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Chemical ecology, fungal interactions and forest stand correlations of the exotic Asian ambrosia beetle, Xylosandrus crassiusculus (Motschulsky) (Curculionidae)

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CHEMICAL ECOLOGY, FUNGAL INTERACTIONS AND FOREST STAND CORRELATIONS OF THE EXOTIC ASIAN AMBROSIA BEETLE, *XYLOSANDRUS CRASSIUSCULUS* (MOTSCHULSKY) (CURCULIONIDAE)

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ABSTRACT

Increasing evidence of non-indigenous ambrosia beetles aggressively attacking hosts in their new environment in the United States has led to concern over the potential for damage to urban trees, nurseries, orchards, and forests. A novel technique of flooding host trees was devised to stimulate ambrosia beetle attacks, with ambrosia beetle attraction peaking four days following flooding. *In-situ* sampling identified significant differences in the composition, quantity and point of release (leaf or bole) of volatiles emitted by the flooded and non-flooded trees. Coupled gas chromatography electroantennographic detection revealed olfactory sensitivity by the ambrosia beetle *Xylosandrus crassiusculus* (Motschulsky) to 29 of these compounds and 12 other compounds apparently not associated with hosts. Traps baited with the combination of ethanol and eugenol showed a mean increase in catches over ethanol baits alone. During a trapping survey of Camp Beauregard, Louisiana, flight periods and biodiversity indices were collected for up to 37 species of ambrosia beetles.. Multiple regression analyses identified significant correlations between forest stand characteristics and ambrosia beetle abundances. In fungal competition and vectoring experiments, *Rafaella* sp., a highly pathogenic, recently discovered fungus associated with the newly-established, exotic ambrosia beetle *Xyleborus glabratus* (Eichhoff), did not provide significant nutritional benefits to *X. crassiusculus.* When *Rafaella* sp. was introduced into a laboratory rearing medium in advance of *X. crassiusculus*, fewer beetle offspring ultimately emerged. Additionally, the ambrosial associate of *X. crassiusculus*, *Ambrosiella*

xylebori, demonstrated superior ability to secure and hold resources against *Rafaella* sp. in differential and spatial separation competition experiments. Relatively earlier addition of *Rafaella* sp. into beetle media decreased the likelihood of gallery construction, suggesting that *X. crassiusculus* could detect the presence of *Rafaella sp*. These three experiments support the hypothesis that these two fungi might compete for spatial and/or nutritional resources, ultimately lowering the fitness of *X. crassiusculus*. There was no evidence that *X. crassiusculus* could transport *Rafaella sp*. in its mycangium, hence *X. crassiusculus* likely cannot serve as a significant vector of *Rafaella sp*. in the field.

CHAPTER I - INTRODUCTION

The bark beetle guild (family Curculionidae; subfamily Scolytinae) comprises the most economically important insect group affecting North American trees (Coulson and Stark 1982, Waters et al. 1985, Paine *et al.* 1997). This guild includes the bark beetles, which feed and reproduce entirely within the host's bark, and the ambrosia beetles, which mine into the sapwood where both adults and larvae feed on the growth of symbiotic fungi. While sometimes capable of killing healthy trees, ambrosia beetles are more often found attacking weakened or felled trees or are secondarily associated with bark beetle attacks (Flechtmann *et al.* 1999). Although ambrosia beetles cause significantly less tree mortality than bark beetles, their habit of mining into felled trees causes physical and aesthetic damage to lumber (Dobie 1978), resulting in the loss of millions of dollars due to wood quality degradation (Lindgren and Fraser 1994) and loss of timber exports (Hosking 1969). Ambrosia beetles are also significant pests of urban forests and ornamental tree nurseries. Like many forms of biotic damage, the severity of ambrosia beetle impact depends on the specific biology of the beetle as well as host and climate factors.

AMBROSIA BEETLE CHEMICAL ATTRACTION

Ambrosia beetles generally prefer stressed and dying hosts, although many species can attack vigorous trees (Kuhnholz *et al*. 2001). Some species arrive four to six weeks after bark beetles or other damaging agents have already killed or severely stressed trees (Flechtmann *et al.* 1999). Ambrosia

beetles exhibit attraction to volatiles derived from host trees and thus are believed to locate suitable hosts mainly via response to host-produced compounds. During the past thirty years, ambrosia beetles have increasingly been found attacking and inhabiting healthy coniferous and hardwood trees in the United States (Arnett 2000, Kuhnholz *et al.* 2001). One recently introduced ambrosia beetle, *Xylosandrus crassiusculus* (Motschulsky) has been causing exceptional amounts of damage to living and stressed trees in the Southeast (Oliver and Mannion 2001).

FUNGAL ASSOCIATION

Ambrosia beetles derive their name from their habit of inoculating their galleries with obligate, mutualistic fungi that are the sole source of nutrition for both the larvae and adults (Arnett 2000). After locating a suitable host tree, a single, mated foundress bores directly into the xylem and constructs a multi-pronged gallery system. Three days following gallery initiation, growth of mutualistic fungi can be observed within the galleries of *X. crassiusculus* (personal observation). Roughly four days after gallery initiation and contingent upon successful fungal inoculation, the foundress begins laying 20-50 eggs (personal observation, Norris 1972) which develop into adults in approximately one month during warm weather. Many ambrosia beetles (e.g., the tribe Xyloborini) are genetically haplodiploid and characterized by strictly inbred, female-skewed sex ratios (Kirkendall 1997).

Ambrosia beetle-fungal interactions have been studied by entomologists, plant pathologists, and ecologists (Norris 1965, Batra 1966, Batra 1967, Norris

1972, Norris 1975, Batra 1979, Bever 1989, Kajimura and Hijii 1992, Six 2003, Six and Klepzig 2004). Research has focused on the systematics of associated fungi (Blackwell and Jones 1997, Harrington *et al*. 2001),as well as the nutritional requirements of beetles and mutualistic nature of the symbiosis (Kajimura and Hijii 1992, Norris 1965, Norris 1972). One study showed intraspecific competition among ambrosia beetle larvae (Beaver 1989), However, little attention has been given to inter- or intraspecific competition between ambrosia beetles and associated fungi.

