PHENOTYPIC VARIATION WITHIN SOUTHERN PINE BEETLE, AND EFFECTS OF PRESCRIBED FIRE ON TREE MORTALITY DURING AN *IPS* BARK BEETLE OUTBREAK

by

BAILEY HOLLAND MCNICHOL

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

ABSTRACT

The southern pine beetle (*Dendroctonus frontalis* Zimmerman) (SPB) and the southern pine engravers, *Ips avulsus* (Eichhoff), *I. calligraphus* (Germar), and *I. grandicollis* (Eichhoff), colonize southern pines and can cause widespread mortality and economic losses when at high population levels. We examined variation in SPB phenotype (body size and hindwing shape), as well as differences in sex-ratios of beetles collected in 2016 and 2017 in the southeastern U.S. Our results showed no differences in SPB size measurements between outbreak and non-outbreak phases, however traps placed in non-outbreak areas caught ~2 times higher proportions of females. We also monitored southern *Ips* infestations between burned versus unburned sites to determine the effects of prescribed fire on loblolly pine (*Pinus taeda* L.) mortality. Overall, we observed 3.6 times higher pine mortality on unburned sites and a higher probability of tree survival on burned sites. At the site-level, models including treatment and survey month best predicted mortality; at the tree-level, a model including treatment, crown mortality level (1-5), *Ips* activity level (none, low, medium, high), and DBH best predicted mortality. Our findings suggest: 1) including the captured proportion of females in SPB outbreak prediction models may allow for more accurate forecasting, and 2) using prescribed fire during active southern *Ips* infestations may increase the resilience of pine forests in the southeastern U.S.

INDEX WORDS: Bark beetles, Forest pests, Forest management, Phenotypic plasticity, Prescription fire, Sex ratio, Sexual dimorphism, Southeastern U.S.

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DEDICATION

For my parents, Tom and Lysa, who have encouraged me to do what makes me happy throughout my life, and have kept me levelheaded with wise words, healthy snacks, and emotional support throughout my degree. Thank you for believing in me and always reminding me that I am loved.

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THESIS INTRODUCTION AND LITERATURE REVIEW

1.1 Economic and Ecological Importance of Pine-Dominant Forests in the Southeastern United States

The southeastern pine forest region extends from Virginia to Texas and is comprised of two major sub-regions: the Coastal Plain and the Piedmont (from northern Georgia to Virginia) (Prestemon and Abt 2002, Schowalter 2012). The Coastal Plain has a flat topography of lowland forests containing poorly drained soils, and historically was comprised of savannah and longleaf pine (*P. palustris* Mill.), shortleaf pine (*P. echinata* Mill.), and oaks (*Quercus* spp.) in the midstory (Varner et al. 2005, Schowalter 2012). The Piedmont lies above the fall line (the location of the prehistoric coastline), consists of hilly upland forests with well-drained soils, and historically featured a mix of loblolly pine (*P. taeda* L.) and hardwood species including oaks,, sweetgum (*Liquidambar styraciflua* L.), and American elm (*Ulmus Americana* L.). Historically, in old-growth forests, loblolly pine was a secondary component of dry longleaf pine forests, and was a prominent species within the upper coastal plain on deeper, moist soils (Wahlenberg 1960). Southern yellow pines comprise most of the economically important conifer species growing in similar plant communities from Virginia to Texas, and are characterized by their strong, durable, high-density wood (USDA Forest Service 1936).

During the 1800s, the southeastern U.S. experienced significant deforestation as a result of land development and colonization, agricultural practices, and intensive logging (Schultz

1997). These practices, along with subsequent fire suppression and planting of loblolly pine at the beginning of the 1900s, resulted in a large-scale transition from longleaf pine-dominant ecosystems to predominantly loblolly pine (Schultz 1997). Conversion of previously harvested forest area and eroded agricultural land to productive timberland was facilitated by improvements in tree genetics and extensive research on applied silvicultural techniques, including nursery management, site preparation and fertilization, and control of weeds using herbicide treatments (Prestemon and Abt 2002, Fox et al. 2007). From the 1950s to the late 1990s, the number of hectares of southern pine plantations increased from <809,000 ha to ~13 million ha, and today, southern pine plantations are some of the most intensively managed forested ecosystems worldwide (Schultz 1997, Fox et al. 2007).

The fast growth rates and short rotation periods of managed pines in the southeastern U.S. (relative to western U.S. timber species) provide substantial economic returns, despite the higher costs associated with intensive management practices (Prestemon and Abt 2002, Fox et al. 2007). Loblolly pine is the most economically valuable species in the Southeast due to its ability to grow on diverse and poor-quality sites, its rapid regeneration, high seed production, substantial yields per hectare, and its use in developing numerous marketable products (Schultz 1997, Fox et al. 2007). Timber is the most important commodity produced by southern pine forests, and the southern region currently accounts for ~60% of all timber products in the U.S., as well as more timber than any other country worldwide (Wahlenberg 1960, Prestemon and Abt 2002). The primary economically important wood products provided by southern pines are lumber, plywood, and paper products (e.g., packaging materials, particleboard, and paperboard) (Prestemon and Abt 2002, Schowalter 2012). Production forestry operations in the southeastern U.S. provide many direct and indirect employment opportunities, and significantly contribute to the growth of state economies (e.g., $35 billion overall economic impact in Georgia alone for 2015) (Georgia Forestry Association 2018).

Southern pine forests also provide numerous ecological benefits, including storing and supplying water resources and maintaining watersheds; preventing and reducing erosion; providing critical food resources and habitat for wildlife; allowing for various recreation opportunities; and sequestering carbon (Wahlenberg 1960, Schultz 1997, Schowalter 2012). Specifically, the rapid growth of loblolly pines gives them an important ecological role in forests, renewing soils damaged during logging operations and minimizing surface erosion (Schultz 1997). Silvicultural treatments that allow for the creation of gaps in the canopy help to encourage regeneration of important mast species for wildlife such as oak seedlings (Wahlenberg 1960). Loblolly pine seeds can also supplement food resources for species of songbirds and small mammals. Particularly when the maintenance of a hardwood midstory component is emphasized, loblolly pine forests feature nesting sites and shelter for many wildlife species, such as white-tailed deer [*Odocoileus virginianus* (Zimmermann)], gray squirrels (*Sciurus carolinensis* Gmelin), eastern cottontail rabbits [*Sylvilagus floridanus* (Allen)], wild turkeys [*Meleagris gallopavo* (L.)], bobwhite quail [*Colinus virginianus* (L.)], mourning doves [*Zenaida macroura* (L.)], woodpeckers (*Picoides* spp.), and numerous songbirds (Wahlenberg 1960, Schultz 1997, Tchakerian and Coulson 2011). Loblolly pine ecosystems also provide habitat for endangered and threatened wildlife species, including the red-cockaded woodpecker (*Picoides borealis* Vieillot) and gopher tortoise (*Gopherus polyphemus* Daudin) (Schultz 1997). The diversity of flora and fauna and the natural beauty of southern pine forests also promote a variety of recreational activities, including hiking, backpacking, angling, hunting, and education/outreach opportunities (Wahlenberg 1960, Coulson and Meeker 2011).

One of the most important management tools in promoting and maintaining the ecological diversity of loblolly pine ecosystems is prescribed burning, which is the informed application of fire to forest fuels for an area of land under particular weather conditions to achieve clear, predetermined objectives (Wade and Lunsford 1989). Prescribed fire is an effective tool for numerous management objectives, for example reducing ground fuels or clearing an area of logging debris (Wallin et al. 2003, Fettig and McKelvey 2014); managing wildlife habitat and understory vegetation (Wade and Lunsford 1989, Cain 1993); increasing aesthetics and accessibility (Wade and Lunsford 1989); managing endangered species and nutrient cycling (Wahlenberg 1960); and minimizing the risk of wildfire and subsequent bark beetle attacks (Wilkinson and Foltz 1982, Lombardero et al. 2006, Fettig and McKelvey 2014).

1.2 The Southern Pine Bark Beetle Guild

The southern pine bark beetle guild is a group of five sympatric species (Coleoptera: Curculionidae: Scolytinae) that co-colonize the phloem of yellow pines in the southeastern U.S. (Connor and Wilkinson 1983, Nebeker 2011). The bark beetles included within this guild are the southern pine beetle, *Dendroctonus frontalis* Zimmermann; the black turpentine beetle, *D. terebrans* (Olivier); the six-spined ips, *Ips calligraphus* (Germar); the eastern five-spined ips, *I. grandicollis* (Eichhoff); and the small southern pine engraver, *I. avulsus* (Eichhoff) (Stephen 2011a). The most susceptible host trees are loblolly (*Pinus taeda* L.) and shortleaf (*P. echinata* Mill.) pines, but members of the guild are known to infest at least 16 species including eastern white pine (*P. strobus* L.), slash pine (*P. elliottii* Engelm.), pitch pine (*P. rigida* Mill), longleaf pine (*P. palustris* Mill.), and Virginia pine (*P. virginiana* Mill.) (Connor and Wilkinson 1983, Coyle et al. 2016). This thesis assesses the role of some biotic and abiotic factors in facilitating successful colonization, reproduction, and initiation of infestations by four members of the southern pine bark beetle guild (*D. frontalis*, *I. avulsus*, *I. calligraphus*, and *I. grandicollis*).

Dendroctonus frontalis are light to dark brown as adults, about 2-3 mm in length, and have rounded elytra (Nebeker 2011). They are typically the primary colonizer of hosts, and tend to initiate attacks on the tree at around three meters and then move into the upper and lower bole (Hain et al. 2011, Stephen 2011b). *Ips calligraphus* are brown to black in color and identified by the six spines present along the margins of their elytra, which appear hollowed-out (Nebeker 2011, Stephen 2011a). This species is the largest of the southern *Ips*, around 4-6 mm in length, and preferentially colonizes fallen debris, the lower bole, or large branches of weakened host trees (Nebeker 2011, Eickwort et al. 2015). *Ips grandicollis* are also brown to black and recognizable by the five spines along their hollowed-out elytral margins (Nebeker 2011, Stephen 2011a). They range from 3-4.5 mm long and will colonize larger limbs, the upper and mid-bole, and recently felled pines (Stephen 2011a, Eickwort et al. 2015). *Ips avulsus* are the smallest of the three *Ips* species, averaging 2-3 mm long, and have four spines along their scooped-out elytral margins (Connor and Wilkinson 1983, Nebeker 2011). They transition from light brown to dark brown or black as the adults mature, but their elytra remain lighter in color than the thorax (Nebeker 2011). *Ips avulsus* typically colonize the upper bole and branches of the host tree, as their small body size allows them to utilize the thinner phloem resource and potentially reduce competition with other bark beetles (Gouger et al. 1975, Bryant et al. 2006, Nebeker 2011). There is partitioning of the woody portions of host pines among the species for example, *I. avulsus* and *I. grandicollis* both tend to target attacks on larger branches to reduce competition with *D. frontalis* and *D. terebrans*, but guild intermixing frequently occurs along the bole

(Birgersson et al. 2012), and spatial colonization patterns change depending on which *Ips* species are present (Ayres et al. 2001).

Species in the *Dendroctonus* genus are monogamous and females are the "pioneer" sex; they search for a suitable host tree and upon landing, release a pheromone that attracts both sexes (Nebeker 2011, Sullivan 2011). Mating occurs under the bark and female *D. frontalis* create sinuous S-shaped egg galleries, a pattern which allows for an increased beetle density when population levels are high (Hain et al. 2011, Stephen 2011a). Females deposit eggs in niches on both sides of the gallery along with spores of one of two species of mycangial fungi (*Entomocorticium* sp. A or *Ceratocystiopsis ranaculosus*) that provide nutrition to the developing brood, and produce up to nine generations per year when populations are high (Hain et al. 2011). When the eggs hatch, *D. frontalis* larvae tunnel short galleries extending from the egg niches and terminating in ovoid feeding cells (Stephen 2011a). The beetles undergo four larval instars, the last being completed in the outer bark where pupation occurs (Stephen 2011a). Pines attacked by *D. frontalis* are characterized by white to reddish pitch tubes in crevices of the bark, round "shotgun" exit holes, and reddish-white boring dust visible on the bark while beetles are active (i.e., before it is removed by wind or rain) (Clarke and Nowak 2009).

Bark beetle species in the *Ips* genus have a different reproductive strategy and life history. The three southern *Ips* species are polygamous, and gallery systems typically contain a single male and multiple females (Nebeker 2011). Male *Ips* beetles are the pioneer sex, arriving first to the host, excavating the nuptial chamber, and releasing pheromones to attract female conspecifics (Nebeker 2011). One to four females join each male, reproduction occurs, and the females construct egg galleries stemming from the central chamber that follow the grain of the wood, resulting in vertical Y- or H-shaped galleries (depending on the number of females)

(Stephen 2011a, Coyle et al. 2016). Oviposition occurs in egg niches, and the egg galleries are generally free of frass so that both sexes can move throughout the phloem for mating purposes (Nebeker 2011). *Ips calligraphus* and *I. grandicollis* can have up eight generations per year, and *I. avulsus* can produce up to ten generations in a year, but the exact number depends on temperature and location (Dixon 1984, Wagner et al. 1988a, Ayres et al. 2001). *Ips* infested pines are characterized by reddish-brown boring dust in the bark crevices, yellow to white dime-sized pitch tubes (which do not occur on severely weakened trees and slash), and shotgun entrance/exit holes (Wilkinson and Foltz 1982, Connor and Wilkinson 1983). The size of the pitch tubes and gallery entrances generally correlate with the adult *Ips* size, but the galleries within the phloem must be examined to determine which of the three species are active in an infested host tree.

Dendroctonus frontalis are generally the first member of the southern pine bark beetle guild to colonize host trees, as they are capable of initiating "mass attacks" on live host trees by producing the aggregation pheromone component frontalin and attracting numerous conspecifics (Sullivan 2011, 2016). Females also produce *trans*-verbenol through the oxidation of α-pinene, and both this compound and host volatile compounds act as synergists in the presence of frontalin, attracting more *D. frontalis* to suitable hosts (Sullivan 2011). Male *D. frontalis* produce *endo*-brevicomin, which is an attractive synergist for frontalin at low concentrations but can inhibit attraction near its point of release at high concentrations (Sullivan et al. 2011). *endo*-Brevicomin can reduce attraction to trees and portions that are heavily colonized while enhancing attraction to adjacent trees, and thereby likely plays a role in stimulating "switching" of attack focus to adjacent trees. Males also produce verbenone, which may function as an anti-aggregation pheromone and play a role in short-range interactions of males on the bark surface

(Sullivan 2011). No host attractants have been identified that are attractive to *D. frontalis* in the absence of pheromone components.

Male *Ips* beetles are the only sex that can produce aggregation pheromones to attract conspecifics to a host tree (Nebeker 2011, Stephen 2011a). *Ips* generally use monoterpenoid compounds for communication, which also are an important component of the chemical defense systems of attacked pines (Seybold et al. 2000). *Ips calligraphus* release the attractant pheromones ipsdienol and *cis*-verbenol (Birgersson et al. 2012), while the principal pheromone component for *I. grandicollis* is ipsenol, which is synergized by *cis*-verbenol (Ayres et al. 2001, Birgersson et al. 2012). *Ips avulsus* release the synergistic aggregation pheromone components lanierone and ipsdienol (Hedden et al. 1976, Birgersson et al. 2012).

As the members of the southern pine bark beetle guild tend to co-colonize host trees, cross-attraction, synergism, and inhibition among each species' semiochemicals are important in mediating infestation dynamics. The two *Dendroctonus* species may be cross-attractive since they are attracted to the combination of host odors and frontalin, which are released by attacks of both species (Delorme and Payne 1990, Sullivan 2011). *Ips avulsus*, *I. grandicollis*, and *I. calligraphus* are known to be attracted to several pheromone components of sympatric bark beetle species (Payne et al. 1991, Ayres et al. 2001, Miller et al. 2005). Although one study found no cross-attraction of *I. avulsus* to ipsenol produced by *I. grandicollis* (Birgersson et al. 2012), *I. avulsus* responded in significantly higher numbers to various combinations of ipsdienol, ipsenol, and lanierone than any one compound alone (Hedden et al. 1976, Payne et al. 1991, Miller et al. 2005). Ipsenol is likely also a synergist for *I. calligraphus*, as its presence has been shown to significantly increase trap catches of this species (Birgersson et al. 2012), particularly in conjunction with *cis*-verbenol and *trans*-verbenol (Payne et al. 1991). Although *I. avulsus* is not readily inhibited by any of the sympatric *Ips* semiochemicals, its pheromone component lanierone inhibits the activity of *I. calligraphus* and *I. grandicollis* (Birgersson et al. 2012).

Other than a weak attraction of *I. grandicollis* to frontalin (Aukema and Raffa 2005), there is no other documented cross-attraction between *D. frontalis* and the three *Ips* species in the guild (Payne et al. 1991, Staeben 2014, Sullivan 2016). Male attacks by *I. grandicollis* were found to reduce male *D. frontalis* arrival on bolts infested with *D. frontalis* females (Sullivan 2011), but overall there is limited cross-inhibition between the three southern *Ips* and *D. frontalis* (Payne and Richerson 1985, Sullivan 2016). During *D. frontalis* mass attacks, *I. calligraphus* tend to become most abundant in the host tree three weeks after initiation of the attack (Stephen 2011a). *Ips grandicollis* exhibit a gradually increasing attack density for ~18 days following the initiation of *D. frontalis* mass attack, at which point the number of attacking adults peaks and is maintained for up to 30-50 days (Nebeker 2011, Stephen 2011b, Birgersson et al. 2012). The lack of a concentrated attack pattern for this species may enable its success, as *I. grandicollis* normally attack extremely stressed trees (Birgersson et al. 2012), which generally have a small percentage of available phloem resource not already colonized by more aggressive bark beetles or fungi. Compared to the other two southern *Ips*, *I. avulsus* exhibit concentrated colonization periods, with peak arrival within ~12 days of mass attack initiation (Wagner et al. 1988a, Nebeker 2011, Stephen 2011a). This species often undergoes a shorter and more synchronous reemergence period compared to the other two *Ips* species (Wagner et al. 1988b, Stephen 2011a).

1.3 Biotic and Abiotic Factors Associated with Infestations by the Southern Pine Bark Beetle Guild

Population outbreaks can be described as rapid shifts in population density for a group of organisms, often over several orders of magnitude, that can have significant economic, ecological, and health consequences (Sharov 1997). A relatively small number of species undergo outbreaks (e.g., pathogens and rodents), but several important insect pests including bark beetles in the *Dendroctonus* genus experience significant fluctuations in population levels. In the southeastern U.S., periodic regional outbreaks of *D. frontalis* have caused widespread pine mortality and triggered more than a billion dollars in economic losses over the past three decades (Clarke and Nowak 2009, Hain et al. 2011, Pye et al. 2011). The three southern *Ips* species can also initiate sizeable infestations, targeting pines that are stressed, damaged, or dying (Wilkinson and Foltz 1982, Connor and Wilkinson 1983, Eickwort et al. 2015). While *D. frontalis* infestations are characterized by expanding groups of contiguous trees, or "spots", *Ips* infestations most often appear as patches of trees with red "flagging" of needles interspersed with healthy trees (Stone et al. 2007, Clarke 2012, Eickwort et al. 2015). Under normal conditions southern *Ips* activity is isolated to one or a few of the weakest trees, but factors that cause trees physiological stress have been shown to render pines susceptible to larger infestations with economic consequences (Wilkinson and Foltz 1982, Connor and Wilkinson 1983, Bryant et al. 2006). However, the economic impacts of pine mortality driven by southern *Ips* infestations have rarely been quantified because mortality is typically less concentrated (more dispersed) within forests, and is often associated with other environmental stressors, making assessment of tree death caused by *Ips* activity more difficult.

Previously, the onset of *D. frontalis* outbreaks has been attributed to either biotic, density-dependent variables such as low population levels of natural enemies, or abiotic, density-independent factors such as favorable climatic conditions (Friedenberg et al. 2008, Birt 2011a). Abiotic factors that are thought to predispose stands to outbreaks of *D. frontalis* or the southern *Ips* species include drought, fire, lightning, wind events, ice storms, management activities that cause mechanical damage to standing pines, offsite planting, and poor soils (Hain et al. 2011, Nebeker 2011, Nowak et al. 2015). Warmer temperatures increase the reproduction, development, and survival ability of all four species, dictating the number of generations per year (Yearian and Wilkinson 1967, Haack et al. 1987, Wagner et al. 1988a, Birt 2011b). Weather conditions (drought, heat, etc.) have been shown to directly affect *D. frontalis* flight thresholds and survival during dispersal as well as host vulnerability to bark beetle infestation, and intense droughts have historically been associated with large-scale southern *Ips* outbreaks (e.g., severe drought in South Georgia in 1954) (Wilkinson and Foltz 1982, Birt 2011b, Coyle et al. 2016).

While thinning operations that reduce stand basal area can help prevent *D. frontalis* outbreaks, *I. grandicollis* is attracted to volatile compounds released by pines with mechanical damage from such treatments, and all three southern *Ips* species are known to breed and build their population levels in residual piles of slash and cut logs (Wilkinson and Foltz 1982, Eickwort et al. 2015, Nowak et al. 2015). Some of the more recent major *D. frontalis* outbreaks have occurred in pine stands with less intensive management, no genetically improved stock, overstocking, and longer rotation periods (older trees) (Nowak et al. 2015, Asaro et al. 2017). However, several authors have concluded the role of abiotic factors in driving changes in *D. frontalis* population phases remains unclear (Turchin et al. 1991, Birt 2011a), indicating density-dependent variables may play a greater role in driving population dynamics.

Predisposing biotic agents that may assist *D. frontalis* and the southern *Ips* species in initiating infestations include Heterobasidion root rot [*Heterobasidion irregularae* (Fr.) Bref], littleleaf disease (*Phytophthora cinnamomi* Rands), and other forest pathogens (Hain et al. 2011, Nebeker 2011). Population levels of insect predators (e.g., the clerid beetle, *Thanasimus dubius* Fabricius) and parasitoids (e.g., several species of braconid and chalcidoid wasps) that help to control the abundance of members of the southern pine bark beetle guild are also important biotic agents in determining the infestation ability of *D. frontalis* and the three *Ips* beetles (Berisford 2011, Reeve 2011). Vertebrate predators, in particular the downy woodpecker [*Picoides pubescens* (*L.*)], hairy woodpecker [*Picoides villosus* (*L.*)], and pileated woodpecker [*Hylatomus pileatus* (*L.*)], may have an impact on bark beetle populations (Stephen 2011b). Several studies have proposed that delayed density-dependent shifts in *D. frontalis* populations are primarily attributed to predation by clerid beetles (Turchin et al. 1991, Hain et al. 2011). However, while predators undoubtedly play a primary role in regulating populations of *D. frontalis* and the southern *Ips*, numerous interacting biotic/abiotic agents are likely to play an important role in enabling populations to increase to outbreak levels as well.

1.4 Thesis Objectives

As our climate changes and temperatures, precipitation patterns, and storm events become less predictable and more extreme, factors that cause physiological stress to pine trees over large geographic scales are becoming more common in the southern and eastern United States. Accordingly, it is critical to continue improving upon our understanding of bark beetle population dynamics and elucidating drivers of their life cycles and outbreaking potential. This thesis aims to explore some of biotic and abiotic factors related to the ability of *D. frontalis*, *I.*

avulsus, *I. calligraphus*, and *I. grandicollis* to successfully colonize, reproduce, and initiate infestations in southern pine trees.

The second chapter examines phenotypic variability across *D. frontalis* populations collected from six states across the southeastern U.S. Our objectives were to: 1) quantify variation in metrics of body size (elytral, hind femur, and hindwing lengths) and hindwing shape between non-outbreak and outbreak *D. frontalis* population phases; 2) determine if similar variation is apparent between male and female *D. frontalis*; and 3) assess differences in the captured proportion of female *D. frontalis* between non-outbreak and outbreak population phases. We hypothesized that: 1) *D. frontalis* collected from outbreak-phase populations will be larger in size (larger body size, and wider and longer hindwings), as related to increased overall fitness and flight ability; 2) we will observe sexual size dimorphism, with female *D. frontalis* being larger than males (following Foelker and Hofstetter 2014); and 3) there will be a higher proportion of females in non-outbreak phase traps (as more females are actively searching for suitable hosts), and a lower proportion of females in outbreak phase traps (indicating males are actively locating on female pheromones).

