# FOREST HEALTH IMPLICATIONS OF RESTORING LONGLEAF PINE (*PINUS PALUSTRIS* MILL.) ECOSYSTEMS WITH PRESCRIBED FIRE

by

#### HALEY MARGARET WALTMAN RITGER

(Under the Direction of Kamal J.K. Gandhi)

#### ABSTRACT

The once vast longleaf pine (Pinus palustris Mill.) forest ecosystems in the southeastern U.S. have dwindled to small remnants, often lacking frequent prescribed fire required to realize maximum ecosystem services and benefits. Reintroducing fire to restore these ecosystems can be associated with increased risk of subcortical insect (Coleoptera: Curculionidae: Scolytinae) colonization and tree mortality, necessitating an understanding of how beetles and tree defenses respond to restoration and management with prescribed fire. We evaluated the impacts of various fire regimes and site conditions in longleaf pine forest stands on subcortical beetles and tree defenses using a long-term fire regime experiment in southwestern Georgia, U.S. By sampling trap catches of over 140,000 beetles across three fire regime treatments and two edaphic site types and measuring stand characteristics, we found that effects of fire exclusion and site type varied at the species level for bark beetle trap catches and altered stand conditions. We observed higher resin flow in longleaf pine trees in stands that had been fire-excluded for 17 years compared to frequently burned stands, but we did not observe differences in total monoterpene concentration among the fire regime treatments. However, we found

higher total monoterpene concentrations in xeric than mesic sites, indicating site-level differences in resin properties. We additionally investigated short-term effects on resin quantity and quality following dormant and growing season prescribed burns in mature, dominant longleaf pine trees. There was a temporary increase in resin flow following the dormant season prescribed burn, no effect on resin flow following the growing season burn, and no differences in total monoterpene concentration following either season burn. These novel experiments and results detailed herein provides restoration guidance to managers and directions for future research on endangered longleaf pine ecosystems in the southeastern U.S.

INDEX WORDS:bark beetles, forest management, *Ips*, longleaf pine, mesic,prescribed fire, resin, restoration, terpenes, xeric

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### HALEY MARGARET WALTMAN RITGER

BA, Indiana University, 2010

MPA, Indiana University, 2014

MSES, Indiana University, 2014

A Dissertation Submitted to the Graduate Faculty of The University of Georgia in Partial Fulfillment of the Requirements for the Degree

DOCTOR OF PHILOPSOPHY

ATHENS, GEORGIA

2023

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### HALEY MARGARET WALTMAN RITGER

Major Professor:

Kamal J.K. Gandhi

Committee:

Steven T. Brantley Kier D. Klepzig Clinton T. Moore Joseph J. O'Brien

Electronic Version Approved:

Ron Walcott Dean of the Graduate School The University of Georgia December 2023

## DEDICATION

For my parents, Tim and Angie Waltman; my husband, Robert Ritger; my dog, Cheddar; and coffee—without you it would not have been possible.

#### ACKNOWLEDGMENTS

I would like to acknowledge all of the academic professionals who have guided, challenged, supported, and believed in me through this winding journey. I especially thank my doctoral advisors, Drs. Kamal Gandhi and Steven Brantley, who stuck with me through so many years and provided mentorship and encouragement. I also thank the members of my committee, Drs. Kier Klepzig, Clint Moore, and Joe O'Brien, whose advice and feedback throughout this lengthy pursuit contributed greatly to my development and this dissertation. I also thank my co-authors Drs. Brian Sullivan and Caterina Villari for contributions to this work and my professional development.

I thank Dr. Lindsay Boring for his vision that enabled much of the research included in this dissertation. I would like to acknowledge all of those who made parts of this work possible with assistance in the field and lab: research professionals and technicians from the University of Georgia (Arya Aghdassi, Brittany Barnes, Lea Clark, Sarah Forget, Meghan McMillan, Afaq Niyas, and Holly Munro) and the Jones Center at Ichauway (Michael Belovitch, Crystal Bishop, Denzell Cross, D. Alex Gordon, Riley Green, Phoebe Judge, Stephen Lewis, Sierra Perez, Robert Ritger, Sean Reynolds, Brandon Rutledge, Tom Sheehan, and Stribling Stuber). Dr. Rick Hoebeke (University of Georgia Natural History Museum) provided guidance and confirmation for insect identifications. Dr. Kimberly Novick (Indiana University), Dr. Ken Keefover-Ring (University of Wisconsin), and Drs. O. Sander Denham and Charles Mason (USDA Agricultural Research Service) contributed training and expert advice on methodologies employed in this dissertation.

I acknowledge financial support provided by the University of Georgia D.B. Warnell School of Forestry and Natural Resources and the Jones Center at Ichauway. An Interdisciplinary and Innovative Research Grant from the University of Georgia Graduate School supported portions of this dissertation. I would also like to acknowledge financial support during my doctoral studies from the Martha Love May Memorial Scholarship, the Robert W. and June C. Porterfield Memorial Scholarship, and University of Georgia Graduate Student Travel Awards.

And last but certainly not least, a tremendous and heartfelt thanks to my family, friends, and work colleagues, who never once doubted that I could do this thing: Mom, Dad, Robert, Dayton, Phoebe, Laura, Izzy, Stribling, Monica, Bailey, Holly.

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#### CHAPTER 1

#### INTRODUCTION AND LITERATURE REVIEW

#### 1.1 The longleaf pine ecosystem and management with prescribed fire

Longleaf pine, *Pinus palustris* Mill. (Pinaceae), forests once dominated the landscape of the southeastern United States, covering approximately 36.4 million hectares (Barnett 2002). Longleaf pine characteristics and life history, including thick bark, highly flammable needles, and resistance to fire in the seedling and sapling stages, indicate a long history of coevolution with frequent fire (Stambaugh et al. 2011). Historically, longleaf pine forests were maintained by Native Americans using prescribed fire for multiple benefits such as promoting game habitat and making clearings for small scale agriculture (Barnett 2002, Van Lear et al. 2005). After European colonization, longleaf pine cover was reduced to about 1.6 million hectares due to expanding human settlement, logging and clearing for agricultural land, and, in the 20th century, conversion to faster growing loblolly pine for timber (Barnett 2002).

Longleaf pine's ability to grow in a wide array of site conditions from the coastal plain to the montane foothills of the southern Appalachians meant these ecosystems covered much of the southeastern U.S (Harper 1913, Boyer 1990, Gilliam et al. 1993, Frost 1993, Brockway et al. 2005). These vast longleaf pine forests provided myriad ecological and economic benefits through habitat for wildlife and other plant species, timber, and non-wood products such as turpentine. Remnants of these open savannah forests continue to provide hotspots of biodiversity, forest products, and other ecological benefits like carbon storage (Kirkman et al. 2001, Barnett 2002, Kush et al. 2004). Longleaf pine forests host the highest plant species richness in North America (Drew et al. 1998). These ecosystems also support rare endemic vertebrates such as the keystone species gopher tortoise (*Gopherus polyphemus* [Daudin]) and the endangered red cockaded woodpecker (*Leuconotopicus borealis* [Vieillot]) (Noss 1989). Longleaf pine forests additionally support culturally important game wildlife species, particularly the northern bobwhite (*Colinus virginianus* [L.]), the popularity of which promoted the maintenance of the ecosystem remnants with frequent fire in the southeastern U.S. as most North American forests were being subjected to strict fire suppression management policies (Way 2006, van Wagtendonk 2007).

Longleaf pine forests are dependent on frequent, low-intensity surface fires to maintain their characteristic structure and biodiversity (Barnett 2002, Varner et al. 2005, Van Lear et al. 2005, Stambaugh et al. 2011). This fire regime is necessary for longleaf establishment by exposing bare mineral soil for germination, and fire maintains the forest's low basal area. Fire is also required for the successful growth and reproduction of the diverse understory herbaceous layer (Stambaugh et al. 2011). Fire promotes growth of longleaf pine through removal of litter and competing vegetation, increasing soil organic matter, and improving soil fertility (Gilliam and Platt 1999). Frequent fire maintains and promotes biodiversity in the herbaceous layer in two important ways: preventing overstory development and reducing overuse of the limited soil moisture (Van Lear et al. 2005). As such, multiple species associated with longleaf pine forests are also adapted to these fire regimes across the range. As the importance of longleaf for biodiversity, wildlife, and other ecosystem services is well-established, a number of organizations have been working to restore millions more acres to longleaf forests through the America's Longleaf Initiative (Lopez et al. 2009). With such ambitious goals, and myriad stakeholders ranging from small landowners to conservation easement organizations and the timber industry, there is a need for both basic and applied research to support the rationale and methodology for restoration. Much of that research centers around the complex interactions between longleaf pine, fire, and other variables that might affect short- and long-term longleaf restoration.

#### 1.2 The southern pine bark beetle guild

In addition to frequent fire, longleaf pines coexist with and have coevolved over millions of years with invertebrate herbivores such as bark beetles (Coleoptera: Curculionidae: Scolytinae). These beetles play an ecologically important role in decomposition and nutrient cycling and act as a disturbance agent (Campbell et al. 2008). Some species of bark beetles are classified as forest pests and can cause tree mortality in small "spots" (localized clusters of trees within a stand) or even widespread mortality during outbreaks. At endemic population levels, these native bark beetles maintain overall stand vigor by preferentially feeding on weakened trees (Waring and Pitman 1985, Christiansen et al. 1987). When many beetles successfully colonize a single tree, the tree is effectively girdled by the galleries excavated by the beetles for egg-laying and introduced beetle-vectored fungi that clog the vascular system (Waring and Pitman 1985, Lorio 1986, Franceschi et al. 2005). When large numbers of beetles colonize many trees, reproductive success increases, and populations can become large enough to overwhelm large numbers of healthy trees. This phenomenon is considered an outbreak, and under such conditions, widespread tree mortality can occur (Franceschi et al. 2005, Campbell et al. 2008).

In southeastern U.S. pine forests, southern pine beetle (Dendroctonus frontalis Zimmerman, SPB) is the most destructive bark beetle species, causing extensive economic and ecological losses during outbreak years (Pye et al. 2011, Tchakerian and Coulson 2011). While SPB can attack and cause mortality in longleaf pine, historically longleaf pine forests have not had extensive SPB outbreaks (Friedenberg et al. 2007, Martinson et al. 2007, Coulson and Klepzig 2011, Nowak et al. 2015, Clarke et al. 2016). Other bark beetle species associated with forest health issues in southeastern U.S. pine forests include *Dendroctonus terebrans* (Olivier), *Ips avulsus* (Eichhoff), *Ips* calligraphus (Germar), and *Ips grandicollis* (Eichhoff). These beetles are generally considered secondary colonizers and often co-occur, preferentially feeding on trees weakened by predisposing factors, such as lightning strike or fire (Wood 1982, Drooz 1985, Bryant et al. 2006, Nebeker 2011, Munro et al. 2019). Additionally, Orthotomicus *caelatus* (Eichhoff) often co-occurs with the aforementioned species utilizing weakened or dying trees, though it has not been associated with economic impacts in southern forests (Wood 1982, Drooz 1985). The root-feeding weevils Hylobius pales (Herbst), Pachylobius picivorus (Germar), and beetles in the genus Hylastes Erichson can also contribute to mortality in pine seedlings and saplings (Beal and McClintick 1943, Wood 1982, Nord et al. 1982, Hanula et al. 2002, Zanzot et al. 2010, Schowalter 2018). Hence,

a complex of various subcortical beetles, both above- and below-ground can affect longleaf pine tree health and sustainability.

#### 1.3 Tree defenses to bark beetles

Pine trees are not defenseless against herbivorous insects. Their primary defense is resin, a viscous, sticky liquid that exists under pressure within the resin duct system of the inner bark of the tree (Vite and Wood 1961, Franceschi et al. 2005, Krokene 2015). When the bark is punctured, resin flows out of the wound, and this exudation can prevent a beetle from successfully entering the bark by acting as a physical barrier (Hodges et al. 1979, Franceschi et al. 2005, Krokene 2015). There is also evidence that the chemical properties of resin can prevent successful beetle attacks and/or reproduction (Stark 1965). Standing resin pools present in trees under ambient conditions make up trees' "constitutive resin," and this resin is expelled during initial and low-level attacks. When a tree experiences a sudden increase in wounding activities, and infection by beetle associated fungi, the tree may begin to increase resin synthesis. This resulting pool of resin is considered "induced resin" (Lombardero et al. 2000). The primary line of defense for a single tree against bark beetle mortality is to prevent successful entry of a large number of beetles by exuding resin. This tree-level resistance has important implications for the overall health of the stand because it is the first line of defense to prevent successful colonization and reproduction of bark beetles, thus preventing the rapid population growth that can lead to outbreak populations large enough to overwhelm defenses of many trees.

#### 1.4 Interactions among bark beetles, longleaf pine trees, and fire

Although longleaf pine is particularly well-adapted to frequent fire, and prescribed fire is an irreplaceable tool in longleaf pine management and restoration, longleaf pine is vulnerable to fire under unfavorable conditions. Longleaf pines can experience detrimental impacts from fire, especially when fire has been suppressed for an extended period of time resulting in a denser midstory with higher fuel loads (Varner et al. 2005). Post-fire tree death can be caused directly by canopy damage (canopy scorch or consumption), root damage, or basal cambium damage (either direct char or heat damage) (Varner et al. 2005, O'Brien et al. 2010). Fire can also indirectly cause tree mortality by inducing physiological tree stress that leads to susceptibility to pathogens or pests (Varner et al. 2005, O'Brien et al. 2010).

Bark beetles interact with fire in complex ways and through direct and indirect mechanisms. Under limited circumstances, beetles can be directly killed by fire if they are in a vulnerable development stage and location (McCullough et al. 1998). However, some beetles are also equipped to detect new areas for colonization by sensing radiant heat through infrared receptors or visually orienting to a smoke plume (McCullough et al. 1998). This attraction may be beneficial to beetles in that scorched and wounded trees are more susceptible to bark beetle attack; however, trees may increase their defenses against insects following a fire (Lombardero et al. 2000, Campbell et al. 2008).

Interactions among bark beetles, pine trees, and fire are complex and depend on many variables related to fire frequency, intensity, and severity; site conditions; and burn season. This complexity can lead to seemingly conflicting conclusions about what may happen regarding tree-bark beetle interactions following the reintroduction of prescribed fire in pine stands. It is possible that there may be increases in tree defenses, fire damage, and/or bark beetle numbers, and hence, the respective levels will determine the survivorship of trees in fire-introduced stands.

Although conventional wisdom holds that longleaf pines are not susceptible to widespread bark beetle damage like other southern pines, formal research is quite limited. A historical examination of bark beetle caused mortality in the West Gulf Coastal Plain provides evidence that non-SPB beetles have played an important role as disturbance agents in longleaf pine forests prior to European settlement and subsequent intensive forest management practices (Clarke et al. 2000). Experimental studies have produced mixed but limited effects of various prescribed and wildfire treatments in longleaf pine on these subcortical beetle taxa. Following a wildfire in mixed stands of longleaf pine and slash pine (*P. elliottii* Engelm.), Hanula et al. (2002) found lower trap catches of *I*. grandicollis and D. terebrans in burned stands compared to unburned stands, higher trap catches of *H. pales* and *P. picivorus* in high severity burn stands compared to unburned stands, and higher catches of *P. picivorus* in moderate burn stands compared to unburned stands. In longleaf pine stands in South Carolina, Sullivan et al. (2003) applied a range of burn severity treatments and measured trap catches compared to an unburned control, finding higher trap catches in the burned treatments for *Hylastes salebrosus* Eichhoff, Hylastes tenuis Eichhoff, and P. picivorus, but no difference in catches among the treatments for *D. terebrans*. In a study comparing various restoration treatments, including various combinations of thinning, burning, and herbicide application, in longleaf pine forests in Alabama, Campbell et al. (2008) found no differences among treatments for trap catches of *I. grandicollis* and *H. pales* but saw higher trap catches of

*D. terebrans* and *P. picivorus* in burned treatments compared to controls. In a study of the effects of various fire regimes in longleaf pine forests in Florida, Hanula et al. (2009) found no effect on trap catches of *Ips* spp. and *H. pales*. Further investigation of these complex interactions under fire exclusion and fire reintroduction for restoration may provide guidance and understanding to support restoration of ecologically important longleaf pine forests.