INVASIVE AND EXOTIC AND NATIVE BEETLES

The exotic ambrosia beetles, *X. crassiusculus*, *X. compactus*, *X. glabratus* and *X. germanus*, have a broad host range and will attack apparently healthy trees (Weber 1978, Atkinson *et al*. 1988). In particular, members of the genus *Xylosandrus* (including *X. germanus*, *X. compactus*, and *X. crassiusculus*) have caused considerable damage since their introductions in 1932, 1952, and 1974, respectively (Felt 1932, Anderson 1974, Ngoan *et al*. 1976, Wood 1977, Anderson and Hoffard 1978, Weber 1982, Mitzell *et al*. 1994). Native host plants often display decreased resistance to introduced insects and pathogens. Further problems arise when introduction of a pest species into a new environment is associated with a release from natural predators. The threat posed by exotic ambrosia beetles demands further research to quantify their effects and develop effective management techniques.

MANAGEMENT OF STEM-INFESTING BEETLES

Losses from bark and ambrosia beetles can often be reduced through adjustment of silvicultural practices, insecticide application, sanitation, treatments with behavior-modifying semiochemicals, and biological control (Aukema et al, 1999). Silvicultural guidelines for minimizing risk of ambrosia beetle attacks prescribe maintaining tree health and vigor through proper watering, pruning and fertilizing (Coyle 2005). Baited traps are commonly used to monitor ambrosia beetle population levels, detect incipient populations, predict attacks and outbreaks, and plan control measures. Successful trapping requires an effective bait for the target pest, and, for bark and ambrosia beetles, baits commonly consist of blends of synthetic host volatiles and/or insect-produced compounds.

My objectives for this study were to:

- Calculate biodiversity indices (abundance, evenness, biodiversity) for exotic ambrosia beetles in central Louisiana.
- Document seasonal variation in ambrosia beetle abundance and correlate ambrosia beetle abundances with forest stand characteristics.
- Identify volatile compounds utilized by ambrosia beetles in distinguishing stressed trees and develop an improved trapping bait for *X. crassiusculus*
- Determine whether *X. crassiusculus* can vector pathogenic *Rafaella sp*. and if *Rafaella sp.* can have an effect upon *X. crassiusculus* fitness.

CHAPTER II – FOREST STAND CORRELATIONS

INTRODUCTION

Insects have been shown to be important ecological indicators in aquatic (Resh and McElravy 1993; Terrell and Perfetti 1989) and terrestrial systems (Peck et. Al 1998; Holloway and Stork 1991; Kromp 1990). Ambrosia beetles play a vital role in the decomposition of dead and dying trees by introducing and opening pathways for fungi and decay-associated organisms (French and Roeper 1972; Zhong and Schowalter 1989). The importance of decaying logs has been demonstrated in long-term nutrient cycling, forest composition, and wildlife (Boddy 1983; Harmon *et al.* 1986; Swift 1977). Significant changes in the ambrosia beetle community may have serious implications to the decomposition of dead and dying trees, influencing many aspects of forest ecosystem regulation and health.

As a part of a cooperative agreement between the USDA Forest Service and the Louisiana Army National Guard a trapping survey was used to detect ambrosia beetle species responding to standard attractant baits. The goal of this portion of the work was to provide forest managers with better diagnose and prescriptions of and for any ambrosia beetle problems. It was hoped that a more complete knowledge of the ambrosia beetle species present on their sites and their relative seasonal populations would give the managers insight into predicting and solving problems.

Biodiversity indices such as abundance, richness, evenness are important in measuring changes over time in community ecology. With the increase in

ambrosia beetle introductions, it is important to record a baseline of abundances in Louisiana for each species to determine their changes over time. Changes in the relative proportion of species over time and in response to subsequent exotic introductions can signal ecosystem changes and degradation.

Biodiversity indices such as abundance, richness, evenness are also useful in comparing communities across global and regional scales (Magurran 2004). Documenting baseline ecosystem biodiversity indices of ambrosia beetles will allow community comparisons across spatial boundaries. This is important in comparing functions and integrity of ambrosia beetle communities. By comparing similar communities with different ambrosia beetle species of native and exotic origin we can gain insight on the effect of exotics. For example, comparisons of decomposition rates between ambrosia beetle communities with few exotics and many exotics could provide important insight into the role of exotic ambrosia beetles in similar ecosystems. Comparisons can also be made between ecosystem functions between native Asian communities and the United States forest communities for further insights into exotic ambrosia beetle effects on forest functions.

Objectives

- Calculate biodiversity indices (abundance, evenness, biodiversity) by season and forest stands. Use existing studies to compare biodiversity of Louisiana ambrosia beetles to other regions of the country.
- Record flight patterns of all ambrosia beetle species throughout the entire year.

- Correlate ambrosia beetle abundances with forest stand characteristics.

A primary objective of my survey was to detect incipient populations of exotic and invasive populations and correlate these ambrosia beetle populations to stand conditions and seasonal patterns on the Louisiana National Guard bases. The correlation between some stand conditions and ambrosia beetle species could also be useful in predicting and minimizing forest problems analyzing and manipulating stand composition.

METHODS

Site

My experimental site was located within Camp Beauregard military base in central Louisiana (latitude = 31.439, longitude = -92.319). This site lies within the Southern Hardwood Forest Region and Southern Pine Region (Barrett 1995). The Southern Hardwood Forest Region can be further categorized as the Bottomland Hardwoods Subregion:

"The Bottomland Hardwood Subregion and Southern Pine Region are characterized by relatively flat topography with slight variations in elevation and considerable differences in soils, conditions, and forest species. The Bottomland Hardwood Subregion soils tend to be vertisols. The Southern Pine Region soils tend to be podzolics.