Dendroctonus frontalis outbreaks and factors potentially regulating fluctuations in population levels have been thoroughly investigated in the ecological literature. However, there is a notable lack of studies addressing phenotypic (i.e., morphological, developmental, or behavioral) variation corresponding to shifts in population densities for *D. frontalis*, and very few for bark beetles generally. While researchers have anecdotally observed distinctions, there is no published literature directly comparing *D. frontalis* adults collected from non-outbreak and outbreak populations to determine if and how traits differ between the two population phases. If variation in *D. frontalis* adult size, hindwing morphology, and/or trap-level proportions of females is found that coincides with population phase, these data may improve the accuracy of current outbreak risk prediction models to inform preventative forest management and reduce catastrophic losses of southern pines.

In the third chapter, we surveyed active southern *Ips* (*I. avulsus, I. calligraphus*, and *I. grandicollis*) infestations on sites treated with prescribed fire and those that remained unburned, and assessed the condition of loblolly pines within the sites over an eight-month period. Our objectives were to: 1) determine the influence of low-intensity prescribed fire on southern *Ips* activity and loblolly pine mortality; and 2) evaluate which variables provide the best overall predictions of loblolly pine mortality at the tree-level and site-level. We hypothesized that: 1) we will observe higher loblolly pine mortality on burned as compared to unburned sites as a result of short-term increases in host tree stress post-burn; 2) at the site-level, treatment (burned or unburned), basal area, and time since burn will be significant predictors of mortality; 3) at the tree-level, we will find significant interactions between treatment, *Ips* activity level, and crown mortality level, and these variables will be predictors of mortality; and 4) there will be an interaction between tree DBH and the level of *Ips* activity, with increased *Ips* activity occurring in smaller DBH pines. As few large-scale *Ips* infestations have been reported in the southeastern U.S., there is no literature addressing the effects of prescribed fire on active southern *Ips* infestations in loblolly pine stands. Chapter three will contribute quantitative data on some of the variables associated with loblolly pine mortality during such infestations, and provide recommendations on the use of low-intensity prescribed fire as a management tool in stands experiencing ongoing southern *Ips* activity.

1.5 References

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CHAPTER 2

PHENOTYPIC DIFFERENCES BETWEEN OUTBREAK AND NON-OUTBREAK PHASES OF *DENDROCTONUS FRONTALIS* POPULATIONS IN THE SOUTHEASTERN UNITED STATES[1]

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[1] McNichol, B.H., B.T. Sullivan, C.R. Montes, H.L. Munro, J.T. Nowak, C. Villari, and K.J.K. Gandhi. To be submitted to *Ecological Applications*.

Abstract

The southern pine beetle (*Dendroctonus frontalis* Zimmerman) (SPB) can cause widespread tree mortality and significant economic losses in pine stands when at high population levels. SPB undergoes large fluctuations in population density with distinct outbreak and non-outbreak phases. Phenotypic plasticity between population phases can allow organisms to alter traits affecting their survival and reproduction in response to environmental conditions, and thus may have predictive value for outbreaks. Our research objective was to investigate phenotypic variation (elytral, hind femur, and hindwing lengths, and hindwing morphology) and the sex-ratios of SPB populations collected in 2016 and 2017 in the southeastern U.S. We used landmark-based geometric morphometrics to compare hindwing morphology between SPB populations in outbreak and non-outbreak phases. Results showed no differences in body size measurements that corresponded to SPB population phase in either year. However, we observed sexual size dimorphism, with female SPB having slightly longer elytra than males in both years, and females having longer and larger hindwings than male SPB in 2017. Interestingly, traps placed in non-outbreak areas caught approximately a two times higher proportion of females compared to traps in the outbreak phase both years. Our findings suggest that the inclusion of the captured proportion of females may enhance current outbreak prediction models for SPB, allowing for more effective monitoring of one of the most economically important insect pests in southeastern U.S. forests.

INDEX WORDS: Bark beetles, forest pests, geometric morphometrics, hindwing morphology, phenotypic plasticity, sex ratio, sexual dimorphism

2.1 Introduction

Density-dependent phenotypic plasticity, the capacity of one genotype to exhibit variable phenotypes in different environments, is essential in allowing certain pest insects to produce periodic outbreaks (e.g., locusts) (Uvarov 1966, Anstey et al. 2009, Whitman and Agrawal 2009). Plasticity in developing morphological characteristics is a strategy that has been shown to increase the fitness of organisms (e.g., fishes and amphibians) in uncertain environments (Leclaire and Brandl 1994). Phenotypic traits affecting the survival and reproduction of insects have been observed to change as a result of environmental conditions (Wallner 1987, Anstey et al. 2009). Plasticity of characteristics regulated by temperature such as growth rate and body size may allow for a rapid population size increase as the thermal environment shifts (Bentz et al. 2011), an important response for insects that tend to have outbreak phases. However, it is important to note that while some phenotypic plasticity is adaptive (e.g., a beetle directly responding to increased population density), plasticity in traits can also be incidental (e.g., reduced body size from lack of available nutrients) or potentially deleterious (e.g., a deformity resulting from a pollution source). Ultimately, observed plasticity in traits that is correlated with or indicates a shift in population phase is useful in enhancing forecasting and outbreak predictions for insect populations.

Traits including but not limited to hindwing size and morphology, insect body size and mass, and wing loading, or the ratio between body mass and wing area, may affect the dispersal ability of insects (Angelo and Slansky 1984, Kölliker-Ott et al. 2003, Tan et al. 2010, Bouget et al. 2015, Kalberer and Kölliker 2017). Reduction or increases in the size of physical characteristics are often representative of a trade-off between reproductive capacity and propensity for dispersal within an insect (Simmons and Thomas 2004, Homburg et al. 2013, Fraser et al. 2014). The nutritional quality of an insect’s environment may also affect development and influence adult size, with nutrient-rich habitats generally allowing for larger, fast-growing organisms and resource-poor environments typically limiting overall size and decreasing growth rate. Transient resources and ephemeral insect habitats have been hypothesized to select for faster development time and larger adult body size (Roff 1991). This may be especially true for bark beetles (Coleoptera: Curculionidae), which tend to persist on relatively rare and low quality host trees at low population levels, but on higher quality hosts at higher population densities, when they can overcome a tree’s defensive system (Stephen 2011, Clarke et al. 2016).

Southern pine beetle (SPB), *Dendroctonus frontalis* Zimmerman, is a phloem-feeding bark beetle that colonizes southern pines for reproduction and development, causing major economic damage (Hain et al. 2011, Stephen 2011). Dispersing pioneer SPB adults accomplish this by initially locating hosts that are susceptible due to weakened defenses (e.g., lightning-struck trees) (Clarke 2012). Once on a suitable host, the beetles produce aggregation pheromones to attract other SPB and increase attack densities. When they reach a critically high population density, they initiate a mass attack strategy and become capable of attacking and overcoming the defenses of more resilient nearby trees (Hain et al. 2011). SPB bore through the outer bark, copulate, and deposit their eggs in sinuous galleries in the living phloem, thus girdling the tree and killing it. This native pest has caused significant destruction of pine trees in the southeastern U.S, particularly loblolly pine [*Pinus taeda* (L.)] and shortleaf pine (*P. echinata* Mill.), causing over a billion dollars in economic losses over the past three decades (Clarke and Nowak 2009, Pye et al. 2011).

Regional SPB outbreaks have historically occurred at irregular intervals between 8-12 years (Pye et al. 2011), though more recent research suggests local population dynamics may not be cyclical (Weed et al. 2017). Recently, synchrony in population dynamics was observed over groups of multiple adjacent states, suggesting that regionally occurring fluctuations in SPB population density may exist (e.g., a coastal group including Alabama, Florida, Georgia, and South Carolina) (Reeve 2018). SPB outbreaks consist of localized infestations ("spots") that appear as a contiguous zone of dead, dying, and/or recently infested pines and range from a few stems to hundreds of acres. An area is considered to be in "outbreak" status when there is one spot (with at least 10 infested pines) per 1,000 acres of suitable host type per county (Clarke 2012, Asaro et al. 2017). Latent (non-outbreak) phase SPB can be difficult to detect in forests because they secondarily colonize weakened or dying trees and do not cause significant mortality. SPB outbreaks and factors potentially regulating fluctuations in population levels have been investigated in previous studies (Beal 1933, Turchin et al. 1991, Turchin et al. 1999, Friedenberg et al. 2008, Clarke and Nowak 2009, Reeve 2018). However, there is a notable lack of studies addressing phenotypic variation (i.e., morphology, development, physiology, and behavior) that may coincide with shifts in population densities for SPB, and very few for bark beetles in general.

SPB traits such as hindwing size and shape may fluctuate depending on the nutritional quality of the phloem and conspecific population density. If populations are high and SPB can attack and colonize vigorous hosts, an optimal nutritional environment (i.e., high-quality phloem and less competition with blue-stain fungi) could lead to increased adult body size and little need for dispersal, reducing selection pressure for large wing size and flight muscles (Brown et al. 2017). Alternatively, access to more nutritious host phloem could trigger adaptive increases in

SPB adult size as well as wing muscle size and dispersal ability (Tan et al. 2010). Larger adult insects that can consume more calories, store more fuel, and have a higher metabolic rate may have higher potential for dispersal over longer distances (Roff 1991). For SPB in this scenario, larvae developing in high-quality hosts would become more robust and potentially more fecund adults capable of long dispersal flights, possibly far beyond the pheromone plume of a mass attack. Two alternative hypotheses exist for selection of phenotypic traits at low population densities, when SPB are generally restricted to feeding in sub-optimal host phloem and experience competition with fungi and other bark beetles during development: 1) adults may have an overall smaller body size with increased capacity for dispersal, detectable via larger hindwings (and therefore decreased wing loading); or 2) both adult size and hindwing size could be reduced due to lack of adequate nutrition for growth and/or a trade-off of investment in reproductive efforts to increase population size.

Skewed sex ratios of attacking populations of insects also directly influence the effective population size (James et al. 2016). The sex ratio was reported to be ~1:1 when SPB emerge from host trees (Stephen 2011), though this conclusion is not well supported in the literature and may differ with population size. A slightly higher ratio of female pioneer beetles may occur initially, favoring a higher ratio of males later as they locate hosts via female pheromone attractants (Stephen 2011). Further, sex ratios of SPB caught in pheromone-baited traps may diverge strongly from 1:1. A male-biased sex ratio of SPB was found during mark-recapture experiments (63% male), but there was no difference in the dispersal distance of each sex following emergence from the host (Turchin and Thoeny 1993).

Risk models quantify the overall likelihood of damage in one location versus another and are typically based on historical data (Birt 2011a,b). A variety of statistical models have been developed to predict the likelihood of SPB infestation using characteristics such as soils, host tree age, and basal area in a particular forest. However, it has been argued that the environmental variables incorporated into these models cannot be accurately measured over large scales, are not always relevant to SPB damage, and that the models do not account for the beetle's dynamic life history and the nature of forested landscapes (Birt 2011a,b). An additional limitation of existing models for predicting SPB outbreaks is that most natural populations are not strictly periodic in their non-outbreak – outbreak phase dynamics (Dwyer et al. 2004, Weed et al. 2017). Ultimately, prediction models for SPB activity may be more accurate if they account for biological features of the beetle (Turchin et al. 1991).

A prediction model was developed that utilizes a network of pheromone-baited traps throughout the Southeast to capture and monitor SPB and its primary predator, the clerid beetle *Thanasimus dubius* (Fabricius) (Billings 1988). This trapping network is the basis for the United States Forest Service, Forest Health Protection (USFS-FHP) Annual Springtime SPB Trapping Survey. The system provides a useful method for forecasting SPB infestations the following summer, and whether population levels of SPB are changing (increasing, static, or declining) (Billings and Upton 2010). Trapping data are compiled to identify which regions have the propensity for SPB outbreaks during the months following the survey so that management efforts can be focused in the most vulnerable locations. The predictions are made using two metrics: the mean SPB count per trap per day, and this count divided by the number of clerid beetles plus total SPB per trap (Billings 1988). These two parameters form an index that can be plotted onto an SPB outbreak prediction chart to assess the overall regional risk of an outbreak (Billings 1988). Analyses of the effectiveness of this risk assessment tool showed that between 1999-2005, the mean accuracy of predicted SPB spots in the 12 surveyed states was 82% (but ranged from 71%-100%), and predictions of anticipated relative population levels (low, moderate, or high) were 74% correct on average (but ranged from 43%-100% by state) (Billings and Upton 2010).

Although the Billings (1988) predator-prey ratio model has been moderately accurate and useful in managing forests to suppress outbreaks, particularly in conjunction with aerial and ground SPB surveys, outbreaks that are not prevented or detected still result in significant losses of economically important southern pines. If biological traits that vary between outbreak and non-outbreak phase SPB populations are identified, measuring these attributes on beetles collected during the trapping survey may allow for a more robust assessment of the sum of effects of density-dependent factors on outbreak dynamics. Incorporating body size measurements of SPB and the captured proportion of females may augment the trap-catch-based forecasts and other predictive models by exploiting phenotypic indicators of population phase.

As linked to enhancing the prediction of population phases of SPB, our primary objectives were to: 1) quantify the phenotypic variation (body size and hindwing shape) between non-outbreak and outbreak population phases of SPB; 2) assess if such phenotypic variation is present between males and females; and 3) determine differences in the captured proportion of female SPB between the two population phases. We predict that SPB in outbreak phases will be larger in size (hindwing, elytral, and hind femur length) than those in non-outbreak phases. Additionally, we predict that there will be sexual dimorphism with female SPB larger in size than males (Foelker and Hofstetter 2014). Further, at the trap-level, there will be a higher proportion of females in non-outbreak (low population density) traps, and a lower proportion of females (stronger male bias) in outbreak (high population density) traps.

2.2. Methods

2.2.1 Field Trapping

For this study, we examined phenotypic variation in SPB collected by the United States Forest Service, Forest Health Protection (USFS-FHP) trapping survey. The survey uses 12-unit Lindgren multiple-funnel traps that include a collection cup at the bottom funnel that is partially filled with diluted antifreeze to retain and preserve captured insects (Lindgren 1983). Each trap is baited with devices releasing the SPB pheromone frontalin (two 400 µl polyethylene microcentrifuge tubes filled with liquid frontalin and passively releasing the compound through the walls), and a high rate of the pine odors alpha- and beta-pinene (70:30; from a sealed polyethylene bag), which is synergistic with frontalin (Billings and Upton 2010, Billings 2011). Starting in 2017, the survey lure additionally began to include an *endo*-brevicomin lure (dispersed from a 2.5 cm long flexlure and displaced 3-4 m from each trap) (Billings 2017). The *endo*-brevicomin component has been identified as having a synergistic effect on the other lure components, particularly when it is placed adjacent to the baited trap, and it substantially increases catches in endemic, low population density areas of SPB (Sullivan and Mori 2009, Sullivan et al. 2011).

The trap collection cups are positioned 1.5-2 m above ground height, and placed approximately >20 m away from susceptible pines to minimize the possibility of SPB attacks on live trees (Billings 2017). Recommended forested areas for trapping consist of sawtimber-aged pine stands either mixed with hardwoods or having a hardwood understory component (i.e., with relatively low pine basal area), and stands are surveyed prior to trap placement to ensure there are no active multi-tree SPB infestations within ~300 m (Billings 2017). The locations selected for traps are dispersed throughout USDA Forest Service districts for wide coverage, and individual traps (3-6 per district) are placed in approximately the same locations across years to enhance detection of inter-annual trends. Trapping is initiated at the start of blooming for eastern redbud (*Cercis canadensis* L.). Catch collections are made on an approximately weekly basis (to prevent decay of the captured insects), and the trapping is continued for 4-6 weeks, depending on the location (Billings 2017). The total number of SPB and their primary predator, the clerid beetle [*Thanasimus dubius* (F.)] is counted in each trap. SPB are identified by their elongated, cylindrical body shape, distinct frontal groove, and rounded elytral declivity (Hain et al. 2011). For our study, we were only interested in investigating phenotypic variation in SPB; no analyses were performed on *T. dubius*.

2.2.2 Selection of SPB Adults

Specimens collected from Alabama, Georgia, Mississippi, North Carolina, South Carolina, and Tennessee trapping surveys during 2016 and 2017 were used to assess phenotypic variation in SPB. Represented forest districts differed somewhat between years due to zero catches and the lack of available samples in some of the districts (Table 2.1). All SPB in each trap were counted and sexed to determine the total number and captured proportion of females (number of females divided by the number of males) at the trap-level. SPB were sexed by the presence of mycangia in females, which appears as a bulge on the anterior pronotum, and frontal tubercles on the heads of males (Wood 1982). Five beetles were randomly selected from each trap to broadly assess characteristics of specimens from across the southeastern range. Trap contents were emptied into a petri dish containing 95% ethanol and arranged in a line. Starting from the right end of the line, the first two males and two females identified under the microscope were selected, and the fifth beetle was chosen randomly from the petri dish. For traps with multiple weeks of catches, five beetles were selected in the same proportions over all weeks, and for traps without enough female SPB, additional males were sampled to ensure five SPB per trap. A total of 79,982 SPB specimens were available and sexed from the 2016 and 2017 trapping surveys. For our analyses, 152 male and 89 female SPB were sampled from the 2016 trapping data, and 146 male and 105 female SPB were sampled from the 2017 data.

2.2.3 Measurements of SPB size

Pronotum width has previously been used as a size measurement for *Dendroctonus* spp. (Armendáriz-Toledano et al. 2014a, Esch et al. 2016), but there is no literature demonstrating that this is a good proxy for overall body size, whereas in Coleoptera elytra length and hind femur length have been shown to correlate closely with overall body size (Knapp and Knappová 2013). Additionally, including measurements of multiple features from each individual can account for variability in proportions among individuals of the same species and provide a more robust assessment of overall body size (Knapp and Knappová 2013). Accordingly, a dissecting microscope with an ocular micrometer was used to measure the right-side elytra length from the anterior (elytral humerus) to the posterior end (elytral tip), and hind femur length from the proximal to the distal end, to the nearest 0.1 μm (Figure 2.1).

To facilitate hindwing removal, the beetles were held in place in a petri dish of distilled water with a pair of forceps. By using a second pair of sharp forceps, the right elytron was opened and removed, and the right hindwing was gently unfolded and smoothed. Once the hindwing was fully extended in the water, it was detached from the beetle's thorax with the forceps and placed on a glass microscope slide. Semi-permanent slide mounts (i.e., with the hindwing compressed under a coverslip) were made with 95% ethanol to highlight features (veins and wing margin). Micrographs were taken using a Leica S6D microscope camera, and the total hindwing lengths (mm) were measured using the Leica software's built-in measurement tool (Leica Microsystems Inc., Wetzlar, Germany).

2.2.4 SPB infestation data

Each summer, the USFS and state forestry commissions carry out aerial surveys to assess whether any SPB infestations have been initiated. If infestations are observed (identified by groups of pines with fading/red needles), the latitude and longitude are recorded to allow for ground surveys, monitoring of infestation growth, and preventative forest management. The spatial dataset is maintained by the USFS-FHP and is available from their Information Technology Specialist upon request. For our study, we used the latitudes/longitudes of identified spots to classify SPB sampled from the USFS-FHP survey traps as outbreak or non-outbreak phase, based on the proximity of a trap to the nearest spot.

2.2.5 Hindwing landmark and semi-landmark acquisition

Landmark-based geometric morphometrics (GM) is a technique that uses measured distances between positions of landmarks, or homologous morphological features that can be defined as a single point in space, to quantify variation in shape (Bookstein 1986, Webster and Sheets 2010, Zelditch et al. 2012). Removal of location, scale, and rotational effects from a digitized image with assigned landmarks (LMs) allows for the analysis of aspects of shape alone, which can provide insight on morphological differences between specimens (Webster and Sheets 2010, Zelditch et al. 2012). Landmark-based GM has previously been used on SPB to analyze spermatheca and seminal rods, and provided evidence of a cryptic species (Armendáriz-Toledano et al. 2014a). However, this technique has not been used to analyze variation in hindwing shape in any species of bark beetle (Curculionidae: Scolytinae).

When selecting an LM, the two most important considerations are the homology of the feature across all specimens and its biologically relevant geometric properties (i.e., the tip or maxima of the curve of a feature) (Bookstein 1986). In studies on hindwing morphology in Coleoptera, LMs have typically been chosen along the costal, radial, medial, and cubitus veins (Bai et al. 2011, Benítez et al. 2014, Su et al. 2015). In the case of SPB, there are several readily visible homologous LMs along the medial, cubitus, and radial veins of the hindwing that can be identified for analysis. Because discrete LMs do not offer information about the shape of the curve between them, additional points called semilandmarks (SLMs) can be utilized to analyze the curvature of a structure on a given specimen (Zelditch et al. 2012, Armendáriz-Toledano et al. 2014b). SLMs are assigned at evenly distributed increments along a curve or outline of a given feature, and can be incorporated into a landmark-based analysis to provide a more robust characterization of shape (Webster and Sheets 2010, Zelditch et al. 2012). Hindwings were chosen specifically for landmark-based GM aimed at identifying adaptive plasticity in SPB because 1) the membranous wings of insects are excellent features for GM analysis because of their two-dimensional shape and numerous suitable LMs, and 2) hindwing shape is related to flight and dispersal ability (Kölliker-Ott et al. 2003), and potentially adapted to conditions associated with differing population densities.

Eleven LMs on the SPB hindwing were chosen for GM analyses, including ten "type 1" landmarks (which occur at the discrete juxtaposition of features such as terminations or intersections of veins; LM1-9 and LM11), and one "type 2" landmark (which are geometric constructs including minima/maxima of curves, for example, the most distal point of the wing margin; LM10) (Zelditch et al. 2012). LMs were defined in homologous locations that could be readily and accurately identified on all specimens (Webster and Sheets 2010, Klingenberg 2011). As hindwing structures have not been labeled for SPB, terminology for hindwing features followed the nomenclature used in original illustrations of the anatomy of adult *Dendroctonus valens* Leconte (Figure 2.2a) (Hopkins 1909). Descriptions of the LM positions are as follows: LM1: intersection of the media and costa; LM2: intersection of the radius and median fold; LM3: lower median fold; LM4: proximal terminus of radius 2; LM5: proximal terminus of media 1; LM6: junction of the distal terminus of media and proximal terminus of media 2; LM7: distal terminus of cubitus 1; LM8: distal terminus of media 2; LM9: distal terminus of media 1; LM10: hindwing tip; and LM11: distal terminus of radius 2 (Figure 2.2a).

When there are a limited number of homologous points that can be assigned as LMs, semilandmarks (SLMs) can be arbitrarily placed at equally spaced increments along the outline of a specimen to capture additional shape information. The software MakeFan8 (Sheets 2014) allows users to assign SLMs by overlaying a grid of evenly distributed lines that intersect the hindwing margins on specimen photographs. For SPB hindwings, a “comb” was applied between LM1 and LM10, and 16 SLMs were assigned where the gridlines intersected the anterior and posterior margins of the wings, beginning at the base of the costa and ending near the cubitus 1 vein (Figure 2.2b) (Hopkins 1909, Sheets 2014).

2.2.6 Statistical Analyses

2.2.6.1 Classification of SPB population phase

The mean number of SPB caught per trap per day, the density of spots within a given radius of each trap, and the distance from a trap to the nearest SPB spot were all considered as potential metrics to classify traps as outbreak or non-outbreak phases. Distance from each survey trap to the nearest SPB spot identified the following summer was calculated using the 'sp' package in R (Bivand et al. 2013). Based on the range of these distances and the proximity of each trap to an infestation, traps were categorized as being in outbreak (i.e., higher SPB population levels) or non-outbreak (i.e., lower SPB population levels) phase. Additionally, the density of spots within a given radius of each trap was calculated, and a Pearson's correlation ('ggpubr' package in R) was used to assess whether there was a correlation between the distance from a trap to the nearest spot and the spot density (Kassambara 2018). Years of sampling were kept separate because of the use of different lures on traps.

