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An Integrated Approach to Ambrosia Beetle Management in Ornamental Tree Nurseries: Biology of and Control Measures for Exotic Xyleborina

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AN INTEGRATED APPROACH TO AMBROSIA BEETLE MANAGEMENT IN ORNAMENTAL
TREE NURSERIES: BIOLOGY OF AND CONTROL MEASURES FOR EXOTIC XYLEBORINA

A Dissertation

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ABSTRACT

Ambrosia beetles have been a challenge to profitable nursery production for decades, with management recommendations focused on population monitoring and properly-timed insecticidal applications. Beetles disperse from forests starting in early spring, but few studies have determined the extent of this flight period, or how far they will fly into a nursery. And while the use of semiochemicals by ambrosia beetles has been widely examined, their use of visual cues including colors represents another gap in our knowledge. In addition to these under-studied behavioral traits, the available chemical control measures for ambrosia beetles are not completely effective, and repeated applications become costly for growers. Additional options are needed to reduce treatment frequency and to provide acceptable protection.

The first experiment from 2012-13 determined beetle response to thirteen different trap colors. Mean beetle capture from opaque and red traps was significantly higher than from yellow or white traps, but we recommend that industry-standard black traps are adequate for ambrosia beetle monitoring.

The second experiment from 2013-14 determined the timing of beetle flights and dispersal distance, as well as optimal trap and crop location. In addition to the well-documented spring flight, southeastern nursery managers need to be aware of a second, late-summer flight. Captures from traps placed at various distances (-25 to 200 m) from the forest/nursery interface showed a significant decreasing trend in numbers of beetles captured over increasing distance from the forest. Susceptible tree cultivars may gain added protection when placed deeper within nursery interiors and when baited traps line adjacent nursery edges.

The third experiment from 2014-15 tested four treatments (kaolin clay, bifenthrin, kaolin + bifenthrin, and an untreated control) applied to ethanol-baited trees, with counts of new ambrosia beetle galleries compared roughly every other day for two weeks. While kaolin trees were better-protected than untreated trees at one day after treatment, subsequently there was no significant difference from untreated controls. And while there was a numerical reduction in attacks on kaolin + bifenthrin trees vs. bifenthrin trees, the effect was not statistically significant at any time.

CHAPTER 1: INTRODUCTION

Starting early in the 20th century and continuing to the present day, dozens of species of exotic ambrosia beetles (Coleoptera: Scolytinae) have been accidentally introduced to the United States, often in wood packing materials (Wood 1977, Haack 2001). Ten exotic species have become established in just the past 30 years, likely the result of increases in shipping volume and speed, and in the number of international trade partnerships (Haack 2006, Atkinson *et al.* 2010, Riggins *et al.* 2010, Eskalen *et al.* 2013). Now spreading in forests throughout the eastern U. S., exotic-invasive ambrosia beetles are degrading natural habitats and have even disrupted commercial forestry operations due to timber staining (Lindgren and Fraser 1994, Fraedrich *et al.* 2008, Kolarik *et al.* 2011, Thompson 2011, Formby *et al.* 2012). It also seems likely that, given their rapid and highly successful spread, exotic-invasive ambrosia beetles may be outcompeting native forest species, with important but unknown consequences for natural ecosystems. But it is in the monoculture of ornamental nurseries, with the high concentrations of stress volatiles and highly-apparent host trees, that ambrosia beetles have caused the most damage. In fact, ambrosia beetles were recently listed as the third-worst arthropod pests of nursery operations in the southeastern U. S. (Fulcher *et al.* 2012).

The better-known bark beetles from the tribes Scolytina and Ipina feed and reproduce within the two-dimensional confines of the phloem, occasionally surpassing release thresholds and causing significant economic damage to commercial forest monocultures. These beetles have a relatively limited area available for colonization, and

to prevent excessive competition for this resource, they maintain necessary spacing between galleries by using olfactory and acoustic cues (Lindgren and Borden 1983). Ambrosia beetles (*Xyleborina*), however, tunnel past the phloem into the wood of trees as they construct their three-dimensional larval galleries. This allows them to populate a host at higher densities with minimal intraspecific competition, lessening the need for metabolically-expensive pheromones. Lacking the symbiotic gut organisms that pure xylophages possess, ambrosia beetles do not digest the wood as they tunnel, but rather kick it out behind them, creating the characteristic "toothpick" that makes for an easy diagnosis of attack for nursery managers (Beaver 1989). While constructing larval galleries in stressed or recently dead trees, ambrosia beetles initiate a symbiotic fungal farm with spores carried with them from their own larval gallery; this symbiosis represents the origin of their common name.

There are many factors that contribute to the success of ambrosia beetles in their march across the eastern U. S., not the least of which is their exotic origin. Exotic organisms are often able to thrive in new locales due to the paucity of density-dependent control factors that existed in their native habitats. These include competitors, predators, parasitoids or pathogens that are adapted for coexistence with ambrosia beetles within their native ecosystem. Without these population checks in place, resource depletion may become the primary limiting factor in their population growth, along with the density-independent abiotic conditions and disturbances that would affect all other organisms in that ecosystem. And with virtually no fidelity to any particular host clade, resource

depletion is unlikely for ambrosia beetles, allowing large populations to build up (Hulcr *et al.* 2007).

In addition to their exotic origin, ambrosia beetles possess several attributes that contribute to their success. Even within class Insecta, they are of a very small size, and smaller insects are able to exploit smaller scales of habitat and food resources. As with other Coleoptera, they have a tough exoskeleton that maintains internal water content, and also provides protection against predation. Some ambrosia beetles possess remarkable spines on their prothorax and posterior portions of their elytra which assist in gallery construction, but they also may provide additional predator deterrence. Ambrosia beetles complete immature development within the protective confines of tree galleries, natural fortresses that can prevent detection and predation. These galleries also facilitate the metamorphic process that enables beetles to survive density-independent mortality factors, including adverse environmental conditions. For example, overwintering beetles enter diapause within the safety of their gallery while outside temperatures may be too low for requisite metabolic reactions. This allows adequate time for completion of pupation, and helps pupae avoid abiotic damage until eclosure and emergence in early spring (Saunders and Knoke 1967, Werle *et al.* 2012). Requiring only three molts through larval instars, ambrosia beetles are bivoltine throughout much of the southeastern U. S. (Weber and McPherson 1983, Werle *et al.* 2012, 2015).

In cases where beetle galleries are not sufficiently protective from adverse abiotic conditions or disturbances, an opportunity is presented for selection of more tolerant

beetle phenotypes, which increases overall species fitness. More frequent or severe disturbances, including fire or extended freeze periods, will select for this tolerance more quickly by eliminating beetles that construct galleries in more exposed areas. As environmental conditions become more benign, beetles complete maturation and begin dispersing from the harbor of their galleries through the use of another adaptive survival mechanism; that of flight.

The ability of ambrosia beetles to fly permits rapid, long-distance dispersal, allowing emerging females to escape from suboptimal conditions and discover new resources. This is especially important in light of their particular need for new habitat, as ambrosia beetles require stressed or freshly dead hosts to complete development. Ambrosia beetles alter their habitat in such a way that it can rarely support more than one generation; symbiotic fungi require 40-50% moisture content in their host tree, which may not be maintained after tree death when vascular systems have been compromised (McLean and Borden 1977). When hosts are in decay class I, while bark is still intact, host moisture content can be initially stable, but will eventually drop below this threshold as wood dries out. And in the subsequent decay classes II through V, bark integrity becomes increasingly compromised and woody materials can actually become too waterlogged during wet seasons. Consequently, rapidly expanding populations of exotic ambrosia beetles rely on effective population dispersal, from crowded to less crowded habitats, and from degraded hosts to new hosts with relatively fresh wood. It is during dispersal that mortality is highest for ambrosia beetles, away from the protective confines of their tree galleries, and

dispersal may even represent a key factor of ambrosia beetle ecology that can be exploited for population management.

In general terms, given a choice of equally-attractive hosts at short vs. long distances, ambrosia beetles will preferentially choose the nearer host. This represents an example of a trade-off that can maximize population fitness, whereby even lower-quality resources that are more apparent will be chosen, as opposed to a continued search for higher-quality resources. Successful ambrosia beetle dispersal depends on suitable temperature, light intensity and wind direction and speed, as all of these factors can affect the ability of the female to detect and orient towards stress volatiles from a new host, and then fly to the host. This is known as directed dispersal, and it provides the greatest opportunity for successful colonization. Temperature can be important in that if it is too cold, metabolic reactions necessary for flight may be compromised, and the ectothermic beetles may be unable to adequately operate flight muscles. Conversely, in periods of extreme heat, other risk factors including desiccation may become problematic for dispersal success. Wind direction and speed are critical to dispersal success, because of the important role that plant stress volatiles play in long-range host detection (Ranger et al. 2010). Beetles need to be downwind from a new host in order to detect the ethanol released by stressed hosts, but if wind speeds are too high, flight can become difficult and they will be increasingly subject to predation or simply being "blown off course". Light intensity is also an important factor in dispersal success, because as the beetles approach their new host, visual cues including color, size, host position and silhouette become increasingly important.

Ambrosia beetle dispersal could potentially be measured using several methods, including mark/recapture experiments, laboratory wind tunnel tests, or by using trap captures at varying distances from source populations in infested wood lots. There are a variety of reports on dispersal patterns for other closely-related bark beetles, and while some researchers used dyes or transmitters to mark their test subjects, Jactel (1991) used an elytral engraving on *Ips* spp. (Coleoptera: Scolytidae) to determine flight distances of up to 4 km. However, there is a lack of information concerning ambrosia beetle dispersal, both when and how far they will fly from their forest refuge into nurseries. Consequently, the research presented in chapter three of this dissertation is devoted to this ecologically-important topic, and in chapter five there is a discussion of the role that increased knowledge of dispersal patterns can play in an ambrosia beetle IPM program.

Semiochemical and visual cues used by ambrosia beetles can be effectively exploited in IPM programs. Ethanol-baited traps positioned within 0.5 and 1.7 m above the ground are most effective at capturing *Xylosandrus germanus* (Blandford) and *Xylosandrus crassiusculus* (Motschulsky) (Coleoptera: Curculionidae: Scolytinae), respectively, and should be placed at the lower height when monitoring for multiple ambrosia beetle species (Reding *et al.* 2010). This is likely related to the resource partitioning exhibited by the relatively synchronous ambrosia beetles, whereby multiple species can occupy the same habitat without exclusionary competition. The resource partitioning practiced by intraspecific ambrosia beetles might be described as random, with no discernible pattern to gallery construction along a tree trunk or stem. The presence of an individual in a

sample unit has no effect on the likelihood of encountering another individual. Due to their fungal farming, each female is responsible for producing a garden for her family, without depending on resources provided by the host tree. With space being the only limiting factor for a population of ambrosia beetles in a given tree, and given the large amount of space available to their three-dimensional gallery construction, colonizing females can construct galleries in close proximity to other galleries without risk of resource depletion. In infested trees, while most galleries are completely isolated from others, I occasionally find two or even three “toothpicks” exuded within very close proximity. With larvae eventually leaving the “cradles” constructed by their mother, roaming about and even helping to enlarge the gallery, the chances of encounter with other families of ambrosia beetles are possible.

When considering the larger community of ambrosia beetles, the dispersion pattern could be described as aggregated, as they preferentially attack stressed trees. But when considering the interspecific partitioning described by Reding *et al.* (2010), there is a more regular population dispersion pattern. In general, the larger *X. crassiusculus* requires more space for larval galleries and will preferentially attack lower, larger-diameter tree sections, while the smaller *Xylosandrus compactus* (Eichhoff) and *Xyleborinus saxesenii* (Ratzeburg) (Coleoptera: Curculionidae: Scolytinae) will colonize upper, smaller-diameter sections. In this way, resource partitioning can limit interspecific competition and increase fitness within the various species of ambrosia beetles, leading to a highly-intense population structure. But in an exception to this trend, Stone *et al.* (2007) report *Cnestus mutilatus* (Blandford) (Coleoptera: Curculionidae: Scolytinae), though much larger than *X.*

crassiusculus, preferentially attacks the upper stems of trees. In unpublished data that I collected from a large block of over 100 ambrosia beetle-infested redbud (*Cercis canadensis* L.) trees at a commercial nursery in 2014, I also found the majority of *C. mutilatus* in upper stems, where the adult females could be seen tunneling straight down through the pith of these smaller branches.

Similarly, visual cues play an important role in ambrosia beetle captures (Reding *et al.* 2010). When these visual cues including silhouette and color are factored into trapping design, trap efficacy can be maximized. Trap silhouette appears to have a strong positive correlation with beetle capture, likely due to the resemblance to tree boles (Lindgren *et al.* 1983). Significantly more redbay ambrosia beetles, *Xyleborus glabratus* Eichhoff (Coleoptera: Curculionidae: Scolytinae), were collected from red, black and blue sticky traps than from yellow traps at one Florida site, whereas less distinct differences were observed at a second site (Hanula *et al.* 2011). Diurnal periodicity has been observed in dispersing ambrosia beetles, with most species showing a peak in activity at or before dusk, when low wind speed and adequate light may increase potential for successful dispersal and colonization (Saunders and Knoke 1967). Due to the importance of visual cues to ambrosia beetle host selection, research devoted to this topic will be discussed in chapter two.

As with any organism, resource acquisition is a key factor in ambrosia beetle ecology, and they belong to the functional group of detritivores. In terrestrial habitats, the insect community of detritivores obtain nutrients by consuming decaying plant or animal

materials, and can include dung beetles (Scarabaeidae: Scarabaeinae), carrion beetles (Silphidae) and bark beetles (Curculionidae: Scolytinae), just to name a few. Detritivores contribute to the important function of nutrient recycling, where vital minerals and organic compounds that might otherwise stay locked up in the form of dead trees or animal carcasses are quickly and efficiently converted back to living tissue. In cases of environmental disturbances, where stable communities can be traumatically disrupted due to fire, storm, or other abiotic events, large amounts of dead and declining organisms can accumulate. This can create a bottleneck that detritivores are perfectly adapted to exploit, and can trigger population outbreaks that begin the process of recovery to habitat stability. Scolytine burrowing can significantly increase the surface area of dead or dying trees, enhancing fungal colonization and critical nutrient recycling rates (Zhong and Schowalter 1989). In addition, detritivores are often preyed upon by carnivorous insects or vertebrates, thus moving the recycled materials further up the food chain. And of course, when a predator dies, the nutrients held within that carcass will again be liberated by a detritivore.