#### **1.5 Research objectives**

There are basic questions remaining about the relationship between longleaf pine and bark beetles, and the influence of longleaf pine's unique interaction with fire on this relationship and forest health risk. To complement efforts to restore millions of acres of longleaf pine, it is important to provide an evaluation of the interactions among longleaf pine, bark beetles, and prescribed fire. Research in other pine forests and on the physiology of some bark beetle species has shown that fire-impacted trees can attract bark beetles (McCullough et al. 1998, McHugh et al. 2003), but studies of this type on longleaf pine and its associated insects are rare. Some pine species respond to fire with induced defenses, but those fires are usually different than the low-severity frequent fire employed in longleaf pine management. The goals of this dissertation are to offer insight into these important and complex interactions and to provide information that is useful to scientists and managers alike.

To evaluate the potential impacts of various fire regimes in longleaf pine forests on subcortical beetles, we sampled beetle trap catches in experimental burn blocks with three fire regime treatments: frequent fire, fire exclusion, and reintroduction of prescribed fire after a period of exclusion (Chapter 2). We also included a representative range of site conditions with mesic and xeric sites, differing in soil moisture availability and thus exhibiting varied forest stand characteristics.

To evaluate how management with prescribed fire may impact tree defenses to bark beetles, we sampled physical and chemical defense components in various experimental prescribed fire treatments (Chapter 3). We conducted experiments using these metrics in the short-term by sampling repeatedly after the application of dormant season and growing season prescribed burns. We also evaluated long-term fire regime treatments and site types for potential impacts to tree defenses by using the same experimental design from Chapter 2.

Overall, the purpose of this dissertation is to enhance our understanding of the complex interactions among bark beetles, tree defenses, and management with prescribed fire and to inform strategies for forest health risk mitigation in restoration. In the conclusion (Chapter 4), I synthesize how the results presented herein provide baseline information that future research can build on and managers can use to inform decision-making in the restoration of these valuable, rare longleaf pine ecosystems.

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### CHAPTER 2

# DIFFERENTIAL EFFECTS OF FIRE REGIME AND SITE CONDITIONS ON BARK BEETLES (COLEOPTERA: CURCULIONIDAE) IN A LONGLEAF PINE (*PINUS PALUSTRIS* MILL.) ECOSYSTEM<sup>1</sup>

<sup>&</sup>lt;sup>1</sup> Ritger, H.M.W., S.T. Brantley, K.D. Klepzig, and K.J.K. Gandhi. 2023. Forest Ecology and Management. 549:121488. Reprinted here with permission of the publisher.

#### Abstract

Longleaf pine (Pinus palustris Mill.) ecosystems in the southeastern U.S. are firedriven. Frequent prescribed fire or lack thereof through shifts in fire regimes may have consequences for tree health and associated herbivorous subcortical beetles (Coleoptera: Curculionidae). Yet, interactions between these ecologically and economically important beetles and prescribed fire in the southeastern U.S. are not well documented. We assessed the effects of various fire regimes, specifically of frequent fire, fire exclusion, and fire reintroduction, on bark and root-feeding beetles in mature longleaf pine stands in two edaphic types (mesic and xeric) in southwestern Georgia. We employed an information theoretic statistical approach to determine the effects of site conditions, as defined by both soil properties and stand structure, and prescribed burning on trap catches of subcortical beetles by species. We trapped a total of 141,611 beetles, indicating that subcortical beetles are abundant in this ecosystem. While the highest trap catches for three *Ips* beetle species were in the xeric fire-exclusion treatment, catches of *I*. grandicollis (Eichhoff) were similar at both sites for the fire-exclusion treatment. For the fire-exclusion treatment, *I. avulsus* (Eichhoff) and *I. calligraphus* (Germar) catches were 2-10 times higher at the xeric than mesic sites. Orthotomicus caelatus (Eichhoff) and Dendroctonus terebrans (Olivier) catches were 1.1-2.4 times higher in frequent-fire than fire-exclusion treatments. Root-feeding weevils, Pachylobius picivorus (Germar) and Hylobius pales (Herbst), exhibited opposite trends with catches higher and lower in burned treatments, respectively. *Ips calligraphus* were positively associated with percent basal area of understory trees, indicating this species may benefit from fire exclusion. Ips avulsus catches were negatively correlated with total basal area, suggesting this species

may benefit from more open habitats like those facilitated by frequent fire. We conclude that both soil properties and stand conditions interact with the fire regime to differentially affect the catches of southeastern bark and root-feeding beetle species in a longleaf pine ecosystem.

**INDEX WORDS:** forest management, *Ips*, mesic, prescribed fire, xeric

#### **2.1 Introduction**

Longleaf pine (*Pinus palustris* Mill.) forests are fire-dependent biodiversity hotspots that provide ecological benefits such as critical habitat for threatened and endangered species, high quality timber and other forest products, and recreational opportunities (Barnett 2002, Brockway et al. 2005). These forests can occupy a range of diverse site types present in the Southeast U.S. (e.g., wet-mesic flatwoods, xeric sandhills, intermediate uplands, montane environs, etc.) and tolerate a broad array of soils (Boyer 1990, Gilliam et al. 1993, Harper 1913). At the time of colonial settlement, longleaf pine forests dominated the landscape of the southeastern U.S., covering ~37 million hectares (Barnett 2002, Frost 1993, Landers et al. 1995). Expanding human settlement, logging, clearing for agricultural land, and conversion to loblolly pine (*P. taeda* L.) for timber reduced longleaf pine forest cover by 1994 to only ~1.3 million hectares (Barnett 2002, Brockway et al. 2005, Landers et al. 1995).

This contraction of an ecologically, economically, and culturally valuable natural resource spurred various stakeholders to action (Noss et al. 1995, Outcalt and Sheffield 1996). Numerous organizations and governmental agencies are working to promote the restoration and reforestation of longleaf pine forests through various cost-share programs, other tax and financial incentives, coalitions such as the Longleaf Alliance, and coordinated efforts like America's Longleaf Restoration Initiative (Brockway et al. 2005, Lopez et al. 2009). Effective conservation of the longleaf pine forest ecosystem requires increasing our ecological understanding of these forests, and assessing the forest health impacts of specific management and restoration activities, especially prescribed fire (Christensen et al. 1996, Van Lear et al. 2005).

Longleaf pine forests are dependent on fire regimes specifically of frequent, lowintensity surface fires to maintain their characteristic open-canopy structure and biodiversity (Barnett 2002, Stambaugh et al. 2011, Varner et al. 2005, Van Lear et al. 2005). Fire exposes bare mineral soil, required for longleaf pine seed germination, and creates canopy gaps for the shade-intolerant plant species (Boyer 1990, Harper 1913, Kirkman and Mitchell 2006). In the absence of frequent burning, longleaf pine forests experience encroachment from hardwoods and faster-growing pines, developing a woody midstory that decreases herbaceous species richness and diversity, increases risk of higher intensity and severity burning in uncontrolled wildfire, and inhibits longleaf pine regeneration (Boyer 1990, Brockway et al. 2005, Gilliam and Platt 1999, Haywood et al. 2001). Longleaf pine characteristics and life history, including its thick bark, highly flammable needles, and resistance to fire in the seedling and sapling stages, indicates a long history of adaptation to the frequent fire regime (Stambaugh et al. 2011).

Simultaneously, longleaf pine coevolved with above- and belowground herbivores such as bark beetles (Coleoptera: Curculionidae: Scolytinae). These subcortical insects are important ecological community members at endemic levels, but they can threaten forest health at higher population levels by directly causing tree mortality or vectoring pathogenic fungi. Historically and anecdotally, longleaf pine forests are characterized as resistant to many biotic disturbance agents, but studies on longleaf pine forests' relationships to these insects are scant (Sullivan et al. 2003, Brockway et al. 2005, Martinson et al. 2007, Campbell et al. 2008, Diop et al. 2009).

Forest management practices such as thinning have been shown to mitigate risks associated with these insects in southeastern pine forests, with most research focused on loblolly pine. Frequent prescribed fire may have similar effects to mechanical thinning by keeping basal area low and promoting overall tree vigor (Fettig and McKelvey 2014). Frequent fire may also affect tree physiology and phytochemistry, enhancing tree defenses or host attractiveness and altering tree interactions with bark beetle populations (Hood et al. 2015, Raffa et al. 2016).

Many existing longleaf pine stands have not experienced frequent fire for an extended period of time due to the exclusion and suppression of fire (Frost 1993). These stands tend to exhibit higher basal area and increased competition for resources (Kirkman et al. 2016). The resulting stress may increase incidence of insect attacks on trees (Parker et al. 2006). While fire exclusion introduces certain stresses to longleaf pine and ultimately results in stand replacement due to regeneration failure, reintroducing fire to fire-excluded stands also brings risks due to accumulated fuels (Haywood 2009). Increased tree mortality, often associated with bark beetles, occurs following the reintroduction of fire after a period of exclusion (Varner et al. 2007). This increased mortality is primarily driven by fire injury to trees' vital tissues with insects as secondary agents, colonizing the weakened trees (Sullivan et al. 2003, O'Brien et al. 2010).

Evaluating the risks and benefits of various prescribed fire management strategies is important for managers tasked with meeting multiple management objectives and constrained by limited resources (Hiers et al. 2003, Ryan et al. 2013). Managers have significant concern that reintroduction of prescribed fire in these stands may increase the colonization of bark beetles thus compromising the health of pine trees and negating the primary management objective. Hence, our study aimed to address gaps in knowledge by directly investigating the effects of frequent prescribed fire, fire exclusion, and fire reintroduction on subcortical insect activity and/or abundance as assessed by trap catches. Our specific research questions were: 1) how does bark beetle catch differ between frequently burned (2-year fire return interval) and fire-excluded (>15 years) longleaf pine stands; 2) how does fire reintroduction after a period of fire exclusion (13 years) affect bark beetle catch; and 3) are bark beetle catches mediated by varying site conditions as defined by stand condition and soil properties? Results from this study can inform management decisions related to managing frequent fire forests for long-term resilience, especially as more longleaf pine stands are being restored over time in the Southeast U.S.

#### 2.2 Materials and methods

#### **2.2.1** Site description and fire treatments

This study utilized a long-term fire exclusion experiment initiated in 2001 at the Jones Center at Ichauway (Ichauway henceforth). Ichauway is an 11,500-ha private reserve located in Baker County, Georgia, in the Eastern Gulf Coastal Plain at 31°13'N, 84°28'W with ~7,000 ha of naturally regenerated second-growth, mature (~80–100 years) longleaf pine forests that have been managed with frequent fire and the Stoddard-Neel approach of forest management since the 1930s (McIntyre et al. 2010). The wiregrass-dominated understory (*Aristida stricta* Michx. var. *beyrichiana* [Trin. & Rupr.] D. B. Ward) indicates a site-use history with relatively minimal soil disturbance and includes diverse perennial warm-season grasses, shrubs, and forbs (Kirkman et al. 2016).

Ichauway lies within the Dougherty Plain ecoregion (Edwards et al. 2013). Mean elevation is 48 m above sea level. Soils are primarily classified as ultisols (95%) with some entisols (5%) and range from excessively well-drained to poorly drained types.

Climate is humid subtropical, consisting of an extended summer season and a short winter season. Mean monthly temperatures are highest in July (28 °C) and lowest in January (10.5 °C). Ichauway receives ~1300 mm annual precipitation distributed evenly throughout the year (Drew et al. 1998, Kirkman et al. 2016).

Ichauway is divided into 177 burn units, discrete tracts of land separated by firebreaks or roads, to facilitate the application of prescribed fire. All burn units included in the study were dominated by a naturally regenerated mature longleaf pine overstory (>80 years old) and ranged in size from 10 to 196 ha (Figure 2.1). Prior to the onset of the long-term experimental treatments in 2001, these burn units had been maintained on a ~2-year fire return interval since the late 1980s when detailed records started, and generally a 1- or 2-year fire return interval since at least back to the 1950s.

Burn units utilized in this study occupy two site types on Ichauway that encompass the range of soil moisture availability typical in the Coastal Plain. Xeric sites are characterized by excessively well drained sandy soils, with mean volumetric water content in the top 30 cm of soil (VWC<sub>30</sub>) ranging from ~3% to ~14%, with a mean of ~7.7%. Mesic sites are characterized by somewhat poorly drained loamy sand soils and have VWC<sub>30</sub> ranging from ~4.4% to ~28%, with a mean of ~12% (S. Stuber, unpublished data).

Starting in 2001, two each of xeric and mesic burn units were taken out of the prescribed fire rotation and put into a fire exclusion treatment. Reference burn units in both the mesic and xeric sites continued on a biennial burn rotation to serve as experimental controls. In 2015, prescribed fire was reintroduced to one each of mesic and xeric fire-excluded burn units. Thus, we have experimental burn units for each of the
three fire regime treatments (fire exclusion, frequent fire, and fire reintroduction after a period of exclusion) at both the mesic and xeric sites; years of prescribed burns for all treatments varied from 2001 to 2017 (Table 2.1).

#### 2.2.2 Sampling plot establishment and characterizing stand conditions

To characterize forest stand conditions and sample trap catches of bark beetles in each burn unit, we established two 40 m radius (0.5 ha) sampling plots. Plot locations were selected using the random location generator in ArcGIS ("ArcGIS" 2015). We established plots  $\geq$  250 m apart to prevent plot-level interactions and  $\geq$  15 m from firebreaks to reduce edge effects. While beetles can fly long distance on flight mills under artificial conditions, other studies have indicated that the attraction radius of baited traps is generally a few meters or < 200 m (e.g., Schlyter 1992, Dodds and Ross 2002). Hence, our plots are considered independent units in the study. We recorded tree species and diameter at breast height (DBH) for every tree > 5 cm DBH within each plot (Table 2.2). We used these measurements to calculate various indices such as basal area, composition, and tree density for use in statistical analyses.

### 2.2.3 Beetle sampling

As our study aimed to address forest management concerns and risks, we focused our sampling efforts on bark beetle species known to be correlated with forest health issues in southern pine forests, such as the genera *Dendroctonus*, *Ips*, and *Hylastes*. *Dendroctonus frontalis* Zimmerman (southern pine beetle, SPB) is the most destructive forest insect in the southeastern U.S. However, we did not include it in this study because SPB is rare in our study area (Forest Health Protection 2005–2017), and while SPB can cause mortality of longleaf pine, it historically does not outbreak on this tree species (Friedenberg et al. 2007, Martinson et al. 2007, Coulson and Klepzig 2011, Nowak et al. 2015, Clarke et al. 2016). The Dougherty Plain region is classified as low-hazard for SPB outbreaks with projections of SPB-caused mortality remaining low into the near future (Krist Jr. et al. 2014).