2.2.6.2 SPB sex ratio

Trap-level proportions of female SPB (number of females divided by the number of males) were compared between outbreak versus non-outbreak status traps to assess if proportion of females differed by population phase. To account for the under-dispersion of the data both years (due to strong male biases for numerous traps), a generalized linear model (GLM) with a quasipoisson distribution was selected to assess this relationship (R Core Team 2016). To accommodate the binomial independent variable (trap status – outbreak or non-outbreak), dummy variables were coded to assign each level a numeric value (i.e., 1 for outbreak and 0 for non-outbreak trap) (Cohen et al. 2003). The mean number of SPB caught per trap per day (total SPB caught per trap/number of days that trap was operational) was also used as a surrogate for estimating population abundance, since absolute number of beetles in a pheromone-baited funnel trap is not equivalent to abundance. The proportion of female SPB data for both 2016 and 2017 were non-normally distributed due to a high proportion of strongly male-biased trap catches, violating the assumption of normality for linear regression. To account for the under-dispersion of the data, a GLM with a quasipoisson distribution was chosen to assess the relationship between proportions of females and mean number of SPB caught per day at the trap-level (R Core Team 2016). All traps that caught <1 SPB per day were excluded due to insufficient individuals for analysis (low trap catches result in a low level of confidence in calculating sex ratios).

2.2.6.3 SPB size measurements

Two extreme outliers that likely resulted from measurement error were removed from the 2017 samples before SPB body size analyses were completed. Pearson's correlations were run using the 'ggpubr' package to verify the size relationship between SPB elytra length, hind femur length, and hindwing length for both years of samples (Kassambara 2018). Prior to analyses, measurements were averaged at the trap level, thus the unit of replication for comparing size measurements was the trap.

Dummy coding was used for all regression analyses that included SPB sex or population phase (i.e., 1 and 0 were assigned for male/female and outbreak/non-outbreak, respectively) (Cohen et al. 2003). To account for the fact that SPB collected from a given trap may be genetically related (i.e., from the same brood), SPB elytral length, hind femur length, and hindwing lengths were averaged at the trap-level, and outbreak versus non-outbreak status traps were compared to determine if there were size differences between the two population phases. Additionally, regressions of the mean trap-level SPB elytra length, hind femur length, and hindwing lengths were conducted to determine if there were differences in size that corresponded to the mean number of SPB per trap per day. The mean body size measurements were normally distributed for both 2016 and 2017, thus simple linear regressions were appropriate for testing both of these relationships (R Core Team 2016).

To assess whether SPB sex was able to predict body size, regressions of elytra length, hind femur length, and hindwing length were conducted on sex, with each SPB adult as our unit of replication. Mean trap-level size measurements for males and females could not be taken for these analyses due to missing data (i.e., traps without females), thus analyses were run comparing individual SPB sizes. For the 2016 data, all body size variables were normally distributed and linear regressions were appropriate to test these relationships. For the 2017 data, the elytra lengths and hindwing lengths followed a normal distribution (linear regression), but the hind femur length data were non-normal and a GLM with a quasipoisson distribution provided a better fit (R Core Team 2016). All analyses were performed in R 1.0.136 (R Core Team 2016), and data were read using the ‘readxl’ package (Wickham and Bryan 2017).

2.2.6.4 SPB hindwing shape

For geometric morphometrics, all hindwing photographs were consolidated into a single dataset using tpsUtil32 version 1.74 software (Rohlf 2017a). The datasets were imported into tpsDig2 version 2.30 software (Rohlf 2017b) and the locations of LMs and SLMs were manually marked on the wing in each image, resulting in a TPS file with raw x and y coordinates associated with each digitized point. TPS files were then uploaded into MorphoJ version 1.06d software (Klingenberg 2014) to quantify differences in hindwing shape between all SPB samples for a given year. In MorphoJ, a full Procrustes superimposition was conducted to remove all non-shape variation associated with the raw landmark data (including size, position, and rotation of the hindwings) (Webster and Sheets 2010, Klingenberg 2011). The Procrustes superimposition also provided the centroid size of the landmark configuration, which is an isometric (i.e., all dimensions are equal) estimation of overall size, calculated as the square root of the sum of squared distances of each LM and SLM from its mean position (Zelditch et al. 2012, Mikac et al. 2013). A covariance matrix was generated from the raw data containing Procrustes coordinates, which provides a statistical measure of shape difference and can be utilized in multivariate statistical analyses (Klingenberg 2011, Klingenberg et al. 2012).

In MorphoJ, a principal component analysis (PCA) was run on the Procrustes coordinates for the 27 LMs and SLMs to examine the major patterns of variation in overall hindwing shape between sampled SPB (Klingenberg 2011). To test for separation in hindwing shape between SPB captured in outbreak versus non-outbreak traps, a discriminant function analysis (DFA) was used to maximize the differences in shape between the two population phases (Konigsberg and Frankenberg 2018). In contrast to PCA, DFA maximizes the separation of group means relative to within-group variation, and is a useful method to determine if groups that are known *a priori* can be readily distinguished (Klingenberg et al. 2012). In MorphoJ, DFA uses Fisher's cut-off point (i.e., a score of zero) to separate shape scores into two groups, and Hotelling's T^2 statistic to test for significance differences between two groups (Klingenberg 2011, Mikac et al. 2013). A permutation test with 10,000 iterations was included to test the null hypothesis of equivalent group means, and the validity of the outbreak – non-outbreak discrimination was measured using leave-one-out cross validation (Lachenbruch 1967).

To test for the presence of sexual dimorphism in hindwing shape between female and male SPB, DFA was again used to maximize the morphological differences between the sexes. DFA was an appropriate technique to assess the accuracy of hindwing shape in correctly classifying an individual as male or female because it is intended for maximizing differences between two identified groups. To assess whether hindwing shape scores for male versus female SPB were significantly different, a permutation test with 10,000 iterations was run, and the strength of the female-male discrimination was tested with leave-one-out cross validation (Lachenbruch 1967).

To test for allometry, or the fact that size can affect shape, a multivariate, pooled within-group regression of shape variation was run against centroid size, which is a good indicator of overall hindwing size (Klingenberg et al. 2012, Mikac et al. 2013). The regression scores (dependent variable) are variables associated with changes in shape that are predicted by the regression, and include the residual variation in the shape tangent space (i.e., direction of the shape change vectors) (Drake and Klingenberg 2008). Untransformed centroid size was selected as the independent variable because it had a better linear relationship than the log-transformed centroid size (Klingenberg et al. 2012). To assess the significance of the regression, a permutation test with 10,000 runs was included to test against the null hypothesis of independence between hindwing size and shape.

Centroid sizes were exported from MorphoJ and analyzed in R software to assess whether there were differences in size between males versus females, and between outbreak versus non-outbreak phases of SPB. The centroid sizes were normally distributed, thus simple linear regressions with dummy coding were appropriate to determine the relationships between centroid size and sex, and centroid size and population phase (R Core Team 2016).

2.3 Results

2.3.1 Classification of SPB population phase

In 2016, the mean number of SPB per trap per day ranged from ~0.1 (numerous traps) to ~21 (Homochitto); distances of traps from the nearest SPB spot identified the following summer ranged from 0.32 km (Oakmulgee) to 283.5 km (Croatan); and densities of SPB spots within a 20 km radius of each trap ranged from 0 (numerous traps) to 293 spots (Homochitto) (Table 2.2). In 2017, the mean number of SPB per day ranged from ~0.03 (Savannah River) to ~345 (Homochitto); distances from traps to the nearest SPB spot ranged from 0.22 km (Tombigbee) to 500.5 km (Croatan); and densities of SPB spots within a 20 km radius of each trap ranged from 0 (numerous traps) to 1,482 spots (Bienville) (Table 2.3). The synergist *endo*-brevicomin was added to SPB survey lures in 2017, explaining the significant increase in trap catches (visible in the mean SPB per day). For both years of data, there was a negative correlation between nearest spot distance and spot density (2016: $r = -0.85$, $T = -2.73$, $p = 0.009$; 2017: $r = -0.93$, $T = -2.10$, $p = 0.04$), with traps for which the nearest SPB spot was located within 2-3 km having higher densities of SPB spots within 20 km (for visualization, variables were log-transformed, and 1 was added to the spot densities to account for zero-counts) (Figures 2.3 a,b).

For both years of data, the cut-off point used to determine SPB population phase for a given trap was ≤ 20 km to the nearest identified SPB spot (classified as "outbreak") or > 20 km to the nearest spot (classified as "non-outbreak"). These data were partitioned because there was natural separation of the traps into two groups at this distance in both 2016 and 2017. Additionally, given the nature of the data that were available and collected (i.e., only 3-6 traps per district, lack of data throughout entire states), larger-scale analyses of outbreaking SPB populations were not feasible.

2.3.2 SPB sex ratio

The trap-level proportion of female SPB ranged from 0.034 to 0.64 in 2016, and from 0.012 to 0.46 in 2017 (excluding traps that caught <1 SPB per day). There was a difference in the mean proportion of females captured by outbreak versus non-outbreak traps in 2016 ($F_{1,51} = 5.18$, $p = 0.027$). Non-outbreak traps caught a 1.9 times higher proportion of females than outbreak traps (Figure 2.4a). Similar trends were observed in 2017 ($F_{1,54} = 15.75$, $p < 0.001$), with a 2.4 times higher proportion of females in non-outbreak traps (Figure 2.4b). However, no relationship was found between the proportion of females and the mean SPB captured per day in 2016 ($p = 0.91$) or 2017 ($p = 0.34$).

2.3.3 SPB size measurements

In 2016, there were positive correlations between the trap-level hindwing length and elytra length ($r = 0.93$, $T = 18.24$, $p < 0.001$), hindwing length and hind femur length ($r = 0.85$, $T = 11.31$, $p < 0.001$), and elytra length and hind femur length ($r = 0.84$, $T = 10.99$, $p < 0.001$) (Figure 2.5 a-c). In 2017, there were also positive correlations between hindwing length and elytra length ($r = 0.93$, $T = 18.00$, $p < 0.001$), hindwing length and hind femur length ($r = 0.81$, $T = 10.20$, $p < 0.001$), and elytra length and hind femur length ($r = 0.84$, $T = 11.35$, $p < 0.001$) (Figure 2.6 a-c).

For both years of data, relationships were not found between the trap status (non-outbreak versus outbreak) and elytra length, hind femur length, or hindwing length (p-values ranged from 0.58 - 0.99 in 2016 and 0.14 - 0.40 in 2017). Similarly, there was no relationship between elytra length and SPB per day ($p = 0.41$ in 2016 and $p = 0.24$ in 2017), hind femur length and SPB per day in 2016 ($p = 0.39$), or hindwing length and SPB per day ($p = 0.87$ in 2016 and $p = 0.06$ in

2017). The only significant relationship found was between hind femur length and SPB per day in 2017 ($F_{1,54} = 4.42$, $p = 0.04$), but it had a low value of $R^2 = 0.058$.

There were differences in elytral length between the sexes in both 2016 ($F_{1,239} = 6.68$, $p = 0.01$) and 2017 ($F_{1,249} = 23.02$, $p < 0.001$). On average, female elytra were 0.05 and 0.07 mm longer than male elytra in 2016 and 2017, respectively (Figures 2.7 a,b). No differences were found in hind femur lengths between males and females in 2016 ($p = 0.208$) and 2017 ($p = 0.173$). In 2016, no relationship was found between hindwing length and sex ($p = 0.344$). However, in 2017, female hindwings were an average of 0.085 mm longer than male hindwings ($F_{1,249} = 7.56$, $p = 0.006$) (Figure 2.8).

2.3.4 SPB hindwing shape

For 2016, the PCA showed that the first five principal components explained 64% of the overall variation in mean hindwing shape. The DFA analyses for 2016 revealed there were differences in shape between non-outbreak and outbreak beetles ($T^2 = 108.9$, $p = 0.005$; Figure 2.9a). However, a relatively small Procrustes distance (0.00465) between the average hindwing shapes for the two groups indicated minimal overall shape dimorphism between the population phases. Leave-one-out cross-validation testing the ability of the DFA to discriminate between non-outbreak and outbreak using hindwing shape scores resulted in the misclassification of 42% (40 of 96) of non-outbreak SPB as outbreak phase, and of 39% (57 of 145) of outbreak SPB as non-outbreak phase (Figure 2.9b).

The DFA analyzing hindwing shapes of the sexes in 2016 showed there were differences in shape between female and male SPB ($T^2 = 105.5$, $p < 0.001$) (Figure 2.9c). Nevertheless, the small Procrustes distance (0.00846) between the average hindwing shapes for female and male

SPB suggests minimal sexual dimorphism in overall hindwing shape. The leave-one-out cross-validation resulted in misclassification of 35% (31 of 89) of female SPB as males, and of 31% (47 of 152) of male SPB as females (Figure 2.9d).

Results from the 2017 PCA showed that the first five principal components explained ~66% of the overall variation in mean hindwing shape. The DFA analyses for 2017 revealed differences in hindwing shape between non-outbreak and outbreak SPB ($T^2 = 101.5$, $p = 0.01$; Figure 2.10a). However, the small Procrustes distance (0.0027) between average hindwing shapes for the two groups indicates minimal overall shape dimorphism between the population phases. Cross-validation testing how accurately the DFA differentiated between non-outbreak and outbreak SPB by their hindwing shape scores resulted in the misclassification of 44% (43 of 98) of non-outbreak SPB as outbreak phase, and of 39% (60 of 153) of outbreak SPB as non-outbreak phase (Figure 2.10b).

The DFA assessing the hindwing shape scores for the sexes in 2017 showed differences in shape between female and male SPB ($T^2 = 133.0$, $p < 0.001$; Figure 2.10c). However, a minimal Procrustes distance (0.0069) between average hindwing shapes for female and male SPB suggests only slight sexual dimorphism in overall hindwing shape. Cross-validation resulted in the misclassification of 36% (38 of 105) of female SPB as males, and of 33% (48 of 146) of male SPB as females (Figure 2.10d).

Pooled within-group regression testing for allometry within the hindwing data for 2016 revealed that centroid size explained 22.4% of the total variation in hindwing shape ($R^2 = 0.224$; $p < 0.001$). Regression assessing whether SPB sex and population phase were good predictors of centroid size did not show differences in hindwing centroid size between males and females ($F_{1,239} = 0.94$, $p = 0.33$), or between outbreak and non-outbreak SPB ($F_{1,239} = 0.12$, $p = 0.73$). In

2017, within-group regression to determine if there was allometry within the data showed that hindwing centroid size explained 31.7% of the total variation in shape ($R^2 = 0.317$; $p < 0.001$). Regression analyzing the effect of SPB population phase on centroid size did not show differences between outbreak and non-outbreak status hindwing sizes ($F_{1,249} = 2.82$, $p = 0.094$). However, contrary to 2016, there were differences in hindwing centroid size between male versus female SPB in 2017 ($F_{1,239} = 7.07$, $p = 0.008$). On average, female centroid size (the square root of the sum of squared distances of landmarks from the object centroid) was 42 units larger than male centroid size, which indicates that female hindwings were larger than males and corroborates the finding of longer hindwings for females than males in 2017 (Figures 2.8, 2.11).

2.4 Discussion

We are aware of no published studies comparing multiple phenotypic traits of SPB adults in outbreak and non-outbreak populations, although plasticity has been observed in attributes of SPB and other *Dendroctonus* species that could vary with population density (e.g., female SPB body size, oviposition rate, gallery length and density, development time) (Bentz et al. 2011, Stephen 2011, Foelker and Hofstetter 2014). We classified captured SPB as non-outbreak or outbreak based on the proximity of their trap of origin to the nearest identified SPB spot the following summer, selecting a uniform cut-off distance (20 km) for all traps in both years to partition the SPB into the two population phases. Contrary to our hypotheses, we did not find any evidence for variation in SPB body size (elytra length, hind femur length, or hindwing length) that coincided with outbreak versus non-outbreak population phases. The greatest propensity for an insect outbreak should occur when the prevailing phenotype favors individuals with a high reproductive potential (Wallner 1987). In the case of SPB, large males have previously been shown to produce considerably (~52%) more progeny than smaller males, but little evidence was found supporting a correlation between female body size and fecundity (Foelker and Hofstetter 2014). Our experimental design was limited in that we could not assess the reproductive capacity of the measured SPB; however, the lack of observed size differences supports the hypothesis that outbreak-phase SPB are not necessarily smaller/larger than non-outbreak beetles (although they may be more fecund or have higher dispersal ability which we did not test).

A variety of traits have been used to assess body size for *Dendroctonus* species, including weight (Pureswaran and Borden 2003, Graf et al. 2012, Liu et al. 2017), body length (Pureswaran and Borden 2003, Elkin and Reid 2005, Lachowsky and Reid 2014), and pronotum width (Lanier et al. 1988, Elkin and Reid 2005, Bentz et al. 2011, Graf et al. 2012, Esch et al. 2016). Although pronotum width is commonly selected, there is minimal literature demonstrating that this measurement is a good indicator of overall body size. Assessing the structural body size of an insect is important in characterizing its overall body condition (i.e., fitness), and using numerous size measurements provides a more robust characterization of total size (Moya-Laraño et al. 2008, Knapp and Knappová 2013). In this study, we measured SPB elytra length, hind femur length, and hindwing length to assess overall body size, and found that all three measurements were positively correlated with each other in both 2016 and 2017. This finding follows the general principle that the sizes of appendages of an individual insect are proportional to its overall body size (Kofuji et al. 1992, Nijhout and Callier 2015).

Overall, no differences were found in SPB body size measurements or the captured proportion of females that correlated to the mean number of SPB caught per trap per day. Although the absolute number of SPB captured in a baited funnel trap is not equivalent to the abundance, we hypothesized that mean SPB per day could be used as a surrogate for estimating population abundance, and accordingly, that we would observe phenotypic differences in SPB captured in traps with a high (i.e., outbreak-level) versus low (i.e., non-outbreak-level) mean catch rate. This hypothesis followed the well-understood mechanism of mass aggregation that SPB undergo when initiating an infestation to reach the threshold population density required to successfully colonize a host pine (Stephen 2011, Schowalter 2012). However, the lack of any compelling variation in observed beetle phenotype that coincided with mean SPB per day suggests that either mean number of SPB captured per day is not a good indicator of population phase, or that there were no measured differences in phenotype that correspond with mean SPB per day.

In Coleoptera, landmark-based geometric morphometrics (GM) has been applied to analyze hindwing shape in taxa including Chinese dung beetles (Scarabaeinae) (Bai et al. 2011), *Nebria* ground beetles (Carabidae) (Palestrini et al. 2012), the western corn rootworm (Chrysomelidae) (Mikac et al. 2013, Benítez et al. 2014), cantharid beetles (Cantharinae) (Su et al. 2015), and the leaf beetle *Oreina cacaliae* (Schrank) (Chrysomelidae) (Kalberer and Kölliker 2017). However, our study is the first to use landmark-based GM to analyze variation in hindwing morphology for a bark beetle species. We discovered there were subtle shape differences between outbreak and non-outbreak phase SPB hindwings in both 2016 and 2017. On average, outbreak-phase SPB hindwings were slightly elongated and narrower in both years compared to non-outbreak phase beetles. Similar results were found between western corn rootworm (*Diabrotica virgifera virgifera* LeConte) collected from rotated versus continuous maize, with beetles from rotated crops (i.e., reflecting ephemeral habitats) having larger hindwings (Mikac et al. 2013). However, cross-validation indicated that SPB population phase was not a highly accurate predictor of hindwing shape either year (56-58% accuracy in classifying non-outbreak SPB, and 61% accuracy in classifying outbreak SPB). Despite our finding of existing hindwing variation, the small difference in average shape and significant overlap in hindwing shape scores between non-outbreak and outbreak SPB suggests that this phenotypic trait may have limited use in predicting outbreaks.

However, our results provide strong support for the existence of sexual size dimorphism in SPB, with female beetles being slightly larger than males (longer elytra both years; longer hindwings and larger hindwing centroid sizes in 2017). Results from the landmark-based GM analyses also showed subtle differences between male and female hindwing shape, with female hindwings being slightly more elongated and wider in both years as compared to male SPB. Previous studies have shown that female beetles, on average, are larger than males for several species in the *Dendroctonus* genus (Bentz et al. 2011, Graf et al. 2012, Foelker and Hofstetter 2014, Lachowsky and Reid 2014, Liu et al. 2017). There are several biological explanations for why this trend is consistently found within *Dendroctonus*. First, as females are the pioneering sex for this genus, they should generally spend more time flying than males as they search for acceptable hosts for colonization (Elkin and Reid 2005). Accordingly, females should have a larger overall body size to allow for increased fuel storage (i.e., lipid concentrations), providing sufficient energy for long-distance dispersal (Coppedge et al. 1994, Graf et al. 2012). Female SPB would also benefit from having wider, longer wings to increase their hindwing surface area and their capacity for extended periods of flight as they search for suitable hosts (Gutiérrez and Menéndez 1997, Brown et al. 2017). Second, female insect body size has been linked to increased fitness and overall capacity for reproductive activities (Nylin and Gotthard 1998, Calvo and Molina 2005). Female mountain pine beetles (*D. ponderosae* Hopkins) with nutritional limitations have been observed to produce smaller eggs (Elkin and Reid 2005), and had increased overall growth within the same time period as male conspecifics (Bentz et al. 2011). In the case of SPB, larger females were found to excavate longer galleries within the phloem, although this metric is not necessarily indicative of increased brood production (Foelker and Hofstetter 2014). Ultimately, it may be more important for female SPB to be larger than males because they are the determinant sex in finding hosts and thereby select the nutritional environment for brood feeding and development (Elkin and Reid 2005, Bentz et al. 2011), which could influence phenotypic traits of their progeny (Attisano and Kilner 2015).

As we hypothesized, there was a higher captured proportion of female SPB in non-outbreak traps as compared to outbreak traps for both years of data. Funnel traps deployed during the USFS-FHP SPB survey use lures that include the female SPB pheromone component frontalin, which is known to attract a heavily male-biased sex ratio (Sullivan 2016). Accordingly, baited survey traps should have a uniformly male bias in the captured SPB, but the overall proportion of females may vary temporally and spatially. A prior study tested the sex ratio biases of various types of traps, and found that, for funnel traps baited with frontalin and turpentine and placed ~100 m from an active infestation, the captured proportion of females was 0.49 (the proportion of males, 0.51, was reported) (Cronin et al. 2000). By comparison, the outbreak phase traps (i.e., relatively closer to infestations) in our study with the maximum proportions of females were 0.42 in 2016 and 0.37 in 2017, whereas the highest proportions for non-outbreak traps were 0.64 in 2016 and 0.46 in 2017. Based on the data available for our study, the vast majority of SPB survey traps were male-biased, but these biases were less strong for non-outbreak traps. Moreover, the addition of the male-produced SPB pheromone component *endo*-brevicomin to the survey in 2017 did not appear to have a notable effect on the trap-level proportion of females, supporting the finding that this lure component is primarily an aggregation synergist to frontalin and does not enhance preferential attraction of either sex (Sullivan and Mori 2009, Sullivan 2016).

Overall, we found little evidence for phenotypic variability in terms of body size metrics between outbreak versus non-outbreak populations of SPB. However, there were distinct sex-level differences in hindwing shape and especially in sex ratio. The detection of a high captured proportion of female SPB in non-outbreak than outbreak traps has important implications for managing SPB and predicting outbreaks. Traps with a higher proportion of females may represent SPB populations where females are actively dispersing and searching for available hosts (Coster et al. 1980), whereas traps with lower proportions of females (i.e., strongly male-biased catches) may indicate that infestations have already been initiated nearby and males attracted to frontalin in the trap lure are actively locating on female pheromone components (Clarke and Nowak 2009, Sullivan 2016). In future years of the USFS-FHP SPB surveys, the addition of the trap-level captured proportion of females (which can be easily distinguished) in predicting outbreaks and fluctuations in local and regional SPB population levels merits further consideration as it may allow for more robust considerations of the population dynamics for this important bark beetle species.

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Table 2.1. Number of southern pine beetle sampled in 2016 and 2017 in six states and twenty-one districts in the southeastern U.S.