As detritivores, ambrosia beetles attack dying or dead trees, which are particularly vulnerable to colonization. In the biochemical arms race between plants and insects, the stressed plants that are attacked by ambrosia beetles have lower concentrations of defense chemicals than do healthy plants. Very slight differences in host vigor are detected by *X. germanus*, and trees with slower growth rates are preferentially colonized (Weber and McPherson 1984). This increases the fitness of "predatory" ambrosia beetles, as they do

not have to dedicate their assimilated resources to detoxification; rather, they can focus on growth and reproduction, and the respiration required by these activities.

Ambrosia beetles have been classified as extreme resource generalists, because they can gain access to a wide variety of tree species without needing to adapt to plant defenses, and this in part has promoted the rapid diversification of their clade (Jordal *et al.* 2000). Being of tropical origin, many of the invasive ambrosia beetles in the U. S. have been shown to be even less host-specific than their temperate counterparts (Beaver 1979). Of 67 species of ambrosia beetles collected in a survey of tropical New Guinea, 95% were broad generalists with no preference for a particular host species or clade (Hulcr *et al.* 2007). This extremely wide host range has been observed not only in field conditions, but also in the laboratory, where sawdust from pear, beech, oak, white ash, black locust black walnut, European buckthorn and red maple have all been used to successfully rear lab colonies of *X. germanus* (Castrillo *et al.* 2012).

Rather than the xylophagy exhibited by the closely-related bark beetles, ambrosia beetles use a relatively unique practice among the detritivores known as xylomycetophagy, or fungal farming (Beaver 1977). During gallery construction, symbiotic fungi are inoculated on the brood chamber walls, with mycelial growth evident as soon as three days later, regardless of brood success (Castrillo *et al.* 2012). Fungal mycelia efficiently extract nutrients from the relatively nutrient-poor wood, providing the beetles with an easily-digested, readily-available food source. After the female lays her eggs, she may remain in the gallery to assist with grooming and defense of her progeny, feeding on the growing

fungal hyphae alongside the larvae (Norris 1979). The origin of this mutualistic agricultural practice in class Insecta is ancient, having been extant for over 100 million years, as evidenced by collections of ambrosia beetles trapped in amber from the Lower Cretaceous (Cognato and Grimaldi 2009, Kirejtshuk *et al.* 2009). Despite its early origins, and its success as evidenced by this longevity through the eons, xylomycetophagy is a relatively unique trait, and has developed independently in three very distinct insect orders: once in ants (Hymenoptera: Formicidae), once in termites (Isoptera) and seven times in the ambrosia beetles (Mueller *et al.* 2005). Xylomycetophagy represents a perfect example of convergent evolution, due to (1) its occurrence in single genera from diverse beetle tribes (e.g., *Camptocerus* from the Scolytini and *Bothrosternus* from the Bothrosternini); (2) by the varying styles of fungal utilization (e.g., larvae of Scolyplatypini, Corthylini, Trypodendrini and *Camptocerus* live and feed in individual cradles adjacent to the parental gallery, while those of Xyleborini and Platypodinae live freely in the parental gallery); and (3) by the wide variance in mycangial physiology.

Mycangia are specialized pores that are typically located on the exoskeleton of dispersing females, and are used to store, protect, feed and transport the vulnerable fungal spores. Mycangia can occur on the head in pouches behind the mandibles (*Xyleborus*), a mentum-prealar pouch (*Monarthrum*) or pharyngeal pouches (*Premnobius*); laterally in shallow thoracic depressions (*Hypothenemus*) or in the proepimeron (*Trypodendron* and *Corthylus*); dorsally in pronotal (Platypodinae, *Xylosandrus*) or mesonotal pores (*Anisandrus*), or pouches in the base of the elytra (*Xyleborinus*); or ventrally in precoxal (*Corthylus*), procoxal (*Gnathotrichus*) or mesocoxal (*Platypus*) cavities (Beaver 1977, 1989).

While there are cases of “horizontal transmission” of fungal spores to a new host, through a strategy known as fungal crop stealing, most transmission occurs vertically through the mycangia of newly-eclosed adult females (Hulcr and Cognato 2010). Spores of the ambrosia fungi are particularly adapted to storage and dispersal via phoresy in these mycangia, which contain oily secretions that protect the thin-walled spores from desiccation and assist with germination upon introduction to the new host tree (Francke-Grosmann 1967). Even within the gallery, the symbionts are reliant upon the beetles. While symbionts can inhibit the growth of other fungi, including wood-rotting species that may be detrimental to beetle shelter or diet, adult and larval beetles alike need to protect their symbionts by grooming out potentially competing microorganisms.

The term “ambrosia fungi” does not refer to a monophyletic group of fungi, as they belong to a heterogeneous group of endophytic Ascomycotes in the order Ophiostomatales, typically represented by the genera *Ambrosiella*, *Rafaellea* and *Dryadomyces* in temperate systems (Batra 1966). Little is known of the tropical ambrosia systems, which may include the genera *Geosmithia*, *Gondwanamyces*, and many other unidentified strains (Hulcr *et al.* 2007). The case has been made that standard morphological traits and molecular analyses based on single genes may simply be inadequate to describe the taxonomy of these unique organisms (Alamouti *et al.* 2009). In an assessment of 56 species of ambrosia beetles now endemic in the continental U. S. and Canada, 32 (57%) had fungal symbionts listed as undescribed, with several more identified only to genus (Roeper 1996). What we do know is that these fungi are polymorphic, producing long filaments within host trees. It is in this stage that the fungi act as efficient extractors of nutrients from the wood, breaking down

large molecules such as cellulose and lignin and providing essential vitamins, amino acids and sterols that beetles would otherwise be unable to obtain from nutrient-poor host tissues. During transport within the mycangia of dispersing beetles, the ambrosia fungi take on a yeast-like monilioid form (Alamouti *et al.* 2009). Due to their phoresy within the mycangia, the ambrosia fungi appear to have lost their ability/need for sexual reproduction.

Unlike most anthropogenic agriculture, ambrosia beetles rarely cultivate pure monocultures; there is typically a primary “ambrosia” fungus fed upon by the larvae, along with several auxiliary fungi, yeasts and bacteria that are fed upon by the adults (Batra 1966). Very little is known about these assemblages, but in some instances the bacteria may represent an essential and ubiquitous component of the fungal garden. The bacterium *Wolbachia* was found in 100% of *X. germanus* test subjects (n=120), with five distinct alleles identified (Kawasaki *et al.* 2010). Other incidental fungal isolates collected from ambrosia beetles include species of *Fusarium*, *Penicillium* and *Trichoderma*, and some of these may have no more than a commensal relationship with the beetles (Beaver 1989; Weber and McPherson 1984). However, this diverse microbial assemblage may actually account for the ambrosia beetle response to the "nutritional heterogeneity hypothesis", whereby the amount of nutritionally-unbalanced food that is eaten will reflect the probability of future encounter with a contrasting unbalanced food. When probability is high, ingestion should also be high, since the cost of over-ingesting the abundant nutrients will be balanced by consuming a complementary food. In contrast, if probability is low, then ingestion should be lower due to greater fitness costs of an unbalanced diet. The

trade-off of maximizing dietary equilibrium while minimizing assimilation costs may be resolved among ambrosia beetles by the diversity of their microbial assemblages, as different symbionts are likely adapted to extraction of different wood nutrients. In addition, it has been theorized that the yeast microbes may stimulate the growth of the primary ambrosia fungi (Francke-Grosmann 1967). However, the roles played by the various microbial assemblages in symbiosis with ambrosia beetles, including my speculation regarding the nutritional heterogeneity hypothesis, are largely understudied. This represents an opportunity for further scientific inquiry, and one that I hope to explore as my research career progresses.

Once resources have been acquired, that portion which has been assimilated is then allocated according to a specific resource budget balancing foraging and dispersal, mating and social behavior, and interactions with other organisms. The efficiency with which assimilated resources are allocated to growth and reproduction determines fitness. Female ambrosia beetles dedicate a majority of assimilated resources towards dispersal, gallery construction and egg production, while males divert the majority of their assimilated resources to reproduction, yielding a high degree of fitness. Nutritional status strongly affects ability of females to disperse and accomplish the laborious challenge of chewing out a new larval gallery. Female fecundity is also tied to her nutritional status, but in general they can lay 1 egg per day, with male eggs laid first (Weber & McPherson 1983). Relative to these activities, females expend very little energy on mating activities, as ambrosia beetles practice a unique type of inbreeding called sib-mating. Females mate with siblings soon after eclosure, thus minimizing energetic output on this task (Weber and McPherson 1983).

This negates the need for producing metabolically-expensive pheromones, and eliminates the time and hazards of locating a mate. It seems intuitive that reduced genetic heterogeneity would decrease a population's ability to recover from adversity, but in haplodiploid organisms like ambrosia beetles, deleterious alleles are rapidly purged and offspring do not experience reduced fitness (Peer and Taborsky 2005). In contrast, outbreeding can reduce subsequent egg viability and occurs only in rare circumstances when gallery systems interconnect within a tree (Peer & Taborsky 2004, 2005).

The asynchronous gender development of ambrosia beetles requires males to have a sufficient lifespan and energy reserves to inseminate multiple females (Castrillo *et al.* 2012). There is extreme sexual dimorphism, as males are smaller, do not possess functional elytra and rarely leave their own larval gallery. These flightless males might be considered reproductive-specialists, eclosing prior to and mating with as many of their sisters as they are able, and then dying shortly after female dispersal (Peer and Taborsky 2004, 2007). This polygynous life history trait of males is a perfect example of a trade-off; maximized sexual capacity at the expense of locomotive ability and lifespan. In this respect, achieving multiple-mating success is the males' sole adult contribution to the population, and is strongly determined by his nutritional status.

The discovery of this unique reproductive system has led to some interesting research on ambrosia beetle sex ratios. Weber and McPherson (1983) report a female to male ratio of 10:1, with more variability reported from laboratory conditions, depending on brood size, host tree species or substrate, and growth rate of the fungal mycelia (Peer

and Taborsky 2004). Castrillo *et al.* (2012) found that as the number of progeny increases, sex ratio of F:M increases; from 4:1 in low-natalty broods, up to 20:1 in high-natalty broods. This female-biased sex ratio is known as spanandry, and while there are few males, each family usually has one, because of the important consequences of producing unmated females (Hamilton 1967). Ambrosia beetles exhibit arrhenotokous reproduction, where mated females produce diploid female and haploid male offspring, while unmated females produce only male offspring (Peer and Taborsky 2004). If there is a lone male in a brood, and he dies, that brood is essentially genetically extinct, as has been revealed by tests conducted on *X. saxesenii* (Hosking 1973). While continued development of unfertilized eggs into adult males may be dependent in part on the presence of a symbiotic bacterium (*Wolbachia*), a successfully mated female will produce mostly daughters, and at least one son (Castrillo *et al.* 2012, Peleg and Norris 1972, Kawasaki *et al.* 2010).

The close inbreeding found in spanandrous populations promotes speciation with a fast rate of evolution, which may explain the large numbers of closely related species in genera such as *Xyleborus* (Beaver 1977). This adaptive radiation may have allowed ambrosia beetles to become acutely pestiferous, as any change in the environment of a recent ancestor may make new resources available to the offspring. Environmental changes are occurring in many habitats, whether due to climate change or accidental transport of exotic organisms through global commerce. As rate of evolution increases, so too might the development of ecologically or agriculturally-problematic traits, including insecticide resistance. These closely related species may then occupy the same habitat, with interspecific competition reduced by variable feeding habits and host species or size

preferences. Sib-mating also yields an increased biotic potential of the species, as nearly all offspring will reproduce due to the availability of mating prior to dispersal. Thus, colonization of isolated habitats is made easier when it is not necessary for both males and females to locate an acceptable resource at the same place and time. Of the 50 species of exotic scolytids established in the U. S., 74% are inbreeding species, providing evidence that this strategy is more advantageous to the establishment of new populations (Haack 2001).

With the risk of genetic extinction of a family when females are unmated, male survival takes on greater importance. Thus, spanandrous systems are only successful when juvenile mortality is low, which may account for the development of brood care and primitive social behavior exhibited by ambrosia beetles. The evolution of social behavior in insects can be defined by cooperative brood care, overlapping generations and, at its highest level of eusociality, a division of labor within a caste system, generally reproductive and worker guilds. While the development of social behavior is well documented in a number of insect families, it is not common for labor to be shared between multiple generations of insects. Ambrosia beetles do not classify as eusocial, but the larvae do engage in brood care, gallery hygiene and enlarging of the gallery alongside any adults present, which may be unique among Coleoptera (Biedermann and Taborsky 2011). The development of intergenerational cooperation may have coevolved with xylomycetophagy, because a lone individual would likely be incapable of maintaining a fungal garden (Mueller *et al.* 2005). While the larvae make a valuable contribution, the ultimate success of a gallery is largely dependent on the foundress. In some cases she will die, but may still

remain at the gallery entrance, essentially "plugging" the hole, providing protection from predation and desiccation alike. This could be considered an example of an altruistic behavior known as kin selection, whereby individual fitness is augmented through the protection of relatives. At the same time, if she does die, the fungal garden can be quickly overrun by contaminating fungi and bacteria, and her brood can be lost. As is the case in the establishment of many new environments with adverse conditions, there is considerable selection for the protection of offspring.

Due to the success of this social system, ambrosia beetle mortality is likely highest in the dispersal and attack phases of the life cycle, and lowest in the immature stages. While dispersing adults are subject to a range of risk factors including predation, host defenses and abiotic environmental conditions, the primary source of mortality for the immature stages may be non-establishment of symbionts, which seems unlikely given successful initiation of a gallery by a foundress. This may represent a Type-I survival curve, with low juvenile mortality and increasing adult mortality over time. Despite the apparent Type-I population curve, I believe ambrosia beetles to be a unique case where they are also opportunistic R-strategists. Typically, a Type-I curve would be associated with an equilibrium-based K strategist, while a Type-III curve would be associated with the opportunistic R-strategists. But ambrosia beetles seem to fit on a Type-I survival curve while having a high reproductive capacity, a combination which may further contribute to their success as invasive species. In a native, naturally forested ecosystem, there would likely be greater equilibrium. But in a non-native agricultural system, with an abundance of stressed and highly-apparent hosts and few biotic control factors, an opportunistic

strategist with Type-I survival might certainly achieve the irruptive population fluctuation that can be associated with invasive organisms.