The area is humid or subhumid with 1.07m to 1.63 meters of rain well-distributed throughout the year. Late summer to early fall is generally the driest part of the year. Moderate droughts occur every few years, while severe prolonged droughts may occur every two or three decades. The area is

characterized by a relatively long frost-free season ranging from 210 to 300 days. mean January temperatures range from about 4.4° C to 12.8° C mean July temperatures range from 27.2° C, with maximum summer temperatures over 37.8° C. Unseasonably early autumn frost and late spring freezes sometimes occur. Abrupt temperature changes are especially characteristic during the winter months.

Commercially important tree species of the Bottomland Hardwood Subregion include; eastern cottonwood (*Populous deltoides*), black willow (*Salix nigra*), baldcypress (*Taxodium distichum*), water tupelo (*Nyssa aquatica*), swamp tupelo (*Nyssa biflora*), sweetgum (*Liquidambar styraciflua*), water oak (*Quercus nigra*), willow oak (*Quercus phellos*), nuttall oak (*Quercus nuttallii*), swamp chestnut (*Quercus michauxii*), cherrybark oak (*Quercus falcata* var. *Padgodaefolia*), green ash (*Fraxinus pennsylvanica*), hackberry (*Celtis occidentalis*), American elm (*Ulmus americana*), overcup oak (*Quercus lyrata*), and water hickory (*Carya aquatica*). Commercially important tree species of the Southern Pine Region include; slash pine (*Pinus elliottii*), loblolly pine (*Pinus taeda*), pond pine (*Pinus serotina*), Virginia pine (*Pinus virginiana*), and eastern red cedar (*Juniperus virginiana*) (Barrett 1995).” Loblolly is the primary pine present on Camp Beauregard.

Experimental Design and Data Analysis

A single Lindgren multiple funnel trap positioned less than 0.5 meter above the ground was hung on each selected forest stand. All traps were baited with a single ethanol pouch bait (Synergy Semiochemical Inc., Burnaby, British

Columbia) attached to the side of the funnel trap. The manufacturers stated release rate is 380 mg/24 hours at 25°C. Baits were replaced before they were found to be near empty. Trapping began May 18, 2005 and terminated Jul. 31, 2006. Traps were checked 1 time per week from May 18, 2005 to Jun. 13, 2005. Traps were checked 1 time every 2 weeks from Jun. 13, 2006 to Sep. 15, 2005. During the winter (Sep. 15, 2006 to Jan. 26, 2006) traps were checked 1 time per month. A total of 30 traps in 30 forest stands were employed.

To choose stands, an initial Pearson correlation was run on the following forest stand characteristics: forest type, pine basal area, pine trees/hectare, pine volume/hectare, hardwood basal area, hardwood trees/hectare, hardwood volume/hectare, total volume/hectare, total trees/hectare and origin date, to identify forest stand characteristics that were not collinear and offered the widest range of stand characteristics. Noncolinear models for testing were chosen by the correlation coefficients and associated p-values more than 0.05 and amount of significantly noncolinear stand characteristics in each Pearson correlation comparison.

The origin date was not collinear with any other variables. Total volume/hectare and total trees/hectare were collinear as were; hardwood basal area, hardwood trees/hectare, hardwood volume/hectare (Table 2.5). These variables were tested in all possible combinations with origin date for a total of six models tested. Abundance of individual species was calculated as the average daily catch for each stand over the entire trapping interval. The trap catch

numbers were then $\log_{10}(X+1)$ transformed to normalize data and help minimize effects of skewed data, outliers, and unequal variation.

The 6 full model multiple regressions were run on the log transformed trap catch per day of the four most abundant species; *Xylosandrus crassiusculus*, *Xyloborinus saxesini*, *Xyleborus ferrugineous*, *Hypothenemus sp*., and total ambrosia beetles. Other species were ignored due to trap counts too low to provide meaningful statistical analysis.

Scolytinae biodiversity, *H,* was calculated using the Shannon-Weiner diversity index (Shannon 1948, Zar 1999).

$$H = [n \log n - \sum_{i=1}^{k} (f_i \log f_i)] / n,$$

Where *n* is total number of beetles captured, *k* represented the total number of species (richness), and *f* is number of beetles in species *i.* Evenness, *J,* was calculated as the ratio of *H* to H_{max} (H_{max} being the theoretical maximum possible diversity for a set of data with *k* categories; Zar 1999), where $H_{max} = \log_{10} k$.

Sample Processing

Trap checking involved emptying trap catch contents, trap maintenance such as cleaning trap of debris, checking/rebaiting the ethanol pouch, and recharging the propylene glycol (low-toxicity antifreeze, Prestone Co. Palatine, Illinois) in each trap cup. Each week's trap catch from a single trap was emptied into a labeled vial and filled with 90% ethanol for storage until further processing. Trap samples were brought back to the lab, sorted and ambrosia beetles identified to species (Wood 1982, http://xyleborini.tamu.edu/keys.php,

http://entomology.lsu.edu/lsam/scolytinae/). *Hypothenemus* specimens were identified to genus. Non-ambrosia beetle species identified and counted included; *Ips* (Coleoptera; Curculionidae) and *Xylobiops basilaris* (Coleoptera; Bostrichidae).

Voucher Specimens

Pinned specimens from trap catch were placed into the collection housed at the USDA Forest Service Southern Research Station at Pineville and LSU Entomology collection. Vouchers were sent to Dr. Robert Rabaglia (USDA Forest Service, Forest Health Protection, Washington, DC) to confirm identities of specimens.

RESULTS

Correlations with Stand Characteristics

Several stand characteristics were statistically significant ($P < .05$) in the multiple regression analysis for their ability to predict abundance of the four most abundant ambrosia beetle species and total trap catch (Table 2.4).

I eliminated forest stand characteristics that were significantly correlated using a Pearson correlation (Table 2.5). Six full selection multiple regression models were tested to determine which independent variables could best describe the four most abundant ambrosia beetle species and total trap catch (Table 2.4). Highly significant correlations between some of the stand conditions and ambrosia beetle species were determined ($p<.05$). I arrived at the most appropriate model by comparing the R^2 values and p-values of the six models.

The model selected to use was composed of the independent variables; origin date, total volume/hectare and hardwood trees/hectare (Table 2.4).