State	**Forest Service District**	**2016** Females	**2016** Males	**2017** Females	**2017** Males	**Total SPB**
Alabama	Bankhead	3	12	NA*	NA*	15
Alabama	Oakmulgee	12	18	15	15	60
Alabama	Shoal Creek	7	8	NA*	NA*	15
Alabama	Talladega	2	8	NA*	NA*	10
Georgia	Chattooga River	7	8	8	7	30
Georgia	Conasauga	4	7	5	5	21
Georgia	Fort Stewart	5	10	7	8	30
Georgia	Oconee	3	7	7	8	25
Mississippi	Bienville	5	10	5	9	29
Mississippi	Chickasawhay	0	0	2	10	12
Mississippi	DeSoto	0	0	1	1	2
Mississippi	Holly Springs	4	3	5	10	22
Mississippi	Homochitto	13	17	14	15	59
Mississippi	Tombigbee	7	8	6	9	30
North Carolina	Appalachian	0	5	5	5	15
North Carolina	Cheoah	5	10	4	11	30
North Carolina	Croatan	6	4	7	8	25
South Carolina	Francis Marion	1	2	4	8	15
South Carolina	Long Cane	3	8	6	8	25
South Carolina	Savannah River	1	1	2	5	9
Tennessee	Ocoee	1	6	2	4	13

* NA = Not Applicable since trap catches were not available. Indicated for districts where trap catches were not available for analyses. Districts with uneven numbers of samples (i.e., not a multiple of 5) did not have adequate samples for 5 samples per trap. Districts with few/no female SPB were supplemented with additional males for a total of five samples per trap, where possible.

Table 2.2. Mean number of SPB caught per trap per day and the spot density within a 20 km radius of each trap for 2016, ranked by the distance (km) from a trap to the nearest spot identified the following summer, for six states and 19 districts in the southeastern U.S. Traps before the dashed line are outbreak phase, traps after the dashed line are non-outbreak phase.

State	Forest District	Mean SPB per day	Number of Spots in 20-km radius	Distance from trap to nearest spot (km)
Alabama	Oakmulgee	5.1	218	0.32
Mississippi	Homochitto	2.0	223	0.44
Mississippi	Bienville	4.0	117	0.87
Mississippi	Bienville	4.6	76	1.00
Mississippi	Tombigbee	4.0	2	1.01
Mississippi	Homochitto	4.2	190	1.02
Alabama	Oakmulgee	1.1	140	1.09
Mississippi	Bienville	0.8	197	1.13
Alabama	Oakmulgee	6.9	238	1.14
Mississippi	Homochitto	13.0	293	1.42
Georgia	Oconee	1.2	10	1.74
North Carolina	Cheoah	4.5	17	1.84
Alabama	Oakmulgee	7.2	84	1.87
Mississippi	Homochitto	21.0	116	2.00
Alabama	Oakmulgee	5.0	235	2.20
Alabama	Oakmulgee	3.9	117	2.41
Alabama	Shoal Creek	1.8	1	3.20
Alabama	Talladega	1.9	14	3.87
Georgia	Oconee	2.6	2	5.05
Mississippi	Tombigbee	2.2	1	6.07
Mississippi	Homochitto	6.4	76	7.65
Mississippi	Holly Springs	6.8	1	9.36
Mississippi	Tombigbee	1.7	1	10.19
Alabama	Bankhead	1.2	1	10.30

Alabama	Bankhead	1.2	1	10.59
Alabama	Talladega	0.9	10	11.76
Alabama	Shoal Creek	4.1	2	12.73
Alabama	Shoal Creek	4.1	2	12.73
Alabama	Shoal Creek	1.2	6	14.27
Georgia	Fort Stewart	2.9	1	19.57
Alabama	Bankhead	0.5	0	23.06
Mississippi	Holly Springs	0.1	0	28.60
Georgia	Fort Stewart	2.0	0	29.36
Georgia	Fort Stewart	1.5	0	35.48
Tennessee	Ocoee	1.0	0	37.49
Tennessee	Ocoee	1.0	0	37.49
Georgia	Conasauga	0.1	0	41.50
Georgia	Conasauga	4.6	0	41.75
Georgia	Conasauga	4.6	0	41.75
North Carolina	Cheoah	1.7	0	42.21
North Carolina	Cheoah	7.3	0	50.48
Mississippi	Homochitto	3.5	0	58.96
Georgia	Chattooga River	3.3	0	65.09
Georgia	Chattooga River	4.2	0	94.63
Georgia	Chattooga River	4.2	0	94.63
North Carolina	Appalachian	0.9	0	105.97
South Carolina	Long Cane	1.6	0	111.03
South Carolina	Long Cane	2.7	0	112.63
South Carolina	Savannah River	0.3	0	149.67
South Carolina	Francis Marion	0.4	0	187.61
South Carolina	Francis Marion	0.1	0	194.19
North Carolina	Croatan	1.6	0	283.43
North Carolina	Croatan	0.7	0	283.45

Table 2.3. Mean number of SPB caught per trap per day and the spot density within a 20 km radius of each trap for 2017, ranked by the distance (km) from a trap to the nearest spot identified the following summer, in six states and 18 districts in the southeastern U.S. Traps before the dashed line are outbreak phase, traps after the dashed line are non-outbreak phase.

State	Forest District	Mean SPB per day	Number of Spots in 20-km radius	Distance from trap to nearest spot (km)
Mississippi	Tombigbee	4.0	1166	0.22
Mississippi	Tombigbee	23.4	1167	0.25
Mississippi	Bienville	3.1	1432	0.28
Alabama	Oakmulgee	49.4	506	0.32
Mississippi	Bienville	28.1	1073	0.32
Alabama	Oakmulgee	64.7	566	0.39
Mississippi	Homochitto	140.8	381	0.42
Mississippi	Homochitto	104.8	392	0.53
Mississippi	Homochitto	65.1	457	0.53
Alabama	Oakmulgee	98.1	318	0.67
Mississippi	Homochitto	159.1	479	0.72
Mississippi	Holly Springs	6.6	147	0.73
Mississippi	Tombigbee	31.0	247	0.74
Alabama	Oakmulgee	122.4	535	0.77
Mississippi	Homochitto	344.6	599	0.81
Mississippi	Bienville	1.8	479	0.82
Mississippi	Homochitto	69.5	329	0.93
Georgia	Oconee	47.8	26	1.04
Georgia	Oconee	49.0	393	1.18
Alabama	Oakmulgee	11.3	449	1.23
Alabama	Oakmulgee	276.9	404	1.25
North Carolina	Cheoah	1.7	48	2.45
Georgia	Oconee	17.5	129	2.60
North Carolina	Cheoah	2.0	7	3.87

Georgia	Chattooga River	73.7	7	5.25
Georgia	Conasauga	29.9	1	8.35
Georgia	Conasauga	1.8	1	9.62
Georgia	Chattooga River	70.2	7	10.45
North Carolina	Cheoah	2.8	1	11.52
Mississippi	Holly Springs	0.5	1	17.61
Georgia	Fort Stewart	8.5	2	19.05
Georgia	Fort Stewart	7.0	0	21.58
South Carolina	Savannah River	0.03	0	23.22
Mississippi	Holly Springs	1.0	0	24.10
Tennessee	Ocoee	0.04	0	25.71
Georgia	Chattooga River	81.3	0	28.21
South Carolina	Long Cane	0.1	0	28.45
North Carolina	Appalachian	23.3	0	28.46
Georgia	Fort Stewart	10.8	0	31.98
South Carolina	Savannah River	0.1	0	32.39
Tennessee	Ocoee	0.2	0	32.71
South Carolina	Long Cane	2.4	0	37.15
Mississippi	Chickasawhay	0.5	0	40.58
South Carolina	Long Cane	0.1	0	41.48
South Carolina	Savannah River	0.1	0	41.93
Mississippi	Chickasawhay	0.5	0	43.75
South Carolina	Long Cane	3.6	0	43.84
Mississippi	DeSoto	0.1	0	47.53
Mississippi	Chickasawhay	1.3	0	48.37
North Carolina	Appalachian	3.5	0	55.12
South Carolina	Francis Marion	0.3	0	180.00
South Carolina	Francis Marion	0.9	0	193.03
South Carolina	Francis Marion	0.1	0	205.91
North Carolina	Croatan	138.0	0	443.85
North Carolina	Croatan	0.7	0	489.14

North Carolina	Croatan	55.5	0	500.49

Figure Legend

Figure 2.1. A southern pine beetle adult. Lines indicate where measurements were taken for elytral (dashed line) and hind femur (solid line) lengths.

Figure 2.2. a) Hindwing vein nomenclature and positions of the eleven true landmarks on southern pine beetle. a: Median fold; C: costa; Sc: subcosta; R: radius; R1: radius 1; R2: radius 2; M1: media 1; M2: media 2; Cu1: cubitus 1; Cu2: cubitus 2. b) Southern pine beetle hindwing with "comb" fan overlaid and labeled locations of true landmarks (1-11) and semi-landmarks (12-27).

Figure 2.3. Pearson correlations between distance (log) from each survey trap to the nearest southern pine beetle spot identified the following summer versus the density (log) of spots within 20 km of each trap in: a) 2016 and b) 2017.

Figure 2.4. Mean (± SE) proportions of female southern pine beetle in outbreak versus non-outbreak traps in: a) 2016; b) 2017.

Figure 2.5. Pearson correlations between mean (± SE) trap-level southern pine beetle size measurements for 2016. a) Mean elytra length versus mean hindwing length; b) Mean hind femur length versus mean hindwing length; c) Mean hind femur length versus mean elytra length.

Figure 2.6. Pearson correlations between mean (± SE) trap-level southern pine beetle size measurements for 2017. a) Mean elytra length versus mean hindwing length; b) Mean hind femur length versus mean hindwing length; c) Mean hind femur length versus mean elytra length.

Figure 2.7. Mean (± SE) elytra lengths for male versus female southern pine beetle in: a) 2016; b) 2017.

Figure 2.8. Mean (± SE) hindwing length for male versus female southern pine beetle in 2017.

Figure 2.9. a) Discriminant scores for hindwing shape for the 2016 non-outbreak (less than zero) versus outbreak (greater than zero) SPB, with overlap occurring where both population phases have the same hindwing shape scores; b) discriminant hindwing shape score classification for the 2016 non-outbreak (less than zero) versus outbreak (greater than zero) SPB based on leave-one-out cross-validation; c) discriminant scores for hindwing shape for the 2016 female (less than zero) versus male (greater than zero) southern pine beetle, with overlap occurring where both sexes have the same hindwing shape scores; d) discriminant hindwing shape score classification for the 2016 female (less than zero) versus male (greater than zero) southern pine beetle based on leave-one-out cross-validation.

Figure 2.10. a) Discriminant scores for hindwing shape for the 2017 non-outbreak (less than zero) versus outbreak (greater than zero) southern pine beetle, with overlap occurring

where both population phases have the same hindwing shape scores; b) discriminant hindwing shape score classification for 2017 non-outbreak (less than zero) versus outbreak (greater than zero) southern pine beetle based on leave-one-out cross-validation; c) discriminant scores for hindwing shape for the 2017 female (less than zero) versus male (greater than zero) southern pine beetle, with overlap occurring where both sexes have the same hindwing shape scores; d) discriminant hindwing shape score classification 2017 female (less than zero) versus male (greater than zero) southern pine beetle based on leave-one-out cross-validation.

Figure 2.11. Mean (± SE) hindwing centroid size for male versus female southern pine beetle in 2017.

Figure 2.1

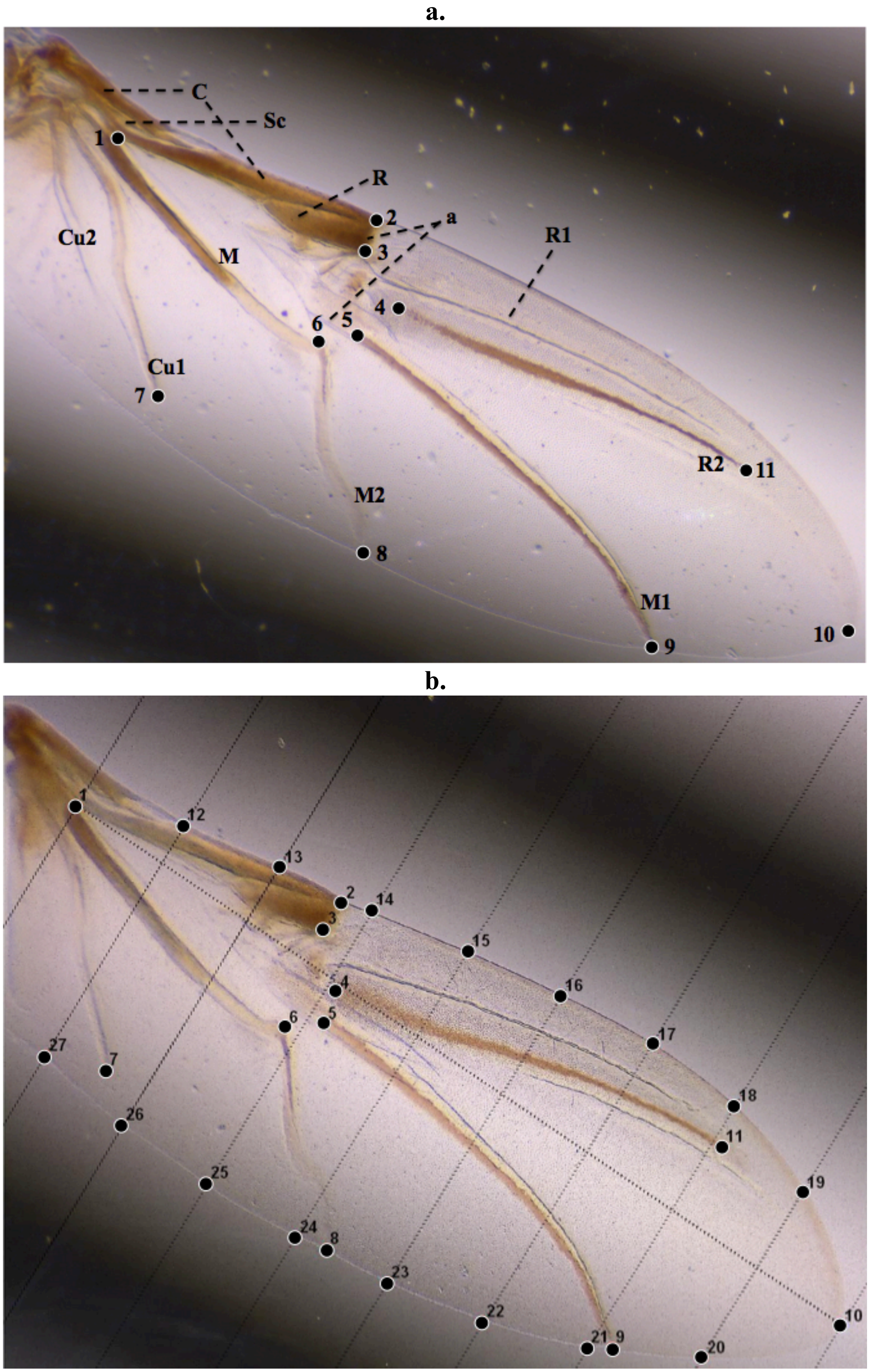
a.
C
Sc
1
R
2
a
R1
3
Cu2
M
4
6
5
Cu1
7
R2
11
M2
8
M1
10
9
b.
1
12
13
2
14
3
15
4
16
6
5
17
27
7
18
26
11
25
19
24
8
23
22
10
21
9
20

Figure 2.2

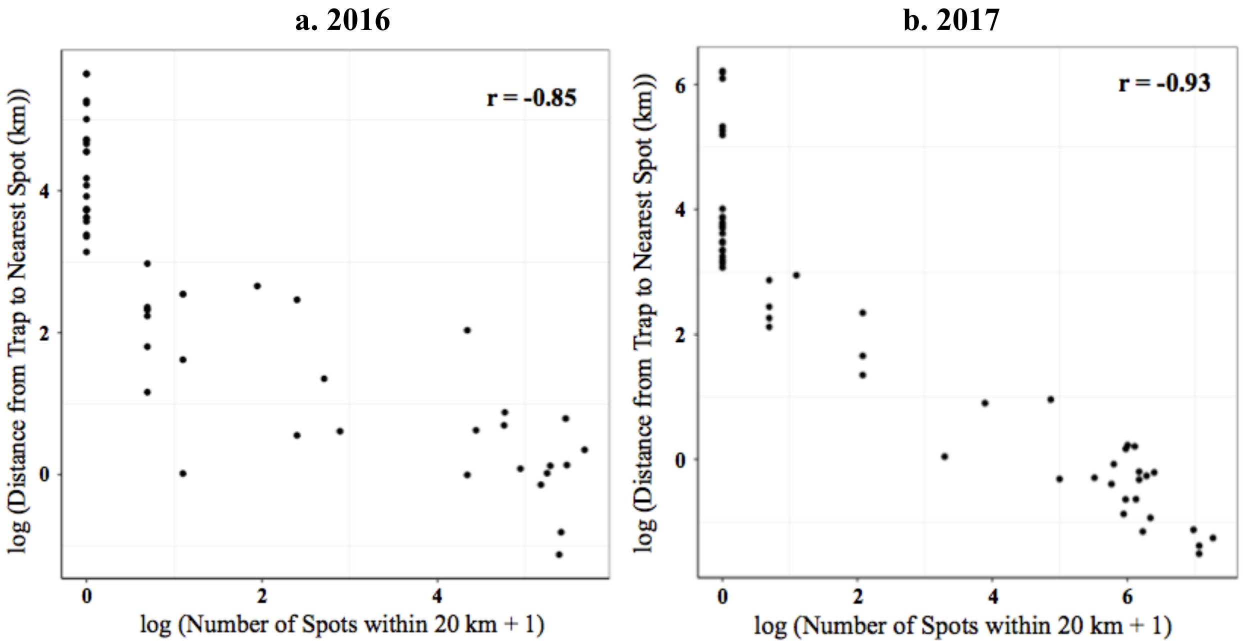
a. 2016
b. 2017
r = -0.85
r = -0.93
log (Distance from Trap to Nearest Spot (km))
log (Number of Spots within 20 km + 1)
0
2
4
6

Figure 2.3

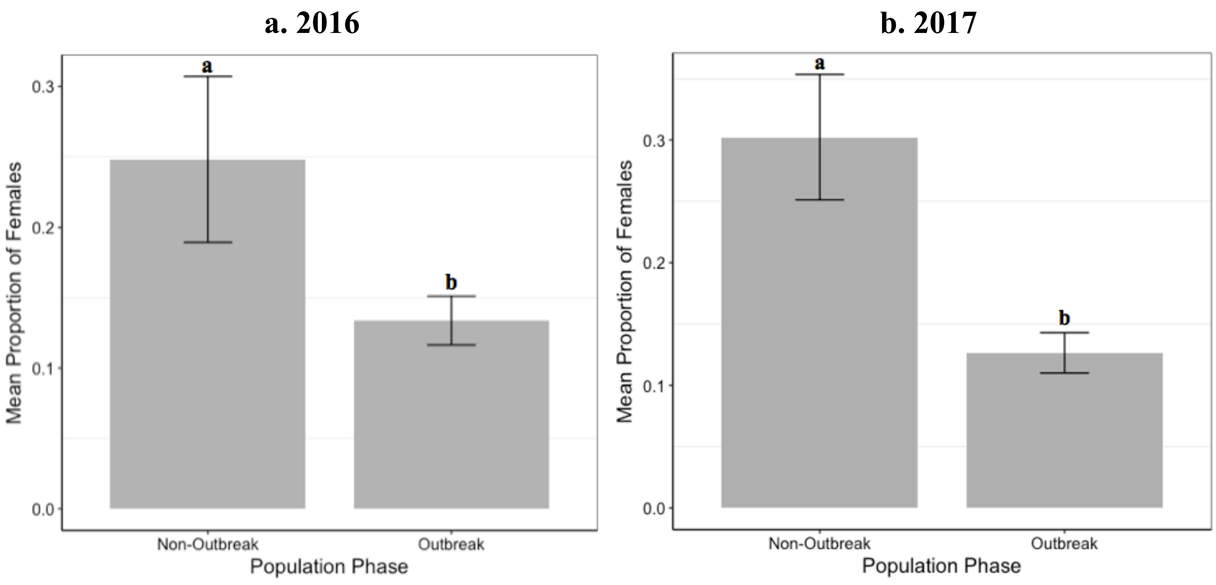
a. 2016
b. 2017
Mean Proportion of Females
0.0
0.1
0.2
0.3
a
b
Non-Outbreak
Outbreak
Population Phase

Figure 2.4

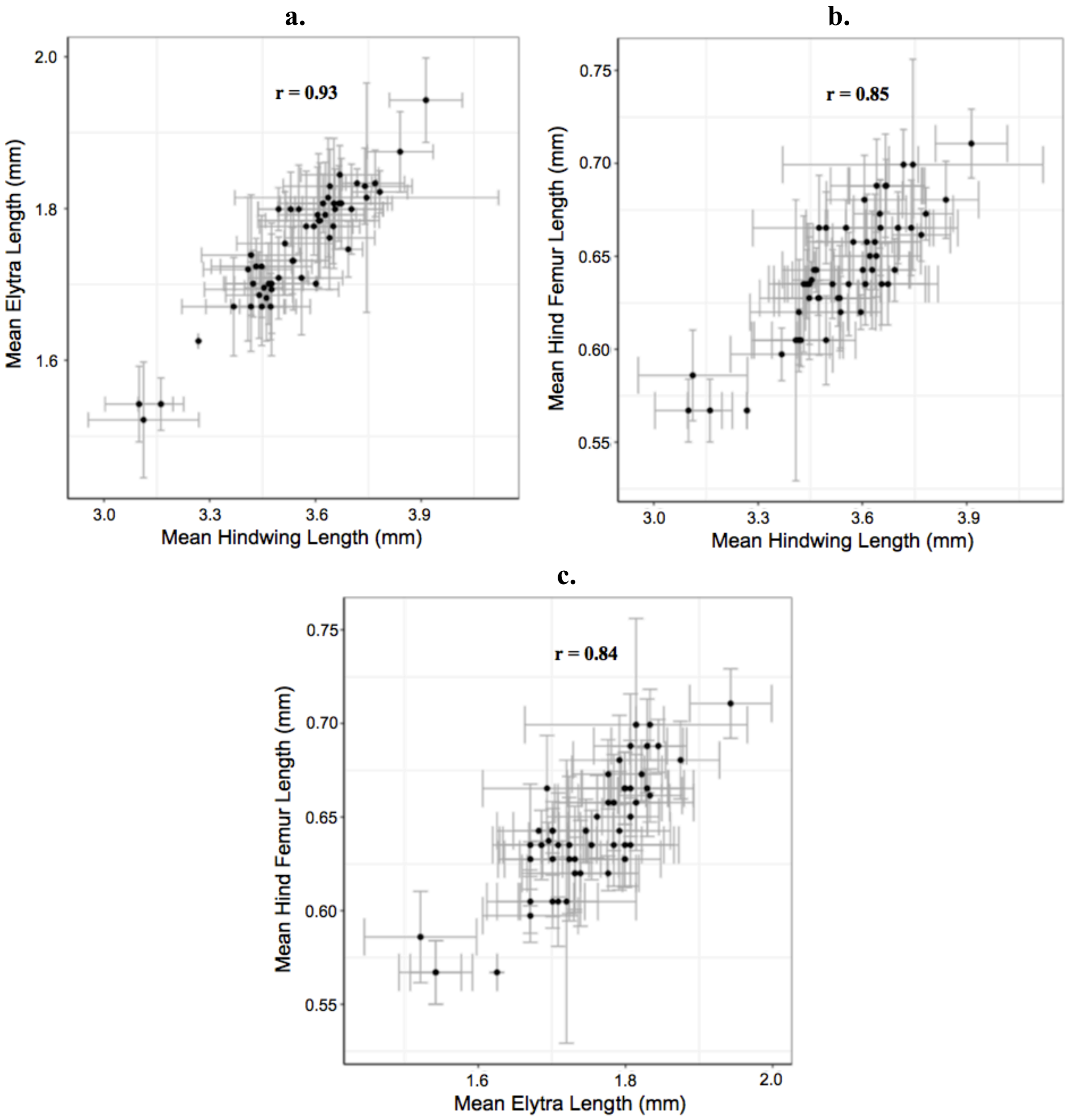
a.
r = 0.93
Mean Elytra Length (mm)
2.0
1.8
1.6
3.0
3.3
3.6
3.9
Mean Hindwing Length (mm)
b.
r = 0.85
Mean Hind Femur Length (mm)
0.75
0.70
0.65
0.60
0.55
3.0
3.3
3.6
3.9
Mean Hindwing Length (mm)
c.
r = 0.84
Mean Hind Femur Length (mm)
0.75
0.70
0.65
0.60
0.55
1.6
1.8
2.0
Mean Elytra Length (mm)