While ambrosia beetles represent a fascinating and valuable component of their native ecosystems, nursery trees are a high-value commodity with multiple years invested in their care before they are saleable. The economic injury level is quite low for this crop, depending on the size of the nursery, and therefore the action threshold is also low; typically management actions are initiated when the first spring beetle is identified in a trap (Hudson and Mizell 1999). Of paramount importance is the maintenance of optimal tree health, as ambrosia beetles preferentially attacked stressed trees. But even apparently-healthy trees are known to be attacked by ambrosia beetles, and chemical inputs are inevitably required for adequate control (Ranger *et al.* 2013). Due to the long ambrosia beetle flight period, repeated applications of insecticide are necessary for maintaining protection on a nursery tree crop (Hudson and Mizell 1999, Werle *et al.* 2015). This can be costly for the grower and can impact natural enemies of a variety of arthropod pests, leading to secondary pest outbreaks that reduce plant vigor and decrease marketability of a crop (Frank and Sadoff 2011). In addition, some classes of insecticides are currently being reviewed by environmental protection organizations for potential non-target impacts, particularly on pollinating insects, with the possibility of further limiting or banning their applications (Campbell 2013). Therefore, effective alternatives are needed to prevent an overdependence on insecticides and development of both resistance and secondary pest emergence (Pimentel *et al.* 1992). Use of particle film technology centered on kaolin clay has become widespread in pest control for certain crops, and the research

presented in chapter 4 will discuss the potential for kaolin as an ambrosia beetle deterrent (Glenn *et al.* 1999).

With this comprehensive examination of ambrosia beetle ecology, it is easy to appreciate the fascinating nature of the ambrosia beetle community, while still maintaining concern for the significant economic impact that the invasive species have made on the horticultural industry. The following chapters will present my research on ambrosia beetle response to visual cues, seasonality and dispersal behaviors of ambrosia beetles, and the potential role for applications of kaolin clay in ambrosia beetle deterrence.

CHAPTER 2: EFFECTS OF TRAP COLOR ON AMBROSIA BEETLE CAPTURE

Introduction

The importance of olfactory cues to ambrosia beetle host-finding is well understood, exemplified by the standard ethanol-baited traps which facilitate monitoring efforts for a variety of species (Oliver *et al.* 2004, Ranger *et al.* 2010). In addition to semiochemical attraction, which occurs over longer distances, bark and ambrosia beetles are known to utilize visual cues such as host position, silhouette and color in close proximity to their host (Prokopy 1986). Baited traps positioned within 0.5 and 1.7 m above the ground are most effective at capturing *X. germanus* and *X. crassiusculus*, respectively, and should be placed at the lower height when monitoring for multiple ambrosia beetle species (Reding *et al.* 2010). Increased effectiveness of bark beetle traps has been observed when appropriate trunk-shaped trap silhouette is presented (Lindgren *et al.* 1983, Goyer *et al.* 2004, Mayfield and Brownie 2013). And greater attractiveness of Scolytinae and Platypodinae to red traps over other colors has been exhibited, while eight other xylophilous curculionids have shown a negative response to light-colored (yellow or white) traps (Entwistle 1963, Dubbel *et al.* 1985, Mizell and Tedders 1999, Goyer *et al.* 2004, Campbell and Borden 2006, Chen *et al.* 2010).

Despite this wealth of information, a definitive trap color preference for Scolytinae ambrosia beetles remains to be determined. A prominent pest of Asian mango (*Mangifera indica* L.) plantations, *Hypocryphalus mangifera* (Stebbing) (Coleoptera: Curculionidae: Scolytinae: Cryphalina), was found to be preferentially collected by green sticky traps and

secondarily by black traps, as compared with white, red, blue and yellow traps (Abbasi *et al.* 2007). Significantly more redbay ambrosia beetles (*X. glabratus*) were collected from red, black and blue sticky traps than from yellow traps at one Florida site, whereas less distinct differences were observed at a second site (Hanula *et al.* 2011).

In contrast with the simple categorical value of trap color, spectral reflectance can provide a quantitative measurement, and can be used as a substitute for trap color when comparing insect captures. Captures of *Dendroctonus frontalis* Zimmermann (Coleoptera: Curculionidae: Scolytinae: Tomicina) were significantly higher at low-reflectance (<30%) black and blue traps than at high-reflectance (>70%) white and yellow traps (Strom and Goyer 2001). Aside from these findings, there has been no analysis of the interaction of spectral reflectance with bark beetle captures.

In addition to the benefits of refined population monitoring, colored traps may be incorporated into a mass-trapping control strategy. Prominent timber pests including the ambrosia beetles *Trypodendron lineatum* Olivier (Coleoptera: Curculionidae: Scolytinae: Xyloterina), *Gnathotrichus sulcatus* (LeConte) and *G. retusus* (LeConte) (Coleoptera: Curculionidae: Scolytinae: Corthyliina) have been effectively managed using a barrier strategy, where baited traps are placed at strategic locations surrounding vulnerable timber yards (Lindgren and Borden 1983, Lindgren and Fraser 1994). While populations of the primary pest *T. lineatum* varied with weather conditions, declining populations of *G. sulcatus* over a twelve-year trapping program in British Columbia accounted for savings in timber degradation estimated at \$500,000. While *X. germanus* exhibits a higher mobility

compared to native ambrosia beetle species, it disperses from similarly-local sources, thus making itself susceptible to a mass-trapping approach (Gregoire *et al.* 2001). The use of appropriately-colored traps may improve mass-trapping programs, making them economically beneficial to an ambrosia beetle IPM program.

Due to the importance of population monitoring for ambrosia beetle control, a scarcity of knowledge regarding ambrosia beetle response to color, the potential for mass-trapping as part of a push/pull strategy, and the need for updated geographical distribution data for exotic-invasive species, the objective of this study was to determine the effect of trap color on ambrosia beetle trap capture.

Materials and Methods

Two sites were used for this research: the Tennessee State University Otis L. Floyd Nursery Research Center (OFNRC) in central Warren County, TN (35°42'34.81"N, 85°44'27.94"W), and the USDA-ARS Thad Cochran Southern Horticultural Lab (TCSHL) in central Pearl River County, MS (30°50'3.14"N, 89°32'52.32"W).

Trap design. Corrugated plastic sheets in 13 colors were cut and folded into three-paneled prism traps, with each panel measuring 22x28 cm, with a 2.5x15 cm slit cut into the panel centers to facilitate ethanol diffusion. During peak ambrosia beetle flight in spring, traps were baited with slow-release ethanol lures (AgBio, Westminster, CO). Transparency sheets (3M, Austin, TX), also with a 2.5x15 cm slit, were then coated with an

adhesive glue [Tangle-Trap (Contech, Inc., Delta, BC) or Pestick (Phytotronics, Inc., Earth City, MO)] and fastened to the outside of each prism trap panel with small binder clips. Use of replaceable transparencies facilitated keeping the trap surface clean, as well as removal of ambrosia beetles from the traps. Prism traps were suspended vertically from metal trap rods (Contech, Inc., Delta, BC), and deployed in a row along the edge of an infested woodlot. All trap colors were replicated four times in a randomized complete block design with 4 m between traps and 8 m between blocks. Transparencies were removed from the traps biweekly, with traps re-randomized within each block following removal. During 2012, traps were operated from 7 May to 18 June in TN. During 2013, traps were operated from 30 May to 9 July in TN and 7 May to 4 June in MS. All suspected ambrosia beetles were removed from transparencies and soaked in Histo-Clear (National Diagnostics, Atlanta, GA) to dissolve excess insect glue, and were then identified using standard dichotomous keys with voucher specimens deposited at the TCSHL (Arnett, Jr. *et al.* 2002, Rabaglia *et al.* 2006).

Spectral reflectance analyses. Corrugated plastic traps were scanned using a portable Konica Minolta CM-2600d spectrophotometer and SpectraMagic (Version 3.61 Release No. 2; Konica Minolta, Tokyo, Japan) software. The spectrophotometer unit was set in observer illuminant Daylight 65 (i.e., average daylight including UV radiation at 6500K), observer angle at 10° (CIE1964), specular component included (SCI), and ultraviolet (UV) at 100% (i.e., illumination contains all UV components of the Xenon light source). For each trap color, 4 random scans were performed and the L*a*b* and percentage reflectance (nm) values averaged. Solid trap colors (i.e., black, blue, brown, green, grey, lavender, orange,

purple, red, white, yellow) were placed on top of a sheet of Staples (Framingham, MA) white copy paper (SKU: 135855 Model: 135848-WH) before scanning. To minimize background reflectance on transparent (i.e., clear) or semi-transparent (i.e., opaque) trap colors, the spectrophotometer aperture opening was placed on the trap surface and then pointed towards a semi-gray wall ~5 m away. Traps were scanned with and without transparencies and there was a slight difference in reflectance when the transparency was on the trap. It was not possible to scan the traps with the insect glue on the transparency without contaminating the spectrophotometer. However, when insect glue was added to the transparency and placed with the sticky-side towards the colored trap surface, spectrophotometer readings were similar to those taken from trap alone, without either the transparency or insect glue. Therefore, we assume traps with both insect glue and transparencies had spectral reflectance similar to traps alone, and are only reporting reflectance data for the colored traps alone.

Statistical analyses. Data were analyzed for individual ambrosia beetle species and for total Xyleborina using generalized linear mixed models with the negative binomial distribution and log link function with the GLIMMIX procedure of SAS (version 9.3; SAS Institute Inc., Cary, NC). Trap color was the explanatory variable and capture count was the response variable. Experimental site, year, blocks and sample dates were included in the model as random factors. *P* values for simultaneous, pair-wise comparisons of least squares means were adjusted using the Holm-Simulated method ($\alpha = 0.05$).

Results

At the OFNRC, *X. crassiusculus* (64% in 2012, 47% in 2013) was the dominant species, followed by *X. saxesenii* (24% in 2012, 17% in 2013) and *X. germanus* (8% in 2012, 13% in 2013). Other Xyleborina captures from the OFNRC included *Ambrosiodmus rubricollis* (Eichhoff), *A. atratus*, *E. validus*, *X. affinis*, *Xyleborus celsus* Eichhoff and *X. ferrugineus* (Coleoptera: Curculionidae: Scolytinae: Xyleborina). Species composition at the TCSHL included *X. compactus* (41%), *X. saxesenii* (28%) and *X. crassiusculus* (25%). Other species of Xyleborina collected at the TCSHL include *Ambrosiodmus lecontei* Hopkins, *Ambrosiodmus obliquus* (LeConte), *Ambrosiophilus atratus* (Eichhoff), *Euwallacea validus* (Eichhoff), *Xyleborus affinis* Eichhoff and *Xyleborus ferrugineus* (Fabr.) (Coleoptera: Curculionidae: Scolytinae: Xyleborina). The camphor shot-borer (*C. mutilatus*) was present at both sites from this test, although abundance was very low. No collections of other species recently introduced into the U.S were made from either site, though our traps did not include the baits that have been shown to be most attractive to species like *X. glabratus* (Kendra *et al.* 2012).

Despite the variation in species composition at the two sites, no site*color ($F = 0.31$, $df = 12$, $P = 0.9873$) or species*color ($F = 0.46$, $df = 60$, $P = 0.9999$) interactions were observed, indicating that colored-trap captures will be consistent regardless of site or ambrosia beetle species. Due to this trend, we analyzed total Xyleborina captures across sites to determine preference for a color. Mean Xyleborina capture was significantly higher from opaque, red and black traps (60, 54 and 51) than from white traps (28); mean

capture from opaque and red traps was also significantly higher than from yellow traps (30) (Fig. 1).

Mean percentage reflectance values were used to generate a graph, with results similar to those reported by Francese *et al.* (2010) (Fig. 2). While white traps had the highest percentage reflectance (80%), they were not a pure white; likewise, black traps (6%) were not a pure black. Orange, red and yellow traps exhibited a similar spectral reflectance curve, while blue, green, lavender and purple had a similar curve.

Discussion

No differences were detected in ambrosia beetle response to our colored traps by species or site. These results suggest visual cues used by the ambrosia beetle of greatest economic concern at any given location should provide equivalent trapping efficacy for other species of Xyleborina. However, consideration of the interaction between trap placement and trap color at specific sites may influence trap efficacy; reflectivity of traps can vary depending on position of the sun or nearby vegetation.

While we observed no difference in response to our colored traps among species or sites, data from this test and from previous work has indicated some interesting differences in the composition of ambrosia beetle populations at our test sites (Werle *et al.* 2012). The dominant species from TCSHL collections, *X. compactus* has still not been collected at the

OFNRC, while its congener *X. germanus* has still not been collected at the TCSHL. Other species have been collected from both sites, including *X. crassiusculus*, *C. mutilatus* and *X. saxesenii*, with representatives from a variety of other incidental genera in the tribe Xyleborina, including *Ambrosiodmus*, *Euwallacea* and *Xyleborus*.

Xylosandrus crassiusculus has frequently been listed as the most destructive ambrosia beetle in the southeastern U. S., and has been observed attacking weakened or even apparently-healthy hosts in this region since 1974 (Anderson 1974, Hudson and Mizell 1999, Oliver and Mannion 2001). First collected in northern Mississippi in 1999, *C. mutilatus* is now widely distributed across the southeastern U. S., and while sparsely-represented from our collections, it has the potential to become another important pest of nursery and landscape plants (Schiefer and Bright 2004, Oliver *et al.* 2012, Leavengood 2013). In contrast, *X. saxesenii* was likely introduced to North America over 100 years ago, is distributed worldwide and from coast to coast and Hawaii in the U. S., and can be one of the most harmful species of Xyleborina (Rabaglia *et al.* 2006, Burbano *et al.* 2012). Interestingly, this species was highly represented in our collections, with abundance second only to *X. crassiusculus* at the OFNRC and *X. compactus* at the TCSHL. Other more recent ambrosia beetle invaders including *X. glabratus*, *C. pseudotenius*, *C. diadematus* and *E. fornicatus* have still not been collected from either site.