Xylosandrus crassiusculus was significantly correlated to total volume/hectare and hardwood trees/hectare (p=.01, 0.003 respectfully) with a three variable model R^2 value of .022. *Xyleborus ferrugineous* was significantly correlated to origin date only (p=.015) with a three variable model R^2 value of 0.014. *Xyleborinus saxeseni* was significantly correlated to total volume/hectare (p=.031) with a three variable model R^2 value of 0.012. The total ambrosia beetles catch was significantly correlated with origin date, total volume/hectare and hardwood trees/hectare (p=0.052, 0.005, 0.003; respectively) with a three variable model R^2 value of 0.025. *Hypothenemus sp.* was not significantly correlated with any stand characteristics.

Seasonal Analysis

The Shannon-Wiener diversity indices among seasons showed some differences (Table 2.6). The highest peak of diversity (H_{max} = 2.278) was in the winter on Dec. 12, 2006 (Table 2.6). There was a slightly lower peak in the spring (May 5, 2006; H_{max} = 1.998) and fall (Sep. 1, 2006; H_{max} = 2.017). There was also a prolonged peak of diversity in the spring between April, 6, 2006 to May 5, 2006 (H_{max}=2.0166). The peak of diversity in the spring was mirrored by a peak in species richness from Apr. 6, 2006 to May 5, 2006 (R_{max} =17) and a shorter peak of abundance May 5, 2006 - May 26, 2006 (abundance max = 51.63). The average, survey wide, Shannon-Wiener diversity index was 1.46. The average survey-wide, Shannon-Wiener evenness index was 0.645.

Table 2.1. Stand characteristics of forest stands sampled during the course of the ambrosia beetle trapping survey on Camp Beauregard, LA. A single ethanol baited Lindgren funnel trap was placed in each stand listed. Forest type key; NP=Natural Pine, PH=Pine/Hardwood mix, H=Hardwood.

Stand_ ID	Origin_ date	Forest _Type	Pine BA metric	Pine Tree/Ha	Pine Volume/ Ha (m^3/Ha)	Hardwood BA (m^2/Ha)	Hardwood Tree/Ha	Hardwood m^3/Ha	Total m^3/Ha	Total Tree/Ha	Total BA
A-001	1969	NP	15.15	26.30	0.00034	4.13	9.31	0.00011	0.00045	35.61	19.28
A-003	1969	NP	8.72	11.74	0.00019	4.36	8.90	0.00011	0.00030	20.64	13.09
A-087	1940	PH	12.17	19.83	0.00029	6.89	12.55	0.00018	0.00048	32.38	19.05
B-006	1958	PH	4.36	11.74	0.00012	9.18	18.62	0.00023	0.00035	30.35	13.54
B-008	1958	PH	14.69	28.33	0.00036	5.74	15.78	0.00017	0.00053	44.11	20.43
B-009	1960	NP	21.12	42.49	0.00041	2.75	9.71	0.00009	0.00050	52.20	23.88
B-011	1960	H	3.67	3.24	0.00010	16.53	31.57	0.00037	0.00046	34.80	20.20
B-012	1959	PH	10.10	21.04	0.00019	7.12	20.23	0.00018	0.00037	41.28	17.22
C-014	1970	NP	16.53	45.73	0.00048	2.98	10.93	0.00009	0.00058	56.66	19.51
D-015	1930	PH	10.56	20.64	0.00018	4.59	15.78	0.00014	0.00032	36.42	15.15
D-017	1969	NP	16.99	46.54	0.00040	4.59	14.16	0.00015	0.00055	60.70	21.58
D-018	1969	NP	16.76	53.01	0.00040	2.98	11.74	0.00008	0.00048	64.75	19.74
D-019	1950	PH	11.25	17.00	0.00025	14.92	38.85	0.00037	0.00062	55.85	26.17
D-020	1950	PH	13.54	27.11	0.00030	9.87	21.85	0.00029	0.00059	48.97	23.42
E-022	1950	PH	10.10	17.81	0.00025	6.43	15.78	0.00019	0.00044	33.59	16.53
G-028	1940	PH	15.38	35.61	0.00034	5.05	14.16	0.00015	0.00048	49.78	20.43
G-030	1940	PH	16.30	33.99	0.00036	6.89	17.00	0.00019	0.00055	50.99	23.19
H-032	1945	PH	10.56	22.66	0.00024	8.95	25.50	0.00033	0.00057	48.16	19.51
H-033	1950	NP	13.54	31.97	0.00035	6.43	18.21	0.00019	0.00055	50.18	19.97
H-034	1950	NP	11.94	27.11	0.00026	6.89	22.66	0.00024	0.00050	49.78	18.82
H-035	1945	PH	4.82	9.71	0.00014	17.45	45.33	0.00052	0.00067	55.04	22.27
H-037	1960	NP	16.76	31.57	0.00037	2.30	2.83	0.00003	0.00040	34.40	19.05
J-069	1942	H	5.74	19.43	0.00014	8.95	36.02	0.00032	0.00046	55.44	14.69
K-071	1958	NP	18.14	26.71	0.00041	3.67	12.95	0.00014	0.00055	39.66	21.81
L-074	1947	NP	8.26	12.55	0.00019	4.36	15.78	0.00017	0.00035	28.33	12.63
L-075	1947	NP	9.64	20.23	0.00025	3.67	17.81	0.00014	0.00039	38.04	13.31
L-083	1956	NP	15.84	23.47	0.00039	8.03	31.57	0.00032	0.00072	55.04	23.88
L-084	1947	PH	4.59	6.48	0.00009	8.03	32.38	0.00031	0.00041	38.85	12.63

Table 2.2. Trapping results on Camp Beauregard, LA in 2005-2006.