To quantify bark beetle and root-feeding beetle activity and/or abundance, we measured trap catches using baited funnel and pitfall traps at each plot location. Trap catches using baited traps may not represent a true measure of abundance or beetle activity in a stand (Bentz 2006), but catches are useful for comparing relative numbers across different treatment types as in this study. Sampling dates were 25 May–23 June 2016 and 1–23 June 2017 (early-to-mid growing season). Release rates for all lures used were either provided by the manufacturer or measured by weight loss over time (Table 2.3). We spaced all traps ~25 m apart and  $\geq$  5 m from any trees and filled trap cups with ~3 cm propylene glycol as a killing and preserving agent. We emptied traps every 10–14 days and stored insects at -20 °C until we identified them.

### 2.2.3.1 Funnel traps

Each sampling plot contained four 12-unit Lindgren funnel traps suspended from metal posts with lures attached at the fifth funnel from the top (Lindgren 1983). We baited funnel traps with distinct lure combinations selected to optimize catches of *Ips* bark beetles, their associates, and woodboring beetles: (1) ethanol ultra-high release (UHR); (2) ethanol UHR and *Sirex* lure; (3) ipsenol, ipsdienol, and *cis*-verbenol; and (4) ethanol UHR, *Sirex* lure, ipsenol, ipsdienol, and *cis*-verbenol (Allison et al. 2012). We randomly assigned one of each lure combination to the four funnel traps at each plot and changed funnel trap lures every three weeks to maintain effectiveness.

#### 2.2.3.2 Pitfall traps

We captured root-feeding beetles by deploying four pitfall traps in each sampling plot. Pitfall traps consisted of two nested 0.47 L (16-oz) plastic beverage cups and were placed in the ground such that the lip of the cup was flush with the forest floor. We drilled holes in the bottom of the outer cup to prevent cups floating out of place in the event of ground-saturating rain. A plastic plate was suspended ~20 cm above the trap to shelter the open trap cup from rainfall. Pitfall traps were baited with host tree volatiles, 95% ethanol (Decon Labs, Inc., King of Prussia, Pennsylvania, U.S.) and turpentine (W.M. Barr & Co., Inc., Memphis, Tennessee, U.S. [2016]; Sunnyside Corporation, Wheeling, Illinois, U.S. [2017]). Bait was placed in semi-permeable poly vials (WestGreen Global Technologies, Langley, British Columbia, Canada) and a freshly cut longleaf pine twig was suspended from the edge of the inner cup with wire (Phillips 1990, Lalík et al. 2019). These baits were changed each time traps were emptied.

### 2.2.3.3 Insect identification and data handling

We identified specimens of our target taxa using published keys, expert assistance from a systematist, and reference collections (Wood 1982). Voucher specimens from this study were deposited at the Natural History Museum of the University of Georgia, Athens, Georgia. Current taxonomic information and authorities were verified using the Integrated Taxonomic Information System and Catalogue of Life on-line databases (ITIS 2020, Bánki et al. 2021).

Following identification, we counted individuals for each sample collected. We excluded data for species with <50 total catches in each year from statistical analyses. To account for uneven sampling periods and disturbed traps, we standardized trap catches of each species to catches per 14-day trapping periods. We rounded standardized catches to the nearest integer prior to statistical analyses.

## 2.2.4 Statistical analyses

Our experimental set up constitutes a randomized complete block design with plots serving as pseudoreplicates within the burn units (Hurlbert 1984). As preliminary data analyses, we calculated and examined the intraclass correlation coefficient to determine that we could consider plots as independent replicates in our statistical analyses, giving us the statistical power to look for effects of the interaction of site and treatment factors. Additional preliminary analyses revealed that model fit did not improve using a repeated measures model for trap catches in the same plots for different years. In addition to results of these preliminary analyses, the biological factors affecting insect populations in the southeastern U.S. can change over short timescales, supporting our inclusion of insect samples collected in different years from the same plot locations as independent with year as a factor. All statistical analysis was performed in R statistical software (v3.6.3, R Core Team 2020).

## 2.2.4.1 Relating stand characteristics to treatment, site types, and *Ips* species

We explored relationships among forest stand characteristics, fire treatment and site type factors, and trap catches of *Ips* spp. To examine how plot-level stand conditions vary by the site type and fire regime treatment factors, we performed an unconstrained multivariate ordination followed by regression using the 'vegan' package in R (Oksanen et al. 2019). We conducted a principal component analysis (PCA) using the 'rda' function, scaling input stand metrics to equal variance to account for differing units of measurement (Weihs et al. 2005). Broken stick method was used to determine how many principal components to include in further analysis (Jackson 1993). We then used the 'envfit' function to regress our categorical variables of site type and fire regime treatment onto the PCA ordination (Oksanen et al. 2019).

Linear regressions with trap catches for each of three *Ips* species and each forest stand metric were performed to determine if any singular measure(s) could explain any portion of the observed variation in the bark beetle catches. We included year as a factor in each linear regression. Trap catches for these three species were sufficiently large to avoid violating assumptions of normality according to the Central Limit Theorem. For each regression we stipulated an alpha of 0.05 for rejecting the null model and we calculated  $R^2$  as the proportion of variance explained by the model.

# 2.2.4.2 Modeling fire regime and site type effects on insect trap catches

We employed an information theoretic approach to examine multiple hypotheses about the effects of the treatments, site types, and years on the trap catches of our study species in a systematic manner (Burnham and Anderson 2002). While our main objective was to determine if and how fire regime treatments affected bark and root-feeding beetles, we suspected that these effects may be mediated by or interact with other factors present in our study, such as site conditions unrelated to fire regime and/or interannual variation consisting of climatic variation and prescribed burning rotations. Rather than relying on a single alternative hypothesis to model and fit our data to, we investigated multiple models that represented biological hypotheses about the way these factors of fire regime treatment, site type, and year (and their interactions) might affect trap catches of our species of interest.

We used generalized linear models fit with the packages 'lme4' and 'MASS' to test for fixed effects of factors (treatment, site type, year, and their interactions) on the trap catches of each species (Venables and Ripley 2002, Bates et al. 2015). We assumed either negative binomial or Poisson distributions based on examinations of variance, overdispersion, and model goodness of fit metrics (Hilborn and Mangel 1997, Burnham and Anderson 2002).

We built a set of candidate models for all possible combinations of the factors, including null models, and fit them to each species' catch data. Models were ranked by the Akaike Information Criterion corrected for small sample size (AIC<sub>C</sub>, via 'AICc' function from 'MuMIn' package), and we used the threshold of  $\Delta AIC_C \leq 2$  to select the top model or top model set for each species (Burnham and Anderson 2002, Grueber et al. 2011, Bartoń 2020). For the species with multiple models meeting the selection criterion, we used the 'MuMIn' package in R to create and evaluate an averaged model with the natural averaging method (Burnham and Anderson 2002, Bartoń 2020). We employed Nagelkerke's *pseudo*- $R^2$  as a goodness of fit measure for selected models (Nagelkerke 1991, Bartoń 2020).

# 2.3 Results

We captured a total of 141,611 individuals of our species of interest within the study period (Table 2.4). The majority of our catches were *Ips calligraphus* (Germar), *I. grandicollis* (Eichhoff), and *I. avulsus* (Eichhoff) constituting 48.9%, 31.5%, and 17.1% of all catches, respectively. The remaining species we caught in sufficient numbers for statistical analysis made up just 1.2% of overall catches, with 1,804 *Orthotomicus caelatus* (Eichhoff), 486 *Dendroctonus terebrans* (Olivier), 967 *Pachylobius picivorus* (Germar), and 131 *Hylobius pales* (Herbst). Trap catches of two *Hylastes* spp. were inadequate for inclusion in statistical analyses.

# 2.3.1 Stand characteristics related to treatment, site types, and *Ips* species

The PCA ordination revealed which forest stand metrics related to variation in stand conditions observed at the experimental plots (Figure 2.2). The first two PCA components cumulatively explained 77.5% of the variance and met the broken stick method stopping criteria. Variable loadings (Table 5.5) and visual inspection of the ordination graphs (Figure 2.2) showed that the variation was largely driven by differences in tree density and forest composition (conifers vs. hardwoods).

Tree density and understory component measures had the highest loadings (> |0.35|) in the first principal component (PC1), which explained 43.5% of the variance. The second principal component (PC2), which explained 34% of the variance, had the highest loadings (> |0.35|) for the hardwood component and the hardwood-conifer composition. Results from the factor regression showed a significant relationship between the PCA and the fire regime treatments (p < 0.05) (Figure 2.2a), with the site type factors not meeting the significance threshold (p = 0.077) (Figure 2.2b). All three fire regime treatments were distinct with some overlap of stand-level metrics, and the fire reintroduction sites had the most variation (Figure 2.2a).

Linear regressions showed the majority of individual stand metrics to be uncorrelated with trap catches of *Ips* beetles, and none of these metrics showed a correlation with trap catches of *I. grandicollis*. Trap catches of *I. calligraphus* were positively correlated with percent basal area of understory trees (DBH  $\ge$  15 cm), and this model explained a large portion of the variation in trap catches (R<sup>2</sup> = 0.71, p < 0.001; Figure 2.3). *Ips avulsus* trap catches were negatively correlated with total basal area, and this model explained a moderate portion of the variation in trap catches (R<sup>2</sup> = 0.44, p < 0.01; Figure 2.4).

#### 2.3.2 Fire regime and site type effects on insect trap catches

In the final set of selected models (Table 2.6), fire treatment appeared as a factor for all species, and represented strong relative importance compared to other factors in model sets for all species except *D. terebrans* (0.44). Site type appeared as a factor in the final model set for all species except *D. terebrans*, but its relative importance varied among models for those species, with highest relative importance for all three *Ips* beetle species (0.97–1). Year was the most consistently important factor across all the model sets (0.65–1). The interaction term for treatment and site type represented the only interaction term present in any selected models. This interaction term had strong relative importance for the *Ips* spp. (1), low relative importance for *P. picivorus* (0.27), and did not appear in the selected models for the other species.

Coefficient estimates and confidence intervals resulting from the model selection process indicated that the effects of factors differed in direction and magnitude among our study species (Figure 2.5). The effects of the fire regime treatments differed substantially by species and interacted with site type for several species (Figures 2.5– 2.7).

The models showed the highest trap catches for all three *Ips* beetle species in the xeric fire exclusion treatment, with substantially lower catches in the fire reintroduction and frequent fire treatments at xeric site types (Figure 2.6). While both *I. calligraphus* and *I. avulsus* had lower trap catches in the xeric frequent fire treatment compared to xeric fire reintroduction (1.05 and 1.83 times lower, respectively), the reverse trend was observed for *I. grandicollis* in the xeric site type with 1.26 times lower catches in fire reintroduction compared to frequent fire treatments.

There were differing trends for the *Ips* spp. catches in mesic sites (Figure 2.6). For *I. calligraphus*, there were similar trap catches in the fire reintroduction and fire exclusion treatments in the mesic sites (Figure 2.6). *Ips grandicollis* trap catches were  $\sim$ 1.4 times lower in the frequent fire treatment compared to the similar trap catches in the fire exclusion and fire reintroduction treatments at mesic site types (Figure 2.6). While overall lower trap catches occurred at mesic compared to xeric site types for *I. avulsus*, there was an opposite trend of fire treatment effects in the mesic site types with catches in

the frequent fire treatment 4.92 times higher than in the fire exclusion treatment (Figure 2.6).

The averaged model for *P. picivorus* also showed an interaction between site type and fire regime treatment on trap catches (Tables 2.6 and 2.7, Figures 2.5f and 2.6). The models in the selected set had similar AIC<sub>C</sub> values (Table 2.6), so the averaged model incorporated uncertainty around the effect sizes of the factors (Table 2.7, Figure 2.6). Even with confidence intervals that incorporated this uncertainty, there were distinct effects of some of the fire regime treatments (Figure 2.6). Catches in the reintroduction and frequent fire treatments were ~1.4 times higher than the exclusion treatment in the mesic site type, while in the xeric site type, catches in the frequent fire treatment were 1.43 times higher compared to the fire exclusion treatment.

The averaged model for *O. caelatus* showed highest trap catches in the frequentfire treatment, intermediate catches in the fire-reintroduction treatment, and lowest catches in the fire-exclusion treatment, 1.56 times lower than the frequent fire treatment (Figure 2.7). While the effects of the reintroduction and exclusion fire regime treatments were important in the model for *O. caelatus*, the uncertainty around the size of these effects (Figures 2.5d and 2.7) and the fact that all four top models had very similar AIC<sub>C</sub> values with only two of them including the treatment term (Table 2.6) warrant caution when evaluating this result.

The averaged models for *D. terebrans* showed the highest trap catches in the fire reintroduction treatment, intermediate catches in the frequent fire treatment, and the lowest catches in the fire exclusion treatment, 1.13 times lower than the frequent fire treatment (Figure 2.7). However, the overlapping standard error bars resulting from this

averaged model prediction conveyed uncertainty about the validity of these differences among the fire regime treatments in *D. terebrans* (Figure 2.7). That the two best models for *D. terebrans* had nearly identical  $AIC_C$  values and only one of them included the treatment term as a predictor also suggests caution is appropriate when interpreting results about the effects of the fire regime treatment on *D. terebrans* in our study (Table 2.6).

The averaged model for *H. pales* (Figure 2.7) showed ~2.1 times higher trap catches in the fire exclusion treatment compared to the other treatments with no significant difference between catches in the reintroduction and frequent fire treatments. All four of the top models used in the model averaging for *H. pales* included the treatment factor, indicating its importance as a predictor for patterns observed in our study data (Table 2.6).

The selected models showed strong evidence of an effect of interannual differences in trap catches for all species, but the direction and magnitude of these differences varied (Tables 2.6 and 2.7, Figure 2.5). The models for *I. calligraphus*, *I. avulsus*, and *H. pales* showed expected trap catches 1.6 to 3.1 times higher in 2017 compared to 2016 for the baseline conditions (defined as fire exclusion treatment and mesic site type in the models), while models for the remaining species showed catches in 2017 reduced to various fractions of 2016 catches in baseline conditions: *I. grandicollis* (0.55), *O. caelatus* (0.09), *D. terebrans* (0.35), *and P. picivorus* (0.44).

Only models for *O. caelatus* and *H. pales* provided strong evidence of a site type effect that did not interact with the fire regime treatments (Table 2.6). Models for both species showed lower expected trap catches in the xeric compared to mesic site type

(Table 2.7). The xeric site incident rate ratio compared to baseline conditions was 0.6 for *O. caelatus* and 0.67 for *H. pales* (Table 2.7).

## **2.4 Discussion**

Investigations into sympatric above- and below-ground herbivorous beetle interactions with prescribed fire and site/stand characteristics in the Southeast U.S. are quite limited, and no previous study has included species-level analysis of all three *Ips* beetle species that co-occur in the Gulf Coastal Plain. This unique study included >140,000 beetles and indicated the following major trends in changes in fire regimes in longleaf pine-dominated stands at Ichauway: 1) these stands were differentiated by tree density and composition, and the differences were correlated with the three fire regime treatments; 2) beetles responded to varying stand conditions with catches of *I. calligraphus* positively correlated with percent basal area of understory trees, while *I. avulsus* catches were negatively correlated with total basal area; and 3) beetle species responded to fire regime treatments, site conditions, and year of sampling in varied ways.