There was not a consistent association of ambrosia beetle capture with trap spectral reflectance. While red traps (640 nm) had a similar wavelength peak as compared with yellow traps (620 nm), Xyleborina captures from red traps were significantly higher than

from yellow. The high captures by red traps may be due in part to a similarity to bark coloration of host trees (Entwistle 1963). Although not a pure black, our black traps had predictably-low reflectance (6%) across the electromagnetic spectrum. The high captures of Scolytinae from black traps in the present study is similar to reports by other researchers (Strom and Goyer 2001, Goyer *et al.* 2004, Campbell and Borden 2006, Chen *et al.* 2010). The most intriguing finding was the high capture counts from opaque traps, at both sites and across all species of Xyleborina. The opaque traps were translucent, and therefore had a lower reflectance (22%) than most other trap colors, including yellow (61%) and white (80%). When comparing ambrosia beetle captures, opaque traps were significantly higher than both yellow and white. Reflectance of opaque traps was intermediate between the other high-performing black (6%) and red (53%) traps.

A highly attractive ambrosia beetle trap may improve the collection of early-dispersing individuals, giving advance notice so that tree crops can be preventatively treated before attacks begin. This optimized trap would also allow for early detection of newly-introduced species, and the subsequent rapid-response that is key to a successful eradication program. Federal programs ranging from the United States Department of Agriculture-Animal Plant Health Inspection Service, the United States Forest Service-Cooperative Agricultural Pest Survey and the National Invasive Species Council are focused on providing advance warning on new invasions of ambrosia beetles and other invasive organisms (Myers *et al.* 2000). Our results do not justify a change to standard ambrosia beetle monitoring practices; Baker traps may be the most cost-effective option, and industry-standard black Lindgren funnel traps may be the most beetle-effective option for

ambrosia beetle monitoring (Lindgren and Fraser 1994, Oliver *et al.* 2004, Hanula *et al.* 2011). Furthermore, our results lead us to conclude that spectral reflectance of traps is not useful as an indicator of ambrosia beetle capture. Trap color does not appear to exhibit as much influence on ambrosia beetle capture as other variables tested in previous experiments, including trap design (Lindgren *et al.* 1983, Oliver *et al.* 2004), trap placement (Reding *et al.* 2010) and olfactory cues (Strom *et al.* 2001, Reding *et al.* 2011, Ranger *et al.* 2012). However, other variables not yet tested may still prove to be influential. For example, wavelengths outside of the 400-700 nm range may play a role in ambrosia beetle host selection. Due to the strong olfactory response of ambrosia beetles to ethanol, more striking differences in response to colored traps may be observed if only color treatment alone were the primary treatment factor, or if fewer color treatments were tested. Future experiments testing the ultraviolet or infrared spectra, with fewer treatments, and possibly without the use of ethanol lures, may elicit more conclusive evidence regarding visual host recognition cues used by ambrosia beetles.

Due to their low capture rates, we conclude that highly-reflective yellow or white traps are not effective for ambrosia beetle monitoring. However, due to their pairing with the highly attractive ethanol lures, our yellow and white traps failed to elicit the deterrent response exhibited by some other Scolytinae and their predators to these colors (Strom *et al.* 2001). Further tests for deterring ambrosia beetle attacks are needed, as this may aid in developing a comprehensive push-pull strategy. Mass-trapping with optimally-baited traps could provide a "pull", while cultural, visual and chemical measures that provide deterrence or avoidance protection might provide a "push" for susceptible tree crops. For

example, an application of a kaolin clay product may create a highly-reflective white appearance on tree crops, providing both a gustatory and a visual deterrent to ambrosia beetle attack (Glenn *et al.* 1999); results of an assessment of kaolin as an ambrosia beetle deterrent are presented in chapter four. When using these comprehensive IPM strategies, growers maintaining optimal tree health will limit unnecessary chemical inputs, reducing risk of insecticide resistance-development while maximizing profits and allowing for a healthier surrounding ecosystem.

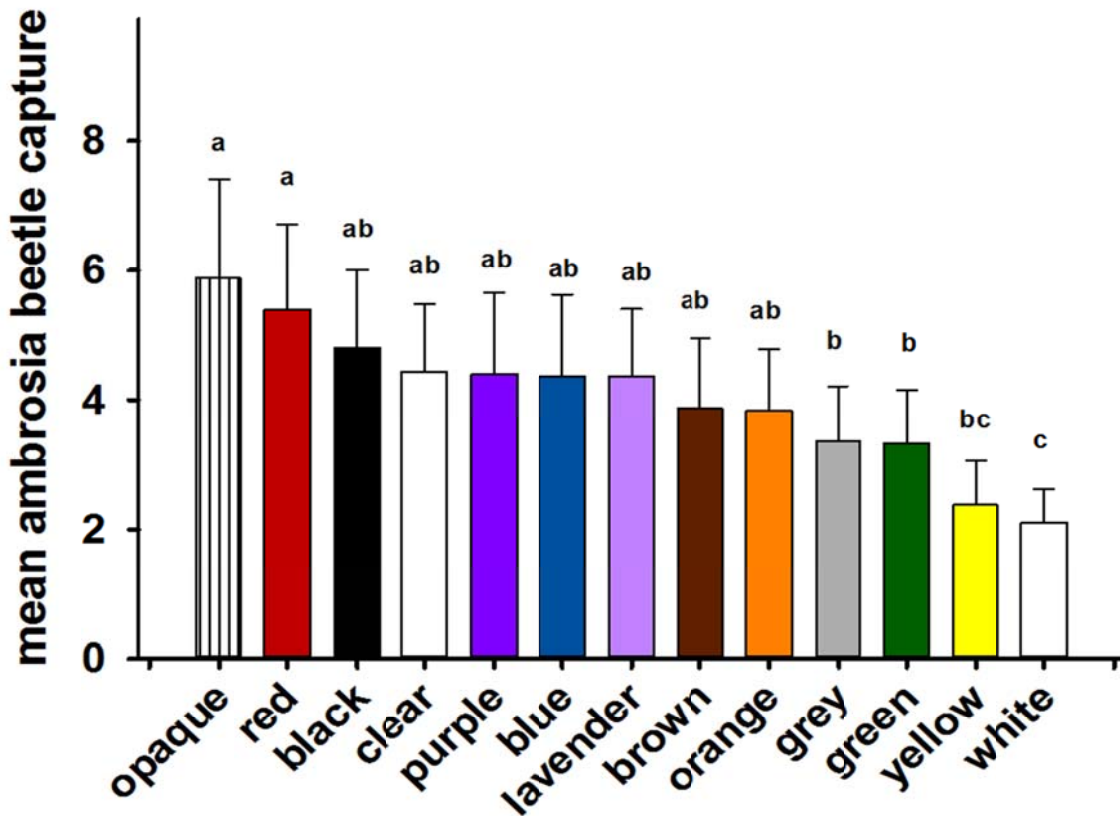


Fig. 1. LS-means of *Xyleborina* capture counts from 13 colors of ethanol-baited prism traps in Tennessee and Mississippi, 2012-2013. Bars that contain different letters are significantly different according to the Holm-Simulated method ($\alpha = 0.05$)

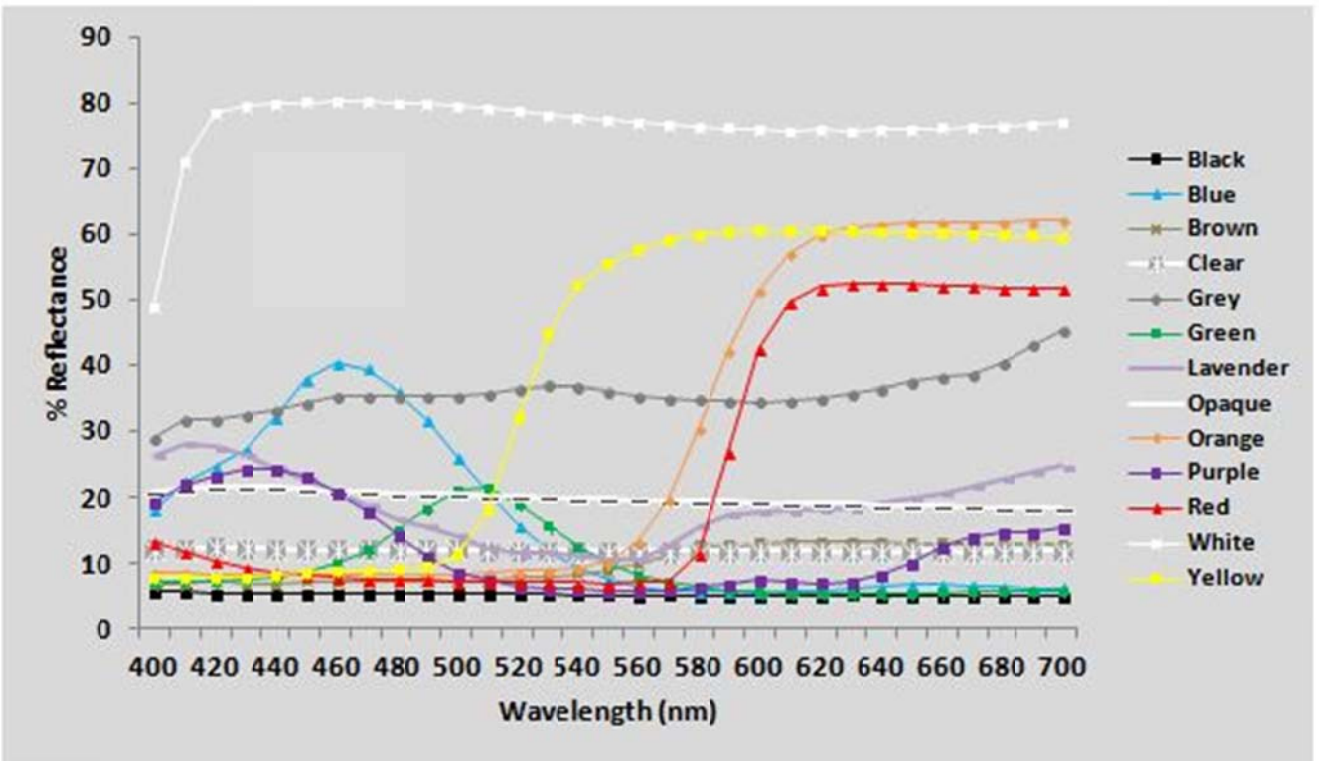


Fig. 2. Spectral reflectance of thirteen colored traps (CM-2600d spectrophotometer and SpectraMagic software; Konica Minolta).

CHAPTER 3: SEASONAL AND SPATIAL DISPERSAL PATTERNS OF AMBROSIA BEETLES

Introduction

Native populations of ambrosia beetles contribute to the important nutrient recycling process in forest ecosystems. For this reason it has been assumed that nursery infestations originate from woodlots surrounding ornamental nurseries, although few studies have fully investigated invasion source prior to this research. In addition to population origin, knowledge of beetle dispersal distance is not well understood. In forest habitats, the directed flight of ambrosia beetles occurs under relatively low wind speeds, particularly close to the ground where most beetle flight occurs (Browne 1961; Reding et al. 2011). But within large open nurseries, where fewer windbreaks exist, higher wind speeds make directed flight significantly more difficult for small beetles (Pasek 1988). In a mark-recapture study, the striped ambrosia beetle, *T. lineatum*, which is a coniferous forest tree pest in the western U. S., only exhibited non-directed flight for distances of 100 m or more, while recaptures at 500 m were primarily downwind of the release point (Salom and McLean 1989). Similarly, *T. lineatum* were recaptured in significantly higher numbers from baited traps in forested as opposed to open settings, likely due to wind speeds roughly four times higher in the open settings (Salom and McLean 1991). Furthering knowledge of ambrosia beetle origin and dispersal patterns may improve available cultural measures; for example, there may be a distance from the forest edge beyond which ambrosia beetles are unlikely to fly to attack trees. In larger nurseries encompassing at least 50 hectares, we hypothesize that locating susceptible cultivars at the interior may provide added protection from ambrosia beetle attack.

Mass trapping is a technique that has been successfully used to suppress or even eradicate incipient populations of invasive insects at their advancing front (Brockerhoff et al. 2010). But even for established populations, mass trapping can offer cost-effective control when an attractant is perceived by a high proportion of the target insects and has a stronger pull than its ambient source (i.e. stressed trees), when traps collect insects throughout the dispersal period, and when traps, lures and labor are cost-effective (El-Sayed et al. 2006). Traps used in conventional ambrosia beetle monitoring programs meet all of these criteria; therefore, by capturing and killing a large proportion of dispersing females, mass trapping could be used as a population management tactic. In some cases, mass trapping can become a stand-alone control measure, but mass trapping also can be effective when combined with a delayed or reduced insecticide application (Huber et al. 1979). In a long-term study in commercial forests, mass-trapping of several western ambrosia beetles [*T. lineatum*, *G. sulcatus* (LeConte) and *G. retusus* (LeConte)] yielded a benefit/cost estimate of five-to-one with associated savings of over \$500,000 (Lindgren and Fraser 1994). Trap position may play an important role in trapping efficacy, since traps placed 15-25 m inside the forest captured significantly more ambrosia beetles than did traps placed at the forest margin (Lindgren et al. 1983).

Standard management recommendations for ambrosia beetles include using ethanol-baited traps to monitor adult flight in early spring, followed by applications of pyrethroid insecticides every three to four weeks after the first beetle flights are detected (Hudson and Mizell 1999; Ranger et al. 2010, 2012; Reding et al. 2010, 2011). Some prior

studies describe an early spring population peak followed by a summer decline, while others include a possible second, late summer peak for southern populations (Hudson and Mizell 1999; Oliver and Mannion 2001; Reding et al. 2010; Werle et al. 2012). Because ambrosia beetle population monitoring is important for properly-timed insecticide applications, for choosing the best location within a nursery for tree crops, and for the development of a push-pull management strategy, my study objectives included: 1) determining the source and timing of ambrosia beetle flights; 2) estimating dispersal distances into ornamental nurseries; and 3) identifying the best location for trap placement based on capture rate and convenience.

Materials and Methods

Experimental locations. Four commercial nurseries were used as research sites, including Tangipahoa Parish (30°47'30.39"N, 90°20'37.91"W), LA; Stone County (30°47'59.92"N, 89°15'21.64"W), MS; Georgetown County (33°14'40.78"N, 79°22'52.80"W), SC; and Pickens County (34°45'50.34"N, 82°39'47.75"W), SC. All nurseries were large (>60 ha), open landscapes with diverse arrays of containerized crops and greenhouses (Tangipahoa Parish, Stone County and Georgetown County sites) and field-grown ornamental trees (Pickens County site). The LA site was surrounded by a combination of managed pine and natural mixed hardwood stands on three sides, with a road and residential area on the fourth. The pine stand at the LA site was subjected to a prescribed burn during our study in February 2014. The MS site was bordered by

managed pine forest on two sides, a barren sand/gravel pit on the third side, and a road with residential areas on the fourth. The pine stand at the MS site last received a prescribed burn in 2011. The two SC sites were surrounded by pine-hardwood mix on all four sides and had not been burned within 5 yr of the experiment.