Tribe	Subtribe	Species	Total caught	% of total
Hylesinini	Hylastina	*Hylorigops rugipennis pinifex*	14	8
Hylesinini	Bothrosternina	*Cnesinus strigicollis*	1	0
Scolytini	Bothrosternina	*Hylocurus bionodatas*	1	0
Scolytini	Bothrosternina	*Micracisella nanula*	20	0
Scolytini	Ipina	*Orthotomicus caelatus*	1	0
Scolytini	Ipina	*Ips spp.*	7	0
Scolytini	Dryocoetina	*Dryoxylon onoharaensum*	115	1
Scolytini	Dryocoetina	*Coccotrypes distinctus*	16	0
Scolytini	Xyleborina	*Ambrosiodmus obliquus*	2	0
Scolytini	Xyleborina	*Ambrosiodmus rubricolis*	3	0
Scolytini	Xyleborina	*Ambrosiodmus tachygraphus*	6	0
Scolytini	Xyleborina	*Xyleborinus saxeseni*	1089	10
Scolytini	Xyleborina	*Xyleborus affinis*	91	0
Scolytini	Xyleborina	*Xyleborus atratus*	13	0
Scolytini	Xyleborina	*Xyleborus californicus*	7	0
Scolytini	Xyleborina	*Xyleborus ferrugineus*	850	8
Scolytini	Xyleborina	*Xyleborus gracilis*	1	0
Scolytini	Xyleborina	*Xyleborus impressus*	254	2
Scolytini	Xyleborina	*Xyleborus intrusus*	1	0
Scolytini	Xyleborina	*Xyleborus pubescens*	75	0
Scolytini	Xyleborina	*Xyleborus sayi*	10	0
Scolytini	Xyleborina	*Xyleborus xylographus*	1	0
Scolytini	Xyleborina	*Xyleborous valvidus*	2	0
Scolytini	Xyleborina	*Xylosandrus compactus*	341	3
Scolytini	Xyleborina	*Xylosandrus crassiusculus*	6302	63
Scolytini	Xyleborina	*Xylosandrus germanus*	32	0
Scolytini	Cryphalina	*Hypothenemus dissimulus*	138	1
Scolytini	Cryphalina	*Hypothenemus sp.*	326	3
Scolytini	Pityophthorina	*Pityophthorus pulicarius*	1	0
Scolytini	Pityophthorina	*Pityophthorus sp.*	2	0
Scolytini	Corthylina	*Monarthrum fasciatum*	1	0
Scolytini	Corthylina	*Monarthum mali*	27	0
Scolytini	Corthylina	*Gnathotrichus materiarius*	3	0
Scolytini	Corthylina	*Corthylus sp.*	17	0
Platypodini	Platypodini	*Platypus compositus*	3	0
Platypodini	Platypodini	*Platypus flavicornus*	2	0
Bostrichidi	Xylobiopa	*Xylobiops basilaris*	156	1
		TOTAL	**9931**	

Table 2.3. Shannon-wiener biodiversity indices (H), evenness (J), Richness (S) and abundance for ambrosia beetles by date in the trapping survey of Camp Beauregard using ETOH baited Lindgren funnel traps in 2005-2006.

Date	5/26/2005	6/4/2005	6/13/2005	6/26/2005	7/13/2005	7/24/2005	8/5/2005	8/17/2005
Shannon-Wiener index (H)	0.82	0.7369	1.0035	0.5803	1.0591	1.7725	1.7597	0.9003
Shannon Evenness (J)	0.33	0.3073	0.4358	0.2520	0.4129	0.7392	0.7082	0.8194
Richness (# Species) (S)	12.00	11.0000	10.0000	10.0000	13.0000	11.0000	12.0000	3.0000
Abundance (all species; trapped per day)	15.45	10.6250	5.8750	3.9904	2.1905	0.5800	0.5345	0.3333

Date	9/1/2005	9/15/2005	10/13/2005	10/31/2005	12/12/2005	1/26/2006	3/11/2006	3/23/2006
Shannon-Wiener index (H)	1.9983	1.7677	1.7133	1.4477	2.276667489	1.4805	1.1852	1.5657
Shannon Evenness (J)	0.8042	0.7677	0.7797	0.7440	0.840703576	0.5772	0.5394	0.8046
Richness (# Species) (S)	12.0000	10.0000	9.0000	7.0000	15	13.0000	9.0000	7.0000
Abundance (all species; trapped per day)	0.8700	0.6300	0.3696	0.3490	0.413793103	0.3578	1.4828	1.3482

Date	4/6/2006	4/20/2006	5/5/2006	5/26/2006	6/4/2006	6/19/2006	7/13/2006	7/31/2006
Shannon-Wiener index (H)	2.0077	1.8926	2.0166	0.4036	1.0337	1.4422	1.3778	2.0499
Shannon Evenness (J)	0.7086	0.6826	0.7273	0.1837	0.4030	0.6264	0.8561	0.7569
Richness (# Species) (S)	17.0000	16.0000	16.0000	9.0000	13.0000	10.0000	5.0000	15.0000
Abundance (all species; trapped per day)	1.3857	1.1524	2.0083	51.6250	9.6500	0.7400	0.3250	0.6652

Table 2.4. Summarized results of 6 models correlating 4 species of ambrosia beetles and the total caught to forest stand characteristics at Camp Beauregard, LA in 2005-2006. dependant variables key: crass = *Xylosandrus crassiuculus*, ferr = *Xyleborus ferrugineous*, sax = *Xyleborinus saxesini*, hypo = *Hypothenemus sp*. Significant t-values are in red. Independent variables 1-3 correspond to the superscripts, denoting the variables of each model.

