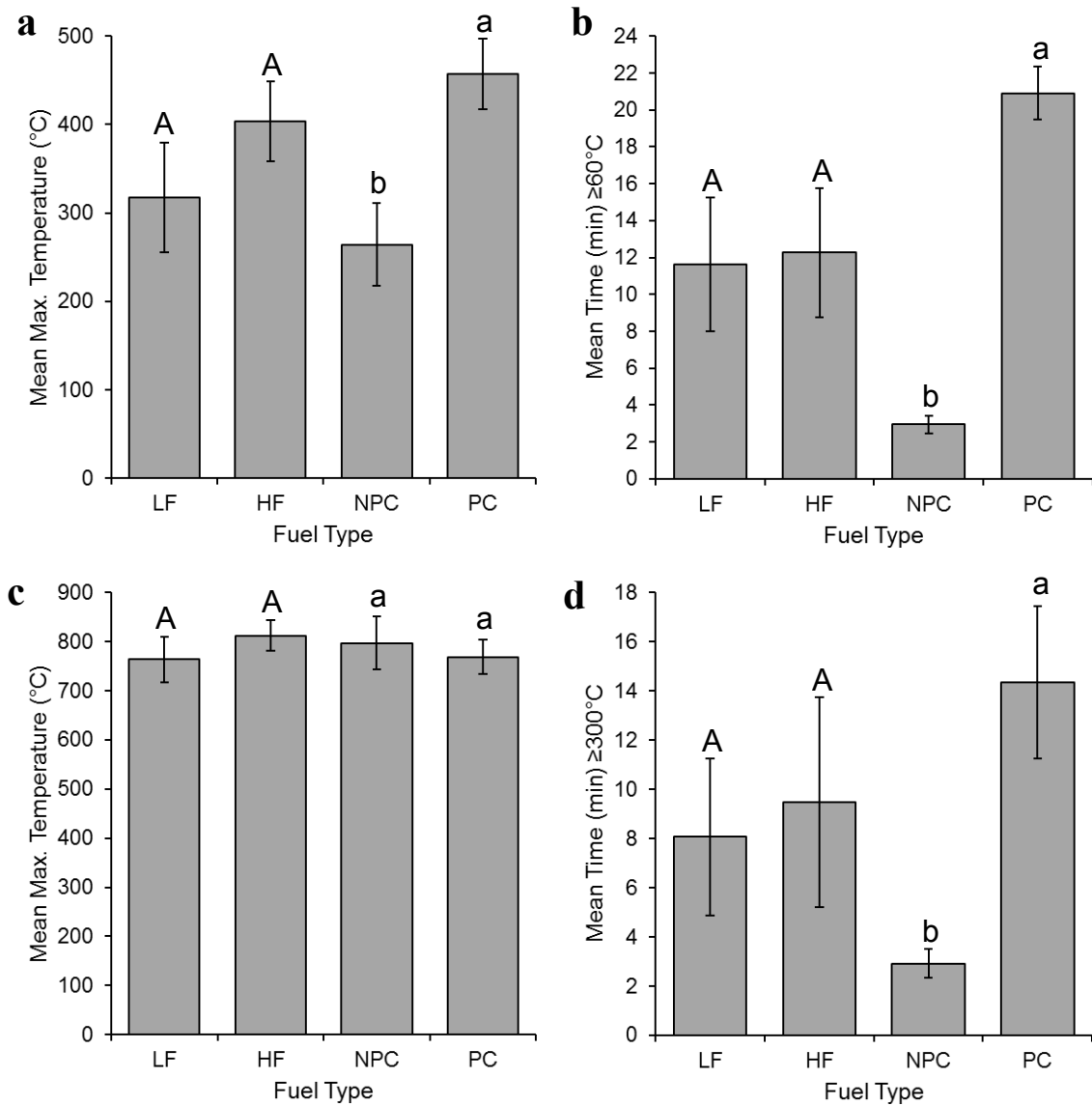
Figure 3.3. Effect of fuel treatments on mean maximum temperatures and mean durations of elevated temperatures for the laboratory study (a & b) and field study (c & d). Error bars represent 1 SE. Different uppercase letters indicate significant differences ($P < 0.05$) between fine fuel treatments as indicated by ANOVA comparisons. Different lowercase letters indicate significant differences ($P < 0.05$) between pine cone treatments as indicated by ANOVA comparisons. No significant interaction ($P > 0.05$) between fine fuel loads and pine cones was found for either study. LF = low fine fuel, HF = high fine fuel, NPC = no pine cones, PC = pine cones.