Trapping methodology. Baker traps were constructed using 2 recycled soda bottles attached with a Tornado Tube (Steve Spangler Science, Englewood, CO) (Oliver et al. 2004; Ranger et al. 2010; Reding et al. 2011). The upper 2 L bottle had 3 rectangular openings (length 15 cm, width 6 cm) in the sides to allow beetle entry, while the lower 592 mL bottle was partially filled with propylene glycol to kill and preserve insects. Traps were baited with a slow-release (65 mg/day at 25 C) ethanol lures (AgBio, Westminster, CO) and suspended about 1 m above the ground with Japanese beetle trap stands (Tanglefoot, Grand Rapids, MI) (LA and MS sites) or stands constructed of lumber and metal shelf support brackets (SC sites). The experimental design was randomized complete block. Treatments tested were traps placed at distances into the nursery from the edge of: -25 m, 25 m, 50 m, 100 m and 200 m (LA and MS sites in 2013), or -13 m, 0 m, 13 m, 25 m, 50 m and 100 m (SC sites in 2011 and 2012; LA and MS sites in 2014) (Fig. 1). Each treatment was a trap placed within its own row at a randomly assigned distance, with rows separated laterally from neighbors by 20 m (SC sites) or 25 m (LA and MS sites) to lessen the interference from adjacent treatments within the block. The number of blocks at each site was limited by nursery size and number of treatments tested. In 2013, each site held 5 blocks with 5 distance treatments in each for a total of 25 traps, whereas in 2011, 2012 and 2014 there were 4 blocks with 6 treatments each for a total of 24 traps. Research plots also

were separated from the lateral and distal nursery edges by at least 200 m (LA and MS sites in 2013) or 100 m (SC sites in 2011 and 2012, LA and MS sites in 2014).

Traps were deployed in the spring with samples collected every 2 wk, and lures were replaced every 8 wk. Collections were made 1 April to 16 December 2011, 13 January to 28 December 2012, 22 April to 26 August 2013, and 21 February to 23 October 2014. The Scolytinae collected from individual traps were brought back to the laboratory for abundance and species determination using standard keys (Rabaglia et al. 2006).

Statistical analysis. Data from each collection year and site were analyzed separately because of variation in experimental design among the research sites and years. The effects of trap distance and collection time period were analyzed for the pooled numbers of *C. mutilatus*, *X. compactus*, *X. crassiusculus* and *X. germanus* because these are the major pestiferous ambrosia beetle species in ornamental tree nurseries. Other ambrosia beetles captured were identified to species and counted, but not used in analysis. Mean captures of the 4 target species per trap per 2 wk sampling period were analyzed using repeated measures analysis of variance (ANOVA), with distance and collection time period as main factors (PROC MIXED, SAS Institute 2011). A first-order autoregressive covariance structure was included in the repeated measures statement. A trend analysis using polynomial contrasts was conducted to properly interpret significant distance effects. Because the distances were unequally spaced, a coefficient matrix for orthogonal contrasts was generated using PROC IML (SAS Institute 2011). The coefficient matrix was then used

in contrast statements in PROC GLM to detect significant linear, quadratic and cubic trends (SAS Institute 2011).

Results

Including all other non-target ambrosia beetle species, a total of 2,345 and 1,961 specimens were collected from the Georgetown County (SC) and Pickens County (SC) sites from 2011-12, whereas 1,671 and 1,702 specimens were collected from the Tangipahoa Parish (LA) and Stone County (MS) sites from 2013-14, respectively. Ten, 11, 11 and 13 ambrosia beetle species were captured in ornamental tree nurseries located in Stone County, Tangipahoa Parish, Georgetown County and Pickens County, respectively (Table 1). When pooled together, the 4 target species (*C. mutilatus*, *X. compactus*, *X. crassiusculus* and *X. germanus*) composed 86.4% (Tangipahoa Parish, LA), 91.7% (Stone County, MS), 69.6% (Georgetown County, SC) and 63.7% (Pickens County, MS) of the total ambrosia beetles collected over 2 years. *Xylosandrus crassiusculus* was consistently one of the most abundant species at all research sites. Similar to findings from other regional studies, *X. germanus* was not recovered from nurseries located in Stone County (MS) and Tangipahoa Parish (LA), whereas *C. mutilatus* was not collected from the nursery located in Georgetown County (SC) (Werle et al. 2012, 2014).

The numbers of ambrosia beetles captured biweekly were significantly different ($P < 0.05$) among distances from the nursery edges and sampling times at all nurseries and in

all years (Table 2). The two-way interactions between distances and sampling times also were significant for all nurseries and years (Table 2).

Ambrosia beetles from our 4 target species were active from March to November at all sampled nurseries (Figs. 2, 3). Populations in LA and MS did not appear to begin flight activities earlier than the more northerly populations in SC. In the first years of this research in LA (2013), MS (2013) and SC (2011), the sampling efforts began too late to detect the initiation of spring flight. In the second years, we detected the initiation of spring flight in late February (at LA and Georgetown County, SC sites) to early March (at MS and Pickens County, SC sites), which quickly developed into peaks in late March in SC (Fig. 2) and early April in LA and MS (Fig. 3). At nurseries in SC, the numbers of ambrosia beetles slowly declined with a second peak in May-June (Fig. 2). Following a summer decline at nurseries in LA and MS, a second surge in ambrosia beetle captures was detected beginning in late-July 2013, as well as at the LA nursery in 2014 (Fig. 3), indicating the possible emergence of a second generation.

Across sampling dates, trap distance from the nursery edge had a significant influence on the numbers of ambrosia beetles captured at all nurseries (Table 2). Trend analysis of the distance effect for ambrosia beetles showed significant linear and quadratic trends ($P < 0.05$) for nurseries located in SC (both years) and the nursery in MS (2014 only) (Table 3). The numbers of beetles captured were greatest at -13 m inside the forest, decreasing sharply at 0 and 13 m into the nursery (Fig. 4). The numbers declined further but at a slower rate or remained similar from 25 to 100 m in SC (Fig. 4) or 13 to 100 m in

MS (2014; Fig. 5). The distance effect showed significant linear, quadratic and cubic trends for the numbers of ambrosia beetles captured at nurseries in LA (2013 and 2014) and MS (2013 only) (Table 3). Similar to nurseries in SC, the greatest numbers of ambrosia beetles were captured at -13 or -25 m inside the forest at the sites in LA and MS (Fig. 5). However, at these sites the numbers captured from 0 to 200 m fluctuated, with the numbers captured at greater distances occasionally higher than those at shorter distances (Fig. 5).

Discussion

Ambrosia beetle trap capture peaks at southeastern nurseries were recorded from March through April, and again in SC from May to June and in LA and MS from late July through August. The timing of the second peak flight in SC agrees with the observations of a May-June emergence of *X. crassiusculus* and May-July emergence of *X. germanus* in middle Tennessee (Oliver and Mannion 2001). Our documentation of this second peak in ambrosia beetle activity should help southern nursery managers to more accurately monitor populations, and alter management strategies accordingly. It may be best for nursery managers to operate a trapping program throughout the spring and summer months as verification of peak flight activity, and potentially as a mass-trapping strategy. Tree crops exposed to the abiotic stress of late summer heat can experience a variety of symptoms including inhibition of growth, reduced ion flux, and production of reactive oxygen species (Wahid et al. 2007), which may increase vulnerability to attack by a second generation of dispersing females.

Each nursery site had a unique ambrosia beetle community (Table 1), likely influenced by the surrounding natural plant communities that serve as hosts. Plant communities are in turn shaped by soil and landscape features, as well as micro-climactic conditions (Ohmann and Spies 1998). While study site differences were likely due in part to natural habitat variability, there also were different forest and nursery management practices at the sites. With no recent prescribed burns at the MS and SC sites, fire-sensitive species including cherry (*Prunus serotina* Ehrh.), sweetgum (*Liquidambar styraciflua* L.), redbud (*C. canadensis*), and sweetbay magnolia (*Magnolia virginiana* L.), all known-hosts of ambrosia beetles, were able to proliferate in adjacent forests (Mizell et al. 1994). However, at the LA site, a prescribed burn in February 2014 occurred before the start of our second year of data collection. This prescribed burn destroyed much of the hardwood undergrowth at the LA site, and with it possibly many of the overwintering ambrosia beetles, contributing to a relatively low spring peak at this site (Fig. 4). Superficially, it may appear that properly timed prescribed fires, by lowering ambrosia beetle population size in surrounding forests, could reduce infestations within nurseries. However, due to greater tree stress, areas subjected to prescribed burns can experience an increase in populations of Xyleborina in subsequent years, and any nursery benefit gained from a fire-induced reduction in ambrosia beetle populations may be temporary (Sullivan et al. 2003; Campbell et al. 2008).

Nursery management practices also can be highly variable, contributing further to study site differences. In 2014, a large block of >100 containerized redbud trees located

between 13-50 m from the edge of the MS nursery was colonized by a pathogenic fungus (*Fusarium lateritium* Nees), as well as a substantial ambrosia beetle population. After the trees were cut in June and brought back to the lab for examination, a mean of >3.5 beetle galleries was observed per tree and >200 total specimens of adult *Xyleborina* were collected. These trees contained a significant portion of the future reproductive capacity of the ambrosia beetles within that area, and when the trees were removed before a second generation could emerge, trap capture data may have been impacted in terms of both trap distance and capture date variables (Figs. 2 and 4). Without the removal of these beetle-infested redbuds, it is possible we might have experienced a more pronounced late summer peak at the MS site in 2014, as well as additional trap captures at or near the forest interface.

A linear and quadratic trend for trap captures can be observed with increasing distance from the nursery edge at nurseries in SC (2011 and 2012) and MS (2014) (Figs. 4 and 5). Although fewer beetles were captured by traps placed at the nursery edge (0 m) as compared with traps within the forest (-13 and -25 m), edge placement was more convenient and easily accessible. When compared with all other traps within the nursery interior (13, 25, 50, 100 and 200 m), the traps at the nursery edge (0 m) did capture more beetles, supporting our hypothesis that the source of the ambrosia beetle population was within the peripheral forested areas as opposed to within the nursery. The effectiveness of the edge traps, combined with the benefit of avoiding daily operations within the nursery as well as the natural obstacles within the forest, would suggest that the optimal trap location would be at the nursery/forest interface.

The effects of weather patterns would certainly play a role in the beetle's detection of ethanol, as well as their ability to fly. A related species, *T. lineatum*, was able to complete upwind oriented flights to baited traps at distances of up to 25 m, but beyond this distance the flights were largely downwind and undirected (Salom and McLean 1989). Similarly, study results support that with increasing distance into the nursery interior, and away from ambrosia beetle source populations, susceptible nursery stock may be subjected to less beetle pressure. The effect of prevailing winds may play an important role, as beetles attracted to volatile emissions may not detect stressed trees that are placed downwind, or conversely may find upwind flight more strenuous (Salom and McLean 1989; Ranger et al. 2014).

The use of a perimeter trapping program may augment the protection offered to nursery trees located at a greater distance from the nursery edge. Significantly more *T. lineatum* were captured in traps placed 100 m from the forest edge when intermediate traps at 5 m or 25 m were not present (Salom and McLean 1989). Therefore, a ring of baited traps at the forest/nursery interface may protect tree crops, as the availability of more proximal perimeter traps would likely intercept dispersing females from longer-distance flights into the nursery interior. Although traps located as close to the forest as 13 m had significantly lower beetle captures than did traps at the edge or within the forest, vulnerable nursery stock may not gain adequate protection from placement at 13 m. At nurseries deploying perimeter traps, placing susceptible cultivars at least 50 m from the nursery edge could help trees escape ambrosia beetle attacks, based on the low trap captures observed in this study at ≥ 50 m.

Perimeter trapping can provide advance warning of ambrosia beetle activity and potentially divert large numbers of dispersing females from susceptible tree crops. When combined with cultural measures, including maintaining tree vigor and placing vulnerable stock at nursery interiors, and a judicious spray program based on monitoring data from the traps, these cumulative efforts may lead to a highly effective, low-cost control program beneficial to nursery owners nationwide.

Table 1. Species composition of ambrosia beetles (Coleoptera: Curculionidae) captured in ethanol-baited Baker traps at ornamental tree nurseries in Louisiana (2013-2014), Mississippi (2013-2014) and South Carolina (2011-2012).

Species	% total specimens			
	Tangipahoa Parish, LA	Stone County, MS	Georgetown County, SC	Pickens County, SC
<i>Ambrosiodmus obliquus</i> (LeConte)	-	-	0.3	0.1
<i>Ambrosiodmus rubricollis</i> (Eichhoff)	0.3	0.2	0.6	0.3
<i>Ambrosiodmus tachygraphus</i> (Zimmermann)	-	-	-	< 0.1
<i>Ambrosiophilus atratus</i> (Eichhoff)	< 0.1	-	-	< 0.1
<i>Cnestus mutilatus</i> (Blandford)	1.3	0.6	-	1.7
<i>Cyclorhipidion bodoanum</i> (Reitter)	-	-	< 0.1	-
<i>Dryoxylon onoharaensis</i> (Murayama)	0.5	0.2	0.9	0.8
<i>Euwallacea validus</i> (Eichhoff)	0.6	0.5	-	-
<i>Xyleborinus octiesdentatus</i> (Murayama)	-	0.2	-	-
<i>Xyleborinus saxesenii</i> (Ratzeburg)	8.5	2.2	14.4	29.5
<i>Xyleborus affinis</i> Eichhoff	1.6	3.0	0.6	0.5
<i>Xyleborus celsus</i> Eichhoff	0.1	-	-	-
<i>Xyleborus ferrugineus</i> (F.)	1.9	2.0	0.4	0.3
<i>Xyleborus pubescens</i> Zimmermann	-	-	13.1	4.7
<i>Xylosandrus compactus</i> (Eichhoff)	13.9	18.3	0.4	0.2
<i>Xylosandrus crassiusculus</i> (Motschulsky)	71.2	72.8	69.1	58.5
<i>Xylosandrus germanus</i> (Blandford)	-	-	< 0.1	3.3

Table 2. Statistics of repeated measure ANOVA for effects of distance from nursery edge and collection time period on the numbers of *C. mutilatus*, *X. compactus*, *X. crassiusculus* and *X. germanus* captured per trap per 2 wk sampling period at ornamental tree nurseries in Louisiana, Mississippi and South Carolina.