		Origin date[1], Total Vol/Ha[2], Hardwood Vol/Ha[3]					Origin date[1], Total Vol/Ha[2], Hardwood Tree/Ha[3]					Origin date[1], Total Vol/Ha[2],		
dependant variable	Independent variable	Parameter estimate	Standard Error	t Value	Pr>t	R^2	Parameter estimate	Standard Error	t Value	Pr>t	R^2	Parameter estimate	Standard Error	t Value
total/day	1	-0.0029	0.0016	-1.82	0.070	0.021	-0.0031	0.0016	-1.95	0.052	0.025	-0.0020	0.0015	-1.30
	2	0.0209	0.0073	2.87	0.004		0.0207	0.0070	2.97	0.003		0.0162	0.0068	2.38
	3	-0.0175	0.0071	-2.45	0.015		-0.0021	0.0008	-2.83	0.005		-0.0018	0.0010	-1.72
crass/day	1	-0.0015	0.0015	-1.00	0.319	0.018	-0.0017	0.0015	-1.12	0.264	0.022	-0.0006	0.0015	-0.44
	2	0.0175	0.0070	2.50	0.013		0.0173	0.0067	2.57	0.010		0.0131	0.0065	2.01
	3	-0.0176	0.0069	-2.57	0.011		-0.0022	0.0007	-2.95	0.003		-0.0019	0.0010	-1.93
ferr/day	1	-0.0012	0.0005	-2.20	0.028	0.012	-0.0013	0.0005	-2.43	0.015	0.014	-0.0010	0.0005	-2.03
	2	-0.0002	0.0024	-0.07	0.948		0.0003	0.0023	0.12	0.902		-0.0010	0.0022	-0.44
	3	-0.0015	0.0023	-0.65	0.517		-0.0003	0.0002	-1.16	0.246		0.0000	0.0003	-0.01
sax/day	1	-0.0002	0.0005	-0.29	0.773	0.013	0.0000	0.0005	-0.09	0.925	0.012	-0.0001	0.0005	-0.17
	2	0.0055	0.0024	2.32	0.021		0.0050	0.0023	2.17	0.031		0.0052	0.0022	2.35
	3	-0.0015	0.0023	-0.62	0.533		0.0000	0.0002	-0.20	0.841		-0.0002	0.0003	-0.50
hypo/day	1	-0.0002	0.0003	-0.66	0.511	0.014	-0.0002	0.0003	-0.94	0.347	0.010	-0.0002	0.0002	-0.73
	2	-0.0014	0.0012	-1.13	0.261		-0.0009	0.0012	-0.75	0.455		-0.0015	0.0011	-1.30
	3	0.0022	0.0012	1.85	0.065		0.0002	0.0001	1.29	0.197		0.0005	0.0002	2.72

		Origin date[1], Total Tree/Ha[2], Hardwood Vol/Ha[3]					Origin date[1], Total Tree/Ha[2], Hardwood Tree/Ha[3]					Origin date[1], Total Tree/Ha[2],		
dependant variable	independent variable	Parameter estimate	Standard Error	t Value	Pr>t	R^2	Parameter estimate	Standard Error	t Value	Pr>t	R^2	Parameter estimate	Standard Error	t Value
total/day	1	-0.0021	0.0016	-1.36	0.175	0.010	-0.0026	0.0016	-1.68	0.093	0.016	-0.0016	0.0015	0.2786
	2	0.0011	0.0006	1.76	0.079		0.0013	0.0006	2.1	0.036		0.0009	0.0006	0.1203
	3	-0.0092	0.0062	-1.48	0.140		-0.0016	0.0007	-2.21	0.027		-0.0009	0.0009	-0.99
crass/day	1	-0.0010	0.0015	-0.64	0.522	0.012	-0.0015	0.0015	-0.97	0.332	0.019	-0.0004	0.0014	-0.29
	2	0.0011	0.0006	1.86	0.064		0.0013	0.0006	2.23	0.027		0.0009	0.0006	1.61
	3	-0.0112	0.0060	-1.87	0.062		-0.0018	0.0007	-2.59	0.010		-0.0013	0.0009	-1.41
ferr/day	1	-0.0011	0.0005	-2.13	0.034	0.013	-0.0012	0.0005	-2.29	0.022	0.014	-0.0010	0.0005	-2.01
	2	-0.0002	0.0002	-0.83	0.406		-0.0001	0.0002	-0.62	0.535		-0.0002	0.0002	-0.99
	3	-0.0012	0.0020	-0.57	0.567		-0.0002	0.0002	-0.97	0.333		0.0000	0.0003	-0.07
sax/day	1	0.0001	0.0005	0.23	0.817	0.002	0.0002	0.0005	0.3	0.762	0.003	0.0001	0.0005	0.14
	2	0.0001	0.0002	0.7	0.483		0.0001	0.0002	0.58	0.561		0.0002	0.0002	0.8
	3	0.0011	0.0020	0.55	0.581		0.0002	0.0002	0.7	0.482		0.0001	0.0003	0.44
hypo/day	1	-0.0002	0.0003	-0.76	0.447	0.015	-0.0002	0.0003	-0.85	0.395	0.012	-0.0002	0.0002	-0.8
	2	-0.0001	0.0001	-1.32	0.188		-0.0001	0.0001	-1.33	0.183		-0.0001	0.0001	-1.27
	3	0.0018	0.0010	1.76	0.078		0.0002	0.0001	1.46	0.146		0.0004	0.0002	2.56

In each model, very low (<.1) R^2 values were found for correlations between beetle abundance and forest stand charactreristics, hence 90% of the residual variability was left unexplained by the model. The model with the highest R^2 values and lowest P-values included the independent variables origin date, total volume/hectare and hardwood tree/hectare.

Averaged results of the Shannon-Wiener diversity and evenness indices showed parallel seasonal patterns (Table 2.6; Fig. 2.1). The Shannon-Wiener diversity and evenness were lowest in the spring (1.1934, 0.4656 respectively), gradually increasing throughout the summer (1.5429, 0.7086) into the fall (1.729, 0.7354) and dropping in the winter (1.3754, 0.6720).

Species Analysis

The survey trapping recorded 37 species of ambrosia beetles (Table 2.2). We caught two Xyleborous valvidus, a new species record for Louisiana. The three most prevalent species and their total percentage of trap catch were *X. crassisculus* (65%), *X. saxeseni* (11%), *X. ferrugineus* (9%) respectively (Table 2.2).

Xylosandrus crassisculus flight reached a peak in mid-May with a higher abundance in 2006 than 2005 (Fig. 2.2). *Xyleborinus saxeseni* exhibited a similar flight pattern as *X. crassisculus* peaking in mid-May (Fig. 2.2). *Xyleborus ferrugineus* flight abundance peaked in early June both years and also had a small slight in the end of August (Fig. 2.2).