The observed stand differences in this study are consistent with previous work that shows a change in fire regime via a lack of frequent fire in longleaf pine forests leads to the development of a woody understory and midstory as hardwoods proliferate (Kush and Meldahl 2000, Kirkman et al. 2016). *Ips calligraphus* catches increased with percent basal area of understory trees, while *I. avulsus* catches decreased with increased total basal area. Bark beetles are known to be responsive to basal area and density. In general, these relationships are positive, although studies examining these trends have been in different forested ecosystems with other *Dendroctonus* and *Ips* species, often using tree mortality rather than trap catches to assess beetle activity (Parker et al. 2006, Fettig et al. 2007, Wallin et al. 2008, Hayes et al. 2009, Hood et al. 2016, Restaino et al. 2019, Tepley et al. 2020). Increased bark beetle activity has been reported in thinned stands, perhaps due to release of tree volatiles in the short-term following thinning activity (Safranyik et al. 2004, Zausen et al. 2005, Powell et al. 2018, Negrón 2019). In our study, increased basal area of understory trees (primarily hardwoods) may have been inducing nutrient or water stress in canopy pines, and hence eliciting higher catches of adult *I. calligraphus*. Because we did not assess tree mortality or observe mortality levels outside the norm, it's unclear whether high populations of beetles were correlated with high colonization on pine trees.

There are several potential factors that could explain our observation of the negative relationship between *I. avulsus* trap catches and total basal area, such as: 1) enhanced reproductive success in stands with lower basal area; 2) increased detection of pheromones and kairomones due to altered site conditions in stands with lower basal area; and/or 3) reduced ability to navigate in stands with higher basal area, especially considering that *I. avulsus* primarily colonize tree crowns (Connor and Wilkinson 1983). Two studies of *I. pini* (Say) in lodgepole pine (*Pinus contorta* var. *latifolia* Engelm. ex S. Watson) in Alberta, Canada, supported the first two hypotheses. Multiple measures of reproductive success and trap catches of *I. pini* were higher among thinned stands, and trap catches were positively correlated to windiness, which was higher in thinned stands (Wood 1982, Hindmarch and Reid 2001a, 2001b). While decreased windiness in denser stands may be a factor leading to reduced ability for beetles to navigate and locate host trees or to aggregate via pheromones, increased presence of non-hosts in these higher

density stands may also interfere with host selection (Zhang and Schlyter 2004, Gitau et al. 2013). In Sweden and China, additions of non-host volatiles to pheromone-baited traps reduced trap catches of *I. typographus* (L.) and *I. duplicatus* Sahlberg, in accordance with the "semiochemicals diversity hypothesis," whereby the presence of non-hosts may offer protection for conifers from bark beetles (Zhang et al. 1999, 2001, Zhang 2003, Zhang and Schlyter 2004). Additionally, a study characterized pheromone plume behavior and dispersal, allowing for inferring potential effects of stand density on pheromone detectability by beetles in southern U.S. pine forests (Thistle et al. 2005). Lower density stands allowed pheromone plumes to disperse into a larger area, increasing the probability of detection by a beetle, while plumes in higher density stands dispersed more slowly and stayed closer to the source, reducing the probability of detection by beetles (Thistle et al. 2005). Especially considering that *I. avulsus* tends to colonize either branches of mature trees or boles of smaller diameter trees (Paine et al. 1981), they may also be more of an open habitat or edge-adapted species.

Beetles responded differentially to fire regime treatments, site conditions, and year of sampling, but these interactive and additive treatment effects varied in importance, magnitude, and direction by species. Our results indicated that the effects of frequent prescribed fire in longleaf pine forests is species-specific and that, for *I. calligraphus* and *I. avulsus*, they differ by site type. Our finding of highest catches of *I. grandicollis* in the fire-excluded treatment supports the hypothesis that excluding fire from longleaf pine forests may increase tree stress and therefore attractiveness of host trees. Trees experiencing stress from increased competition for resources (e.g., nutrients or water) may increase production of ethanol and/or monoterpenes, which enhance

attraction for *Ips* beetles (Werner 1972, Kimmerer and Kozlowski 1982, Mattson and Haack 1987, Erbilgin et al. 2003, Kelsey et al. 2014). That fire-excluded xeric stands also had the highest catches of *I. calligraphus* and *I. avulsus* supports this hypothesis. However, at mesic sites, *I. avulsus* catches exhibited the opposite trend from xeric sites, with higher catches in the frequent-fire treatment compared to fire-exclusion treatment. Only one previous study examined prescribed fire regime effects on southeastern *Ips* spp. and found no difference in trap catches between fire-excluded and frequent-fire stands, although the *Ips* beetles were not evaluated at the species-level (Hanula et al. 2009). Although all the three *Ips* species can be sympatric on the same tree (Paine et al. 1981), they are responding differentially to site, stand, and treatment types and thus, they may require nuanced management systems.

While *O. caelatus* is not well-studied and has not been associated with economic impacts in southern forests, it is considered a secondary pest, usually colonizing weakened or dying trees (Wood 1982, Drooz 1985). *Orthotomicus caelatus* catch was higher in the frequent-fire than the fire-excluded treatment, as similarly found for *I. avulsus*. In Florida longleaf and slash pine (*P. elliottii* Engelm.) forests, Hanula et al. (2002) found no difference in trap catches of *O. caelatus* in burned areas of variable fire severity compared to unburned areas following a wildfire. In Wisconsin, a study investigating bark beetle activity related to Red Pine Decline reported similar trap catches of *O. caelatus* among both healthy and symptomatic stands of red pine (*Pinus resinosa* Aiton) (Wood 1982, Erbilgin and Raffa 2002).

*Dendroctonus terebrans* generally exploits weakened or injured trees and typically co-occurs in trees infested with *Ips* spp. (Munro et al. 2019). Past field studies

of *D. terebrans* did not observe increased catches in burned pine stands compared to unburned stands (Hanula et al. 2002, Sullivan et al. 2003). Campbell et al. (2008) reported increased trap catches of *D. terebrans* when burning was combined with thinning and herbicide treatments. While our results show increased catches in the burned treatments, the effect in the statistical models is not strong. These observations may indicate that none of the stands in our study were experiencing the level of tree injury or stress required to increase attraction of *D. terebrans* or to result in the kinds of *Ips* infestations that may increase the occurrence of *D. terebrans*.

Previous studies of the root-feeding weevils *P. picivorus* and *H. pales* have shown similar trends among varied site conditions, leading some to conclude that they occupy the same ecological niche (Nord et al. 1982, Hanula et al. 2002, Zanzot et al. 2010). However, one study indicated differential responses where *P. picivorus* catches were higher in burned treatments compared to controls, and no effects of treatment were observed for *H. pales* (Campbell et al. 2008). Sullivan et al. (2003) reported increased *P. picivorus* catches in burned versus unburned study plots but did not capture enough *H. pales* for statistical analysis. Our results indicate that *H. pales* and *P. picivorus* responded differently to site conditions, with catches of *H. pales* highest in the fire-exclusion treatment and *P. picivorus* higher in the fire treatments compared to fire-exclusion.

It is possible that the trends we observed for our study insect species may have been different if we had conducted sampling during July to September of each year. However, considering that we trapped >140,000 beetles in 3–4 weeks of sampling each year, our results encompass a period of high beetle catches. The high number of beetles collected combined with low levels of tree mortality in these stands is perplexing. We question whether these longleaf pine stands are acting as "ecological-traps" for beetles where they are highly attractive but also resource-poor habitats (Delibes et al. 2001). However, because bark beetles require tree hosts for reproduction, it is possible that even though suitable host trees may be rarer on the landscape, longleaf pines have more prolific beetle broods due to their nutritional and/or defense qualities. On lightning struck loblolly pine trees, a ~3 m section of *Ips* beetle galleries was found to have 545 eggs and larvae (Anderson and Anderson 1968). On slash pines, broods from trees with thicker phloem were larger and emerged earlier (Haack et al. 1987). Similar data for longleaf pines are unknown, and it would be interesting to compare *Ips* beetle brood development among longleaf, slash, and loblolly pines in the same habitats and climatic conditions.

We acknowledge that in this study we were limited by the number of burn units and stands that were long-term fire-suppressed at Ichauway and were thus limited in replication. Ichauway has an active fire program, and most longleaf pine stands are burned within 1–2-year periods. We would have introduced significant geographic, land management history, and prescribe burning management variation by including burn units elsewhere. While our inferences are limited to these burn units, managers are facing a need to restore longleaf pine stands with prescribed burning without a clear understanding of how bark beetle species may respond to inclusion of fire after a long history of fire exclusion. Hence, our study may provide important baseline data for restoring longleaf stands, while managers, decision-makers, and forest health professionals will further benefit from studies conducted elsewhere in the region.

# **2.5 Conclusions**

Overall, the effects of fire regime on bark beetle catches and the degree to which site conditions interact with those effects vary distinctly by beetle species, with each species exhibiting unique trends in catches among treatments. Often, these bark beetle species are described as secondary colonizers that utilize recently fire- or weather-injured trees, drought-stressed trees, or logging slash (Wood 1982, Nord et al. 1982, Drooz 1985, Nebeker 2011). The differential trends observed in our study provide evidence that complex environmental factors including prescribed fire regime and site type may affect the dynamics of southern pine bark and root-feeding beetles in pine-dominated forests. Further understanding of these effects may aid forest managers in decision-making regarding prescribed burning and forest health conditions in longleaf pine forests.

## 2.6 Acknowledgments

We acknowledge financial support provided by the University of Georgia D.B. Warnell School of Forestry and Natural Resources and the Jones Center at Ichauway. Rick Hoebeke (University of Georgia Natural History Museum) provided guidance and confirmation for insect identifications. We thank technicians from the University of Georgia (Arya Aghdassi and Holly Munro) and the Jones Center at Ichauway (Michael Belovitch, Crystal Bishop, D. Alex Gordon, Riley Green, Phoebe Judge, Stephen Lewis, Sierra Perez, Robert Ritger, and Sean Reynolds) for assistance in the field and lab. We additionally thank Brittany Barnes (University of Georgia), and Tom Sheehan and Stribling Stuber (the Jones Center at Ichauway) for technical assistance and logistics support. Two anonymous reviewers provided valuable comments that greatly improved this paper.

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Table 2.1. Prescribed burn history by treatment since 2001 in experimental longleaf pine stands in southwestern Georgia, U.S. All burns were conducted in the dormant season. Burns occurred in the same year for both mesic and xeric sites.

Treatment	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017
Frequent Fire																	
	•		•		•		•		•		•		•		•		•
Fire Exclusion																	
	•																
Fire																	
Reintroduction	•														•	•	•

Site Type	Fire Exclusion	Fire Reintroduction	<b>Frequent Fire</b>		
Mesic site					
Longleaf pine basal area	$9.49\pm3.45$	$11.54\pm2.94$	$10.13\pm0.24$		
Other conifer basal area	$0.32\pm0.30$	$0.37\pm0.01$	$0.96\pm0.96$		
Oak basal area	$5.32\pm0.52$	$5.95\pm5.36$	$1.03\pm1.03$		
Other hardwood basal area	$0.24\pm0.01$	$0.04\pm0.04$	$0\pm 0$		
Total basal area	$15.37\pm2.61$	$17.90\pm2.46$	$12.12\pm2.23$		
Tree density	$910.17 \pm 269.57$	$997.70 \pm 128.32$	$609.76\pm80.57$		
Xeric site					
Longleaf pine basal area	$3.34\pm0.64$	$12.2 \pm 1.67$	$7.79 \pm 1.74$		
Other conifer basal area	$0\pm 0$	$0\pm 0$	$0.01\pm0.01$		
Oak basal area	$5.63 \pm 1.17$	$4.01\pm0.61$	$6.24 \pm 1.80$		
Other hardwood basal area	$0.03\pm0.02$	$0.30\pm0.13$	$0.78\pm0.78$		
Total basal area	$9.00\pm0.55$	$17.23\pm0.92$	$14.81\pm0.84$		
Tree density	$739.08\pm62.67$	$1744.74 \pm 137.27$	$870.38\pm6.96$		

Table 2.2. Forest stand summary data (means  $\pm$  standard errors, n = 2) of experimental longleaf pine stands in southwestern Georgia, U.S. Basal area (m<sup>2</sup> ha<sup>-1</sup>) and tree density (trees per ha) include all living trees  $\geq$  5 cm diameter at breast height (DBH) within the 40 m radius plot.

Lure	Release Device	Release Rate	Release Rate Manufacturer		Release Rate Source
Ethanol UHR	pouch	0.5 g day <sup>-1</sup> at 23 °C	Contech Enterprises, Inc. (Delta, British Columbia, Canada)	2016	provided by manufacturer
Sirex UHR	pouch	903.56 mg day <sup>-1</sup> at 20 °C	Chemtica USA (Durant, Oklahoma, U.S.)	2016	provided by manufacturer
Ethanol UHR	pouch	300 mg day <sup>-1</sup> at 20 °C	Synergy Semiochemicals Corp. (Burnaby, British Columbia, Canada)	2017	provided by manufacturer
<i>Sirex noctillio</i> lure	pouch	$1 - 3 \text{ g day}^{-1}$ at 20 °C	Synergy Semiochemicals Corp. (Burnaby, British Columbia, Canada)	2017	provided by manufacturer
Ipsenol	bubble cap	300 μg day <sup>-1</sup> at 20 °C	Synergy Semiochemicals Corp. (Burnaby, British Columbia, Canada)	2016 - 2017	provided by manufacturer
Ipsdienol	bubble cap	150 μg day <sup>-1</sup> at 20 °C	Synergy Semiochemicals Corp. (Burnaby, British Columbia, Canada)	2016 - 2017	provided by manufacturer
<i>cis</i> -verbenol	bubble cap	$0.6 - 0.8 \text{ mg day}^{-1}$ at 20 °C	Synergy Semiochemicals Corp. (Burnaby, British Columbia, Canada)	2016 - 2017	provided by manufacturer
Koptec Pure Ethanol, 95%	poly vial	11.08 mg day <sup>-1</sup> at 23 °C	Decon Labs, Inc. (King of Prussia, Pennsylvania, U.S.)	2016 - 2017	measured by weight loss
Klean Strip™ Green™ Pure Turpentine	poly vial	82.53 mg day <sup>-1</sup> at 23 °C	W.M. Barr & Co., Inc. (Memphis, Tennessee, U.S.)	2016	measured by weight loss
Pure Gum Spirits of Turpentine	poly vial	22.17 mg day <sup>-1</sup> at 23 °C	Sunnyside Corporation (Wheeling, Illinois, U.S.)	2017	measured by weight loss

Table 2.3. Lure specifications and manufacturer information. UHR = ultra-high release.

Beetle species	2016	2017	Totals
Ips calligraphus (Germar)	21,930	47,274	69,204
<i>Ips grandicollis</i> (Eichhoff)	32,886	11,788	44,674
<i>Ips avulsus</i> (Eichhoff)	10,402	13,824	24,226
Orthotomicus caelatus (Eichhoff)	1,705	99	1,804
Dendroctonus terebrans (Olivier)	391	95	486
Pachylobius picivorus (Germar)	750	217	967
<i>Hylobius pales</i> (Herbst)	64	67	131
Hylastes salebrosus Eichhoff	57	3	60
Hylastes porculus Erichson	18	-	18
Hylastes tenuis Eichhoff	35	6	41
Totals	68,238	73,373	141,611

Table 2.4. Total catches of species of interest for the study period in experimental longleaf pine stands in southwestern Georgia, U.S.