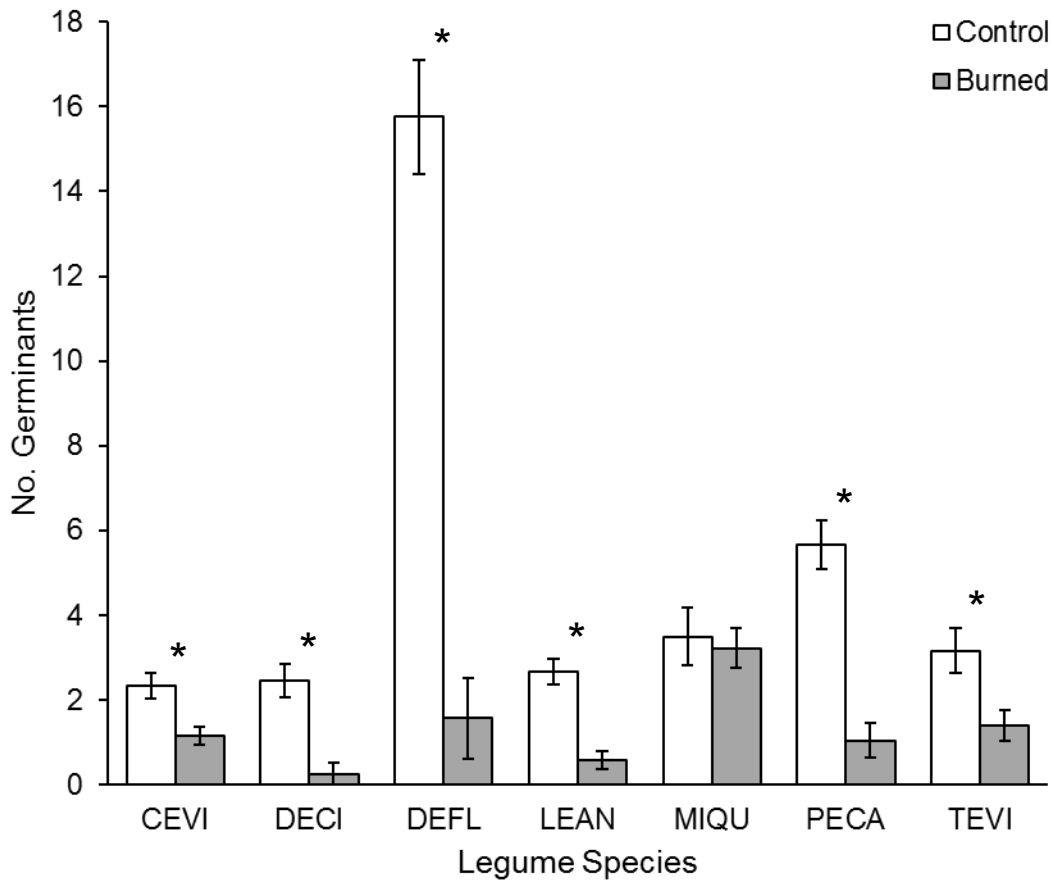
Figure 3.4. Germination of seeds from untreated controls and burned treatments (means; $n = 30$) for the field germination study. Error bars represent 1 SE. Asterisks indicate significant differences as indicated by ANOVAs ($P < 0.05$) presented in Table 3.2. Species codes are: CEVI = *Ce. virginianum*, DECI = *D. ciliare*, DEFL = *D. floridanum*, LEAN = *L. angustifolia*, MIQU = *M. quadrivalvis*, PECA = *P. canescens*, TEVI = *T. virginiana*.
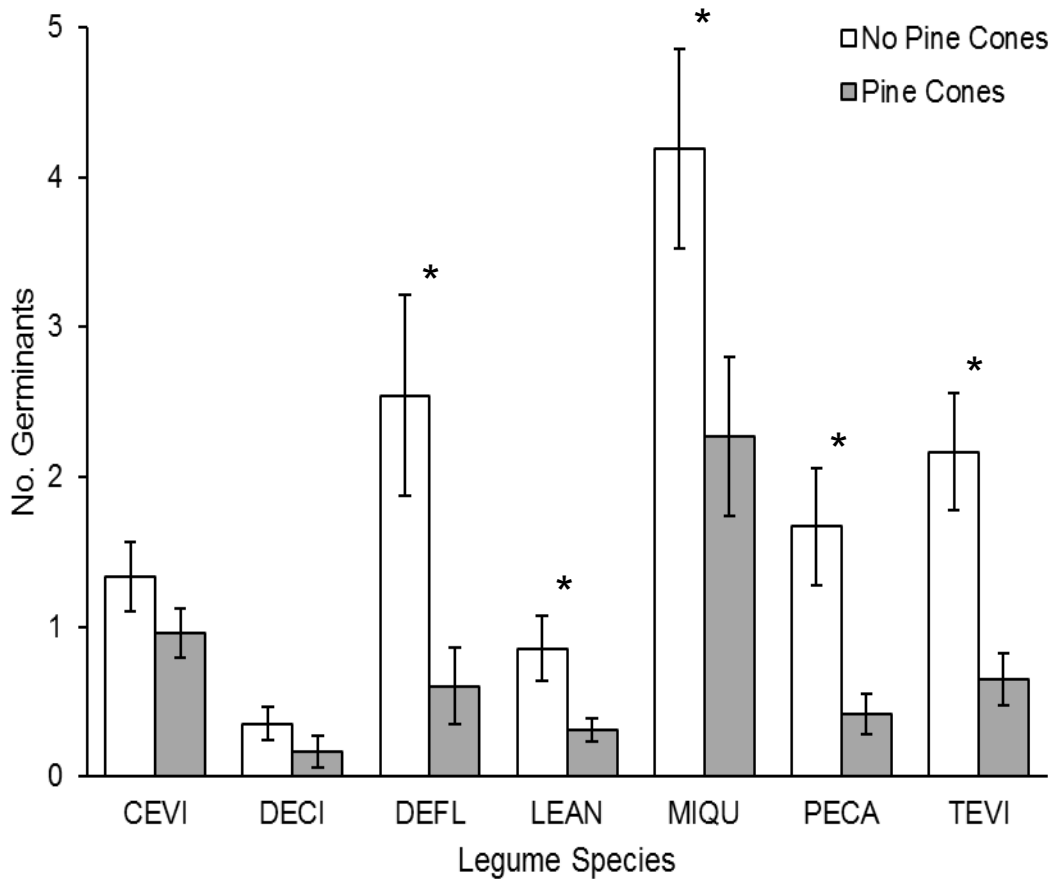