Although the majority of ambrosia beetle species had flight peaks between Apr. 26, 2006 and June 4, 2006, a few species were most abundant in other seasons. *Hypothenemus dissimulus* had a fall flight (Oct. 13. 2005 to Oct. 31, 2006) that was equal to their spring flight (Mar. 23, 2006 to Apr. 6, 2006; Fig. 2.2). *Xyleborus atratus* had its flight in early April. The six *Ambrosidiomus tachygraphus* caught were trapped in late January.

Table 2.5. Pearsons correlation coefficients on 9 forest stand characteristics of 28 stands on Camp Beauregard, LA. Prob > |r| under H0: Rho=0. N=28. Insignificant correlations between independent variables are in red.

	Pine BA	Pine Tree/Ac	Pine Vol/Ac	Hardwood BA	Hardwood Tree/Ac	Hardwood Vol/Ac	Total Vol/Ac	Total Tree/Ac	Origin date
Pine BA	1	0.89441	0.96112	-0.60949	-0.59243	-0.58606	0.44414	0.45686	0.34257
		<.0001	<.0001	0.0006	0.0009	0.001	0.0179	0.0145	0.0743
Pine Tree/Ac	0.89441	1	0.86997	-0.56326	-0.50617	-0.55907	0.38325	0.55572	0.0743
	<.0001		<.0001	0.0018	0.006	0.002	0.0441	0.0021	0.1403
Pine Vol/Ac	0.96112	0.86997	1	-0.62903	-0.58991	-0.57342	0.47893	0.45935	0.43025
	<.0001	<.0001		0.0003	0.001	0.0014	0.0099	0.0139	0.0223
Hardwood BA	-0.60949	-0.56326	0.62903	1	0.87344	0.9508	0.29464	0.07032	-0.40994
	0.0006	0.0018	0.0003		<.0001	<.0001	0.128	0.7222	0.0303
Hardwood Tree/Ac	-0.59243	-0.50617	-0.58991	0.87344	1	0.93078	0.30647	0.24541	-0.462
	0.0009	0.006	0.001	<.0001		<.0001	0.1127	0.2081	0.0133
Hardwood Vol/Ac	-0.58606	-0.55907	-0.57342	0.9508	0.93078	1	0.37088	0.12183	-0.44822
	0.001	0.002	0.0014	<.0001	<.0001		0.052	0.5368	0.0168
Total Vol/Ac	0.44414	0.38325	0.47893	0.29464	0.30647	0.37088	1	0.73255	-0.01432
	0.0179	0.0441	0.0099	0.128	0.1127	0.052		<.0001	0.9423
Total Tree/Ac	0.45686	0.55572	0.45935	0.07032	0.24541	0.12183	0.73255	1	0.07701
	0.0145	0.0021	0.0139	0.7222	0.2081	0.5368	<.0001		0.6969
Origin date	0.34257	0.28586	0.43025	-0.40994	-0.462	-0.44822	-0.01432	0.07701	1
	0.0743	0.1403	0.0223	0.0303	0.0133	0.0168	0.9423	0.6969	

Table 2.6. Shannon-Wiener biodiversity, evenness, richness and abundance values and indices for ambrosia beetles separated by season. Survey total, maximums and minimums and corresponding dates given. All trapping occurred on Camp Beauregard, LA from 2005-2006.

Season	Shannon-Wiener index (H)	Shannon Evenness (J)	Richness (# Species) (S)	Abundance (all species; trapped per day)
Spring	1.1934	0.4656	12.4000	10.2504
Summer	1.5429	0.7086	10.1667	0.8564
Fall	1.7295	0.7354	11.0000	0.3725
Winter	1.3754	0.6720	8.0000	1.4155
Survey average	1.4603	0.6454	10.3917	3.2237
Max	2.277 (12/12/2005)	0.856 (7/13/2006)	17 (4/6/2006)	51.625 (5/26/2006)
Min	0.4036 (5/26/2006)	0.1837 (5/26/2006)	5 (7/13/2006)	0.325 (7/13/2006)