	Forest Stand Metrics	Variable l	Loadings
Attribute	Description	PC1	PC2
BA	total basal area (m <sup>2</sup> ha <sup>-1</sup> )	0.298	-0.065
pineBA	total basal area (m <sup>2</sup> ha <sup>-1</sup> ) for <i>Pinus</i> spp.	0.338	0.277
pinepctBA <sup>†</sup>	percentage by BA of <i>Pinus</i> spp.	0.190	0.434
hardwood $BA^{\dagger}$	total BA (m <sup>2</sup> ha <sup>-1</sup> ) of hardwoods	-0.072	-0.403
understoryBA*	total BA (m <sup>2</sup> ha <sup>-1</sup> ) of trees with DBH $\leq$ 15 cm	0.381	-0.176
understorypctBA	percentage by BA of trees with DBH <15 cm	0.139	-0.213
treedensity*	number of trees per hectare	0.403	-0.181
coniferdensity*	number of conifers per hectare	0.386	0.184
coniferpctdensity <sup>†</sup>	percentage by density of conifers	0.108	0.458
hardwooddensity $^{\dagger}$	number of hardwoods per hectare	0.162	-0.435
understorydensity*	number of trees with DBH < 15cm per hectare	0.409	-0.162
understorypctdensity	percentage by density of trees with DBH <15cm	0.274	0.002
*denotes attribute with loading	g> 0.35  in PC1	<u>Proportion (</u>	of Variance
<sup>†</sup> denotes attribute with loading	> 0.35  in PC2	0.435	0.334

Table 2.5. Forest stand metrics and associated loadings for the first two principal components (PCs) resulting from the Principal Components Analysis (PCA) in experimental longleaf pine stands in southwestern Georgia, U.S. Basal area (BA, m<sup>2</sup> ha<sup>-1</sup>) and tree density (trees per ha) include all living trees  $\geq$  5 cm diameter at breast height (DBH) within 40 m radius plots.

Table 2.6. Factors and selected models resulting from the model selection process for each *Ips* beetle species and their associated attributes: factors, model formulas, corrected Akaike Information Criterion (AIC<sub>c</sub>), Akaike weights ( $w_i$ ), and the Nagelkerke adjusted pseudo-R<sup>2</sup> value. Only factors included in top models are shown. Relative importance is a weighted measure related to the number and fit of models that the factor appears in within the candidate set. AIC<sub>c</sub> is a relative goodness of fit metric, corrected for small sample size. The Akaike weights presented here are calculated for the group of selected models for each species.

	Factors an	d Relati	ve Imp	ortance	Selected Models					
Species	Treatment	Year	Site	Treatment *Site	Model Formula	AIC <sub>c</sub>	w <sub>i</sub>	Pseudo- R <sup>2</sup>		
Ips calligraphus	0.98	1	1	0.96	trap catches ~ treatment*site + year	354.5	1	0.94		
Ips grandicollis	1	1	0.97	0.91	trap catches ~ treatment*site + year	335.7	1	0.84		
Ips avulsus	1	0.92	1	1	trap catches ~ treatment*site + year	333.9	1	0.83		
Orthotomicus caelatus	0.58	1	0.5	< 0.01	trap catches $\sim$ treatment + year	185.6	0.32	0.83		
					trap catches ~ treatment + site + year	186	0.26	0.85		
					trap catches $\sim$ year	186.4	0.22	0.77		
					trap catches $\sim$ site + year	186.6	0.19	0.79		
Dendroctonus	0.44	1	0.29	0.02	trap catches ~ year	149.4	0.53	0.42		
terebrans					trap catches $\sim$ treatment + year	149.6	0.47	0.54		
Pachylobius picivorus	0.72	1	0.47	0.27	trap catches $\sim$ treatment + year	169.8	0.45	0.61		
					trap catches ~ treatment*site + year	170.8	0.28	0.76		
					trap catches ~ year	170.8	0.27	0.47		
Hylobius pales	0.91	0.65	0.7	0.02	trap catches $\sim$ treatment + site + year	101.4	0.37	0.54		
					trap catches $\sim$ treatment + site	101.7	0.31	0.46		
					trap catches $\sim$ treatment + year	102.7	0.19	0.44		
					trap catches ~ treatment	103.4	0.14	0.35		

Table 2.7. Intercepts and coefficient estimates for selected or averaged models for each species. Estimates, unconditional standard errors (SE), and 95% confidence interval (CI) limits have been back-transformed from the log scale so that intercept estimates are on the scale of the response variable (trap catches per 14 days) and parameter estimates are incident rate ratios. The intercept represents the baseline conditions in each model and consists of the fire-exclusion treatment at the mesic site type in 2016. TreatmentRE represents the reintroduction fire treatment, and treatmentRX represents the frequent fire treatment.

	Estimate	SE	CI lower	CI upper	Р		Estimate	SE	CI lower	CI upper	Р
Ips calligraphus						Orthotomicus caelatus					
(intercept)	623.39	1.10	523.36	748.65	< 0.0001	(intercept)	48.27	1.42	23.85	97.71	< 0.0001
treatmentRE	1.24	1.13	0.98	1.57	0.078	treatmentRE	1.80	1.32	1.01	3.19	0.045
treatmentRX	0.99	1.13	0.78	1.25	0.9	treatmentRX	2.24	1.31	1.27	3.94	0.0055
sitexeric	2.15	1.13	1.69	2.72	< 0.0001	year2017	0.09	1.28	0.05	0.14	< 0.0001
year2017	3.09	1.07	2.69	3.55	< 0.0001	sitexeric	0.67	1.26	0.41	1.07	0.096
treatmentRE:sitexeric	0.45	1.19	0.32	0.62	< 0.0001	Dendroctonus terebrans					
treatmentRX:sitexeric	0.53	1.19	0.38	0.74	0.0002	(Intercept)	12.17	1.30	7.13	20.76	< 0.0001
Ips avulsus						year2017	0.35	1.28	0.21	0.58	< 0.0001
(intercept)	93.88	1.25	62.57	147.63	< 0.0001	treatmentRE	2.01	1.33	1.11	3.61	0.02
treatmentRE	3.38	1.34	1.90	6.00	< 0.0001	treatmentRX	1.34	1.34	0.73	2.45	0.35
treatmentRX	4.92	1.34	2.76	8.79	< 0.0001	Pachylobius picivorus					
sitexeric	11.26	1.34	6.35	19.99	< 0.0001	(Intercept)	21.03	1.23	13.89	31.84	< 0.0001
year2017	1.78	1.18	1.28	2.49	< 0.0001	treatmentRE	1.78	1.27	1.10	2.88	0.02
treatmentRE:sitexeric	0.08	1.51	0.03	0.17	< 0.0001	treatmentRX	1.54	1.22	1.02	2.33	0.039
treatmentRX:sitexeric	0.03	1.51	0.01	0.06	< 0.0001	year2017	0.44	1.16	0.32	0.60	< 0.0001
Ips grandicollis						sitexeric	1.04	1.23	0.67	1.61	0.86
(intercept)	1421.84	1.11	1176.64	1736.38	< 0.0001	sitexeric:treatmentRE	0.47	1.32	0.27	0.84	0.011
treatmentRE	0.97	1.14	0.75	1.27	0.83	sitexeric:treatmentRX	1.18	1.31	0.67	2.08	0.57
treatmentRX	0.72	1.14	0.55	0.94	0.014	Hylobius pales					
sitexeric	1.13	1.14	0.87	1.48	0.35	(Intercept)	4.67	1.31	2.70	8.09	< 0.0001
year2017	0.55	1.08	0.47	0.64	< 0.0001	sitexeric	0.60	1.28	0.36	1.00	0.049
treatmentRE:sitexeric	0.43	1.21	0.30	0.63	< 0.0001	treatmentRE	0.51	1.33	0.28	0.93	0.027
treatmentRX:sitexeric	0.74	1.21	0.51	1.08	0.11	treatmentRX	0.43	1.35	0.23	0.81	0.0085
						year2017	1.57	1.27	0.95	2.60	0.08



Figure 2.1. Study site location and experimental burn units. Ichauway is located in southwestern Georgia, U.S. and comprised of burn units, discrete management units bounded by maintained dirt firebreaks (depicted by gray lines). The recent prescribed fire history for the experimental burn units is provided in Table 2.1.


Figure 2.2. Forest stand characteristics ordination results from Principal Components Analysis (PCA). Refer to Table 2.5 for a key to the metrics. Each panel shows importance and direction of metrics (arrows) and relationships (ellipses) with fire regime treatments (a) and site types (b) in experimental longleaf pine stands in southwestern Georgia, U.S.



Figure 2.3. Relationships between *Ips calligraphus* trap catches and basal area percentage of trees with DBH <15 cm in longleaf pine stands in southwestern Georgia, U.S.



Figure 2.4. Relationships between *Ips avulsus* trap catches and total basal area in longleaf pine stands in southwestern Georgia, U.S.



Figure 2.5. Estimates of parameter coefficients with 95% confidence intervals for selected and averaged models for bark beetle species. Parameter estimates and confidence intervals are shown here as returned by the models, giving log-odds compared to the baseline condition, which is the fire exclusion treatment at mesic site types in 2016. RE is the reintroduction fire treatment and RX is the frequent fire treatment. Back-transformed intercept estimates and coefficients are presented in Table 2.7.



Figure 2.6. Trap catches predicted by selected or averaged models in 2016 for mesic and xeric sites with standard error bars for (clockwise from top left) *Ips calligraphus, Ips grandicollis, Pachylobius picivorus,* and *Ips avulsus*. Note that the scale for the y-axes varies by species.



Figure 2.7. Trap catches at the mesic site predicted by the averaged models in 2016 with standard error bars for (from left to right) *Orthotomicus caelatus, Dendroctonus terebrans*, and *Hylobius pales*. Note that the scale for the y-axes varies by species.

### CHAPTER 3

### EFFECTS OF PRESCRIBED FIRE AND FIRE REGIME ON LONGLEAF PINE (PINUS PALUSTRIS MILL.) TREE DEFENSES TO BARK BEETLES<sup>2</sup>

<sup>&</sup>lt;sup>2</sup> Ritger, H.M.W., S.T. Brantley, K. D. Klepzig, B.T. Sullivan, C. Villari, and K.J.K. Gandhi. To be submitted to *Forest Ecology and Management*.

#### Abstract

Climate change is expected to increase the incidence of abiotic stresses, disturbances, and insect and disease outbreaks in forest ecosystems, prompting the need for managers to understand how to increase resiliency. Because longleaf pine (Pinus *palustris* Mill.) ecosystems in the southeastern U.S. are often considered resilient to such disturbances, investments in restoring this rare forest type have increased dramatically. Longleaf pine forests' historical lack of widespread insect outbreaks, long lifespan, and disturbance-adapted life history suggest that longleaf pine trees are particularly welldefended against bark beetles (Coleoptera: Curculionidae: Scolytinae). However, interactions between longleaf pine defenses and prescribed fire, the primary management tool for longleaf stands, are not well documented. Pine forests have co-evolved with subcortical insects, and resin comprises their primary physical and chemical defense against bark beetles. We investigated the short-term effects of dormant and growing season prescribed burns on resin quantity and quality in mature longleaf pine trees in southwestern Georgia, U.S. We also determined the effects of various fire regimes, specifically of frequent fire, fire exclusion, and fire reintroduction, on physical and chemical properties of resin in two edaphic types, mesic and xeric. From 2017 to 2018, we collected 924 resin flow samples ranging from 0 g day<sup>-1</sup> to 32.68 g day<sup>-1</sup> and 512 phloem tissue samples with total monoterpene concentrations ranging from 19.58 ng mg<sup>-1</sup> to 25,897.16 ng mg<sup>-1</sup>, indicating great variability among tree defenses in this ecosystem. The dormant season prescribed burn resulted in a temporary 2.39 g day<sup>-1</sup> resin flow increase from the pre-treatment baseline two weeks after treatment, an increase 29 times higher than the increase of just 0.08 g day<sup>-1</sup> observed in the control treatment. There was

no effect of prescribed fire on resin flow following a growing season prescribed burn or on monoterpene concentration in either short-term prescribed burn treatment. Longleaf pine stands with a frequent fire regime had lower resin flow compared to stands that had been fire-excluded for 17 years. While there was no effect of fire regime on total monoterpene concentration, we found that the xeric sites had 1.49–3.57 higher total monoterpene concentration than mesic sites. We conclude that management with prescribed fire and site conditions like soil water availability affect longleaf pine defenses to subcortical insects. Further investigation of these site effects is warranted to support restoration and management decision-making for longleaf pine stands in the future.

**INDEX WORDS:** bark beetles, longleaf pine, prescribed fire, resin, terpenes

#### **3.1 Introduction**

Climate change will have a substantial effect on many forested ecosystems, including pine (*Pinus* spp.) dominated areas (Gan 2004). Climate change is expected to increase temperatures, increase drought duration and frequency, increase wildfires, and result in larger and more severe pest and disease outbreaks. In addition to climate change, herbivorous subcortical beetles (Coleoptera: Curculionidae) constitute one of the biggest forest health threats to pine ecosystems worldwide. For example, outbreaks attributed to a single beetle species in the southern U.S. forests have caused an estimated \$43 million in losses in the decades between 1980 and 2010 (Pye et al. 2011). Additionally, in British Columbia, cumulative reductions of 57.4 billion CAD are projected for the period 2009– 2054 resulting from outbreaks of mountain pine beetle in 2004–2005 (Corbett et al. 2016). Ecological impacts and economic losses are expected to increase as climate change creates conditions favorable for increased bark beetle outbreaks. Climatic changes may lead to increased beetle abundance by range expansion or altered reproduction dynamics (Trân et al. 2007, Cullingham et al. 2011, Bentz et al. 2014, Dodds et al. 2018) and increased abundance of suitable host trees on the landscape by reducing tree vigor or otherwise compromising tree defenses (Hofstetter and Gandhi 2022).

Pine trees are not defenseless against bark beetles. Pine trees and bark beetles share a long co-evolutionary history and intricate chemical interactions. A pine tree's primary defense is resin, a viscous, sticky liquid composed of organic terpenoid compounds that exists within the inner bark of the tree (Franceschi et al. 2005, Krokene 2015). Resin can act as a physical defense by preventing beetle entry or entombing attacking beetles as it crystallizes (Hodges and Lorio 1975, Strom et al. 2002). Resin can also be a chemical defense, with many of its components (terpenes, resin acids, and phenolics) known to cause toxic and sublethal consequences for beetles and their associated fungi (Hofstetter et al. 2005, Kopper et al. 2005). Compounds contained in resin can repel adult beetles, are toxic to adults and developing larvae, inhibit growth or reproduction of microbial organisms associated with bark beetles, and interfere with aggregation pheromones used by beetles to increase their population numbers to overwhelm tree defenses (Mason et al. 2015).

Unlike many other southeastern U.S. pines, longleaf pines (*P. palustris* Mill.) have been relatively unaffected by insect outbreaks and are hypothesized to be highly resistant to these major abiotic and biotic disturbances (Diop 2009). This resistance is fortunate for conservation efforts because the longleaf pine ecosystem is a biodiversity hotspot that provides critical habitat for threatened and endangered species, high quality and long-lived forest products, and culturally significant recreational opportunities (Barnett 2002, Brockway et al. 2005). These forests dominated the landscape of the southeastern United States, once covering approximately 37 million hectares (Barnett 2002, Frost 1993, Landers et al. 1995). In the time since European colonial settlement >95% of native longleaf forests have been converted to other uses including residential development, loblolly pine (P. taeda L.) plantations, agricultural land, or they have reverted to unmanaged mixed pine-hardwood forest due to a lack of fire. Longleaf pine can occupy a wider range of site types than any other southern pine (e.g., wet-mesic flatwoods, xeric sandhills, intermediate uplands, montane environs, etc.) and tolerate a broad array of soils (Boyer 1990, Gilliam et al. 1993, Harper 1913). Despite its reduced presence on the landscape, the combination of broad tolerance to stress, low density, and adaptations to tolerate fire make the system more resilient to disturbances such as insect outbreaks.