Figure 3.5. Effect of presence of pine cones during fires on post-burn germination of legume seeds (means; $n = 30$) for the field germination study. Error bars represent 1 SE. Asterisks indicate significant differences between treatments as indicated by ANOVAs ($P < 0.05$) presented in Table 3.3. Species codes are: CEVI = *Ce. virginianum*, DECI = *D. ciliare*, DEFL = *D. floridanum*, LEAN = *L. angustifolia*, MIQU = *M. quadrivalvis*, PECA = *P. canescens*, TEVI = *T. virginiana*.
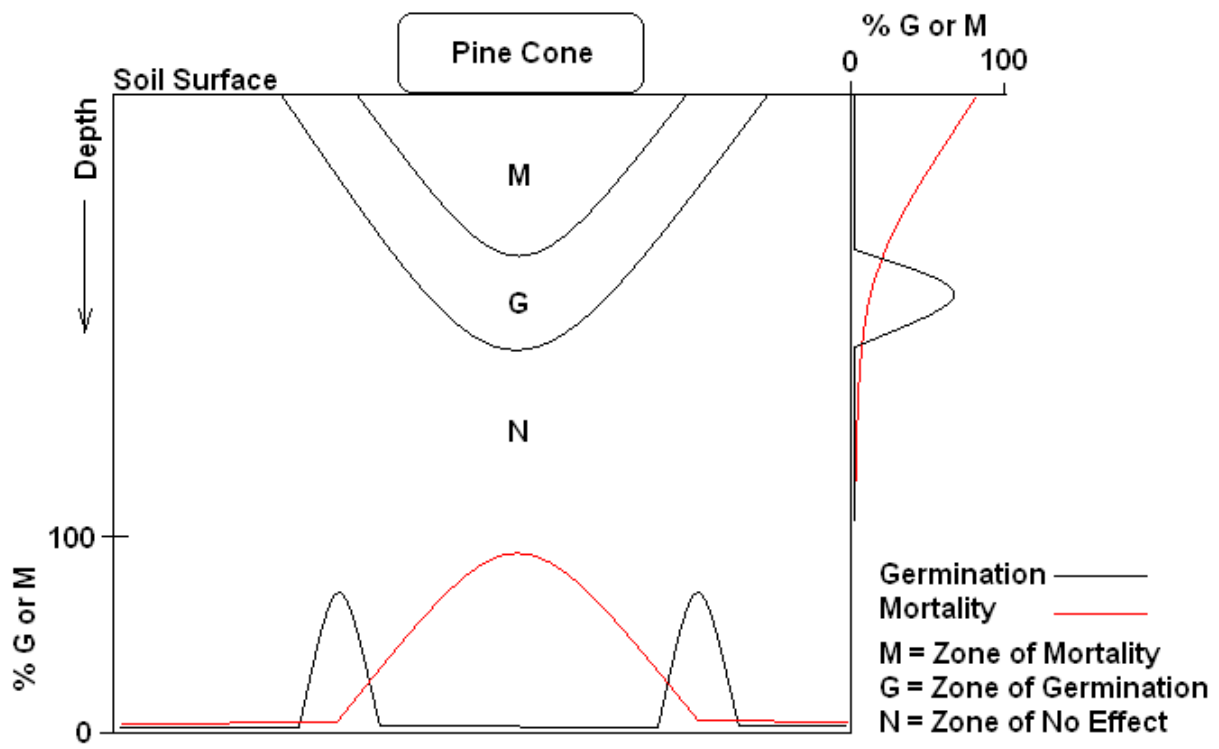
Figure 3.6. Potential impacts of burned pine cones on germination and mortality of seeds.