Figure 2.5

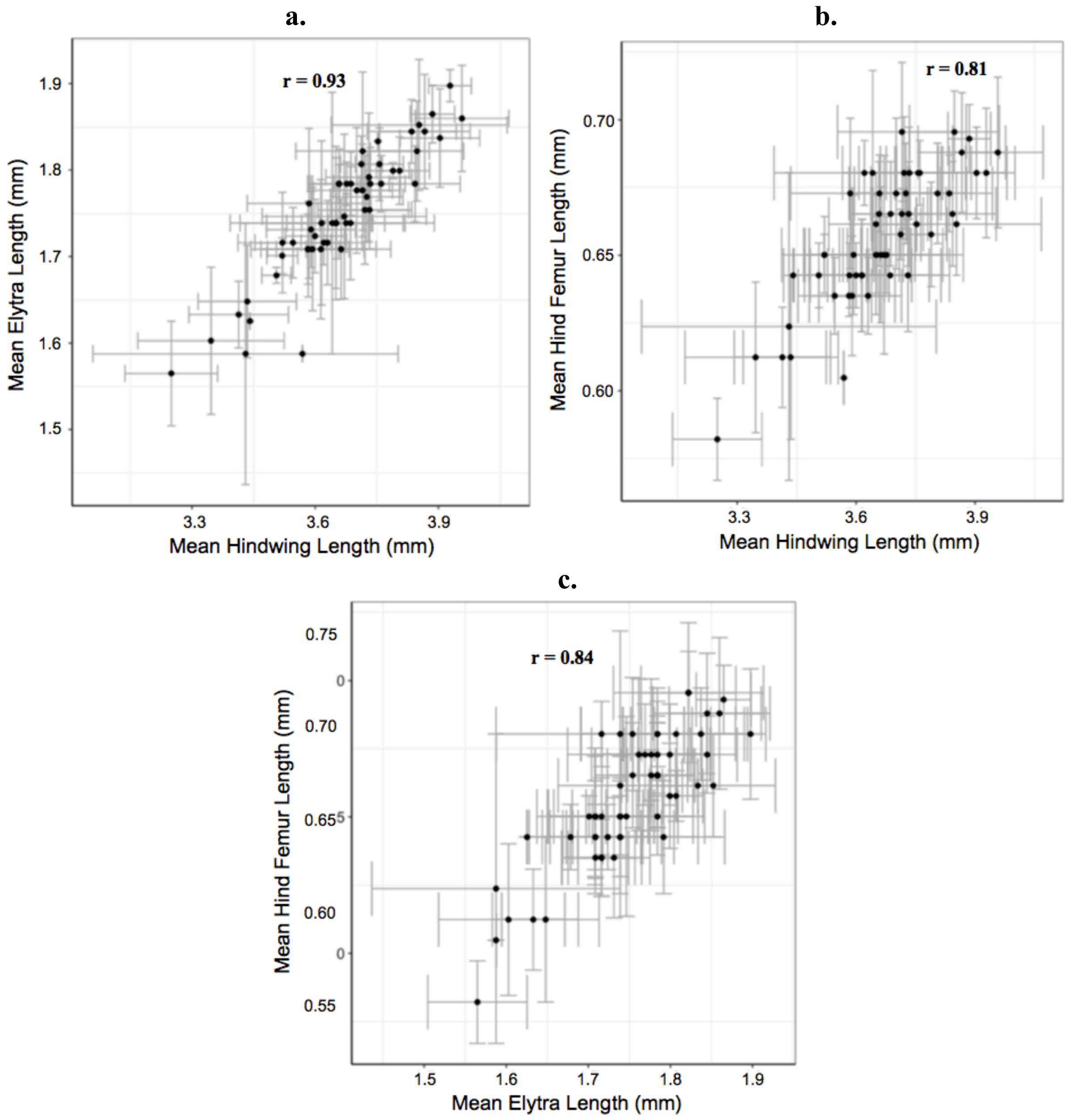
a.
r = 0.93
Mean Elytra Length (mm)
1.9
1.8
1.7
1.6
1.5
3.3
3.6
3.9
Mean Hindwing Length (mm)
b.
r = 0.81
Mean Hind Femur Length (mm)
0.70
0.65
0.60
3.3
3.6
3.9
Mean Hindwing Length (mm)
c.
r = 0.84
Mean Hind Femur Length (mm)
0.75
0
0.70
0.655
0.60
0
0.55
1.5
1.6
1.7
1.8
1.9
Mean Elytra Length (mm)

Figure 2.6

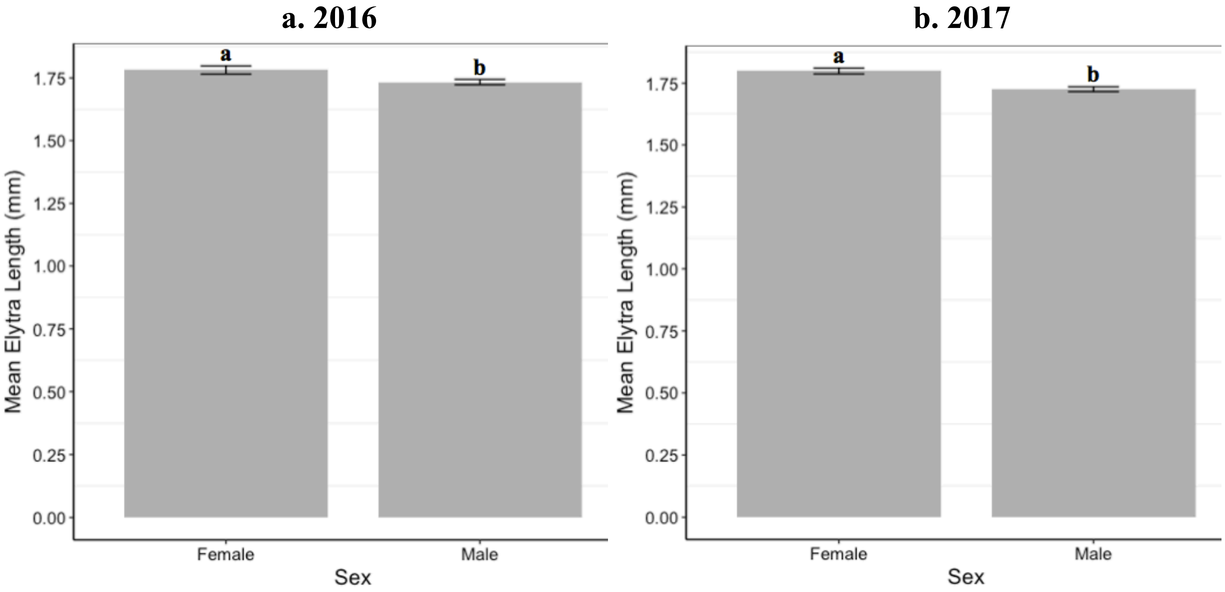
a. 2016
b. 2017
Mean Elytra Length (mm)
0.00
0.25
0.50
0.75
1.00
1.25
1.50
1.75
a
b
Female
Male
Sex

Figure 2.7

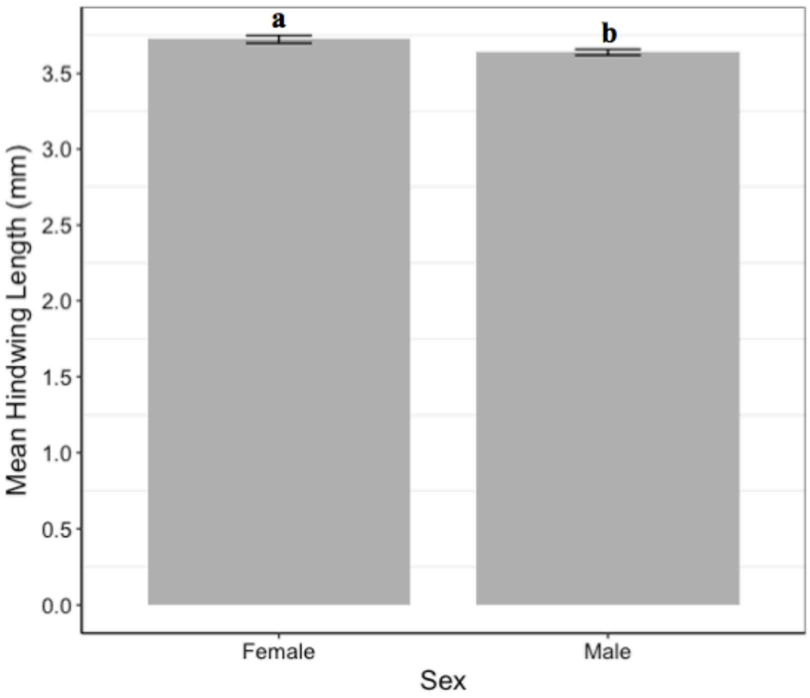
a
b
Mean Hindwing Length (mm)
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
Female
Male
Sex

Figure 2.8

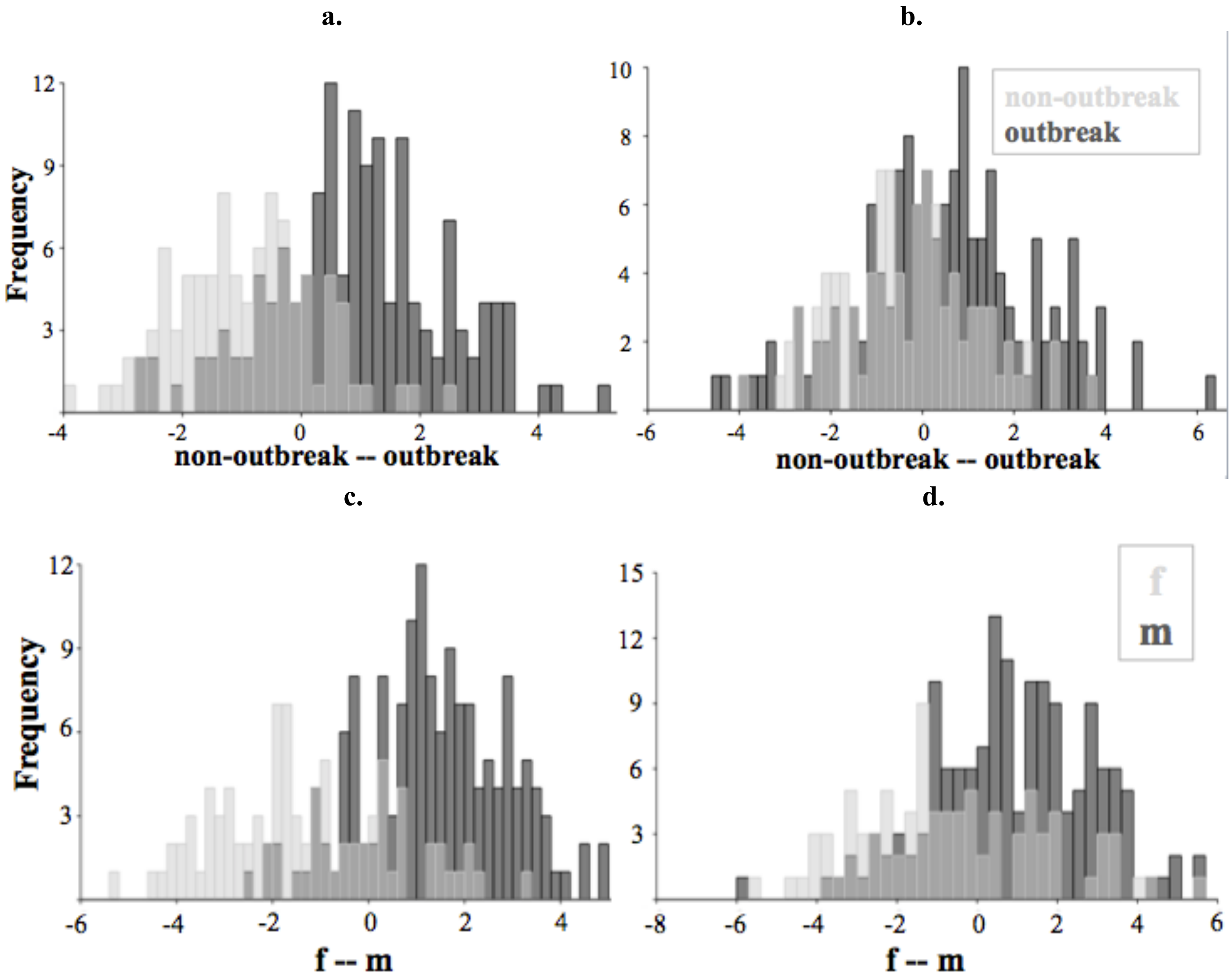
a.
b.
c.
d.
Frequency
12
9
6
3
-4
-2
0
2
4
non-outbreak -- outbreak
10
8
6
4
2
-6
-4
-2
0
2
4
6
non-outbreak
outbreak
non-outbreak -- outbreak
Frequency
12
9
6
3
-6
-4
-2
0
2
4
f -- m
15
12
9
6
3
-8
-6
-4
-2
0
2
4
6
f
m
f -- m

Figure 2.9

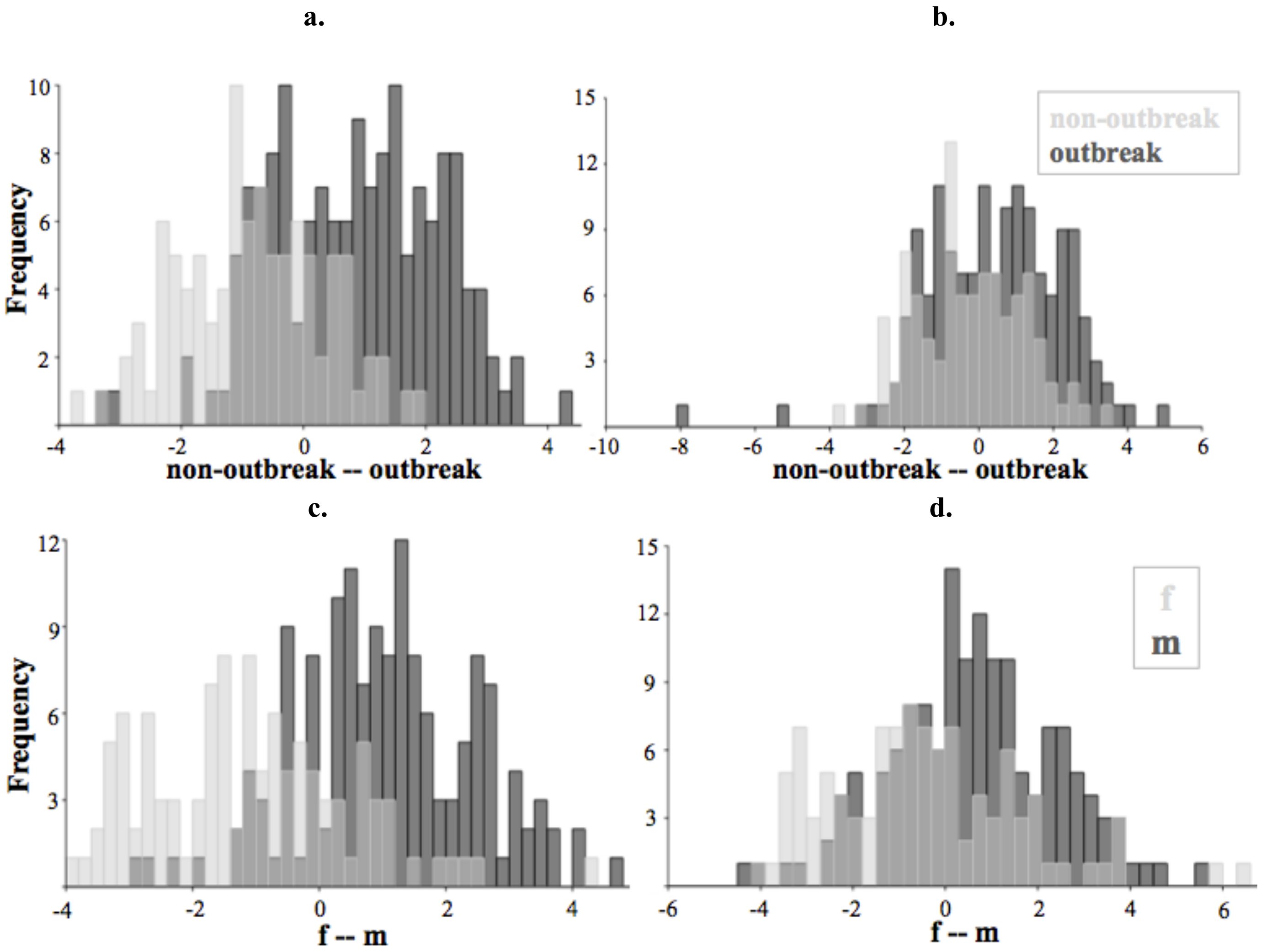
a.
b.
Frequency
10
8
6
4
2
-4
-2
0
2
4
non-outbreak -- outbreak
15
12
9
6
3
-10
-8
-6
-4
-2
0
2
4
6
non-outbreak -- outbreak
non-outbreak
outbreak
c.
d.
Frequency
12
9
6
3
-4
-2
0
2
4
f -- m
15
12
9
6
3
-6
-4
-2
0
2
4
6
f -- m
f
m

Figure 2.10

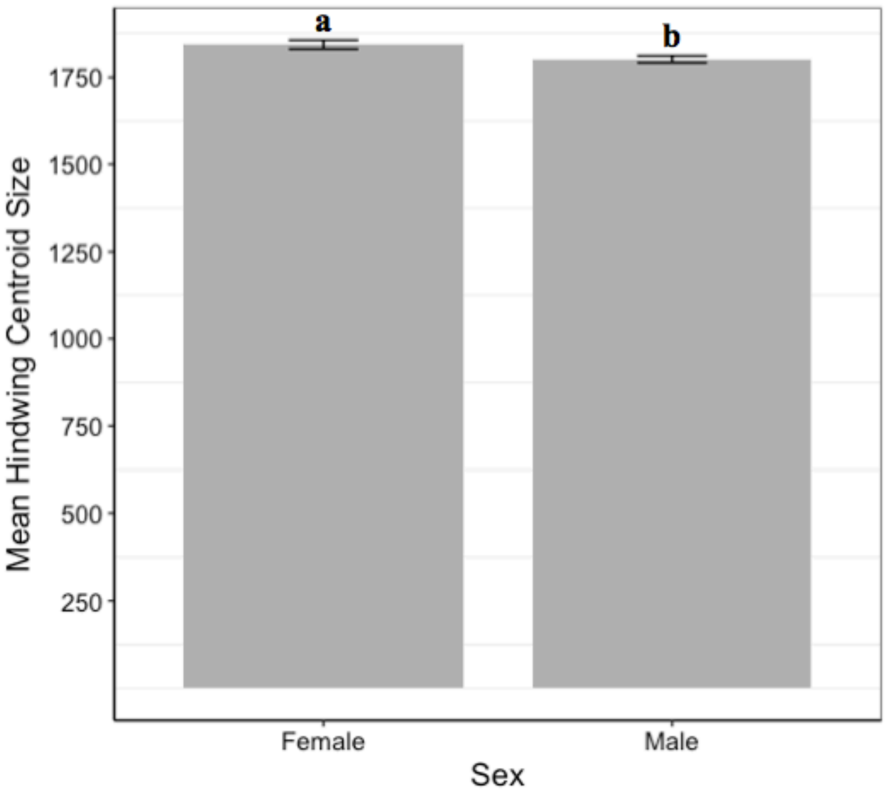
a
b
1750
1500
1250
1000
750
500
250
Mean Hindwing Centroid Size
Female
Male
Sex

Figure 2.11

CHAPTER 3

RELATIONSHIPS BETWEEN LOW-INTENSITY PRESCRIBED FIRE, SOUTHERN *IPS* BARK BEETLE OUTBREAKS, AND LOBLOLLY PINE (*PINUS TAEDA* L.) MORTALITY[2]

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[2] McNichol, B.H., C.R. Montes, B.F. Barnes, J.T. Nowak, B.T. Sullivan, C. Villari, and K.J.K. Gandhi. To be submitted to *Forest Ecology and Management*.

Abstract

The southern pine engraver species, *Ips avulsus* (Eichhoff), *I. calligraphus* (Germar), and *I. grandicollis* (Eichhoff), are considered secondary colonizers of stressed, damaged, and dying pine trees. However, they may undergo outbreaks and colonize live hosts if large-scale environmental conditions that cause physiological stress to trees are present. In 2016, >230 concurrent *Ips* infestations >2 ha in size were documented in Georgia. In these forests, prescribed burning is conducted every 2-3 years to reduce fuel-loads, improve wildlife habitat, and manage understory vegetation. However, burning may increase susceptibility of pines to insects and diseases, and effects of low-intensity prescribed fire on active southern *Ips* infestations are not well studied. Our objectives were to: 1) compare *Ips* infestations between burned versus unburned sites to determine the effects of prescribed fire on loblolly pine mortality; and 2) determine which site and tree-level variables were the best predictors of mortality. We monitored >830 pines on ten sites for eight months following prescribed fire. Overall, 69 (8%) trees died with 3.6 times higher tree mortality on unburned sites, and higher probability of survival on burned sites. At the site-level, binomial logistic regression models including treatment (burned versus unburned) and time since burn were the best predictors of loblolly pine mortality. At the tree-level, model selection showed that treatment, crown mortality level (1-5), *Ips* activity level (none, low, medium, high), and tree DBH provided the best predictions of mortality. Prescribe burning may thus help alleviate high pest pressure and increase tree resilience in pine forests in the southeastern U.S.

INDEX WORDS: Bark beetles, forest management, *Ips*, prescription fire, southeastern U.S.

3.1 Introduction

The southern pine bark beetle guild includes three species of pine engraver, the small southern pine engraver, *Ips avulsus* (Eichhoff); the six-spined ips, *I. calligraphus* (Germar); and the eastern five-spined ips, *I. grandicollis* (Eichhoff) (Stephen 2011). The three southern *Ips* colonize the phloem of southern pine species for feeding and reproductive activities (Yearian and Wilkinson 1967, Nebeker 2011). Unlike the more aggressive southern pine beetle (*Dendroctonus frontalis* Zimmermann), which can colonize live trees, the three southern *Ips* are typically secondary colonizers of the phloem of stressed, damaged, or dying southern pines (Wilkinson and Foltz 1982, Nebeker 2011). While *D. frontalis* infestations are characterized by expanding groups of contiguous live pines ("spots"), *Ips* infestations most often occur in a single host or a few trees, and are much slower in growth (Stone et al. 2007, Clarke 2012, Eickwort et al. 2015).

Factors including windstorms, wildfires, pathogens, and drought may render pine stands susceptible to *Ips* infestations (Connor and Wilkinson 1983, Bryant et al. 2006, Coyle et al. 2016). Numerous *Ips* species are reported to colonize host conifers during or following warmer and drier periods [e.g., *I. confusus* (LeConte) and *I. typographus* (L.)] (Bakke 1983, Breshears et al. 2005, Faccoli 2009, Floyd et al. 2009, Aakala et al. 2011). Widespread, prolonged drought during the growing season is frequently cited as one of the primary factors facilitating high southern *Ips* populations, leading to significant pine mortality in the southeastern U.S. (Wilkinson and Foltz 1982, Connor and Wilkinson 1983). However, most of these observations are anecdotal, as no studies have quantified pine mortality resulting from southern *Ips* infestations during drought periods, or used water deficit data to verify these relationships.

Moisture deficiency has been identified as one of the primary causative factors in decline of tree and forest health, weakening tree defenses and altering the fitness and survival of insects

(Fettig et al. 2007, Jactel et al. 2011). The *Pinus* species most susceptible to moisture stress during water deficit are often growing in shallow or clayey soils, may have shallower root networks, and experience competition from neighboring pines (Wilkinson and Foltz 1982, Kelsey et al. 2014). Trees growing in coarse soils that cannot access water during drought conditions can increase water movement in their rhizosphere by altering the density and hydraulic conductance of fine roots, or their rooting depth (McDowell et al. 2008). The ability of trees to control water loss during drought may explain their capacity for production of secondary compounds, predicting future vulnerability to additional drought, insects, or pathogens (Allen et al. 2010). Elevated temperatures can also increase water stress in forests independent of precipitation rates (Allen et al. 2010, Teskey et al. 2015), thus facilitating infestation of hosts by bark beetles.