Effect	Georgetown County, SC (2011)			Georgetown County, SC (2012)		
	df	F	P > F	df	F	P > F
Distance	5, 18	15.91	< 0.0001	5, 18	9.46	0.0001
Time	16, 288	11.22	< 0.0001	24, 432	11.42	< 0.0001
Distance x Time	80, 288	2.02	< 0.0001	120, 432	2.13	< 0.0001
	Pickens County, SC (2011)			Pickens County, SC (2012)		
	df	F	P > F	df	F	P > F
Distance	5, 18	27.27	< 0.0001	5, 18	31.18	< 0.0001
Time	18, 324	14.06	< 0.0001	22, 396	18.54	< 0.0001
Distance x Time	90, 324	1.91	< 0.0001	110, 396	2.57	< 0.0001
	Stone County, MS (2013)			Stone County, MS (2014)		
	df	F	P > F	df	F	P > F
Distance	4, 20	14.43	< 0.0001	5, 18	4.23	0.0101
Time	10, 200	2.84	0.0025	17, 306	8.02	< 0.0001
Distance x Time	40, 200	1.71	0.0093	85, 306	1.54	0.0043
	Tangipahoa Parish, LA (2013)			Tangipahoa Parish, LA (2014)		
	df	F	P > F	df	F	P > F
Distance	4, 20	5.75	0.0030	5, 18	10.57	< 0.0001
Time	10, 200	3.64	0.0002	17, 306	5.48	< 0.0001
Distance x Time	40, 200	2.42	< 0.0001	85, 306	1.40	0.0214

Table 3. Results of trend analysis for effects of distance from nursery edge on the numbers of *C. mutilatus*, *X. compactus*, *X. crassiusculus* and *X. germanus* captured at ornamental tree nurseries in Louisiana, Mississippi and South Carolina.

Contrast	Georgetown County, SC (2011)			Georgetown County, SC (2012)		
	df	<i>F</i>	<i>P</i> > <i>F</i>	df	<i>F</i>	<i>P</i> > <i>F</i>
Linear	1	28.91	< 0.0001	1	16.21	< 0.0001
Quadratic	1	6.71	0.0099	1	8.66	0.0034
Cubic	1	0.00	0.9904	1	0.06	0.8076
	Pickens County, SC (2011)			Pickens County, SC (2012)		
	df	<i>F</i>	<i>P</i> > <i>F</i>	df	<i>F</i>	<i>P</i> > <i>F</i>
Linear	1	59.23	< 0.0001	1	43.03	< 0.0001
Quadratic	1	26.79	< 0.0001	1	21.27	< 0.0001
Cubic	1	2.47	0.1164	1	2.58	0.1088
	Stone County, MS (2013)			Stone County, MS (2014)		
	df	<i>F</i>	<i>P</i> > <i>F</i>	df	<i>F</i>	<i>P</i> > <i>F</i>
Linear	1	59.78	< 0.0001	1	20.08	< 0.0001
Quadratic	1	26.55	< 0.0001	1	10.98	0.0010
Cubic	1	12.18	0.0006	1	0.50	0.4793
	Tangipahoa Parish, LA (2013)			Tangipahoa Parish, LA (2014)		
	df	<i>F</i>	<i>P</i> > <i>F</i>	df	<i>F</i>	<i>P</i> > <i>F</i>
Linear	1	53.04	< 0.0001	1	9.44	0.0023
Quadratic	1	65.70	< 0.0001	1	38.69	< 0.0001
Cubic	1	17.35	< 0.0001	1	8.40	0.0040



Fig. 3. Satellite image of the MS research site (Google, Mountain View, CA) with an overlay showing a Randomized Complete Block Design of five blocks. Representing the 2013 test, each block shown here had a trap placed at -25, 25, 50, 100, and 200 m from the nursery/forest interface.

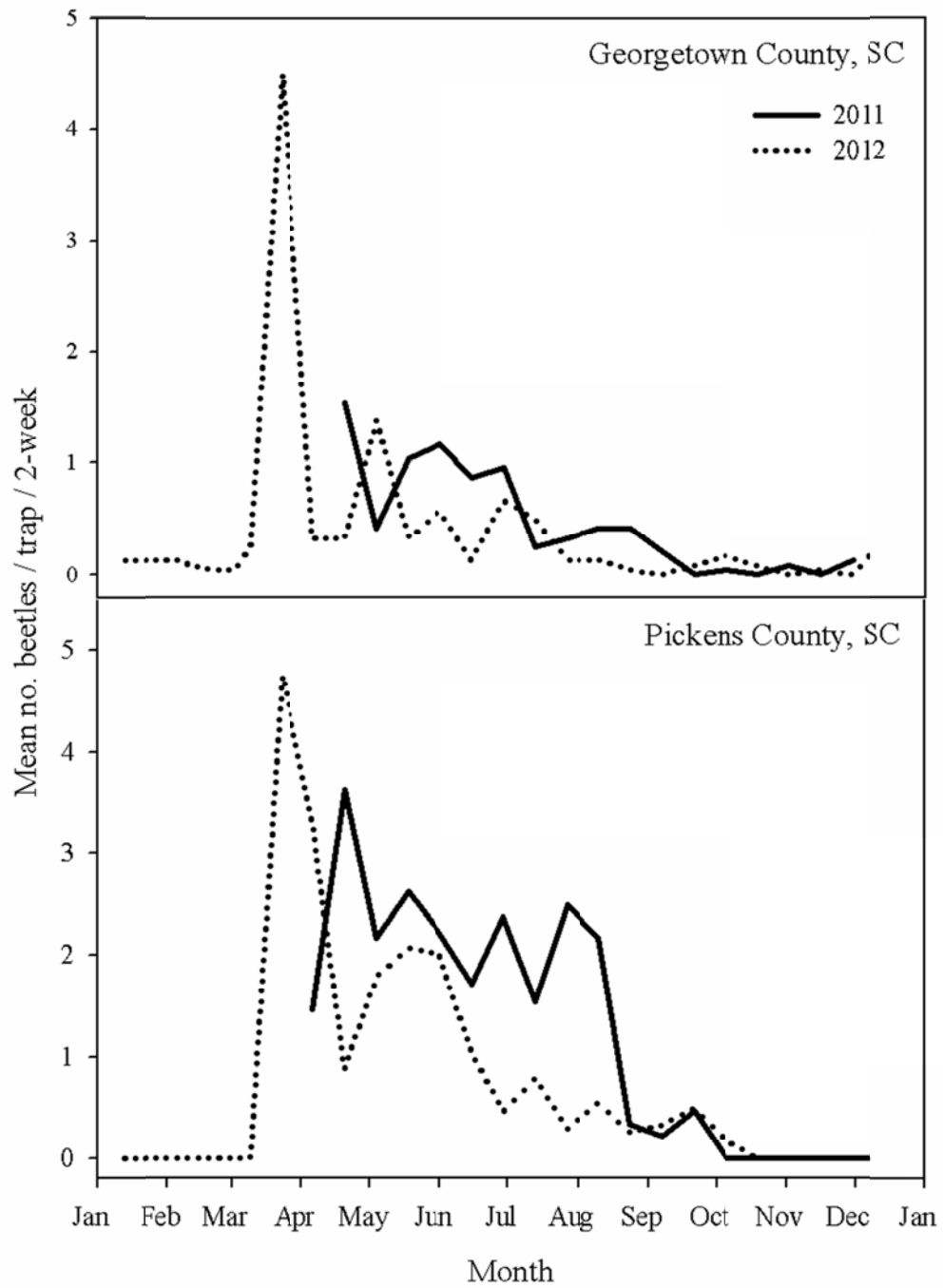


Fig. 4. Mean ambrosia beetles captured in ethanol-baited Baker traps at two sites in South Carolina in 2011 and 2012.

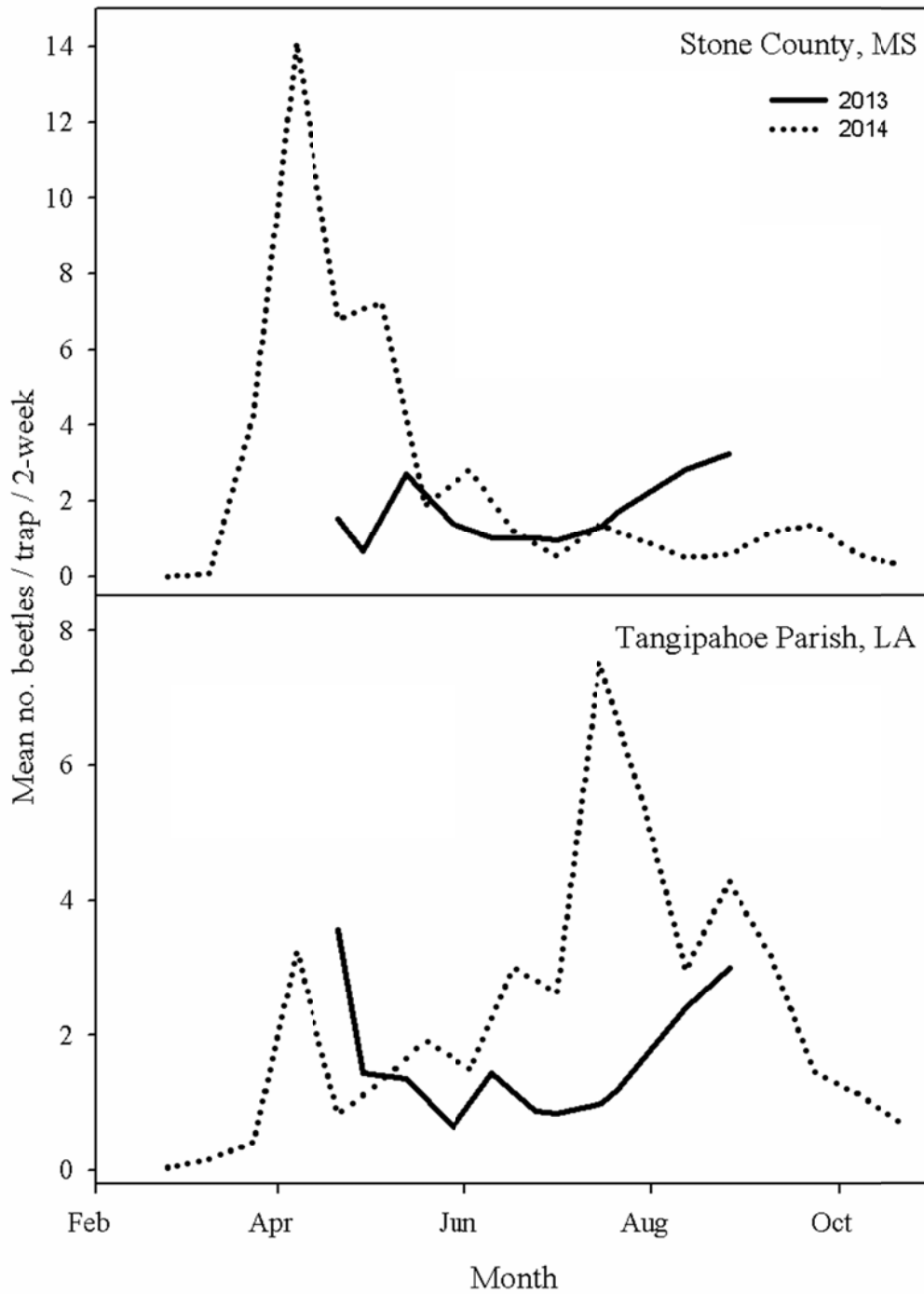


Fig. 5. Mean ambrosia beetles captured in ethanol-baited Baker traps at two sites in Louisiana and Mississippi in 2013 and 2014.

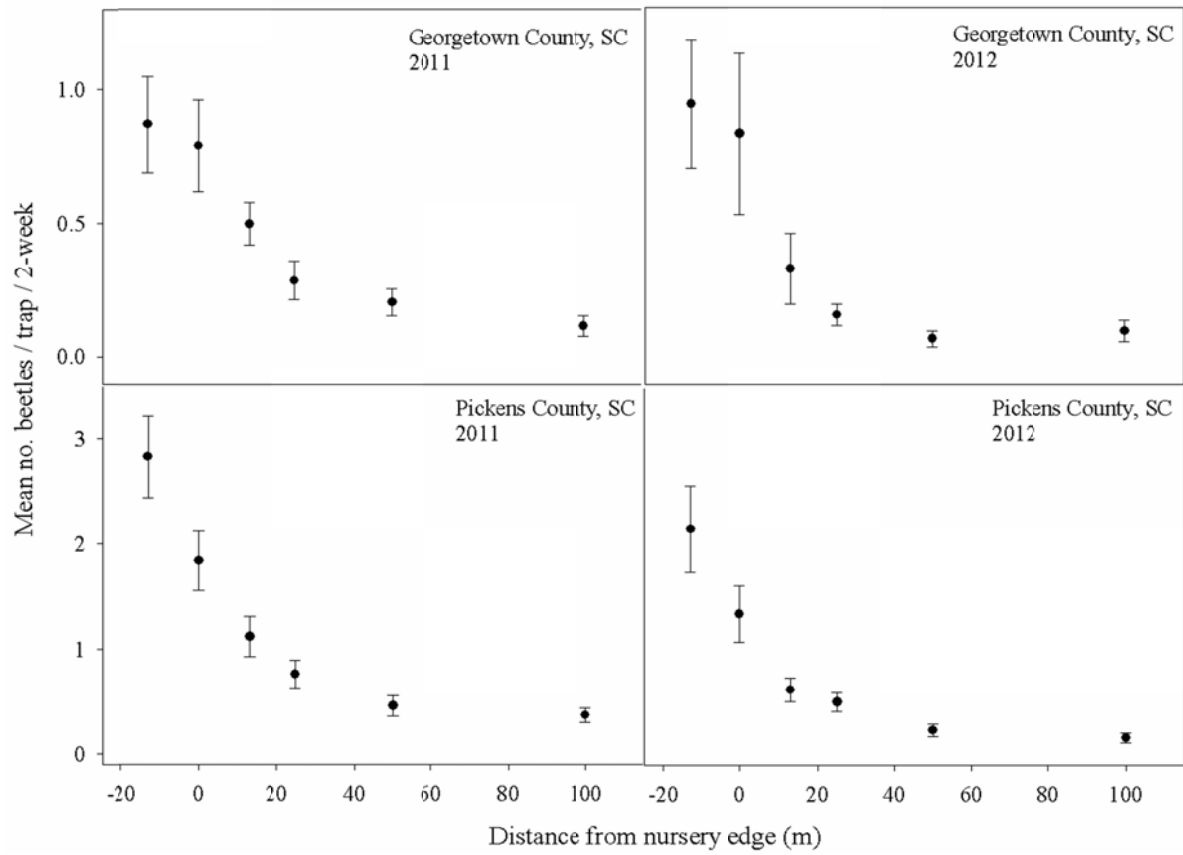


Fig. 6. Mean ambrosia beetles (+/- SEM) captured in ethanol-baited Baker traps deployed at various distances from the nursery-forest interface at two sites in South Carolina in 2011 and 2012.