CHAPTER 4

SUMMARY AND CONCLUSIONS

Results of the studies presented in Chapters 2 and 3 indicate that fires have the potential

to affect germination and mortality of legume seeds. Such impacts may, in turn, affect

recruitment of these and similar species, thereby influencing post-fire vegetation patterns. The

species-specific germination and mortality responses observed indicate that the intensity of each

fire may be an important determinant of post-fire plant assemblages. Furthermore, fine-scale fuel

variation, which in turn affects similarly scaled fire behavior, has the capacity to dramatically

affect recruitment of individual species from seed.

The results presented in Chapter 2 indicate species-specific germination and responses to

the various fire surrogates studied: dry and moist heat, smoke, and manual scarification.

Germination and mortality of most species tended to be relatively unaffected by dry heat, except

when exposed to the most extreme combination of temperature and duration (i.e., 150°C for 10

min), which was characterized by reduced germination and increased mortality. Two species,

however, experienced enhanced germination with increasing heat: the hard-seeded *Lespedeza*

angustifolia and *Crotalaria rotundifolia*; although the effect was most pronounced for *Cr.*

rotundifolia and both species exhibited sharply increased mortality at 10 min and 150°C.

Germination of all species was enhanced by short exposures to moist heat (0.16 to 1 min)

without experiencing increased mortality. Additionally, seeds of all species survived (15-70%)

even after the longest exposure to moist heat (10 min), although germination tended to decline

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following these longer exposure times. Scarification greatly enhanced germination of all species without affecting seed mortality. Generally, germination and seed mortality of the legume species examined in this study do not appear to be influenced by plant-derived smoke. The only exception was *Centrosema virginianum*, which exhibited slightly reduced, but statistically significant, germination after exposure to smoke.

Chapter 3 investigated the potential role of fine-scale fuel heterogeneity on various parameters of fire behavior (maximum temperature and duration of potentially lethal temperatures). Increasing fine fuel loads did not affect intensity of the fire (i.e., maximum temperatures or duration of potentially lethal temperatures). While maximum temperatures were not affected, adding pine cones greatly increased the time seeds were exposed to potentially lethal temperatures. As might be expected, in both studies, germination of all species was substantially reduced by exposure to fire, especially if seeds had pine cones burning over them. Manipulating pine needle fuel (i.e., fine fuel) loads did not affect germination.