Prescribed fire is a forest management tool that may reduce the likelihood of bark beetle infestations if the treatment is timed when beetles are not dispersing and minimizes injury to residual standing pines (Geiszler et al. 1984, Lombardero et al. 2006, Fettig et al. 2007). Regularly prescribing fire in pine-dominant stands limits the risk of damaging wildfires that weaken tree defenses and increase the chance of beetle infestations (Wilkinson and Foltz 1982). However, prescribed fire can still stress healthy pines and make them susceptible to *Ips* bark beetle attacks, particularly if the fire treatment causes bole char and resulting heat damage to phloem tissue (Lombardero et al. 2006, Fettig et al. 2007, Negrón et al 2016), crown scorch or consumption (Wallin et al. 2003, Bryant et al. 2006), or root injury or death (Geiszler et al. 1984, Bryant et al. 2006). Bark beetles may opportunistically colonize individual fire-injured hosts that are releasing attractive, stress-related volatiles, and thus act as a secondary contributor to pine mortality (Manion 1991, Regelbrugge and Conard 1993, McHugh and Kolb 2003, McDowell et al. 2008).

Previous research has found associations between prescribed burning and *Ips* bark beetle presence (Geiszler et al. 1984, Bradley and Tueller 2001, Campbell et al. 2008). *Ips* have been observed to preferentially mass attack dead or dying pines that were moderately to severely fire-damaged during prescribed burns, particularly small diameter pines (Davis et al. 2012, Fettig and McKelvey 2014, Negrón et al. 2016). Conversely, several authors have found no relationships between prescribe fire treatments and the subsequent level of bark beetle attraction and infestation (Sullivan et al. 2003, Elkin and Reid 2004, Lombardero et al. 2006). For instance, no evidence was found of *I. grandicollis* attraction to burned stands of longleaf pine (*P. palustris* Mill.), although this species of *Ips* is known to colonize recently burned trees (Sullivan et al. 2003). Similarly, fire damage to the boles of red pines (*P. resinosa* Aiton) did not influence the landing rates of *I. pini* and *I. grandicollis* (Lombardero et al. 2006), and prescribe fire damage did not influence landing behavior, which trees were infested, attack rate, or the reproductive success of mountain pine beetle (*D. ponderosae* Hopkins) (Elkin and Reid 2004). A lack of consistent relationships between prescribe fire and bark beetle infestation of recently burned hosts suggests that the degree of fire injury and physiology of individual trees (i.e., defensive response following a burn) may play a role in host attractiveness and colonization by bark beetles.

The southeastern U.S. experienced drought conditions in 2016 that accelerated beginning in June and became more severe in September and October (U.S. Drought Monitor 2017). As of the end of November 2016, 60% or more of the land area in Alabama, Mississippi, Georgia, and Tennessee was classified as being in a state of “exceptional drought” or “extreme drought” (U.S.

Drought Monitor 2017). However, it should be noted that the U.S. Drought Monitor classifies drought using the Palmer Drought Severity Index (PDSI), which features a water balance model that does not account for precipitation events occurring over short time scales, differences between potential versus actual evapotranspiration rates, or variation in the depth of soil moisture capacity (Alley 1984). Additionally, the PDSI features drought severity classes that are arbitrarily defined, and does not have a clear method to demarcate the beginning and end of a drought period (Alley 1984, Heddinghaus and Sabol 1991). These limitations make it difficult to determine specifics regarding local soil water balance and water availability, and their relationships to individual tree-level rooting characteristics and physiology. Thus, drought may not indicate availability of water to trees, and we should be cautious in assuming that severe drought unequivocally means water-stressed host pines were available.

Beginning in September 2016, over 300 southern *Ips* beetle infestations were recorded in the Oconee Ranger District of the Chattahoochee-Oconee National Forest (Eatonton, Georgia) during the drought period, and many others were later documented throughout North Georgia and the Southeast (P. Merten, personal communication, 8 February 2017). The infestations mostly occurred in loblolly pine (*P. taeda* L.) and resembled *D. frontalis* infestations with respect to intensity and growth, expanding concentrically outwards and in severe cases killing numerous hectares of pines. Sampling of several trees felled in the Oconee Ranger District revealed colonization by all five members of the southern pine bark beetle guild, with the highest densities of the three *Ips* species. Standing, symptomatic trees exhibited reddening and wilting of the needles, and had many entrance and exit holes and frass in the crevices of the bark. Almost none of the characteristic exudation of resin (“pitch tubes”) was observed at beetle entrance holes, indicating host tree defense mechanisms may have been reduced as a result of drought stress (Allen et al. 2010, Hain et al. 2011, Stephen 2011).

For the Oconee Ranger District, the Piedmont National Wildlife Refuge (Jasper and Jones Counties, Georgia), and several other managed forests in the Southeast experiencing southern *Ips* activity, the recommendation for winter 2017 was to avoid applying prescribed fire in stands with signs or symptoms of *Ips* to prevent potentially exacerbating *Ips* infestations (Georgia Forestry Commission 2017, P. Merten, personal communication, 8 February 2017; C. Schimdt, personal communication, 21 March 2017). However, because so few infestations of this magnitude have been reported in the Southeast (though such infestations have likely occurred), no one has previously investigated the effects of prescribed fire on active *Ips* infestations. Some authors have addressed the risk of southern pine mortality post-burn (Mann and Gunter 1960, Lilieholm and Hu 1987, Campbell et al. 2008), but there is no literature addressing the impact of prescribed fire on infestations of southern *Ips* beetles in loblolly pine stands.

Our aim was to assist with informing forester and landowner decisions on the use of prescribe fire in stands experiencing active southern *Ips* infestations. Our research objectives were to: 1) compare *Ips* infestations in burned versus unburned sites to determine the effects of low-intensity prescribe fire on beetle activity and loblolly pine mortality, and 2) assess which variables were the best overall predictors of loblolly pine mortality at the tree- and site-level. We hypothesized that there would be increased loblolly pine mortality on burned as compared to unburned sites. At the site-level, we expected basal area, treatment, and time since burn to be significant predictors of loblolly pine mortality. We anticipated that there would be interactions between treatment, the level of *Ips* activity, and the level of crown mortality, and that these variables would influence the probability of mortality at the tree-level. Further, we hypothesized there would be an interaction between tree DBH and *Ips* infestation, with increased beetle activity occurring in smaller-sized pines, as previously shown for *Ips* (e.g., Fettig and McKelvey 2014).

3.2 Methods

3.2.1 Study site and prescribed burn treatments

The study was conducted during 3 April – 14 November 2017 in the Bishop F. Grant Memorial Forest, located in Putnam and Morgan Counties in the east-central region of Georgia (33°25'N, 83°27'W), adjacent to the Oconee Ranger District (Figure 3.1). The 4,665-hectare forest is situated in the Piedmont region and features natural pines, pine plantation, mixed pine-hardwood, upland hardwood, and bottomland hardwood stands. Soils within the study site are primarily Davidson clay loam and loams (6-25% slopes) and Vance sandy loam (2-25% slopes) that range from moderately eroded to eroded as a result of previous agricultural activity (National Cooperative Soil Survey, USDA NRCS). In 2017, the local annual mean temperature was 18.4 ± 0.38 °C, and cumulative annual precipitation was 124.54 cm (PRISM Climate Group 2018). The Warnell School of Forestry and Natural Resources manages B.F. Grant Memorial Forest for teaching, outreach, and research in cooperation with the Georgia Department of Natural Resources, which utilizes the forest as a state Wildlife Management Area.

A total of 417 hectares were treated with prescribed fire in March 2017 (Figure 3.1). The primary management objectives of the burns were to: 1) control understory growth (primarily sweetgum, *Liquidambar styraciflua* L.); 2) reduce fuel loads; 3) encourage loblolly and shortleaf (*P. echinata* Mill.) pine growth; and 4) improve wildlife habitat for various species. The pine-dominant stands have not been on a regular burn rotation, but the forest managers are currently working to establish uniform three-year burn rotations throughout the forest (see Table 3.1 for more details regarding the previous management and burn history for study sites). Firebreaks were installed prior to all burns, and primarily backing fires were used in an effort to reduce the heavy fuel loads and minimize rapid movement of fire through stands. The burns were carefully supervised to maintain flame height around a maximum of 1 m when possible, although shifting wind patterns and high ground fuel loads did result in some bole char and needle scorch.

3.2.2 Experimental design

Ten sites that were actively experiencing *Ips* infestations or had signs of recent infestation were treated with prescribed fire or remained unburned in 2017 (five each of unburned and burned sites). A minimum of 500 m was present between each site (Table 3.1, Figure 3.1). In each site, three 0.08-ha (16 m radius) circular plots were placed along a transect line, with at least 75 m between each plot. A total of 30 plots were established in early April 2017 for the study, and plots were revisited in late May, July, September, and November 2017.

All trees within each plot that had a DBH >7 cm were measured and recorded, and pine trees were identified to species and marked with a unique tree tag to allow for temporal monitoring. The total number of dead trees in each plot at the initiation of the study was recorded. Pine basal area was calculated at the tree-level using the DBH measurements, summed over the three plots, and divided by total area (0.24 ha) to determine the mean basal area for each site (Table 3.1). For one each of dominant, co-dominant, and intermediate trees in each plot (nine total per site), cores were taken with an increment borer to estimate mean age within the site (Table 3.1). The same nine pines were measured with a clinometer to obtain an estimate of mean tree height at the site-level (Table 3.1). Overall, our 10 sites and 30 plots included 838 study trees (831 loblolly pines and 7 shortleaf pines). Given that the overwhelming majority of the trees were loblolly pines (99%), our analyses and models aimed to predict loblolly pine mortality.

For every tagged *Pinus* tree, crown class was recorded as suppressed, intermediate, co-dominant, or dominant. The overall crown mortality was assessed for each tree on a scale from 1 to 5: 1 = 0% mortality; 2 = 25% dieback (red and/or fading needles); 3 = 50% dieback; 4 = 75% dieback; and 5 = dead, 100% mortality. In plots occurring on the five burned sites, for each tree the maximum height of char on the bole was estimated, and needle scorch was marked as present or absent. Signs of *Ips* spp. activity on each tree were recorded as presence/absence of entrance and exit holes, frass, and pitch tubes. Based on the number of entrance and exit holes on the lower 2 m of the bole, trees were rated as having either no *Ips* activity (zero holes), low *Ips* activity (1-10 visible holes), medium *Ips* activity (11-20 visible holes), or high *Ips* activity (more than 20 visible holes).

Tree health assessments were performed on the tagged trees every ~6-8 weeks to record the live crown ratio, crown mortality rating, and to observe the current level of bark beetle activity. During each assessment, the total number of dead trees in each plot was counted and recorded, and temporal measurements continued until the fifth visit in November 2017, when no additional *Ips* activity was observed (i.e., infestations were no longer expanding and/or had collapsed).

3.2.3 Statistical Analyses

3.2.3.1 Survival analysis

To determine whether the burned and unburned treatments showed differences in loblolly pine survival, we performed a Kaplan-Meier survival analysis (Kaplan and Meier 1958) using the 'survival' package in R (Therneau 2015). The Kaplan-Meier estimator is a non-parametric statistic that can be used to estimate the probability of survival at various points in time. A log-rank test was applied to compare the survival curves of two groups (burned and unburned sites) over time (April, May, July, September, and November), and was visualized using the 'survminer' package (Kassambara and Kosinski 2018). All analyses were performed in R 1.0.136 (R Core Team 2016), and data were input using the 'readxl' package (Wickham and Bryan 2017).

3.2.3.2 Site-level predictors of loblolly pine mortality

To examine the influence of numerous explanatory variables on *Pinus* spp. mortality, previous studies have employed binary logistic regression models to predict expected tree mortality (Regelbrugge and Conard 1993, McHugh and Kolb 2003). Logistic regression is optimal for modeling probabilities because the model has a Bernoulli distribution (ranges from 0 to 1), and can use continuous or categorical explanatory variables to predict a binary response variable, such as whether a tree is alive or dead (Regelbrugge and Conard 1993). The full logistic regression model form is:

$$P_m = \frac{1}{1 + e^{-(\beta_0 + \beta_1 X_1 + \cdots + \beta_n X_n)}} \quad (1)$$

Where P_m is the probability of tree mortality, e is the natural logarithm base, β_0, β_1, and β_n are the estimated slope parameters (regression coefficients), and X_1 and X_n are the explanatory (independent) variables (Bradley and Tueller 2001, McHugh and Kolb 2003, Negrón et al. 2016).

To account for unmeasured variables that may have influenced overall observed mortality at the site-level (n = 10), we summarized the proportion of dead trees on each site during each month using the 'plyr' package in R (Wickham 2011), and used binary logistic regression models to determine the effects of the following predictor (independent) variables on loblolly pine mortality at the site-level: 1) treatment (burned or unburned); 2) site-level basal area; and 3) time since burn. The response (dependent) variable in Equation 1 for all models was tree status (alive = 0, dead = 1), which allowed for comparison among models. Models were developed to test how the levels of each predictor variable at time 1 influenced loblolly pine mortality (status) at time 2 (e.g., how variables in April affected mortality in May, and so on) (Figure 3.2). Logistic regression models were estimated using generalized linear models (GLMs) with the logit link function (Tsai and Gill 2013). We tested GLMs including each predictor variable individually, as well as the two-way and three-way interactions between variables. If interactions were not significant, they were removed and additive models including the main effects were tested.

3.2.3.3 Tree-level predictors of loblolly pine mortality

To determine if variables measured at the individual tree-level (n = 838) were important predictors of mortality, GLMs (with the logit link function) were used to estimate the binary logistic regression models. Models testing our hypotheses included combinations of the following predictor variables: 1) site; 2) treatment (burned or unburned); 3) level of *Ips* activity (none, low, medium, or high); 4) level of crown mortality (1-5; 1 = 0%, 5 = 100%); and 5) DBH (cm). Similar to the site-level analyses, the response variable for all of our models was tree status (alive = 0, dead = 1), which allowed for meaningful comparison of the relative ability of each model to predict individual loblolly pine mortality. Models were developed to test how the levels of each predictor variable at time 1 influenced loblolly pine mortality (status) at time 2 (Figure 3.2). Due to perfect collinearity between site and treatment that resulted in singularities in the GLM regression matrix (as each site had one associated treatment), these two predictors were not included in the same candidate models (Nielsen et al. 2004). An examination of the variance inflation factors for each model using the 'car' package in R revealed no further evidence of collinearity between predictor variables (Fox and Weisberg 2011).

We tested GLMs including each predictor variable individually, as well as the two-way, three-way, and four-way interactions between the predictors (site or treatment, *Ips* activity, crown mortality, and DBH) (R Core Team 2016). Where interactions were not significant, additive models including the main effects were tested. All significant logistic regression models associated with our tree-level hypotheses were evaluated using Akaike's Information Criterion (AIC), which is an information-theoretic approach to model selection (Burnham and Anderson 2002). AIC values are indicators of the performance of each model included in the comparison relative to the other candidate models (Wagenmakers and Farrell 2004). When all included model parameters are known, AIC values are calculated using the maximized log-likelihood:

$$AIC = -2\log\left(\mathcal{L}(\hat{\theta}|y)\right) + 2K \qquad (2)$$

Where $\log\left(\mathcal{L}(\hat{\theta}|y)\right)$ is the numeric log-likelihood value at its maximum point (corresponding to maximum likelihood estimates), and K is the number of estimable parameters in the model (Akaike 1973, Burnham and Anderson 2002). AIC model selection is a useful technique when comparing models with varying numbers of parameters because models receive a penalty as the number of parameters increases (Kane and Kolb 2010). After AIC values were determined for each model using Equation 2, the Δ AICs – corresponding to each value minus the lowest AIC value – were calculated. To allow for more clear interpretation of the relative likelihood of each of our candidate models, Akaike weights – which are distributed based on the Δ AIC for each model and give the most weight to the best candidate model – were determined using the following expression:

$$w_i = \frac{\exp(-\frac{1}{2}\Delta_i)}{\Sigma\exp(-\frac{1}{2}\Delta_i)} \qquad (3)$$

Where w_i is the Akaike weight, or the weight of evidence in favor of a given model *i* being the best (most likely), and Δ_i is the delta AIC value for that model (Burnham and Anderson 2002). For the model including the best combination of predictor variables (with the highest Akaike weight derived using Equation 3), predicted probabilities of loblolly pine mortality [referred to as P(dead) hereafter] were calculated for different levels of each predictor variable to determine the influence of each variable on mortality. Assessment of the contribution of individual predictor variables to overall P(dead) was important to isolate the relative importance of each variable. Because evaluating the model for every observed DBH from 8-55 cm would be exhaustive, to summarize the influence of DBH on P(dead), we selected three values to generalize the effect of tree diameter in the model: a low (10 cm), medium (25 cm – the mean for our observed data), and high (50 cm) DBH size-class.

3.3 Results

3.3.1 Survival Analyses

Of the 838 pines included in our study, 69 (8%) died by the final set of temporal measurements in November 2017, with 15 (22%) of the dead trees occurring on burned sites and 54 (78%) on unburned sites. The majority of loblolly pine mortality on unburned sites occurred prior to our surveys in May (n = 36), and most mortality on burned sites occurred preceding measurements in November (n = 8) (Figure 3.3a). Kaplan-Meier survival analyses showed there was a difference in the probability of survival of trees occurring on burned versus unburned sites (log-rank $p = 0.02$), with a lower probability of survival in the unburned sites during all survey months (Figure 3.3b).

The crown mortality level was 5 (100%) for all 69 trees that died, as they had stopped producing new green needles. Overall, there were a higher proportion of loblolly pines with crown mortality levels 3 and 4 on burned sites (Figure 3.4a,b). Seven of the dead trees (10%) had no signs of *Ips* activity, seven (10%) had low *Ips* activity, seven (10%) had medium *Ips* activity, and the remaining 48 (70%) had high *Ips* activity levels. In general, there were higher proportions of loblolly pines with a low level of *Ips* activity on the burned sites (Figure 3.5a,b). Interestingly, five out of the seven dead trees with no *Ips* activity died on the burned sites prior to November and had significant crown scorch, suggesting mortality of these trees may have resulted from the prescribed burn treatment or other factors. Trees that died during the study had DBH measurements ranging from 8-55 cm (mean ± SE 25 ± 0.35 cm).

3.3.2 Site-level predictors of loblolly pine mortality

We did not find any significant interactions between treatment, basal area, and time since burn (all *p*-values > 0.05). GLMs including only site-level basal area and only time since burn were not found to be good predictors of loblolly pine mortality ($p > 0.10$). However, the model including only the effect of treatment (burned versus unburned) on loblolly pine mortality was significant ($F_{1,38} = 37.53$, $p < 0.001$), with higher mortality occuring on unburned sites (Figure 3.3a,b). Additionally, a model including the main (additive) effects of treatment and time since burn was found to be significant ($F_{4,35} = 10.99$, $p < 0.001$), indicating temporal differences in mortality between the burned versus unburned treatment. Specifically, there was higher mortality in May on unburned sites, and higher mortality in November on burned sites (Figure 3.3a).

3.3.3 Model selection and influence of predictors on tree-level probability of loblolly pine mortality

None of the hypothesized interactions between predictor variables were significant; accordingly, logistic regression models selected for comparison included only the main effects (Table 3.2). Of all models tested, 14 models including individual predictor variables and the main effects between predictors were found to be significant, and were included in the model selection. Based on the Δ AIC values and associated Akaike weights (w_i), the best candidate model for our data included DBH, treatment, level of *Ips* activity, and level of crown mortality as predictors of loblolly pine mortality (AIC value = 202.25) (Table 3.2). The best model was given substantially more support than the other nine candidate models ($w_i = 0.99$), despite having the largest number of parameters ($K = 6$).

For every combination of crown mortality level, *Ips* activity level, and DBH, the P(dead) was higher for unburned than burned sites (Table 3.3, Figure 3.6). Increasing the value for DBH resulted in a higher overall P(dead), regardless of the crown mortality level, *Ips* activity level, or treatment (Table 3.3). However, the overall influence of DBH as a predictor of P(dead) was not very strong, indicated by the slope of the curves (Figure 3.7) (parameter estimates for the effect of DBH). The highest P(dead) occurred when crown mortality level was 5 and *Ips* activity was medium or high, regardless of DBH and treatment [all P(dead) ≥ 0.89] (Table 3.3). In general, increasing the level of *Ips* activity resulted in a higher P(dead) (Table 3.3, Figure 3.8). For burned treatments, when crown mortality level was 5 and *Ips* activity was low or none, P(dead) decreased considerably, particularly when DBH was low [e.g., P(dead) = 0.24 with no *Ips* activity and P(dead) = 0.63 with low *Ips* when DBH = 10 cm]. For unburned treatments with crown mortality level 5 and low to no *Ips* activity, all P(dead) were ≥ 0.84 [with the exception of P(dead) = 0.61 for no *Ips* activity and DBH = 10 cm] (Table 3.3).

When the crown mortality level was 4, for high levels of *Ips* activity, all P(dead) were ≥ 0.94, irrespective of treatment or DBH. However, decreases in *Ips* activity were associated with corresponding decreases in P(dead) for both treatments and there was an influence of DBH [e.g., for burned treatments with medium *Ips* activity, P(dead) = 0.46 for DBH = 10 cm, and P(dead) = 0.95 for DBH = 50 cm]. A significant drop was observed in P(dead) for unburned treatments when there was no *Ips* activity, but only for low and medium DBH [P(dead) = 0.14 when DBH = 10 cm, 0.35 when DBH = 25 cm, but 0.80 at DBH = 50 cm]. Similar trends were observed for P(dead) for unburned and burned treatments when the crown mortality level was 3. When *Ips* activity level was medium or low, the predictive model showed a significant decrease in P(dead) from crown mortality levels 4 to 3 [at level 3, all P(dead) ≤ 0.50] (Table 3.3). However, this threshold between crown mortality levels 3 and 4 is not as apparent when the level of *Ips* activity is high, suggesting *Ips* activity also has a substantial predictive influence on P(dead). Additionally, we observed that increasing the DBH at crown mortality level 3 when the *Ips* activity level was none only slightly influenced P(dead), suggesting the importance of DBH as a predictor of loblolly pine mortality is dependent on the levels of other predictors.

When the crown mortality level was 2, P(dead) < 0.001 for both treatments and all levels of DBH, indicating a threshold between crown mortality levels 3 and 2 [i.e., P(dead) is much lower when crown mortality is < 3 (50%)]. However, very large confidence intervals surrounding predicted probability estimates at this level of crown mortality indicate there is a lot of uncertainty associate with P(dead) when the crown mortality level is 2 (Table 3.3). Interestingly, P(dead) increased slightly when the crown mortality level was decreased to 1 and *Ips* activity level was high in the unburned sites, suggesting some pine mortality is associated with *Ips* activity [P(dead) = 0.25 at DBH = 25 cm and 0.71 at DBH = 50 cm]. Altering the values of each predictor variable showed that for our study, crown mortality was the overall most important predictor of the probability of loblolly pine mortality (despite uncertainty at crown mortality level 2 indicated by the large confidence intervals).

3.4 Discussion

The three southern *Ips* species typically infest hosts in a dispersed pattern, attacking the most stressed and compromised trees in a given stand (Bryant et al. 2006, Stone et al. 2007, Eickwort et al. 2015). Accordingly, large-scale southern *Ips* outbreaks are relatively uncommon, and there are currently no published studies that provide data on the interactions between *Ips* bark beetle activity, prescribed fire, and loblolly pine mortality. Our study aimed to fill this gap by monitoring active *Ips* infestations and loblolly pine mortality in stands treated with prescribed fire versus unburned stands, and determining the best predictors of tree mortality using quantitative tree-level and site-level measurements. We found that the majority of pine mortality during our study occurred on unburned sites, and in general, trees that succumbed had high levels of *Ips* activity (evidenced by ≥ 20 entrance/exit holes and lots of visible frass) and ~100% crown mortality.

Previously, *Ips* beetles have been observed to preferentially mass attack pines with moderate to severe fire damage that are dead or dying (Geiszler et al. 1984, Hanula et al. 2002, Wallin et al. 2003, Negrón et al. 2016). We found that the probability of survival was higher on burned sites during all months following the study initiation of our study, and no support was found for increased southern *Ips* attraction and infestation of hosts occurring on burned sites, as found in other studies (Sullivan et al. 2003, Lombardero et al. 2006). We recorded a higher proportion of loblolly pines with crown mortality levels 3 and 4 on burned sites. The observation that many of these trees survived indicates some of the crown dieback on burned sites may have occurred as a result of heat damage during the prescribed fire treatments, but did not damage the pines enough to kill them. We also found higher proportions of loblolly pines with low levels of *Ips* activity on the burned sites, which indicates that *Ips* beetles may have been unsuccessful in attacking some of the trees in burned sites, or prescribed fire may have increased host resilience by reducing competition and/or increasing defensive compounds (Wallin et al. 2003, Hood et al. 2015).