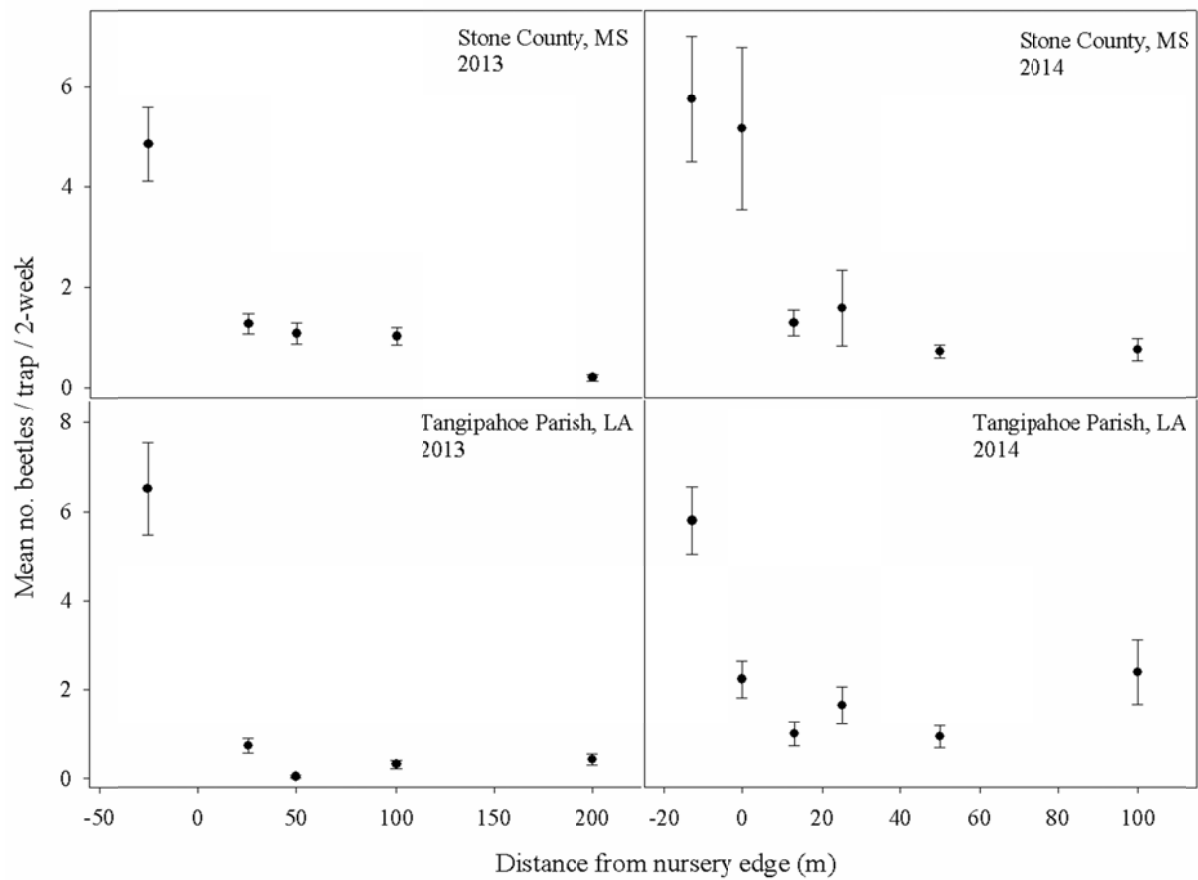


Fig. 7. Mean ambrosia beetles (+/- SEM) captured in ethanol-baited Baker traps deployed at various distances from the nursery-forest interface at two sites in Louisiana and Mississippi in 2013 and 2014.

CHAPTER 4: EFFICACY OF KAOLIN CLAY AS AN AMBROSIA BEETLE DETERRENT

Introduction

Recent discoveries in ambrosia beetle biology are contributing to a more efficient monitoring program, enabling growers to time preventative insecticidal treatments for maximum efficacy (Reding *et al.* 2011, Ranger *et al.* 2014, Werle *et al.* 2015). However, susceptible trees may remain vulnerable after pesticide applications, which are not entirely effective. Due to the long ambrosia beetle flight period, repeated applications of insecticide are necessary for maintaining protection on a nursery tree crop (Hudson and Mizell 1999, Werle *et al.* 2015). Current treatment recommendations include monitoring for population emergence in early spring with applications of pyrethroids every three to four weeks as soon as ambrosia beetles are detected (Mizell *et al.* 1998).

Multiple treatments are costly for growers and can impact natural enemies of a variety of arthropod pests, leading to secondary pest outbreaks that reduce plant vigor and decrease marketability of a crop (Frank and Sadoff 2011). In addition, some classes of insecticides currently are being reviewed by environmental protection organizations for potential non-target impacts, particularly on pollinating insects, with the possibility of further limiting or banning their applications (Campbell 2013). Therefore, effective alternatives are needed to prevent an overdependence on conventional insecticides and the development of both resistance and secondary pest emergence (Pimentel *et al.* 1992). A comprehensive push-pull IPM strategy may offer the best results for managing destructive ambrosia beetles in a cost-effective manner. This strategy might incorporate

visual and olfactory cues as well as optimal trap and crop location, as discussed in prior chapters, and would deter beetles from attacking trees (push) while attracting them into lethal traps (pull) (Cook *et al.* 2007, Ranger *et al.* 2011, 2012, 2013; Burbano *et al.* 2012, VanDerLaan and Ginzel 2013, Reding *et al.* 2015, Werle *et al.* 2015).

Use of particle film technology centered on kaolin clay has become widespread in pest control for certain crops (Glenn *et al.* 1999). Typically utilized in tree fruit protection, kaolin applications can coat foliage, trunk and fruit alike, disrupting existing pest populations or deterring immigrating pests. Treated trees may become visually repellent to pests due to the white reflective surface. While this approach has led to reports of success against aphids, lace bugs, fruit flies, thrips, psyllids, whiteflies and leafhoppers, there is potential for an even wider pest management application for kaolin (Glenn *et al.* 1999, Puterka *et al.* 2000, Mazor and Erez 2004, Saour 2005, Spiers *et al.* 2005, Villanueva and Walgenbach 2007, Marcotegui *et al.* 2015, Nunez-Lopez *et al.* 2015).

While not necessarily a direct cause of adult mortality, a physical barrier of kaolin will impact insect movement, feeding and oviposition by altering tactile recognition of plant surfaces and attachment of particles to the insect body (Glenn *et al.* 1999, Puterka *et al.* 2000, Unruh *et al.* 2000, Larentzaki *et al.* 2008). Even when compared with conventional insecticides such as carbaryl, this barrier effect can make kaolin equally proficient at reducing feeding damage by a wide range of insect pests, including Japanese beetle (*Popillia japonica* Newman) (Mmbaga and Oliver 2007). Applications of kaolin also have been impregnated with pesticides or essential oils to increase the toxicity of the

treatment to insect pests (Marcotegui *et al.* 2015). In addition to the physical barrier presented by kaolin, I discussed in chapter one how ambrosia beetles are less-attracted to traps colored white or yellow as compared to darker colors, so kaolin may also provide a visual deterrent to ambrosia beetles (Entwistle 1963, Dubbel *et al.* 1985, Mizell and Tedders 1999, Goyer *et al.* 2004, Werle *et al.* 2014). For these reasons, we hypothesized that kaolin would provide an effective "push" component to a larger push-pull ambrosia beetle management strategy.

Prior research has revealed some trees attacked by ambrosia beetles have partial holes excavated just past the bark layer, representing galleries that were abandoned before completion. These abandoned galleries can be directly adjacent to completed galleries, or in more isolated parts of the tree. To date, a sufficient explanation has not been made for this phenomenon and further investigation is warranted.

Because of the incomplete knowledge regarding this economically important pest of ornamental tree production, and the need for more diverse and effective control measures, our research objectives were: 1) to determine whether there is any correlation between successful vs. abandoned beetle galleries ; and 2) to assess the efficacy of kaolin, both alone and with an insecticidal additive, for reducing ambrosia beetle attacks over time.

Materials and Methods

Research was conducted at a commercial ornamental nursery in Stone County, MS (30°47'59.92"N, 89°15'21.64"W; 2014) and at the Thad Cochran Southern Horticultural Laboratory in Pearl River County, MS (30°65'96.84"N, 89°63'50.69"W; 2015), and at Tennessee State University's Otis Floyd Nursery Research Center in Warren County, TN (35°42'34.81"N, 85°44'27.94"W; 2014-15). While ambrosia beetle species composition varies between these two States, species responses have been similar in prior experiments (Oliver and Mannion 2001, Werle *et al.* 2012, 2014). To stimulate ambrosia beetle attack, containerized eastern redbud (*C. canadensis*) trees were injected with 75 mL of 5% ethanol using an Arborjet Tree I.V. (Arborjet Inc., Woburn, MA). Following injection, a backpack sprayer (Solo, Newport News, VA) was used to apply three treatments: 1) a pyrethroid typically used in the nursery industry (OnyxPro; FMC Corp., Philadelphia, PA), mixed at a rate of 1.25 mL/L and applied to runoff on the tree trunk and canopy; 2) a kaolin crop protectant (Surround WP; Tessengerlo Kerley, Inc., Phoenix, AZ), mixed at 60 g/L and applied to runoff on the tree trunk and canopy; and 3) a combination of treatments one and two mixed at the same rates. In each case, treatments were sprayed onto the main trunk of the trees until runoff, and after 10 min, treatments were reapplied to runoff. In addition, there was a fourth, control treatment with nothing sprayed. Trees were deployed 20 m apart in a randomized complete block design along the edge of woodlots at each research site. The MS sites had three blocks in 2014 and five blocks in 2015, while the TN site had four blocks in both 2014-15.

Tests were begun during peak ambrosia beetle flight at each site (early April in MS and early May in TN), with new ambrosia beetle galleries on each tree counted at one, four, six, eight, eleven and thirteen days after treatment (DAT). Beetle galleries were circled with a wax pencil to prevent re-counting of previous attacks. In addition to counts of completed galleries, counts were made of abandoned galleries.

Statistical analysis. All statistical analyses were conducted using SAS (SAS Institute 2013). Pearson correlation analysis was conducted on the number of galleries vs. abandoned galleries on each tree to determine if there was a significant relationship ($\alpha=0.05$; PROC CORR). The dependant variable was tested using analysis of variance (REML-ANOVA; PROC GLIMMIX). In order to improve the probability distribution, data were transformed using the lognormal (\log_{10}) function. Preliminary analysis showed there were significant differences for the main effect of time ($P < 0.001$) across the insecticide treatments, but not for site ($P = 0.381$) or year ($P = 0.083$); therefore, separate analyses were conducted for each time interval using the BY statement.

The fixed effects in each model were site, season, treatment and their second order interactions while gallery counts were the response variable. Tree was the random variable within each treatment and the default (containment) degree of freedom method was used. Estimated means, standard errors, and differences of means were calculated using the LSMEANS option. \log_{10} of the LSMEANS were then back-transformed. Least significant differences (LSD) were calculated from the LSMEANS comparisons on a log scale and least

significant ratios (LSR) calculated to determine statistical differences among the four treatments at each observation time.

Results and Discussion

Beetle pressure at the two research sites was disproportionate, with 73% of the total galleries from both years counted on trees from the TN site. However, the disproportion was primarily evident in the first year when galleries at the MS site accounted for only 9.1% of that yearly total. Because of this extremely low ambrosia beetle pressure at the Stone Co., MS research site in 2014, that data set was excluded from analysis and the test was moved to a different location in Pearl River Co., MS for year two. This move yielded a much more proportionate data set, with 49.8% of total galleries counted at the MS site in year two.

Correlation analysis revealed a positive, though non-significant relationship between the number of completed vs. abandoned galleries ($r = 0.2264$; $P = 0.107$). Due to this positive correlation, further analysis was conducted on combined (abandoned + completed) gallery counts. While we now know that this phenomenon is simply density dependent, i.e. greater abandonment on trees with more attacks, we still do not know what causes some ambrosia beetles to cease excavation prematurely. One possible explanation for this potentially important life history trait is predation of beetles during gallery excavation; checkered beetles (Coleoptera: Cleridae) are well known scolytine predators,

and are readily collected from baited traps (Allison *et al.* 2013, Werle *et al.* 2012). Another explanation for gallery abandonment may be a rejection of that region of the tree by the foundress beetle if it is deemed unsatisfactory for brood development. For example, insecticidal treatments may disrupt contact or volatile cues for the foundress, thereby increasing the likelihood of gallery abandonment as compared with control trees. Although our gallery abandonment data is not significant, it is still interesting to note that the kaolin (26.6%) and kaolin + bifenthrin (k+b; 28.6%) trees did receive a higher proportion of abandoned galleries as compared with the bifenthrin (15%) and control (12.1%) trees (Fig. 1). Future tests may still include measurements of gallery abandonment to determine treatment efficacy.

Analysis of variance determined a statistically significant model for between-treatment effects at one, four, six and eight DAT, but not thereafter, so only the data from the first four observation times are presented (Table 1). The treatment effect from the first DAT was significant ($df = 3, 16; F = 11; P < 0.001$) due to greater numbers of beetle galleries on control trees compared with the other three treatments. Kaolin trees performed as well as the bifenthrin trees in this first observation, but galleries on the k+b trees were significantly less than on both control and kaolin trees. At no time were significant differences detected between k+b and bifenthrin-treated trees.