A conceptual model was presented in Chapter 3 describing the potential effects of pine cones, which can smolder long after the fine fuels have stopped burning and cooled. Elevated temperatures from fires have the potential to kill seeds and, in locations where smoldering fuels promote longer durations of these lethal temperatures, such as under pine cones, more (or all) seeds will be killed. Under pine tree canopies, it is logical to assume that all seeds will be killed directly under and adjacent to any pine cones consumed in a fire. One can further assume that potentially lethal conditions decrease with increasing distance from pine cones until, after some distance, lethal conditions are no longer achieved (i.e., seeds are unaffected). By creating small pockets of open space, burned pine cones create potential colonization gaps for seeds stored in the soil seed bank or from other sources (e.g., unburned plants, animal scat, etc.). Within the soil

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seed bank, seeds that are able to survive and germinate closer to the center of burned pine cones may be able to take advantage of the open space, survive, and thrive, while others are killed or, if they survive, are unable to compete. Differential survival and germination of seeds and recruitment of seedlings following fires—mediated by small-scale pre-fire fuel variation—may, therefore, influence fine-scale patterns of plant distribution. Furthermore, within frequently burned systems, small-scale fire variation may be more important for determining localized species distribution—at least during initial stages of (re)colonization—than other factors, such as light or soil nutrients.

The behavior and impact of each fire is unique, affected by fuel loads, type, and distribution, as well as by moisture, weather, and local topography. Similarly, individual seeds will respond to fires based on a number of intrinsic (e.g., moisture content, presence of a hard seed coat, etc.) and extrinsic (e.g., location within soil profile, soil moisture, fire intensity, etc.) factors. Of the intrinsic factors, seed hardness and moisture content may be the most important to survival during, and germination following, fires. It is clear that cracking (i.e., scarifying) the seed coat increases germination of these legume species. Dry heat generated by fires may vaporize internal seed moisture and increase internal pressure or cause cells to differentially swell and contract, thereby cracking the seed coat. Alternatively, steam generated within moist soil or driven off of adjacent biomass during fires may soften or penetrate seed coats, thereby initiating germination. In addition, seeds with low moisture contents may have less damage to cells following water vaporization and may, therefore, more readily survive fires than seeds with high moisture contents. Although high moisture contents may initially buffer seeds against elevated temperatures experienced during fires, increased water content may also increase cellular damage once the water has vaporized. Because intrinsic and extrinsic seed characteristics

do not exist in isolation from one another, knowledge of these factors and their interactions are essential for determining species response to a given fire.

APPENDIX

FIELD OBSERVATIONS OF SOIL SURFACE AND SUBSURFACE TEMPERATURES

AND RESIDENCE TIMES OF SMALL-SCALE EXPERIMENTAL FIRES

Treatment	Temperature (°C)[a]		Residence Time (min)[b]	
	Surface	Subsurface	Surface	Subsurface
March, No Cones	526.3 ± 144.8	30.0 ± 3.8	1.5 ± 0.5	0.0 ± 0.0
March, Cones	675.5 ± 173.5	52.6 ± 11.7	7.0 ± 2.3	0.8 ± 1.3
July, No Cones	525.3 ± 157.1	57.0 ± 8.8	14.8 ± 6.6	1.8 ± 2.6
July, Cones	685.0 ± 83.8	75.7 ± 34.6	35.8 ± 13	8.8 ± 11.9

Temperatures and residence times (mean ± SD) recorded at the soil surface (surface) and 2.5 cm belowground (subsurface) during experimental burns of individual *Tephrosia virginiana* plants in a garden plot at the Joseph W. Jones Ecological Research Center in Bryan Co., Georgia in March and July 2007. Plants were burned with (cones) or without (no cones) two pine cones and a small amount of pine needles (approx. 32 g). Pine cones and pine needles were oven-dried prior to use. Fires were ignited using the portable furnace described in Chapter 3. Three replicates were used for each treatment. [a]Mean maximum temperatures recorded using chromel-alumel (K-type) thermocouples. [b]Mean residence time in minutes of temperatures ≥60°C (i.e., potentially lethal).