While our study used low-intensity prescribed fire treatments that were carefully monitored, some mature pines had significant bole char (4-6 m high) and needle scorch in the lower portions of their crowns post-burn. Additionally, there were a few (4-5) suppressed, low-DBH pines with no observable southern *Ips* activity that may have died as a result of the prescribed fire (Fettig and McKelvey 2014). Nonetheless, we observed 78% of loblolly pine mortality on unburned rather than burned sites. This result is particularly compelling because the burned sites have not been on a regular 2-3 year burn rotation and had increased build-up of ground fuels, which may have resulted in more intense burns and basal cambial damage (e.g., one site had not been burned in the last 20 years, and the other four sites were burned between 2003-2006) (McNab 1977, Varner et al. 2005, Obrien et al. 2010).

Our experimental design was limited in that we were not able to collect any pre-fire data on individual tree condition and the level of *Ips* infestation, however we began monitoring 2-3 weeks post-burn to capture any sizeable growth in *Ips* activity and expansion of infested host pines. Thus, while we cannot conclude that the prescribed fire treatments did not contribute to loblolly pine mortality, these low-intensity prescribed burns did not cause significant additional mortality within active southern *Ips* infestations. Another caveat of our experimental design was that we were not able to fell *Ips*-infested trees to verify which species were present, and we generalized observed activity of the three southern *Ips*. However, the three species have different life histories and tend to colonize different sections of the bole; although all three southern *Ips* species were observed in the pines felled during our preliminary surveys, it should be noted that our sampling technique could not account for additional *Ips* activity in the upper bole and crown of infested trees.

Our finding that site-level basal area was not a good predictor of loblolly pine mortality is supported by a previous study that found no clear relationship between basal area and the susceptibility of Jeffrey pines (*P. jeffreyi* Grev. & Balf.) to *Ips* spp. infestations and resulting increases in mortality (Bradley and Tueller 2001). We found no interactions between any of our tree-level predictors of loblolly pine mortality, but perhaps the most surprising finding was the lack of an interaction between the level of *Ips* activity and host tree DBH. In a study that implemented thinning and prescribed fire treatments in ponderosa pine (*P. ponderosa* Douglas ex C. Lawson) ecosystems, almost all pine mortality caused by *Ips pini* (Say) infestation occurred on burned sites in the smallest DBH class (19-29 cm) (Fettig and McKelvey 2014). Another study found that various *Ips* spp. occurred at higher proportions in *P. ponderosa* hosts with DBH < 32 cm as compared to larger-DBH pines following wildfires (Negrón et al. 2016).

Both findings suggest some *Ips* spp. may preferentially select hosts in smaller DBH classes post-burn, perhaps to avoid competition with other insects, or because these trees may be more stressed following fire (smaller pines have thinner bark and thus, are less well-protected from fire). However, for *I. calligraphus*, new progeny adults have been observed to emerge 3-5 days earlier when they developed in thicker phloem at various temperatures (Haack et al. 1987), suggesting that *Ips* colonization in larger DBH hosts with thicker phloem may be advantageous to accelerate brood development. For our study, the best candidate model predicted higher probabilities of loblolly pine mortality with increasing DBH on both burned and unburned sites. It is also likely that these trees may have either been over-mature, or more vulnerable due to disease or another unmeasured physiological stressor.

While 70% of the dead trees in our study had high levels of *Ips* activity, indicating that the degree of tree-level *Ips* infestation is a contributor to observed mortality, it is important to note that there were potentially other factors contributing to loblolly pine mortality that were not measured in this study. For example, virulent blue-staining (Ascomycetes: Ophiostomatales) fungi assist many bark beetle species with colonization by helping to overwhelm the defenses of conifer hosts (Lieutier et al. 2009). The three southern *Ips* species have been shown to carry the blue-stain fungus species *Ophiostoma ips* (Rumbold) Nannfeldt on their exoskeletons as well as on phoretic mites that hitchhike on the beetles (Gouger et al. 1975, Klepzig et al. 2001, Stephen 2011). Additionally, there may have been other pathogens (e.g., *Phytophthora* spp.) present in the clayey, poorly drained soils that could increase the physiological stress of loblolly pines and their susceptibility to infestation by the southern *Ips* beetles.

Our model selection results show that tree DBH, treatment, level of *Ips* activity, and level of crown mortality were all important predictors of the probability of mortality for an individual loblolly pine. We decided to use categorical (rather than continuous) predictor variables in the models because: 1) categorical measurements are easier to visually assess; 2) categories are more intuitive (e.g., ranking crown mortality levels from 1 – 5, rather than trying to estimate 45% dieback, for example); and 3) categorical rankings do not require specialized training for personnel or expensive equipment (e.g., a laser rangefinder or hyperspectral imaging camera to quantify crown mortality). The tradeoffs of our decision to use categorical variables are that they are somewhat subjective (e.g., two users may interpret the cutoff between level 2 versus level 3 crown mortality differently), and the levels of *Ips* activity were determined using visual assessments primarily in the basal two meters of the tree (i.e., additional *Ips* activity in the crown may have been unobservable). We took these crown mortality measurements over time to enhance our ability to account for the gradual fading of foliage over time and to ensure that we recorded the most accurate levels of tree mortality possible.

Based on our findings, in particular the evident thresholds in the probabilities of loblolly pine mortality predicted by the best candidate model, low-intensity prescribed fire may be a viable forest management tool in stands experiencing active southern *Ips* infestations, unless: A) ≥ 50% of the crowns of numerous loblolly pines have fading or red needles; or B) on pines with significant crown mortality, there is a medium to high level of *Ips* activity, observable via the presence of frass and many entrance/exit holes (~10 holes per 0.1 m^2) on the basal 2 meters. As an additional precautionary measure, the use of prescribed fire may be avoided in *Ips*-infested stands that have older, higher-DBH (≥ 50 cm) loblolly pines, as these trees may be more susceptible to other stressors or damage due to their mature age.

Future research needs to address the implementation of other common management techniques in southeastern pine-dominant forests (e.g., fertilization treatments, thinning, clearcuts) during active southern *Ips* infestations to determine their impact on beetle population levels. Moreover, as silvicultural treatments (i.e., thinning and harvests) that produce slash and logging debris are known to attract and promote colonization of downed materials by the southern *Ips*, additional studies should also assess whether such treatments may also increase the rate of infestation in live, standing pines during ongoing infestations.

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Table 3.1. Summary of forest management activities, prescribed fire history, and stand characteristics for the ten study sites in 2017.

Site (burn date)	Last Recorded Burn	Prior Management	Pine BA (m^2/ha)	Mean Pine DBH (cm ± SE)	Mean Pine Age (yrs. ± SE)	Mean Pine Height (m ± SE)	Latitude and Longitude
1 (burned 7 March 2017)	2003	N/A	22.64	46 ± 3.3	68 ± 5.4	33 ± 6.0	32°26.1'N, 83°26.4'W
2	Before 2003	Thinned in 2016	22.72	21.5 ± 0.47	26 ± 4.5	17 ± 6.8	33°26.0'N, 83°27.8'W
3	Before 2003	Thinned in 2016	23.84	21.5 ± 0.47	23 ± 0.9	16 ± 3.1	33°26.1'N, 83°27.8'W
4 (burned 20 March 2017)	Before 1997	Thinned in 2012	21.40	24 ± 0.84	31 ± 1.7	20 ± 5.3	33°25.8'N, 83°28.4'W
5	Before 2003	Thinned in 2015	17.54	26.5 ± 1.05	40 ± 3.1	21 ± 3.9	33°23.8'N, 83°28.9'W
6	Before 2003	Thinned in 2015	20.14	25 ± 0.82	28 ± 1.2	21 ± 3.0	33°23.9'N, 83°28.3'W
7	2012	Thinned in 2008	20.76	24 ± 0.65	29 ± 1.1	16 ± 3.2	33°24.2'N, 83°27.5'W
8 (burned 21 March 2017)	2004	Thinned in 2013	14.92	29 ± 1.22	37 ± 1.0	31 ± 2.1	33°25.7'N, 83°26.0'W
9 (burned 21 March 2017)	2004	N/A	15.19	26 ± 2.08	52 ± 5.0	30 ± 9.5	33°26.1'N, 83°26.2'W

10 (burned 21 March 2017)	2006	N/A	19.51	26 ± 1.89	48 ± 4.4	29 ± 5.5	33°25.5’N, 83°25.8’W

Table 3.2. List of all significant candidate models predicting individual loblolly pine mortality during active *Ips* infestations, ranked by their AIC value.

Model*	***K***	**AIC**	**Δ AIC**	***w_i***
Status_{T2} ~ DBH + treatment + Ips_{T1} + CM_{T1}	6	202.25	0.00	0.99
Status_{T2} ~ treatment + Ips_{T1} + CM_{T1}	5	211.22	8.98	0.01
Status_{T2} ~ DBH + Ips_{T1} + CM_{T1}	5	215.86	13.61	0.00
Status_{T2} ~ Ips_{T1} + CM_{T1}	4	240.23	37.98	0.00
Status_{T2} ~ DBH + CM_{T1} + treatment	5	285.85	83.60	0.00
Status_{T2} ~ CM_{T1}+ treatment	4	315.93	113.68	0.00
Status_{T2} ~ DBH + CM_{T1}	4	338.91	136.67	0.00
Status_{T2} ~ CM_{T1}	3	415.01	212.76	0.00
Status_{T2} ~ Site +Ips_{T1}	4	513.18	310.94	0.00
Status_{T2} ~treatment +Ips_{T1}	4	540.43	338.18	0.00
Status_{T2} ~ DBH +Ips_{T1}	4	555.45	353.20	0.00
Status_{T2} ~Ips_{T1}	3	564.71	362.46	0.00
Status_{T2} ~Site	3	1520.93	1318.68	0.00
Status_{T2} ~treatment	3	1603.36	1401.11	0.00

*T1 = time 1; T2 = time 2; Status = tree alive (0) or dead (1); *Ips* = level of *Ips* activity; CM = crown mortality level; Treatment = burned or burned; Site = ten total, five burned/five unburned. *K* is the number of parameters for each model. All significant models were included in model selection, and are ranked above by their Akaike's Information Criteria (AIC) and Δ AIC scores. The best candidate model has the smallest AIC and the highest Akaike weight (w_i). No significant interactions were found; all models included only the main effects between predictor variables (additive).

Table 3.3. Predicted probabilities of loblolly pine mortality for burned versus unburned sites with 95% confidence intervals, based on the best predictive model including crown mortality level, *Ips* activity level, DBH, and treatment as independent variables.

Crown mortality level[a]	***Ips* activity level**	**DBH**[b] **(cm)**	**P(dead)**[c]**, burned**	**Lower bound, burned**	**Upper bound, burned**	**P(dead)**[C]**, unburned**	**Lower bound, unburned**	**Upper bound, unburned**
5	high	10	0.993	0.909	1.000	0.999	0.979	1.000
5	high	25	0.998	0.971	1.000	1.000	0.994	1.000
5	high	50	1.000	0.994	1.000	1.000	0.999	1.000
5	medium	10	0.892	0.638	0.975	0.977	0.893	0.995
5	medium	25	0.964	0.832	0.993	0.993	0.962	0.999
5	medium	50	0.995	0.949	1.000	0.999	0.990	1.000
5	low	10	0.635	0.320	0.866	0.899	0.682	0.974
5	low	25	0.851	0.607	0.955	0.967	0.884	0.991
5	low	50	0.976	0.862	0.996	0.995	0.972	0.999
5	none	10	0.238	0.111	0.438	0.615	0.348	0.827
5	none	25	0.505	0.262	0.746	0.840	0.630	0.942
5	none	50	0.881	0.535	0.979	0.974	0.863	0.996
4	high	10	0.940	0.506	0.996	0.988	0.836	0.999
4	high	25	0.981	0.785	0.999	0.996	0.950	1.000
4	high	50	0.997	0.956	1.000	0.999	0.992	1.000
4	medium	10	0.462	0.165	0.789	0.815	0.521	0.947
4	medium	25	0.738	0.381	0.928	0.935	0.784	0.983

4	medium	50	0.953	0.711	0.994	0.990	0.939	0.999
4	low	10	0.153	0.048	0.392	0.481	0.215	0.759
4	low	25	0.372	0.161	0.647	0.752	0.536	0.889
4	low	50	0.811	0.462	0.955	0.956	0.848	0.989
4	none	10	0.031	0.013	0.072	0.142	0.068	0.276
4	none	25	0.096	0.044	0.197	0.352	0.223	0.508
4	none	50	0.434	0.137	0.788	0.797	0.504	0.938
3	high	10	0.410	0.038	0.925	0.781	0.163	0.985
3	high	25	0.695	0.126	0.973	0.921	0.427	0.995
3	high	50	0.943	0.476	0.997	0.988	0.833	0.999
3	medium	10	0.037	0.007	0.184	0.165	0.034	0.525
3	medium	25	0.112	0.021	0.423	0.393	0.111	0.771
3	medium	50	0.477	0.090	0.893	0.824	0.377	0.973
3	low	10	0.008	0.001	0.050	0.040	0.006	0.209
3	low	25	0.026	0.005	0.130	0.120	0.026	0.411
3	low	50	0.161	0.025	0.584	0.495	0.138	0.858
3	none	10	0.001	< 0.001	0.008	0.007	0.001	0.038
3	none	25	0.005	0.001	0.023	0.024	0.005	0.100
3	none	50	0.033	0.005	0.204	0.150	0.027	0.527
2	high	10	< 0.001	< 0.001	1.000	< 0.001	< 0.001	1.000
2	high	25	< 0.001	< 0.001	1.000	< 0.001	< 0.001	1.000
2	high	50	< 0.001	< 0.001	1.000	< 0.001	< 0.001	1.000

2	medium	10	< 0.001	< 0.001	1.000	< 0.001	< 0.001	1.000
2	medium	25	< 0.001	< 0.001	1.000	< 0.001	< 0.001	1.000
2	medium	50	< 0.001	< 0.001	1.000	< 0.001	< 0.001	1.000
2	low	10	< 0.001	< 0.001	1.000	< 0.001	< 0.001	1.000
2	low	25	< 0.001	< 0.001	1.000	< 0.001	< 0.001	1.000
2	low	50	< 0.001	< 0.001	1.000	< 0.001	< 0.001	1.000
2	none	10	< 0.001	< 0.001	1.000	< 0.001	< 0.001	1.000
2	none	25	< 0.001	< 0.001	1.000	< 0.001	< 0.001	1.000
2	none	50	< 0.001	< 0.001	1.000	< 0.001	< 0.001	1.000
1	high	10	0.020	0.001	0.282	0.094	0.005	0.684
1	high	25	0.062	0.004	0.506	0.254	0.021	0.845
1	high	50	0.324	0.032	0.876	0.711	0.147	0.972
1	medium	10	0.001	< 0.001	0.008	0.006	0.001	0.041
1	medium	25	0.004	0.001	0.022	0.018	0.003	0.098
1	medium	50	0.026	0.004	0.164	0.120	0.021	0.467
1	low	10	< 0.001	< 0.001	0.002	0.001	< 0.001	0.009
1	low	25	< 0.001	< 0.001	0.004	0.004	0.001	0.019
1	low	50	0.006	0.001	0.027	0.028	0.007	0.108
1	none	10	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.001
1	none	25	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.003
1	none	50	0.001	< 0.001	0.005	0.005	0.001	0.020

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[a] Crown mortality levels: 1 = 0%, 2 = 25%, 3 = 50%, 4 = 75%, 5 = 100%

[b] DBH: mean = 25 cm, 10 and 50 cm selected to show changes in P (dead) with a low and high DBH relative to the mean (DBH range = 8-55 cm).

[c] P(dead) = predicted probability of loblolly pine mortality, rounded to three decimal places. Upper and lower bounds are 95% confidence intervals around probabilities, and are asymmetric due to inverse logit transformation (i.e., for error associated with binomial logistic regression to follow a normal distribution).

Figure Legend

Figure 3.1. Map showing the locations of B.F. Grant Memorial Forest in the state of Georgia, the ten study sites within B.F. Grant Memorial Forest, and the 2017 prescribed burns. Credit: Dustin Thompson, Forest Resources Manager, B.F. Grant Memorial Forest.

Figure 3.2. Conceptual model demonstrating the relationships tested by logistic regression models regarding the best combinations of predictors of tree-level probability of loblolly pine mortality. Variables in gray circles are predictors, and while tree status (alive = 0, dead = 1), shown in the black boxes, is the response variable.

Figure 3.3. a) Mean (± SE) percent loblolly pine mortality that occurred during temporal monitoring on burned and unburned sites; b) Kaplan-Meier estimation curves for the probability of loblolly pine survival in burned and unburned stands.

Figure 3.4. Temporal changes in the proportions of loblolly pine trees with each level of crown mortality (1 = 0%, 2 = 25%, 3 = 50%, 4 = 75%, and 5 = 100%) for: a) burned sites; b) unburned sites (note scale break on y-axis to show variation between months).

Figure 3.5. Temporal changes in the proportions of loblolly pine trees with each level of *Ips* bark beetle activity (none, low, medium, or high) for: a) burned sites; b) unburned sites (note scale break on y-axis to show variation between months).

Figure 3.6. Probability of mortality (0 = alive, 1 = dead) of individual loblolly pines (n = 838) on burned versus unburned sites in November (end of study), including the mean (± SE) probability of tree mortality for each treatment.

Figure 3.7. Probability of mortality (0 = alive, 1 = dead) of individual loblolly pines (n = 838) in November (end of study) as compared to diameter at breast height (DBH), labeled by treatment (burned versus unburned). Curves show the parameter estimates associated with DBH for burned (solid line) and unburned (dashed line) provided by the model.

Figure 3.8. Probability of mortality (0 = alive, 1 = dead) of individual loblolly pines (n = 838) in November as compared to *Ips* activity level, labeled by treatment (burned versus unburned) and including the mean (± SE) probability of mortality for each *Ips* activity level. No loblolly pines had a medium level of activity at the end of the study.

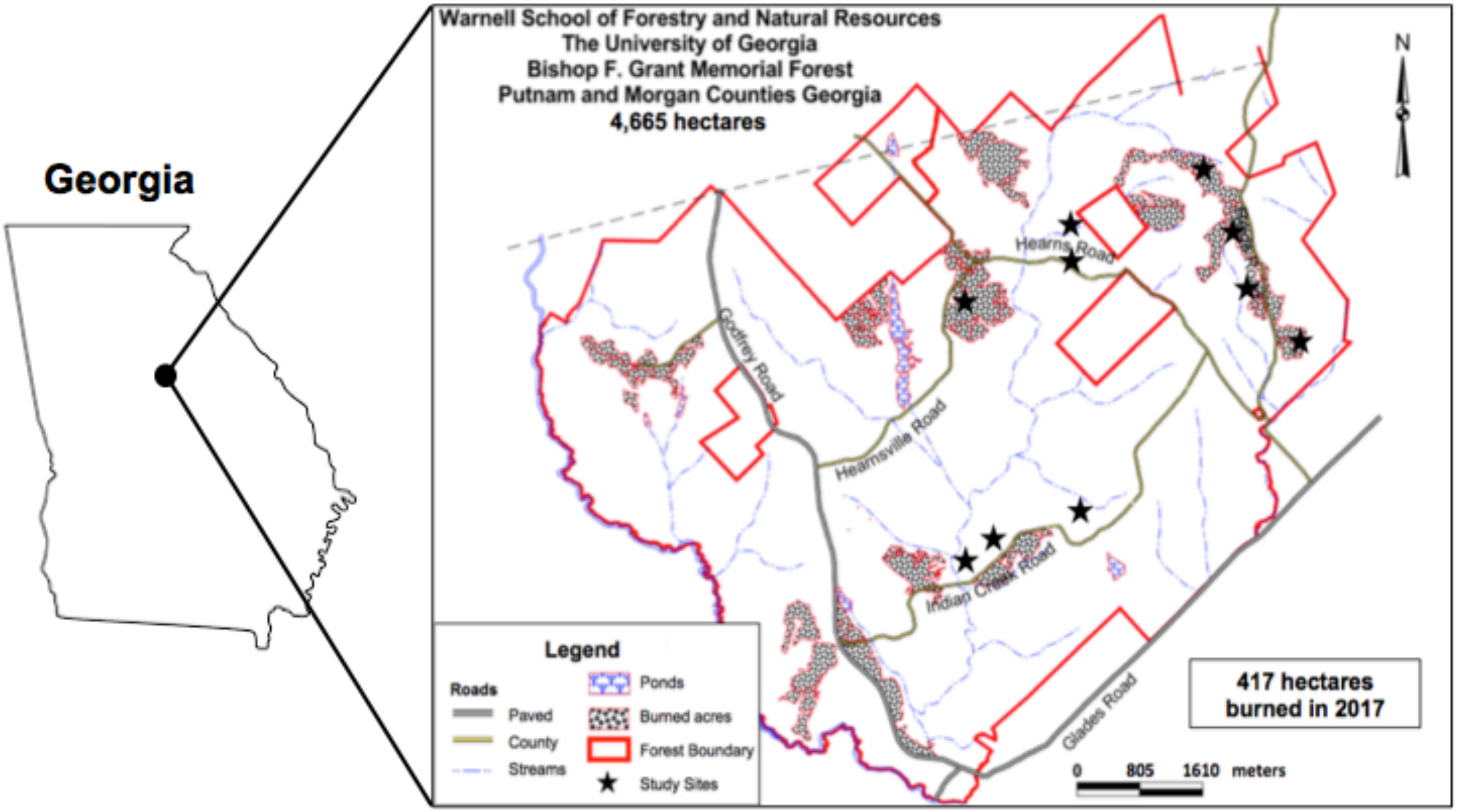
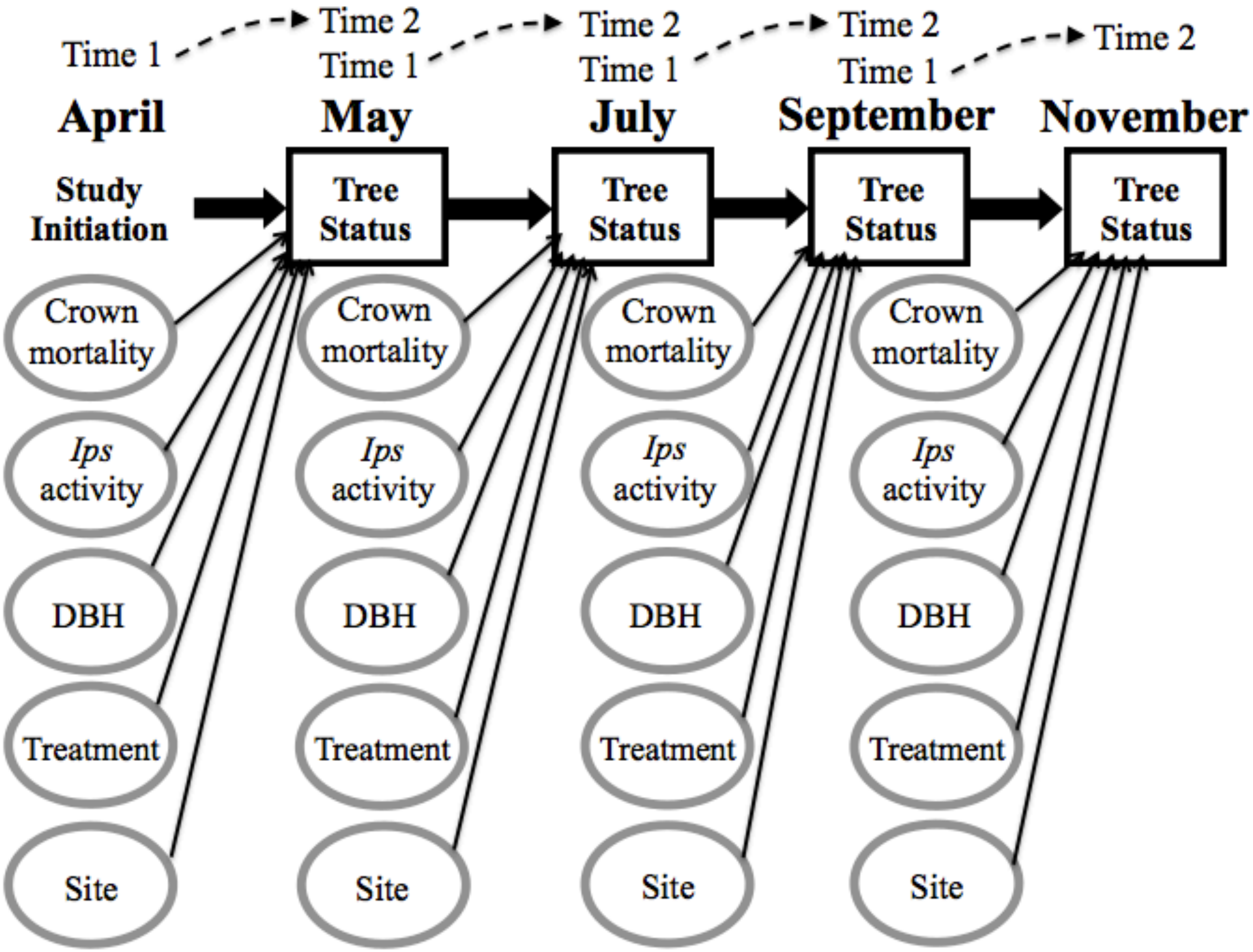