Longleaf pine forests are dependent on frequent, low-intensity surface fires to maintain their characteristic open-canopy structure and biodiversity (Barnett 2002, Stambaugh et al. 2011, Varner et al. 2005, Van Lear et al. 2005). Tree morphological characteristics and life history, including its thick bark, highly flammable needles, and resistance to fire in the seedling and sapling stages, indicate a long history of coevolution with frequent fire (Stambaugh et al. 2011). In the absence of frequent burning, hardwoods and faster-growing pines encroach into the midstory, limiting regeneration, increasing risk of wildfire, and increasing competition for water and nutrients (Boyer 1990, Brockway et al. 2005, Gilliam and Platt 1999, Haywood et al. 2001).

While longleaf pine forests have been characterized as relatively resistant to insect disturbances compared to other southern pines, peer-reviewed studies on longleaf pine defenses are scant (Hodges et al. 1979, Martinson et al. 2007, Slack et al. 2016). Forest management practices such as thinning have been shown to mitigate risks associated with these insects in southeastern pine forests, with most research focused on loblolly pine (Turchin et al. 1999, Schowalter 2012, Nowak et al. 2015). Fire is a natural part of southeastern forests; however, fire can wound and stress trees, attract bark beetles, and alter tree defenses. These effects may only worsen under climate change (Anderegg et al. 2015), and frequent prescribed fire may have similar effects to mechanical thinning by keeping basal area low and promoting overall tree vigor (Fettig and McKelvey 2014). Frequent fire effects on tree physiology and phytochemistry may also interact with bark beetle populations by enhancing tree defenses or host attractiveness (Hood et al. 2015, Raffa et al. 2016).

Because fire is an integral component of longleaf pine forests, frequent prescribed fire is often the primary management tool used to restore and preserve these ecosystems. While fire exclusion introduces certain risks to longleaf pine, reintroducing fire to fireexcluded stands with the goal of restoration also brings risks due to accumulated fuels (Haywood 2009). Many remnant longleaf pine stands do not experience frequent fire and have been fire-excluded for an extended period of time (Frost 1993). These stands tend to exhibit higher basal area (Kirkman et al. 2016), which increases competition for resources and may compromise defenses such as resin production. Such competition and associated stress may increase incidence of insect outbreaks (Parker et al. 2006). Increased tree mortality, often associated with bark beetles, often occurs following the reintroduction of fire after a period of exclusion (Varner et al. 2007). Researchers posit that this increased mortality is primarily driven by fire injury to trees' vital tissues with insects as secondary agents, colonizing the weakened trees (Sullivan et al. 2003, O'Brien et al. 2010). Although past studies have shown that fire, mechanical wounding, and simulated or induced bark beetle attacks prompt changes in resin flow and chemistry (Lombardero et al. 2000, Mason et al. 2015, Keefover-Ring et al. 2016, Kolb et al. 2019), few studies have examined the effect of low intensity fire on longleaf pine.

Evaluating the risks and benefits of various prescribed fire management strategies and regimes is important for managers, often tasked with meeting multiple management objectives while constrained by limited resources (Hiers et al. 2003, Ryan et al. 2013). Our study aimed to address gaps in knowledge by directly investigating the effects of frequent prescribed fire, fire exclusion, and the reintroduction of fire on longleaf pines' resin production and chemical makeup, characteristics known to affect pine trees' ability to defend against bark beetles and associated pathogens. Our specific research questions were:

- What are the immediate (~2-months) effects of dormant and growing season prescribed fire treatments on the production of resin in longleaf pine trees?
- 2) What are the immediate (~2-months) effects of dormant and growing season prescribed fire treatments on monoterpene concentrations in phloem of longleaf pine trees?
- 3) How does resin production differ between frequently burned (2-year fire return interval) and fire-excluded (>15 years) longleaf pine stands; how does fire reintroduction after a period of fire exclusion (13 years) affect resin production; and are differences in resin production responses to fire treatments mediated by varying site conditions?
- 4) How does phloem monoterpene concentration differ between frequently burned (2-year fire return interval) and fire-excluded (>15 years) longleaf pine stands; how does fire reintroduction after a period of fire exclusion (13 years) affect phloem monoterpene concentration; and are differences in phloem monoterpene concentrations related to varying site conditions?

Results from this study may inform effective conservation of the longleaf pine forest ecosystem by increasing our understanding of interacting ecological processes and the potential forest health impacts of specific management and restoration activities in these forests.

#### **3.2 Methods**

#### **3.2.1 Site description**

We used longleaf pine stands at the Jones Center at Ichauway (Ichauway henceforth) as our model system for this study. Ichauway is an 11,500-ha private research and education reserve in Baker County, Georgia, U.S. (31°13'N, 84°28'W). Ichauway has ~7,000 ha of naturally regenerated, mature, second-growth (~80–100 years) longleaf pine forests that are generally managed on a two-year fire return interval (McIntyre et al. 2010). Soils range from excessively well-drained to poorly drained types, primarily classified as ultisols (95%) with some entisols (5%). Mean elevation is 48 m above sea level. Climate is humid subtropical, with a long growing season (mid-March to mid-November) and mild winters. Mean monthly temperatures are highest in July (28 °C) and lowest in January (10.5 °C). Annual precipitation is ~1,300 mm yr<sup>-1</sup> and is distributed evenly throughout the year (Drew et al. 1998, Kirkman et al. 2016).

# **3.2.2 Stand selection and sampling design to assess immediate effects of prescribed** fire

To facilitate the application of prescribed fire, Ichauway is divided into 177 burn units ranging from <10 to ~300 ha, with a median size of ~67 ha. The frequent application of prescribed fire on discreet, but ecologically similar, burn units provided the opportunity to examine effects of fire on tree defenses in the short term (~2 months). We employed a Before-After Control-Impact Paired Series (BACIPS) experiment for two distinct prescribed burn treatments: 1) dormant; and 2) growing season burns. The primary fuels are wiregrass (*Aristida stricta* Michx. var. *beyrichiana* [Trin. & Rupr.] D. B. Ward), other grasses, and longleaf pine litter. Because these landscapes are burned frequently, under careful prescription, and with minimal fuel buildup, the fires are lowintensity surface fires that move quickly across the forest understory.

We selected three burn units with similar forest stand structure and species composition that have been maintained with prescribed fire on a 1–3-years burn interval (Table 3.1). All burn units in the study were dominated by a naturally regenerated mature longleaf pine overstory (> 80 years old) and ranged in size from 65 to 196 ha. We established four 0.5-ha monitoring plots in each burn unit using the random location generator in ArcGIS, stipulating that plots be  $\geq 250$  m apart to reduce inter-plot spatial correlation and  $\geq 15$  m from a firebreak to reduce edge effects (ESRI 2015). To characterize forest stand conditions, we recorded tree species and diameter at breast height (DBH) for every tree > 5 cm DBH within a 40 m radius centered at each monitoring plot location.

To minimize effects of the repeated sampling procedures on measured response variables in the short-term study, we utilized two separate sets of dominant longleaf pine trees (~70 m apart) at each plot location for the different types of resin sampling, physical (resin flow) or chemical (phloem extraction). In each plot, we sampled 11 longleaf pine trees, six for resin flow and five for resin chemistry, for a total of 24 resin flow trees and 20 resin chemistry trees per burn treatment. At the start of each study, we measured DBH, a potentially influencing factor on resin flow and resin chemistry characteristics, on all study trees (Table 3.2). Pre-treatment resin sampling occurred prior to the prescribed burns in each study to identify baseline conditions, and post-burn sampling

occurred for ~6 weeks in each study (Table 3.2). For the growing season prescribed burn treatment, multiple pre-treatment resin flow samples were averaged to calculate each tree's baseline for resin production.

The dormant season burn occurred on 21 March 2017 with mid-morning ignition. Weather conditions for the duration of the dormant season burn consisted of air temperatures 27.8–30.0 °C, relative humidity 37–42 %, westerly surface winds 3.6–5.4 m s<sup>-1</sup>, and atmospheric mixing height of 1760.5 m. There was no crown scorching and patchy fuel consumption resulted in ~50% top-kill for woody understory vegetation and ~80% duff layer consumption. The growing season burn occurred on 1 June 2018 with late-morning ignition. Weather conditions for the duration of the growing season burn consisted of air temperatures 31.7–33.9 °C, relative humidity 48–53 %, westerly surface winds 2.7–4.5 m s<sup>-1</sup>, and atmospheric mixing height of 1354.5 m. There was no crown scorching and clean fuel consumption resulted in top-kill for most of the woody understory vegetation and little remaining duff.

## **3.2.3 Stand selection and sampling design for effects of fire regime and site conditions**

To examine the effects of fire regime on tree defenses, we used burn units that are part of a long-term study on fire exclusion and fire reintroduction (Ritger et al. 2023). All burn units included in the study were dominated by a naturally regenerated mature longleaf pine overstory (>80 years old) and ranged in size from 10 to 65 ha. The burn units were maintained with frequent fire (~2-year return interval) prior to the onset of the long-term experimental treatments in 2001. Burn units in this study also occupy two site types on Ichauway that encompass the range of soil moisture availability typical in the Coastal Plain. Xeric sites are characterized by excessively well drained sandy soils, with mean volumetric water content in the top 30 cm of soil (VWC<sub>30</sub>) ranging from  $\sim$ 3% to  $\sim$ 14%, with a mean of  $\sim$ 7.7%. Mesic sites are characterized by somewhat poorly drained loamy sand soils and have VWC<sub>30</sub> ranging from  $\sim$ 4.4% to  $\sim$ 28%, with a mean of  $\sim$ 12% (Ritger et al. 2023).

Starting in 2001, two each of xeric and mesic burn units were taken out of the prescribed fire rotation and put into a fire exclusion treatment. Reference burn units in both the mesic and xeric sites continued on a biennial burn rotation to serve as experimental controls. In 2015, prescribed fire was reintroduced to one each of mesic and xeric fire-excluded burn units. Thus, we have experimental burn units for each of the three fire regime treatments (fire exclusion, frequent fire, and fire reintroduction) at both the mesic and xeric sites. Years of prescribed burns for all treatments varied from 2001 to 2018, with all burns occurring in the dormant season (Table 3.3). Because soil moisture availability and fire frequency affect forest composition and structure, these sites varied greatly in those characteristics as well as in fuel loads and types throughout the study (Table 3.4).

We established two 0.5-ha sampling plots per site-treatment stand using the random location generator in ArcGIS, ensuring plots were  $\geq 250$  m apart to reduce interplot spatial correlation and were  $\geq 15$  m from a firebreak to reduce edge effects (ESRI 2015). In each plot, seven dominant longleaf pines were sampled for resin flow monthly from July to September in 2018, for a total of 14 trees repeatedly sampled per site type-fire regime treatment combination. A randomly selected subset of three trees per plot

were also sampled for resin chemistry once per year in July 2017 and August 2018, for a total of six trees sampled annually per site type-fire regime treatment combination (Table 3.2). We measured DBH of all study trees as a potential covariate.

#### 3.2.4 Resin sampling

We assessed resin flow using two samples taken from opposite sides of the bole at breast height (~1.37 m) over a 24-hour period (Roberds and Strom 2006). Resin samples were refrigerated, weighed within 10 days of collection, and the two samples were averaged for each tree to determine resin flow as g day<sup>-1</sup>. New samples were taken  $\geq$  5 cm to the right of the previous sample.

We harvested phloem to evaluate monoterpene concentrations. At ~1.37 m height on bole, we scraped bark with a chisel, and then extracted 4–6 discs (~ 1 cm diameter) of phloem with a cork borer or punch. Phloem samples were placed in labeled coin envelopes, put on dry ice in the field, and stored at -80 °C until ground and extracted. Equipment was rinsed with 80% ethanol between trees in the field. For trees that were repeatedly sampled, new samples were taken ~10 cm to the right of the last sample.

Chemical extraction and analyses followed previously published protocols (Keefover-Ring et al. 2016, Raffa et al. 2017). To preserve chemical integrity, all samples were ground in liquid nitrogen using a mortar and pestle. Approximately 100 mg ground tissue was aliquoted into each of two vials, a 2 ml glass vial with PFTE screw cap for monoterpene extraction. Ground samples were kept at -80 °C until extracted.

To extract the monoterpenes, we added 1 ml of 95% n-hexane solution with 0.2  $\mu$ l ml<sup>-1</sup> toluene and naphthalene as internal standards to each sample and placed in a

sonicating water bath for 10 minutes. Each sample was briefly vortex mixed and shaken on an orbital shaker overnight. Samples were centrifuged at 1450 rpm for 10 minutes, and 175 µl of extracted monoterpenes was transferred to a 2 ml glass vial with volumereducing insert and PTFE screw cap. Post-extraction phloem tissue was evaporated under the hood two days and then oven-dried at 70 °C for 48 hours before obtaining the dry weight. Extracted monoterpenes were kept at -20 °C until analyzed using gas chromatography-mass spectrometry (GC-MS) for identification and quantification.

Monoterpenes were identified and quantified on a Hewlett-Packard G1800C GCD system. Two µl of sample was injected splitless onto a HP-5MS capillary column (30 m by 0.25 mm with 0.25 µm film thickness; Agilent Technologies, Wilmington, DE) with helium as the carrier gas and a pulseless constant flow of 0.7 ml min<sup>-1</sup>. The temperature program was an initial temperature of 40 °C, held 0.7 minutes, with a 10 °C min<sup>-1</sup> ramp to 240 °C, held for 12 minutes. The injector temperature was 180 °C and the detector temperature was 220 °C. Monoterpenes were identified by matches of both retention times and mass spectra to identified standards. Compounds were quantified relative to the internal standards naphthalene and toluene and to response curves calculated from analyses of serial dilutions of standards (Appendix A). Concentrations were calculated as ng mg<sup>-1</sup> dry weight of phloem tissue. We distributed samples from the various experiments across multiple blocks of runs through the gas chromatograph-mass spectrometer (GCMS) to prevent introduction of bias from particular runs of the instrument. We tested for any effects of this factor in the statistical analyses but found none.

#### **3.2.5 Statistical analyses**

For all of our studies, our experimental design constitutes a complete block design with plots and individual trees as pseudoreplicates within each of burn units (Hurlbert 1984). During preliminary data analyses, we calculated and examined the intraclass correlation coefficient for sampling plot and individual trees and determined that we could consider trees within plots as independent replicates with repeated measures. This allowed us the statistical power to look for interaction effects of various factors. All statistical analyses were performed in R statistical software, (v3.6.3 R Core Team 2020). For all analyses, significance was assessed with an alpha level of 0.05.

### 3.2.5.1 Assessing the immediate (~2-months) effects of dormant and growing season prescribed fire treatments on resin production and total monoterpene concentrations in longleaf pine trees

To evaluate resin production and total monoterpene concentration responses following the application of the prescribed fire treatment, we used the difference from the pre-treatment baseline measurements as the response variables. We examined histograms (Appendix B) and calculated the Wilks-Shapiro test for normality and determined that the data were not normally distributed. Explorations of data fit to other distributions did not yield a better fitting distribution. Because linear regression models are robust to violations of the assumptions of normality when the sample size is large (n > 10) (Schmidt and Finan 2018, Knief and Forstmeier 2021), we proceeded to analyze these data assuming our results would be insensitive to such violations. Preliminary data analyses explored whether DBH should be included as a covariate. For resin flow, DBH was excluded from final analyses due to lack of significant effect or improved model fit, but DBH was included as a covariate for the total monoterpene concentration response analyses.