At four DAT, we had significant treatment ($df = 3, 16; F = 15.55; P < 0.001$) and site effects ($df = 1; F = 13.17; P = 0.001$), and at this time we had our only significant site*treatment ($df = 3, 24; F = 4.11; P = 0.017$) and season*treatment ($df = 3, 24; F = 7.45; P$

= 0.001) interactions. Beetle pressure at 4 DAT was much greater than at any other observation time, averaging 13.5 beetle galleries per tree as opposed to only 4.8 galleries per tree at the next closest time, 6 DAT. The higher numbers of beetle galleries also were associated with increased variability, so significant site*treatment and season*treatment interactions were not so surprising. All of the other observation times had more consistent beetle pressure, and no significant interactions of treatment with either site or season were detected.

At four DAT, the kaolin-only treatment became less effective at deterring beetle attacks. While galleries on bifenthrin and k+b trees were significantly lower than both the control and kaolin trees, the kaolin trees were no different than the controls at this time. This increase in attacks may be explained by rain events at both sites in 2015, which began to denude coverage on the kaolin-only trees.

At both six (df = 3,16; F = 4.49; P = 0.018) and eight (df = 3,12; F = 6; P = 0.01) DAT, the treatment effects were still significant, but weather conditions at each site in 2015 made data collection temporarily impossible. At the TN site, heavy rain at six DAT prevented counts of beetle galleries, with the damp wood making it difficult to distinguish the tiny holes. In addition, circling the galleries to prevent recounts was not possible on the wet bark, so data were not recorded in TN at six DAT. In MS, while there was rain during that whole second week of the test in 2015, storms were particularly heavy at eight DAT, so no MS data were recorded at this time. For this reason, season and state effects could not be calculated for the six DAT data. While the eight DAT data had a significant season effect

(df = 1,15; F = 11.08; P = 0.005) due to the greater beetle pressure in 2014, no site effect could be calculated. However, these data still reveal some valuable information, with contrasts of treatment ratios the same as they were at four DAT. But by eight DAT, the bifenthrin treatments started to lose some efficacy; while still lower than the control trees, both bifenthrin and k+b trees at eight DAT were not significantly different from the kaolin-only trees. As the industry-standard method for ambrosia beetle management, a reduced number of galleries on bifenthrin and k+b trees compared with control trees might be expected through eight DAT. While attack suppression did drop off after 8 DAT, Hudson and Mizell (1999) suggested repeating bifenthrin applications in 10-14 day intervals during peak beetle flights. While our data supports Hudson and Mizell's recommendation, in cases of heavy rains over extended periods, we might suggest that the bifenthrin treatment interval be shortened.

Because we did not observe any differences between the bifenthrin and k+b treatments at any of our observation times, we surmise that kaolin had little impact on the gallery suppression provided by the k+b treatment. And while kaolin did provide some control early on, coverage on kaolin-only trees became noticeably diminished following rain events, particularly after heavy rain made collection of data impossible during one observation time at each State site in 2015. The additional surfactant present in the bifenthrin product likely increased the k+b persistence through rain at both sites and both years. Our results parallel those from other researchers, who found that kaolin made an excellent alternative to conventional chemicals in a variety of crop settings, but its hydrophilic nature at standard formulations made repeated applications necessary (Glenn

1999, Larentzaki et al. 2008). A technical representative of the kaolin manufacturer did recommend that a layering effect or incorporation of an additional spreader/sticker-type adjuvant may be necessary for adequate persistence (K. Volker, personal communication).

When combined with a supplementary surfactant that can extend coverage on trees, kaolin applications will contribute some deterrent to ambrosia beetle attacks. However, the additional costs of follow-up applications would likely not justify the modest degree of added control. Therefore, we do not recommend kaolin as a "push" component for a larger push-pull ambrosia beetle IPM strategy.

Aside from reducing ambrosia beetle infestations, kaolin is known to have a wide range of horticultural benefits in crop production. Much research has been directed towards kaolins reduction of heat stress and increasing leaf carbon assimilation, as well as fruit weight, quality and yield in a variety of crops (Glenn *et al.* 1999, 2001, 2002, 2003, Melgarejo *et al.* 2004, Saour 2005). Containerized plants at southeastern ornamental nurseries may be subjected to chronic heat stress during summer months, which can lead to water loss, metabolic lesions, decreased photosynthetic and respiration efficiency, loss of membrane integrity and electrolyte leakage. Due to an increased reflection of infrared radiation, applications of kaolin have been shown to significantly reduce tree canopy temperatures and heat stress (Glenn *et al.* 1999, 2001).

Many plant pathogens require a moist surface as well as direct contact with the host tissues for germination of propagules, and a coating of hydrophobic kaolin can obstruct

disease inoculum from infecting host tissues (Marco *et al.* 1994, Glenn *et al.* 1999). Applications of kaolin have been found to be as effective in reducing disease severity on crapemyrtle (*Lagerstroemia indica* L.) as conventional fungicides (Mmbaga and Oliver 2007). The symbiotic fungi associated with many species of ambrosia beetles (*Ambrosiella* spp.) may not be primary pathogens in host trees, but beetle galleries can weaken trees and become an easy entry point for more virulent secondary infections, including *Fusarium* spp. (Kessler 1974, Anderson and Hoffard 1978, Weber and McPherson 1984, Kinuura 1995, Kuhnholz *et al.* 2001, Dute *et al.* 2002).

In recent years, researchers have been noticing an increase in spring mortality of trees apparently caused by ambrosia beetle attacks, and subsequent infection by *Fusarium* spp. Manifesting as numerous orange cankers along the main trunk and branches, one such outbreak of *F. lateritium* (Nees) killed an entire block of >100 trees at a MS nursery in 2014. While more research is needed, it is believed that these *Fusarium* outbreaks, as well as the ambrosia beetle attacks, are secondary problems on trees that have experienced a primary stressor like winter freeze injury (Ranger *et al.* 2016; C. T. W., unpublished data).

Winter injury is more commonly caused by wide temperature fluctuations than by just cold weather (Relf and Appleton 2015). When trees are properly acclimated to cold weather by going dormant, they can survive even severe winter conditions. However, mild daytime temperatures can cause trees to break winter dormancy and begin drawing up water from the roots. Subsequent temperature drops can then expose the tree to risk of freeze injury. In addition to inhibiting pathogen infection, a white layer of kaolin may serve

to reflect heat and delay breaking of dormancy during mild winter days, potentially reducing susceptibility to winter freeze injury and the ensuing *Fusarium* infections and ambrosia beetle attacks.

With temperatures that are projected to continue rising due to global warming, episodes of both chronic heat stress in the summer and winter freeze injury may become more commonplace at ornamental nurseries, leading to an increased susceptibility of crops to ambrosia beetles (Kamata *et al.*2002, Choi 2011). Further research on the impact kaolin can make in reducing tree stress, and subsequent susceptibility to ambrosia beetle attacks and *Fusarium* infections, is warranted.

Table 4. Effects of time and tree insecticide treatments on numbers of ambrosia beetle galleries per tree in MS (2015) and TN (2014-15).

Treatments	Mean beetle galleries ^a			
	1 DAT	4 DAT	6 DAT	8 DAT
1. Control	18.634	140.864	42.993	26.712
2. Kaolin	1.4534	45.635	26.589	2.194
3. Bifenthrin	0.369	0.568	0.817	0.668
4. Kaolin + Bifenthrin	0.200	0.386	1.377	0.564
Ratio 4:1	93.068 - S	364.922 - S	31.232 - S	47.359 - S
Ratio 4:2	7.258 - S	118.223 - S	19.315 - S	3.891 - NS
Ratio 4:3	1.842 - NS	1.471 - NS	0.593 - NS	1.184 - NS
Ratio 3:1	50.5248 - S	248.085 - S	52.648 - S	39.995 - S
Ratio 3:2	0.254 - NS	80.371 - S	32.56 - S	3.286 - NS
Ratio 2:1	12.823 - S	3.087 - NS	1.617 - NS	12.173 - S
LSR ^a	4.474	6.582	10.527	11.05

^aLog LS means were transformed back to the original scale.

^bLSR, least significant ratio. Ratio values are considered significant (S) if they are greater than, and not significant (NS) if they are less than the respective LSR ($\alpha = 0.05$).

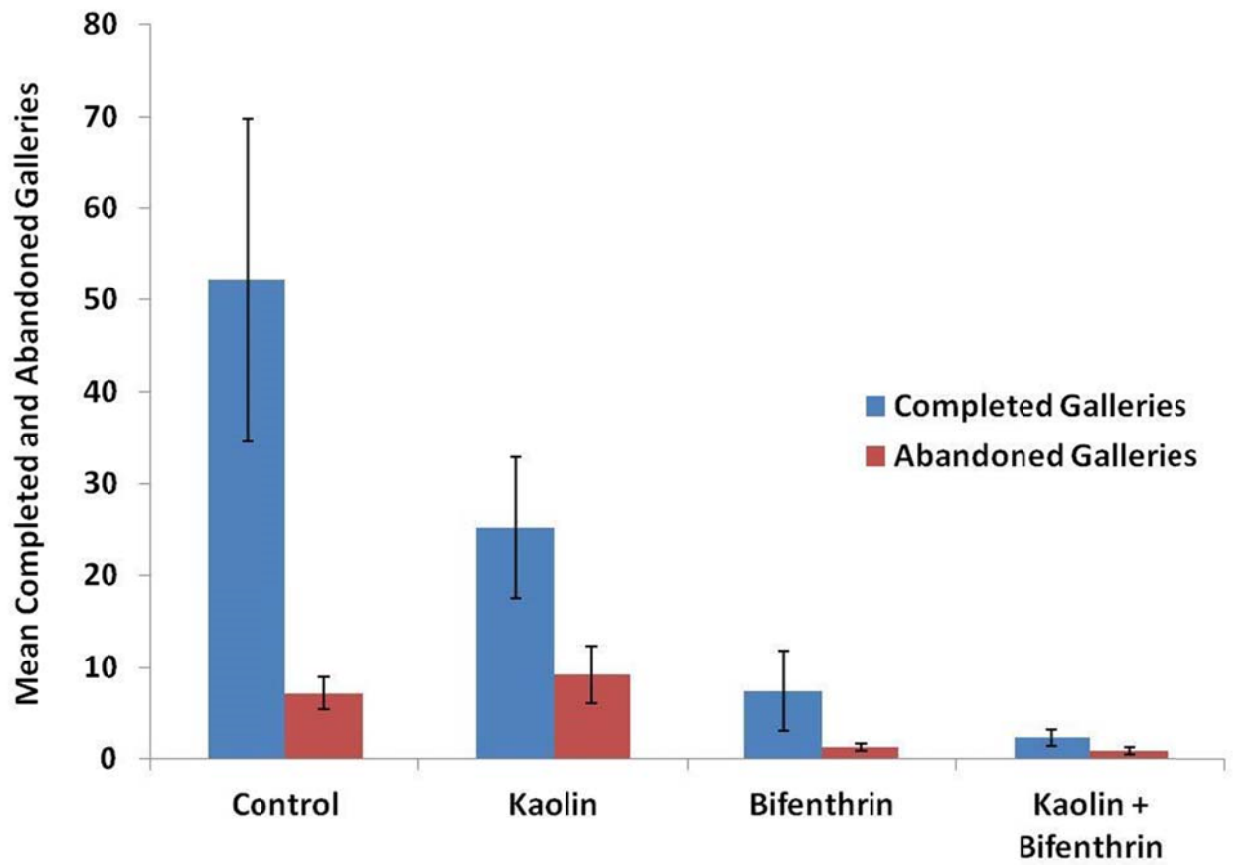


Fig. 8. Mean (\pm SE) number of completed vs. abandoned ambrosia beetle galleries from each of the four tree treatments in MS (2015) and TN (2014-15).

CHAPTER 5: CONCLUSIONS

Conclusions

Even with all of the new information presented in this dissertation, the foundation of the ambrosia beetle IPM strategy remains one of the earliest-discovered, most basic aspects: accurate population monitoring. We are fortunate to have highly effective ethanol lures for use with a variety of traps; from the more expensive black Lindgren funnels that incorporate the characteristic tree-bole silhouette, to inexpensive homemade Baker traps. And we now know that by placing traps at the forest/nursery interface, and checking them regularly from early spring through late summer, a nursery manager will know when a new generation of ambrosia beetles is dispersing to new hosts, and when preventative chemical treatments should be applied. Continued monitoring will also let the manager know to stop spraying when beetles are between flight periods, saving money and keeping both consumer costs and non-target impacts low.

We also learned that placing susceptible cultivars at the nursery interior, further away from source populations in surrounding woodlots, may help vulnerable nursery crops to avoid ambrosia beetle attacks. When using a perimeter trapping program, even placing crops at a modest distance of 50 m from the nursery/forest interface may provide protection.

While we now know that applications of kaolin clay on their own may not yield adequate control of ambrosia beetles, there exists potential for further exploration of

different formulations, particularly in combination with spreader/sticker adjuvants that can extend coverage through rain events. Kaolin may also provide a reduction in heat stress of nursery crops, prevention of early release of dormancy, or even disease prevention, and these avenues are all deserving of further research. It is also helpful to have the additional validation from our research that bifenthrin can be an effective deterrent to ambrosia beetle attacks.

In spite of all these gains, there remains much work to be done on this important horticultural problem. It appears that the dispersal phase of the ambrosia beetle lifecycle represents a key factor that may be exploited for effective pest management, and that development of a mass-trapping technique may intercept large numbers of dispersing beetles before they even detect a vulnerable tree crop. Looking beyond my doctoral work at LSU, I will be testing these theories starting in the spring of 2016, with perimeter-trapping and crop-location variables incorporated into a comprehensive push-pull IPM strategy. This strategy will incorporate visual and olfactory cues as well as optimal trap and crop location, and will deter beetles from attacking trees (push) while attracting them into lethal traps (pull) (Cook *et al.* 2007). A second test starting in 2016 will help to determine optimal spacing of perimeter traps to protect nursery crops in the most efficient manner.

Aside from these crop protection strategies, very little is known about the impact of predators and parasites on ambrosia beetle populations. Biocontrol can make an important contribution to an IPM program, and research directed towards identifying and

assessing the potential of checkered flower beetles (Coleoptera: Cleridae) as ambrosia beetle predators may yield valuable biocontrol information towards this end.

The ambrosia beetle community has been a fascinating group to work on, and while there are still many questions regarding the most effective management strategies, I am confident that as my career progresses we will eventually remove ambrosia beetles from the list of the most-damaging insects at southeastern nurseries.

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VITA

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