Georgia
Warnell School of Forestry and Natural Resources
The University of Georgia
Bishop F. Grant Memorial Forest
Putnam and Morgan Counties Georgia
4,665 hectares
N
Hearns Road
Godfrey Road
Hearnsville Road
Indian Creek Road
Glades Road
417 hectares burned in 2017
0 805 1610 meters
Legend
Roads
Paved
County
Streams
Ponds
Burned acres
Forest Boundary
Study Sites

Figure 3.1


Time 1
Time 2
Time 1
Time 2
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Time 2
Time 1
Time 2
April
May
July
September
November
Study Initiation
Tree Status
Tree Status
Tree Status
Tree Status
Crown mortality
Ips activity
DBH
Treatment
Site
Crown mortality
Ips activity
DBH
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Site
Crown mortality
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Crown mortality
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Treatment
Site

Figure 3.2

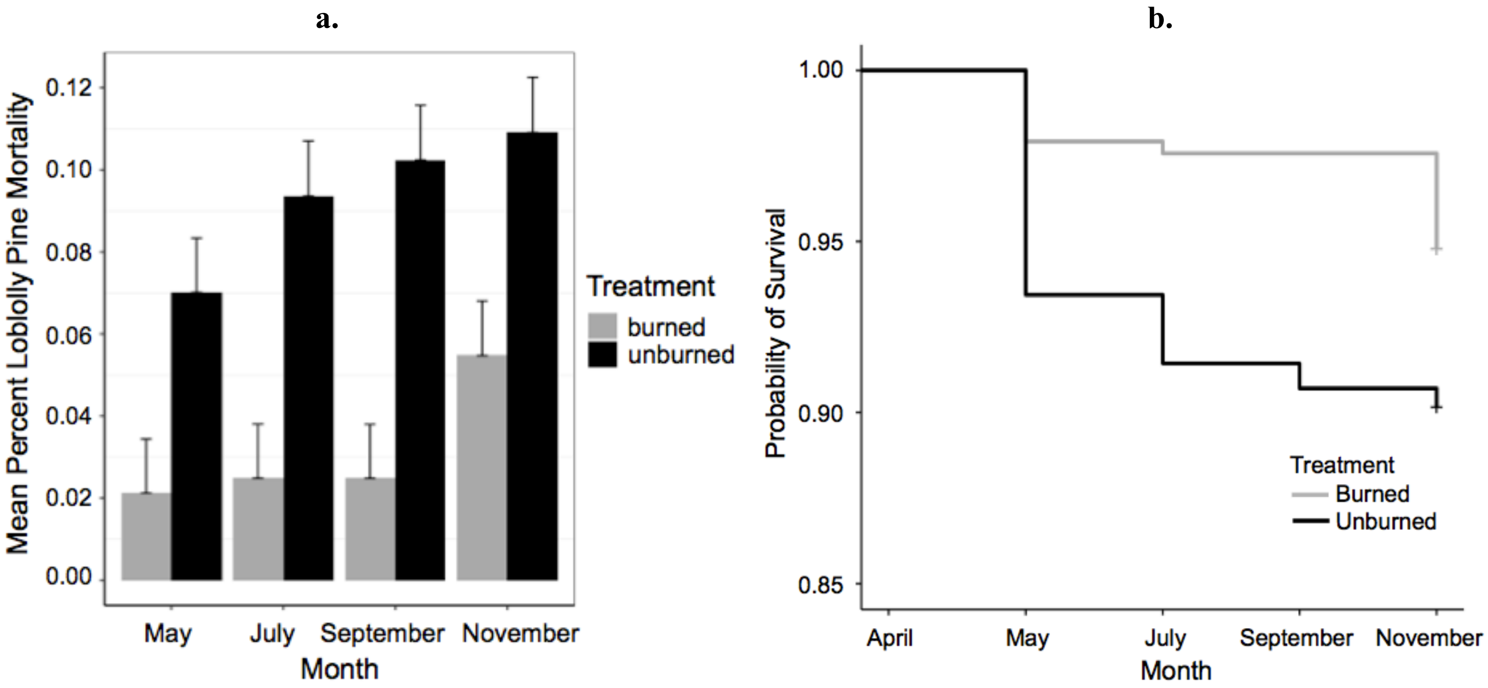
a.
b.
Mean Percent Loblolly Pine Mortality
0.12
0.10
0.08
0.06
0.04
0.02
0.00
May
July
September
November
Month
Treatment
burned
unburned
Probability of Survival
1.00
0.95
0.90
0.85
April
May
July
September
November
Month
Treatment
Burned
Unburned

Figure 3.3

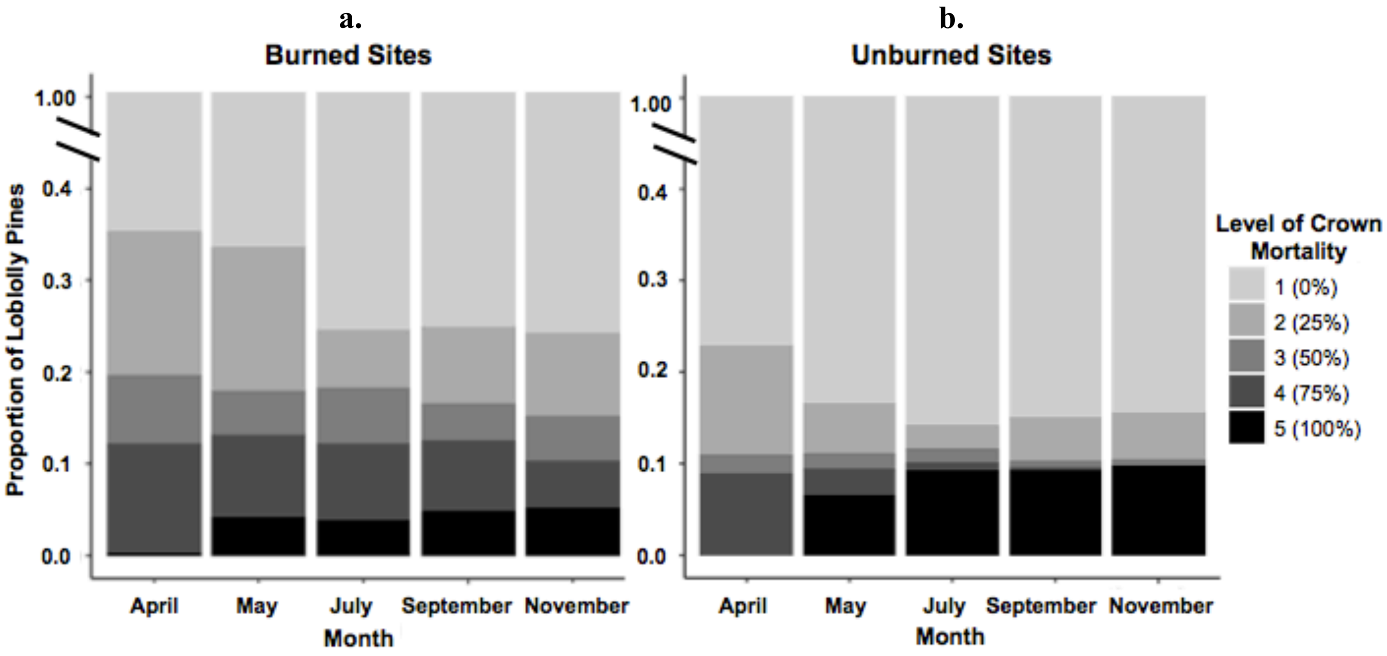
a.
Burned Sites
b.
Unburned Sites
Proportion of Loblolly Pines
1.00
0.4
0.3
0.2
0.1
0.0
April
May
July
September
November
Month
Level of Crown Mortality
1 (0%)
2 (25%)
3 (50%)
4 (75%)
5 (100%)

Figure 3.4

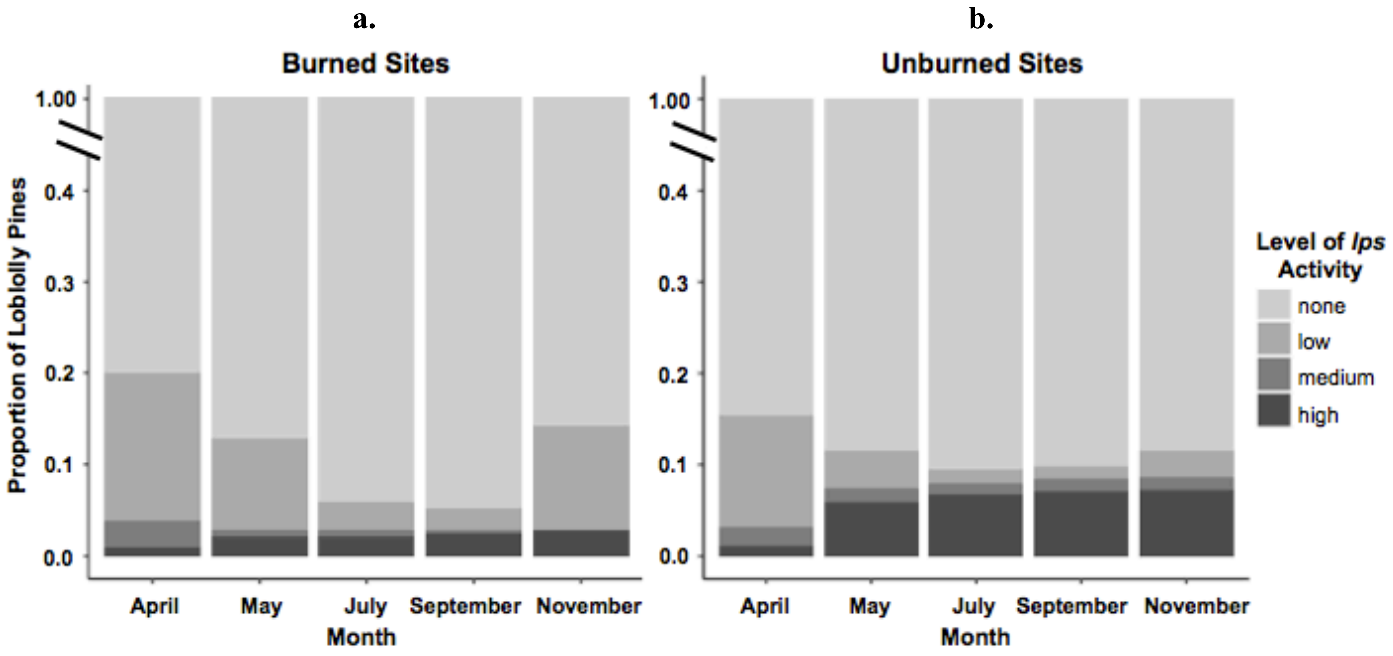
a.
b.
Burned Sites
Unburned Sites
Proportion of Loblolly Pines
1.00
0.4
0.3
0.2
0.1
0.0
April
May
July
September
November
Month
Level of *Ips* Activity
none
low
medium
high

Figure 3.5

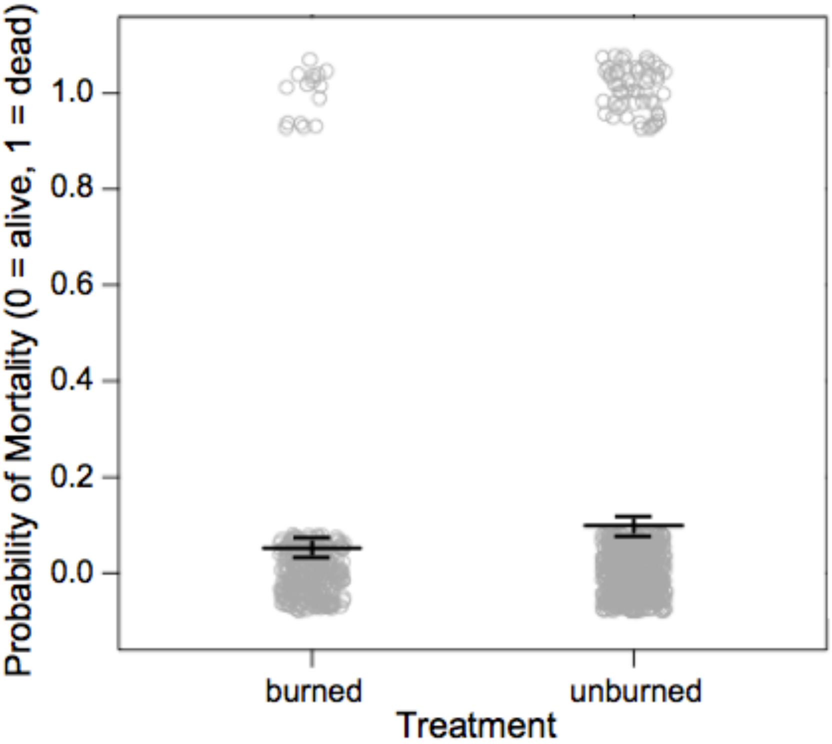
Probability of Mortality (0 = alive, 1 = dead)
1.0
0.8
0.6
0.4
0.2
0.0
burned
unburned
Treatment

Figure 3.6

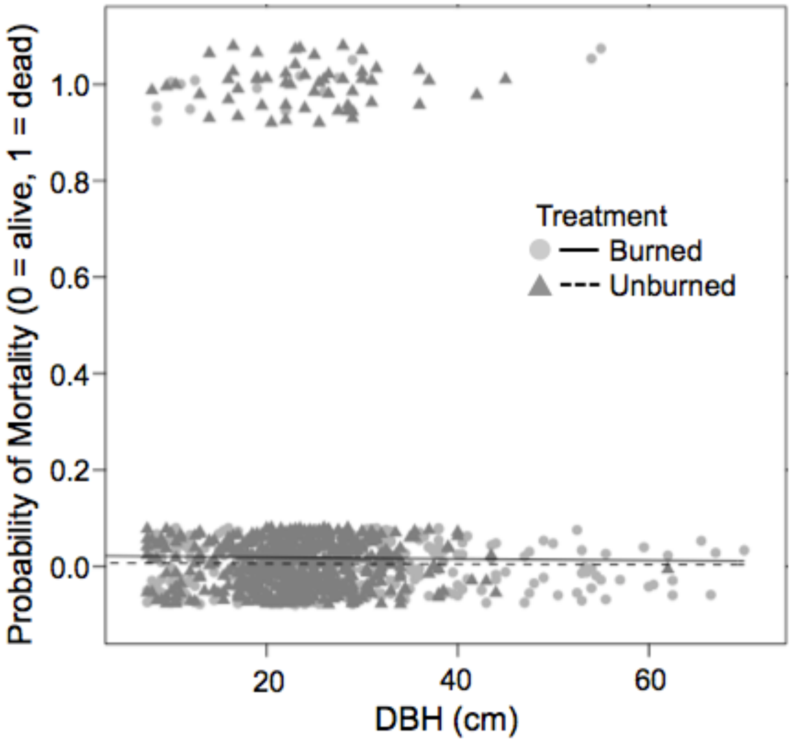
Probability of Mortality (0 = alive, 1 = dead)
1.0
0.8
0.6
0.4
0.2
0.0
20
40
60
DBH (cm)
Treatment
● — Burned
▲ --- Unburned

Figure 3.7

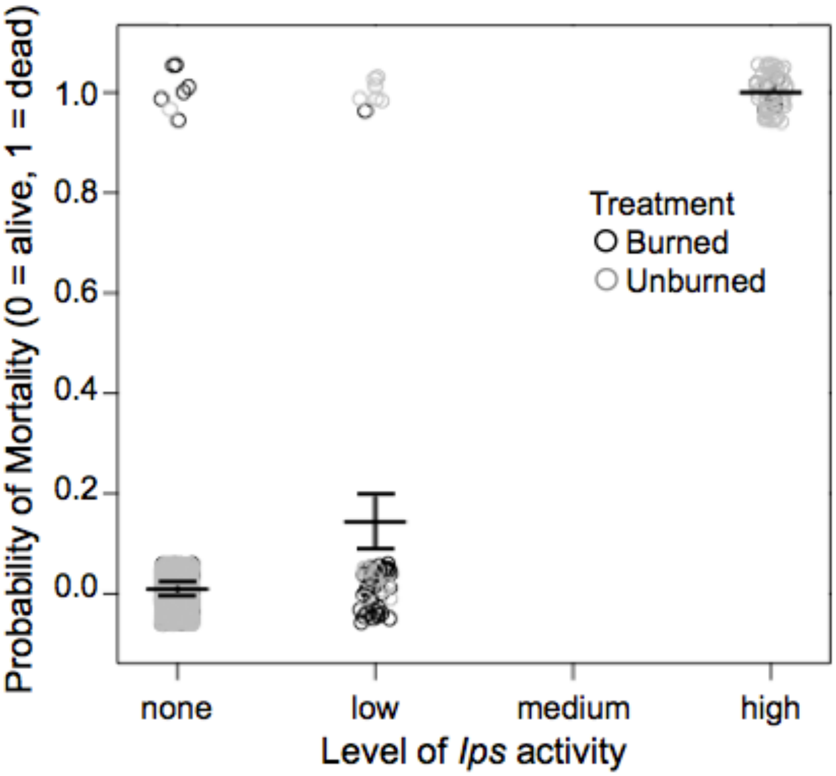
Probability of Mortality (0 = alive, 1 = dead)
1.0
0.8
0.6
0.4
0.2
0.0
Treatment
○ Burned
○ Unburned
none
low
medium
high
Level of *Ips* activity

Figure 3.8

CHAPTER 4

THESIS CONCLUSIONS

4.1 Conclusions

As weather and climate events that cause physiological stress to pine trees in the southern and eastern United States (e.g., warmer temperatures, precipitation anomalies, and storms) become more frequent and intense, it is more important now than ever to improve our understanding of what drives bark beetle population dynamics. This thesis broadly aimed to explore biotic and abiotic factors related to the ability of four members of the southern pine bark beetle guild to successfully colonize, reproduce, and initiate infestations in southern pine trees.

The second chapter examined phenotypic variation in southern pine beetle (*Dendroctonus frontalis* Zimmermann) (SPB) populations collected from six states across the southeastern U.S. Our objectives were to quantify variation in body size and hindwing shape between non-outbreak and outbreak SPB population phases, to determine if this variation was present between males and females, and to assess differences in the captured proportion of female SPB between the two population phases. We hypothesized that: 1) SPB in outbreak phase would be larger in size; 2) there would be sexual size dimorphism, with female SPB being larger than males; and 3) there would be a higher proportion of females in non-outbreak traps as compared to outbreak traps.

We did not find support for variation in SPB body size or hindwing shape that coincided with population phase, or major differences in hindwing morphology between males and females. However, we found evidence for sexual size dimorphism in SPB; female elytra were longer than male elytra in both 2016 and 2017, and female hindwings were longer and larger than male hindwings in 2017. Assessments of the captured proportion of female SPB showed that there were significantly higher proportions of females in non-outbreak traps as compared to outbreak traps. In 2016, non-outbreak traps caught a 1.9 times higher proportion of females (on average), and in 2017, non-outbreak traps had a 2.4 times higher captured proportion of females. The proportion of females caught at the trap-level during the USFS-FHP Annual Springtime SPB Trapping Survey could be included as data to improve upon current SPB outbreak prediction models, allowing for a more robust understanding of SPB population dynamics and ultimately enhancing the sustainability of southern pine forests.

The third chapter monitored active southern *Ips* [*I. avulsus* (Eichhoff), *I. calligraphus* (Germar), and *I. grandicollis* (Eichhoff)] infestations on five burned and five unburned sites, and temporally assessed the condition of loblolly pines (*Pinus taeda* L.) on those sites from April to November 2017. Our objectives were to determine the effects of low-intensity prescribed fire on southern *Ips* activity and loblolly pine mortality, and to assess which variables were the best overall predictors of mortality at the tree-level and site-level. We hypothesized that: 1) there would be increased loblolly pine mortality on burned sites; 2) at the site-level, treatment (burned or unburned), basal area, and time since burn would be significant predictors of loblolly pine mortality; 3) at the individual tree-level, there would be interactions between treatment, *Ips* activity level, and crown mortality level (and that these variables would be important predictors of mortality); and 4) there would be an interaction between tree DBH and *Ips* infestation, with increased *Ips* activity occurring in smaller DBH pines.

Out of 838 total surveyed pines, 69 (8%) died; we observed 3.6 times higher loblolly pine mortality on unburned sites, and we found a higher overall probability of survival on burned sites. At the site-level, basal area was not a good predictor of mortality, and logistic regression models including: 1) treatment and 2) the additive effects of treatment and survey month provided the best predictions of loblolly pine mortality. At the tree-level, AIC model selection showed that the best model included treatment, crown mortality level, *Ips* activity level, and DBH as predictors of individual loblolly pine mortality. Overall, we did not find evidence supporting an increase in loblolly pine mortality as a result of low-intensity prescribed fire treatments, or enhanced attractiveness of burned host trees to active southern *Ips* beetles. Contrary to our hypotheses, we did not find any significant interactions between tree-level or site-level predictors of loblolly pine mortality, or a visible correlation between host DBH and *Ips* activity level. As we did not find any evidence for low-intensity prescribed fire treatments significantly contributing to the level of *Ips* activity or loblolly pine mortality, we conclude that prescribed fire may be a viable management tool in pine-dominant stands experiencing southern *Ips* infestations unless: A) ≥ 50% of many loblolly pine crowns on the burn unit have fading or red needles; or B) numerous trees have a medium to high level of *Ips* activity, observable via the presence of many exit holes (~10 holes per 0.1 m^2 / 1 ft^2) and visible frass.

4.2 Future Research Directions

While the results of our research efforts to gain a better understanding of the drivers of population dynamics for SPB and the three southern *Ips* species provide an important contribution to the existing literature, we recommend that future research endeavors address the following:

1) As our SPB data were from two years of trapping data that differed in their lure components (*endo*-brevicomin was added to the survey in 2017), and we only examined samples from 6 out of the 13 states that employ the annual SPB trapping survey, traps

from all states should be assessed for additional years of data to determine whether the trend of higher captured proportions of female SPB in non-outbreak traps occurs over the entire monitored range.

2) Our analyses did not examine any relationship that may exist between the captured proportion of females and the number of clerid beetles (*Thanasimus dubius* Fabricius). This relationship merits further investigation; if there is a correlation between the overall proportion of females and the abundance of clerid beetles at the trap-level, this may influence the overall effects of predation on SPB population dynamics and potential for an outbreak.

3) While assessing characteristics of host trees during the southern *Ips* infestations, we did not take measurements to quantify tree water use and defenses (e.g., hydraulic conductivity, resin flow rate, and resin volume) and characterize potential differences in defensive responses between individual loblolly pines. Future studies on southern *Ips* outbreaks may incorporate measurements of tree-level physiology and production of defensive compounds to improve our understanding of why specific host trees may be more susceptible to southern *Ips* colonization during outbreaks.

4) Because large-scale southern *Ips* outbreaks are not commonly documented, there is a lack of studies evaluating the effects of other common silvicultural techniques (e.g., fertilization, thinning, and clearcuts) in loblolly pine stands in the southeastern United States on the rate/level of beetle infestation. The southern *Ips* are known to be attracted to slash and logging debris, but assessment of whether forest management activities that create downed material increase the level of infestation in live hosts will help inform decisions on the use of such techniques during ongoing infestations.