We used linear mixed-effects models fit with the packages 'lme4' (Bates et al. 2015) in R to test for fixed effects of factors (treatment, sample week, and their interactions) and the random effects of repeated measures on the resin variables difference from baseline following the prescribed burn treatment. For each regression, we performed an analysis of variance using the function 'anova ()' in R to compare experimental and null models, and we stipulated an alpha of 0.05 for rejecting the null model.

## **3.2.5.2** Assessing the effects of prescribed fire regime treatments and site conditions on resin production and total monoterpene concentrations in longleaf pine trees

Using the package 'lme4', we fit generalized linear mixed models with our response variables of resin flow and total monoterpene concentrations to assess the fixed effects of experimental factors while accounting for random within-tree effects due to repeated measures (Bates et al. 2015). For resin flow, we assumed the gamma distribution based on examinations of variance, overdispersion, and model goodness of fit metrics (Hilborn and Mangel 1997, Burnham and Anderson 2002). For total monoterpene concentration, we determined that the data best fit a lognormal distribution. Due to robustness of generalized linear models to violations of assumptions and to support straightforward interpretation of model results, we used a generalized linear mixed effects model fit to the gaussian distribution with a log link function (Knief and Forstmeier

2021). Preliminary data analyses explored whether DBH should be included as a covariate; lack of significant effect or improved model fit led to its exclusion from final analyses. We performed Likelihood Ratio Tests with a stipulated alpha of 0.05 using the 'anova ()' function in R to assess the probability that the experimental models explained more of the variability in resin flow and total monoterpene concentrations than null models fit to our data without the experimental factors of interest.

Due to the novel nature of our investigation of resin chemistry in longleaf pines, we expanded our analyses to evaluate which, if any, of our experimental factors may have an effect on total monoterpene concentration. We employed an information theoretic approach to investigate multiple hypotheses and identify the best fitting model to our response variable, factors, and potential covariates included in the study. We built a set of candidate models for all possible combinations of the factors as well as a null model and fit them to our total monoterpene concentration response variable data. We ranked models by  $AIC_C$ , (via 'AICc' function from 'MuMIn' package), and we used the threshold of  $\Delta AIC_C \leq 2$  to select the top model (Burnham and Anderson 2002, Grueber et al. 2011, Bartoń 2020). Using the 'MuMin' package, we calculated Nagelkerke's R<sup>2</sup> (Nagelkerke 1991) and conditional and marginal R<sup>2</sup> values (Nakagawa and Schielzeth 2013) for the selected model (Bartoń 2020).

#### **3.3 Results**

Over two years (2017–2018), we collected 924 resin flow measurements from 156 trees and 512 phloem samples from 96 trees between the months of March and September. We observed a high degree of variability in both resin flow and total

monoterpene concentration. For resin flow, the lowest measurement was 0 g day<sup>-1</sup> in the mesic fire reintroduction treatment in July 2018, and the highest resin flow we recorded was 32.68 g day<sup>-1</sup> in the xeric fire reintroduction treatment in September 2018. In the short-term experiments, both the lowest (-8.73 g day<sup>-1</sup>) and highest (18.40 g day<sup>-1</sup>) resin flow difference from baseline were six weeks following the dormant season prescribed burn. For total monoterpene concentration, the lowest measure in our study was 19.58 ng mg<sup>-1</sup> for the growing season burn on three weeks thereafter, and the highest was 25,897.16 ng mg<sup>-1</sup> in the control treatment for the growing season burn about four weeks after the burn. In the short-term experiments, the lowest (-3,529.08 ng mg<sup>-1</sup>) and highest (23,753.53 ng mg<sup>-1</sup>) differences from baseline in total monoterpene concentrations were both from control treatments in the short-term prescribed burns.

## **3.3.1 Immediate** (~2-months) effects of dormant season prescribed fire treatments on the production of resin in longleaf pine trees

For the dormant season prescribed burn, the experimental model for resin flow difference from baseline with the interaction of prescribed fire treatment and sampling week factors differed significantly from the null model (p < 0.05). Thus, we reject the null model and conclude that the dormant season prescribed fire treatment interacted with time following treatment to affect resin flow in our study. The model's estimated coefficients and confidence intervals (Figure 3.1) and observed data (Figure 3.2) show that trees in the burn unit treated with prescribed fire had significantly higher increase in resin production compared to trees in the control two weeks following the prescribed burn, indicating a short-term response. This was an average of 2.39 g day<sup>-1</sup> more resin

flow compared to pre-treatment, while the control trees produced nearly the same amount of resin as pre-treatment (an average increase of just 0.08 g day<sup>-1</sup>).

## **3.3.2 Immediate (~2-months) effects of growing season prescribed fire on the production of resin in longleaf pine trees**

The experimental model for resin flow difference from baseline following the growing season prescribed burn that included the interaction of prescribed fire treatment and sampling week factors did not differ significantly from the null model (p > 0.05). Thus, we cannot reject the null model and concluded that we could not detect effects of growing season prescribed fire treatment on resin flow difference from baseline in our study (Figure 3.3).

## **3.3.3 Immediate (~2-months) effects of dormant season prescribed fire on the total monoterpene concentration in longleaf pine trees**

For the dormant season prescribed burn, the experimental model for total monoterpene concentration that included the interaction of prescribed fire treatment and sampling week factors did not differ significantly from the null model (p > 0.05). Thus, we cannot reject the null model and could not determine effects of growing season prescribed fire treatment on total monoterpene concentration difference from baseline in our study (Figure 3.4).

### **3.3.4 Immediate** (~2-months) effects of growing season prescribed fire on the total monoterpene concentration in longleaf pine trees

The experimental model for total monoterpene concentration difference from baseline that included the interaction of prescribed fire treatment and sampling week factors did not differ significantly from the null model (p > 0.05). Thus, we cannot reject the null model and conclude that we could not detect effects of growing season prescribed fire treatment on total monoterpene concentration difference from baseline in our study (Figure 3.5).

## **3.3.5 Effects of prescribed fire regime treatments and site conditions on the production of resin in longleaf pine trees**

The experimental model for resin flow including the fire regime treatment and site type factors is significantly different from the null model (p < 0.05). Thus, we reject the null model and conclude that treatment and site type affected resin flow in our study (Figures 3.6 and 3.7). The model's estimated coefficients and confidence intervals show that trees in the frequent fire treatment had significantly less resin flow compared to the fire exclusion treatment. Across both site types and all three months sampled, resin flow in the fire exclusion treatment was 1.3–2.5 times higher compared to the frequent fire treatment (Figure 3.7). The model also shows significantly higher resin flow in September an average of 1.4 times higher than in July.

### **3.3.6 Effects of prescribed fire regime treatments and site conditions on total monoterpene concentration in phloem of longleaf pine trees**

The experimental model for total monoterpene concentration including the fire regime treatment and site type factors did not differ significantly from the null model (p > 0.05). The experimental model has similar AIC<sub>C</sub> and conditional R<sup>2</sup> values as the null model (Appendix G). Thus, we cannot reject the null model in favor of the full experimental model. However, our model selection approach produced a top model that included only the site type factor. This selected model is significantly different from the null model (p < 0.05), has lower AIC<sub>C</sub>, but has similar conditional R<sup>2</sup> values as the null model (Appendix G). Although we concluded that total monoterpene concentrations differed significantly by site type in our study, with total monoterpene concentrations 1.49–3.57 times higher in the xeric site type compared to the mesic site type over two years (Figure 3.8), the selected model fit is poor and warrants caution in interpreting these results.

#### **3.4 Discussion**

We found the following major trends in longleaf pine tree defenses in response to site, fire regime, and seasonal prescribed fire treatments at our study site in southwestern Georgia: 1) treatment with prescribed fire in fire-maintained stands only had a small short-term impact on resin production in the dormant season, with no discernable effect on resin production in the growing season or monoterpene concentration in either burn seasons; 2) stands treated with frequent fire regimes had lower resin flow compared to fire-excluded stands; and 3) while fire regime treatments did not have a detectable effect

on total monoterpene concentration, trees at the xeric site had higher total monoterpene concentrations as compared to trees at the mesic site.

The dormant season burn occurred around the time trees are generally transitioning from dormant to growing season in the southeastern U.S., when healthy trees will be switching their allocation of photosynthate from defense to growth (Lorio 1986, Waring 1987). The immediate effects of even a low-intensity burn may interrupt this phenological cycle and may explain the difference in the timing of changes from baseline in control versus treatment trees over the course of several weeks. Despite this potential disruption to the typical pattern of allocating photosynthates to growth and defense, at this time of year, healthy trees should have sufficient reserves of carbon to recover from any depletion of defensive compounds and any resultant defoliation events (Waring 1987).

The results of our study are not consistent with many other studies that have reported increased resin flow in *Pinus* species in the short-term (< 1 year) following either prescribed fire or wildfire (Santoro et al. 2001, Lombardero et al. 2006, Sparks et al. 2017). We suspect this difference may be explained by the nature of the prescribed fires used in longleaf pine forests in the southeastern U.S., as this pine species is a firetolerator as compared to other species (Keeley 2012). A study in red pine (*P. resinosa* Aiton) found that following a prescribed burn increased resin flow was positively correlated with the char height along the bole of the tree (Santoro et al. 2001), while another study in red pine following a wildfire found a significant difference in resin flow of trees with scorched boles compared to trees with unscorched boles (Lombardero et al. 2006). A study in ponderosa pine (*P. ponderosa* Douglas ex. P. Lawson & C. Lawson) (another fire-tolerator species; Keeley 2012) found decreasing resin flow associated with increasing crown scorch following a prescribed burn (Wallin et al. 2003). The low intensity, low severity prescribed burns employed in our study, and generally applied in longleaf pine forest management, typically do not result in extensive charring or crown scorch and that may be one reason we did not observe a difference in resin flow in our prescribed burn treatments compared to the control treatments.

Our finding of higher resin flow in stands where fire was excluded compared to stands receiving frequent prescribed fire is also inconsistent with literature on burning in other pine forests. Studies in western U.S. pine forests have found resin production (or associated physiological traits like resin duct production, size, and area) increases following prescribed burns and decreases in the absence of fire. Studies in ponderosa pine found increased resin production following prescribed burns (Perrakis and Agee 2006, Six and Skov 2009, Perrakis et al. 2011). One study in western U.S. pine forests found that for many, but not all, sites assessed resin duct area decreased by up to 15% following the cessation of fire (Hood et al. 2015). We posit that these differing results in our study compared to western pine forest systems may be explained in part by increased competition for water and other nutrients following the rapid growth of vegetation in the southeastern climate when fire is excluded, which distinguished our system from western forests where vegetation regrowth may be much slower.

Other studies have shown that moderate water stress increases resin production in conifers (Hodges and Lorio 1975, Dunn and Lorio 1993), while severe drought stress or limited water availability leads to decreased resin production (Lombardero et al. 2000, Gaylord et al. 2013, Netherer et al. 2015). We observed increased resin flow at the xeric

sites, indicating that while soil water availability is reduced, the trees are not experiencing extreme water stress. We also observed increased resin flow in the fire exclusion treatments at both site types, indicating that competition for water may be increased in the absence of fire but is not leading to extreme water stress. This evidence from our study supports the growth-differentiation balance hypothesis that when a factor like water availability limits growth but does not inhibit photosynthesis, plants will allocate more photosynthates to defense mechanisms (Lorio 1986, Mattson and Haack 1987, Huang et al. 2020).

Increased monoterpene concentration, as we found in the xeric site, has been found to be associated with moderate water stress and can increase tree attractiveness to bark beetles (Hodges and Lorio 1975, Gershenzon 1984, Mattson and Haack 1987, Kelsey et al. 2014). Specific host volatiles can enhance attractiveness for certain genera or species of bark beetle (Werner 1972, Hunt and Raffa 1989, Erbilgin et al. 2003, Miller and Rabaglia 2009, Hofstetter et al. 2012). Evidence from a study in Louisiana suggests that early colonizing southern pine beetles (*Dendroctonus frontalis* Zimmerman) may selectively choose loblolly pine over longleaf pine when both hosts are available, possibly cuing in on terpenoid compound volatiles to differentiate between the host tree species (Friedenberg et al. 2007). Assessing the full profile of terpenoid compounds for longleaf pine and any effects of management with prescribed fire could provide additional useful information for assessing forest health risks in deciding which species to grow and how to manage their stands.

#### **3.5 Conclusions**

Overall, this is the first study assessing defensive components of southeastern U.S. longleaf pine trees under varying fire regimes and site conditions, and it provides important baseline information about prescribed fire effects on tree defenses in these forests. Future work could focus on assessing potential differences in resin composition (monoterpenes and diterpenes) amongst all these treatments, since resin flow is just one metric to determine defensive capability of pine trees. Finally, understanding how tree defenses are affected in the short and long-term by various prescribed fire management activities provides important information to managers to consider as they work to restore this valuable ecosystem.

#### **3.6 Acknowledgments**

We acknowledge financial support provided by the D.B. Warnell School of Forestry and Natural Resources and the Jones Center at Ichauway. An Interdisciplinary and Innovative Research Grant from the University of Georgia Graduate School supported portions of this project. We thank technicians from the Jones Center at Ichauway (Denzell Cross, D. Alex Gordon, Sierra Perez, Robert Ritger, Sean Reynolds) and the University of Georgia (Afaq Niyas, Lea Clark, Sarah Forget, Meghan McMillan) for assistance in the field and lab. We additionally thank Brittany Barnes (University of Georgia) and Stribling Stuber (the Jones Center at Ichauway) for technical assistance and logistics support. We also thank Brandon Rutledge (the Jones Center at Ichauway) for providing prescribed fire treatment information. We thank Dr. Kimberly Novick (Indiana University), Dr. Ken Keefover-Ring (University of Wisconsin), and Drs. O. Sander Denham and Charles Mason (USDA Agricultural Research Service) for training and expert advice on methodologies employed in this study.

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Table 3.1. Forest stand summary data (means  $\pm$  standard errors, n = 4) of experimental longleaf pine stands in southwestern Georgia, U.S. Basal area (m<sup>2</sup> ha<sup>-1</sup>) and tree density (trees per ha) include all living trees  $\geq$  5 cm diameter at breast height (DBH) within the 40 m radius plot.

	<b>Dormant Season Control</b>	<b>Dormant Season Prescribed Burn</b>	<b>Growing Season Prescribed Burn</b>
		<b>Growing Season Control</b>	
Longleaf pine BA*	$12.55\pm0.27$	$10.79\pm0.48$	$17.49 \pm 1.36$
Other conifer BA	$0.30\pm0.18$	$0.66\pm0.45$	$0\pm 0$
Oak BA	$1.62\pm0.94$	$1.68 \pm 1.10$	$0.25\pm0.25$
Other hardwood BA	$0.06\pm0.05$	$0.09\pm0.09$	$0\pm 0$
Total BA	$14.40\pm0.66$	$13.23 \pm 1.43$	$17.74 \pm 1.56$
Tree density	$130.81\pm9.66$	$427.23 \pm 45.71$	$170.10 \pm 26.11$

\*BA = Basal Area

Dormant Seaso	n Prescribed Burn Experim	ent
Average Tree $DBH \pm SE$ :	$47.21 \pm 0.92 \text{ cm}$	
<u>Sample Week</u>	Sample Date(s)	Sample Type
pre-treatment 1	March 8–10, 2017	phloem, resin flow
post-treatment 1	March 2–30, 2017	phloem, resin flow
post-treatment 2	April 3–6, 2017	phloem, resin flow
post-treatment 3	April 10–13, 2017	phloem, resin flow
post-treatment 4	April 16, 2017	resin flow
post-treatment 5	April 26 – 28, 2017	phloem, resin flow
post-treatment 6	May 2–3, 2017	resin flow
Growing Seaso	n Prescribed Burn Experim	ent
Average Tree DBH ± SE:	$44.80\pm0.82~\text{cm}$	
<u>Sample Week</u>	<u>Sample Date(s)</u>	<u>Sample Type</u>
pre-treatment 1	April 26, 2018	resin flow
pre-treatment 2	May 3-7, 2018	phloem, resin flow
pre-treatment 3	May 21, 2018	resin flow
post-treatment 1	June 4-6, 2018	phloem, resin flow
post-treatment 2	June 12-13, 2018	phloem, resin flow
post-treatment 3	June 19-20, 2018	phloem, resin flow
post-treatment 4	June 28, 2018	phloem
post-treatment 5	July 5, 2018	resin flow
post-treatment 6	July 11-13, 2018	phloem, resin flow
Fire Regime	e and Site Type Experiment	
Average Tree DBH ± SE:	$44.64 \pm 1.29 \text{ cm}$	
<u>Sample Event</u>	<u>Sample Date(s)</u>	<u>Sample Type</u>
2017 Phloem	July 12, 2017	phloem
July Resin Flow	July 25–30, 2018	resin flow
2018 Phloem	August 1, 2018	phloem
August Resin Flow	August 8–9, 2018	resin flow
September Resin Flow	September 17–19, 2018	resin flow

Table 3.2. Sampling dates and average diameter at breast height [(DBH and Standard Error (SE)] for trees in each experiment.

Table 3.3. Prescribed burn history by treatment since 2001 in experimental longleaf pine stands in southwestern Georgia, U.S. All burns were conducted in the dormant season. Burns occurred in the same year for both mesic and xeric sites.

Treatment	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018
Frequent Fire	•		•		•		•		•		•		•		•		•	
Fire Exclusion	•																	
Fire Reintroduction	•														•	•	•	

Table 3.4. Forest stand summary data (means  $\pm$  standard errors, n = 2) of experimental longleaf pine stands in southwestern Georgia, U.S. Basal area (m<sup>2</sup> ha<sup>-1</sup>) and tree density (trees per ha) include all living trees  $\geq$  5 cm diameter at breast height (DBH) within the 40 m radius plot.

	Fire Exclusion	Fire Reintroduction	<b>Frequent Fire</b>
Mesic site			
Longleaf pine BA	$9.49\pm3.45$	$11.54 \pm 2.94$	$10.13\pm0.24$
Other conifer BA	$0.32\pm0.30$	$0.37\pm0.01$	$0.96\pm0.96$
Oak BA	$5.32\pm0.52$	$5.95 \pm 5.36$	$1.03\pm1.03$
Other hardwood BA	$0.24\pm0.01$	$0.04\pm0.04$	$0\pm 0$
Total BA	$15.37\pm2.61$	$17.90 \pm 2.46$	$12.12 \pm 2.23$
Tree density	$910.17 \pm 269.57$	$997.70 \pm 128.32$	$609.76 \pm 80.57$
Xeric site			
Longleaf pine BA	$3.34\pm0.64$	$12.2 \pm 1.67$	$7.79 \pm 1.74$
Other conifer BA	$0\pm 0$	$0\pm 0$	$0.01\pm0.01$
Oak BA	$5.63 \pm 1.17$	$4.01\pm0.61$	$6.24 \pm 1.80$
Other hardwood BA	$0.03\pm0.02$	$0.30 \pm 0.13$	$0.78\pm0.78$
Total BA	$9.00\pm0.55$	$17.23\pm0.92$	$14.81\pm0.84$
Tree density	$739.08 \pm 62.67$	$1744.74 \pm 137.27$	$870.38\pm6.96$



Figure 3.1. Estimates of parameter coefficients with 95% confidence intervals for the resin flow difference from baseline in the dormant season prescribed burn experiment. Parameter estimates and confidence intervals are shown here as returned by the model, giving log-odds compared to the baseline condition, which is the dormant season prescribed burn treatment in the first week of post-treatment sampling.



Figure 3.2. Observed resin flow difference from baseline data for six weeks following the dormant season prescribed burn treatment with standard error bars.



Figure 3.3. Observed resin flow difference from baseline data for six weeks following the growing season prescribed burn treatment with standard error bars.



Figure 3.4. Observed total monoterpene concentration difference from baseline data for five weeks following the dormant season prescribed burn treatment with standard error bars.



Figure 3.5. Observed total monoterpene concentration difference from baseline data for six weeks following the growing season prescribed burn treatment with standard error bars.



Figure 3.6. Estimates of parameter coefficients with 95% confidence intervals for the effects on resin flow in the fire regime and site type experiment. Parameter estimates and confidence intervals are shown here as returned by the model, giving log-odds compared to the baseline condition, which is the fire exclusion treatment at the mesic site in July.



Figure 3.7. Model-predicted and observed resin flow  $(g day^{-1}) \pm standard error by fire regime treatment July – August 2018 at the xeric (a) and mesic (b) site types.$ 



Figure 3.8. Observed total monoterpene concentration (ng mg<sup>-1</sup>)  $\pm$  standard error by site type in 2017 and 2018.

## CHAPTER 4

# CONCLUSIONS AND FUTURE DIRECTIONS

#### **4.1 Dissertation synopsis**

Longleaf pine (Pinus palustris Mill.) forest ecosystems, once widespread throughout the southeastern U.S., provide myriad benefits and ecosystem services such as high value timber, recreation opportunities, biodiversity hotspots, carbon sequestration, and wildlife habitat for rare and endangered vertebrates and popular game species (Noss 1989, Drew et al. 1998, Kirkman et al. 2001, Barnett 2002, Kush et al. 2004, Way 2006). A key component to a quality longleaf pine forest ecosystem that supports all of these resources is frequent fire, but many extant stands of longleaf remain unburned (Barnett 2002, Varner et al. 2005, Van Lear et al. 2005, Way 2006). Many managers cite concerns about the unknown impacts to forest health of reintroducing fire in these longleaf pine stands, particularly as subcortical beetles (Coleoptera: Curculionidae: Scolytinae) may exploit fire-injured or weakened trees (Drooz 1985, Bryant et al. 2006, Varner et al. 2009, Nebeker 2011, Bär et al. 2019, Munro et al. 2019). The overarching goal of this original dissertation research was to gain and report useful and practical insight for both managers and scientists regarding the important and complex interactions among longleaf pine forests, bark beetles, and management with prescribed fire.

Using a previously established long-term fire regime experiment in southwestern Georgia, U.S., we evaluated the potential impacts of various fire regimes and site conditions in longleaf pine forest stands on subcortical beetles (Chapter 2, Ritger et al.

2023). In 2016 and 2017, we sampled over 140,000 beetles caught over approximately three weeks in early summer in experimental burn blocks with three fire regime treatments—frequent fire, fire exclusion, and fire reintroduction after a period of exclusion—in two sites differing in edaphic characteristics and soil moisture availability. These forest stands, which we labelled either as "mesic" or "xeric", varied in forest stand characteristics due to differences in both soil moisture and fire regime. For the three *Ips* beetle species that occur in the coastal plain, we found that fire regime treatments interacted with site type, and the highest trap catches were in the fire-exclusion treatment at the xeric site type. In the fire-exclusion treatment, *I. avulsus* (Eichhoff) and *I. calligraphus* (Germar) catches were 2–10 times higher at the xeric than mesic sites. For the other secondary colonizer species, Orthotomicus caelatus (Eichhoff) and Dendroctonus terebrans (Olivier), trap catches in the frequent fire treatment were 1.1–2.4 times higher than in the fire-exclusion treatments. The root-feeding weevil *Pachylobius picivorus* (Germar) also had higher trap catches in the frequent fire treatments relative to the fire-excluded treatments, while another root-feeding weevil *Hylobius pales* (Herbst) had the opposite trend with lower trap catches in the frequent fire treatments compared to fire-exclusion. Relative to specific forest stand characteristics, *I. calligraphus* trap catches were positively associated with percent basal area of understory trees and I. avulsus catches were negatively correlated with total tree basal area. These relationships indicate that *I. calligraphus* may utilize fire-excluded stands that have greater woody midstory encroachment, while *I. avulsus* may utilize more open stands like those created and maintained by frequent fire regimes.

We evaluated the impacts tree defenses to bark beetles by sampling physical and chemical defense components in various prescribed fire treatments in mature longleaf pine stands in southwestern Georgia, U.S. (Chapter 3). We sampled resin quantity and quality in the short-term following dormant and growing season prescribed burns in mature, dominant longleaf pine trees. Further, we investigated the effects on the physical and chemical properties of resin from mature longleaf pines at two site types, mesic and xeric, managed under various fire regimes, specifically of frequent fire, fire exclusion, and fire reintroduction. Two weeks after the dormant season prescribed burn, we observed a 29-fold increase (2.39 g day<sup>-1</sup>) in resin flow from the pre-treatment baseline, higher than the 0.08 g day<sup>-1</sup> increase as observed in the control treatment. This increase was temporary with no other differences detected in the six weeks of sampling following the dormant season prescribed burn treatment, and no differences in total monoterpene concentration. In the growing season prescribed burn treatment, there was no discernible effect on resin flow or total monoterpene concentration. Interestingly, longleaf pine trees in stands that had been fire-excluded for 17 years had higher resin flow than stands managed with frequent fire, but we found no detectable difference in total monoterpene concentration among the three fire regime treatments. However, we found 1.49-3.57higher total monoterpene concentrations in xeric than mesic sites indicating important site-level differences in resin properties.

#### 4.2 Management recommendations

Although our studies were limited to a small portion of the range of the longleaf pine ecosystems, these novel investigations can provide some recommendations for

managers working to restore longleaf pine stands. Drier site types have often been interpreted as at higher risk for bark beetles due to predisposing factors resulting from lower soil water availability which limits carbon allocation to both growth and defense and may increase concentration of beetle-attracting volatiles (Lorio 1986, Mattson and Haack 1987, Gaylord et al. 2013, Kelsey et al. 2014, Anderegg et al. 2015, Kolb et al. 2019, Gonzalez et al. 2023). In our study of longleaf pine stands at a drier site, we found lower trap catches of *Ips* spp. in stands treated with frequent fire and in those where prescribed fire was reintroduced compared to fire-excluded stands, indicating that treatment with prescribed fire may reduce activity and perhaps, infestation by bark beetles. We also found that the concentration of monoterpenes in the resin of longleaf pines at a drier site was much higher than at mesic sites, making these trees potentially more attractive to bark beetles. Thus, our results especially support reducing the activity of *Ips* bark beetles in longleaf pine stands at xeric sites by using frequent prescribed fire.

In addition, our results support the broadly accepted management practices of continued application of frequent low-severity, low-intensity prescribed fire in longleaf pine stands. The lack of a significant sustained response in tree defenses to prescribed burns in the dormant and growing seasons indicates that this level of disturbance does not negatively impact trees' abilities to defend against bark beetles. The use of frequent prescribed fire maintains a lower basal area of understory trees, and our findings indicate that is correlated with lower trap catches of *I. calligraphus*.

#### **4.3 Future directions for research**

My first recommendation is that studies assess effects of treatments and site conditions on bark beetle catches at the species-level. Many researchers and managers often describe *Ips* spp. generally and their activities in a stand are often lumped together. However, our examination of species-level trap catches in relation to fire regime treatments and site/stand conditions revealed distinct and meaningful ecological differences among the *Ips* spp. that co-occur in the Coastal Plain. A common understanding is that the *Ips* spp. co-occur on colonized trees, partitioning among parts of the tree from branches to bole from smallest to largest body size (Nebeker 2011), but our results indicate that the different *Ips* spp. are correlated with differing stand characteristics. This suggests that it may be prudent for management for *Ips* bark beetles to vary as based on the most abundant species prevalent in infested pine stands.

Future examinations of bark beetles and tree defenses in longleaf pine ecosystems may take into account the site type, specifically the soil moisture availability that contributes to and interacts with forest health responses to prescribed fire. Factors that we were unable to explore in this dissertation research, such as additional restoration treatments like mechanical thinning and herbicides, may also affect bark beetle activity and tree defenses. These treatments are often recommended as ways to reduce fuels prior to reintroducing prescribed fire in longleaf pine stands that have been fire-excluded for extended periods of time. Studies in other pine forest types have shown effects from these treatments that would be important for managers to consider, especially increased mechanical injury leading to tree mortality, logging slash attracting bark beetles, and/or reduced tree defenses (Zausen et al. 2005, Fettig et al. 2007, Campbell et al. 2008). Additional investigation into the resin chemistry of longleaf pine trees sampled in this dissertation could reveal additional insights into key components of defense or host attractiveness to bark beetles. While examinations of the effects of wildfire or prescribed burning on terpenoid compounds are quite limited, other physiological stressors such as drought, insects, and pathogens have been shown to affect the chemical makeup of resin (Werner 1972, Hodges and Lorio 1975, Raffa 2014, Keefover-Ring et al. 2016, Kolb et al. 2019). Individual compounds and their relative proportion have been shown to be important in host attraction, successful beetle entry, and defense against beetles and pathogens (Erbilgin et al. 2003, Boone et al. 2011, Hofstetter et al. 2012, Gitau et al. 2013). Hence, detailed data on the relative chemical constituents of resin (primarily terpenes) will be helpful in understanding if resin chemical properties may vary under various fire regimes or site conditions.

To build on and broaden the inference of this work investigating fire regime treatment effects on bark beetles and tree defenses, additional studies with similar treatments may be undertaken at longleaf pine forests across the southeastern U.S. A broad network of sites would introduce a number of other variables that may be relevant to beetle populations and tree defenses, including climate, soils, site management history, and disturbance history. While this may make interpretation of results challenging, standardized measurement of stand and site characteristics across numerous locales could alleviate the need for shared management history and allow for detection of trends at the regional scale. Understanding the effects of a broad range of characteristics may help managers develop prescriptions for attaining the most resilient stands of longleaf pine especially under climate change.

## **4.4 References**

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Compound	CAS Registry Number	Source	Purity
(+) <b><i>a</i></b> -pinene	7785-26-4	Aldrich, Darmstadt, Germany	
(-) camphene	5794-04-7	Aldrich, Darmstadt, Germany	80%
(-) <b>β</b> -pinene	18172-67-3	Aldrich, Darmstadt, Germany	99%
myrcene	123-35-3	Aldrich, Darmstadt, Germany	95%
<b>α</b> -terpinene	99-86-5	Fluka, Seelze, Germany	97%
(+) limonene	5989-54-8	Aldrich, Darmstadt, Germany	97%
p-cymene	99-87-6	Aldrich, Darmstadt, Germany	99%
terpinolene	586-62-9	Aldrich, Darmstadt, Germany	90%
camphor	464-49-3	Aldrich, Darmstadt, Germany	98%
linalool	78-70-6	Aldrich, Darmstadt, Germany	97%
(-) bornyl acetate	5655-61-8	Aldrich, Darmstadt, Germany	95%
$\alpha$ -terpineol	10482-56-1	Fluka, Seelze, Germany	98.5%
borneol	464-45-9	Aldrich, Darmstadt, Germany	98%

Appendix A. Monoterpene compounds and standards used for identification and quantitation.



# Appendix B. Histograms of response variables for resin defense studies (Chapter 3).