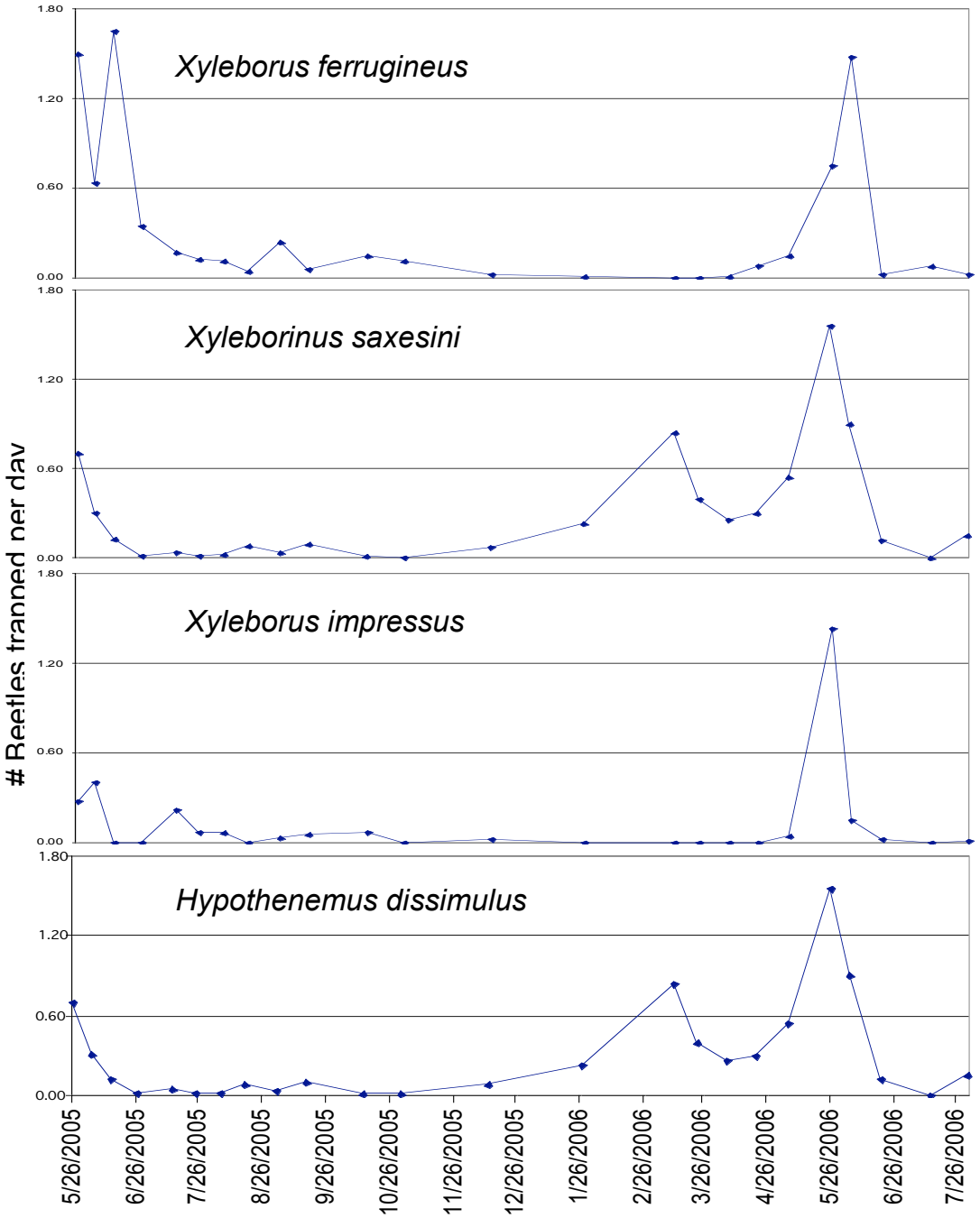

Fig. 2.1a. Flight period on Camp Beauregard in 2005-2006 for the most 9 abundant species. Fig 2.1 cont'd on subsequent pages.

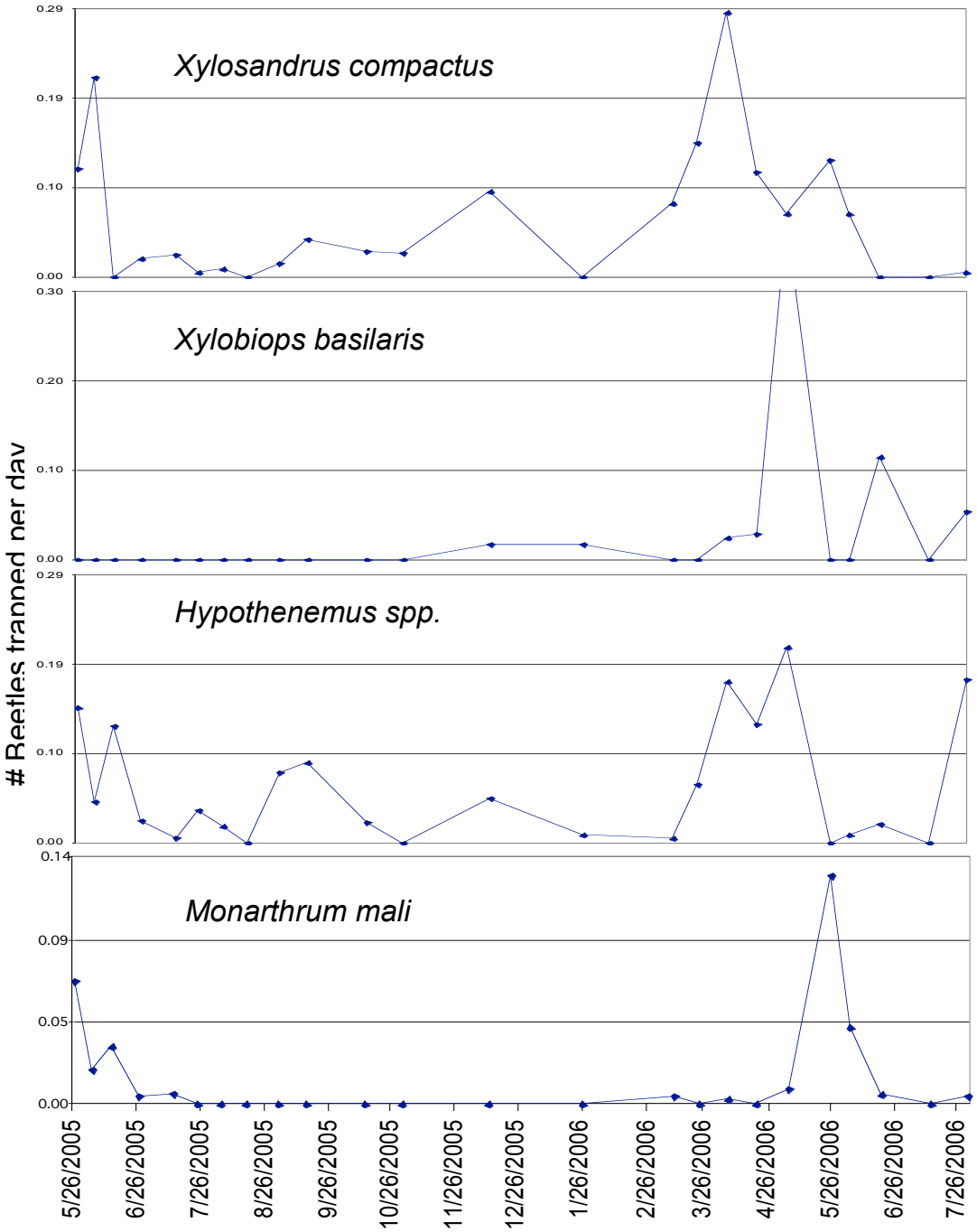

Fig. 2.1b continued. Flight period on Camp Beauregard in 2005-2006 for the most 9 abundant species.

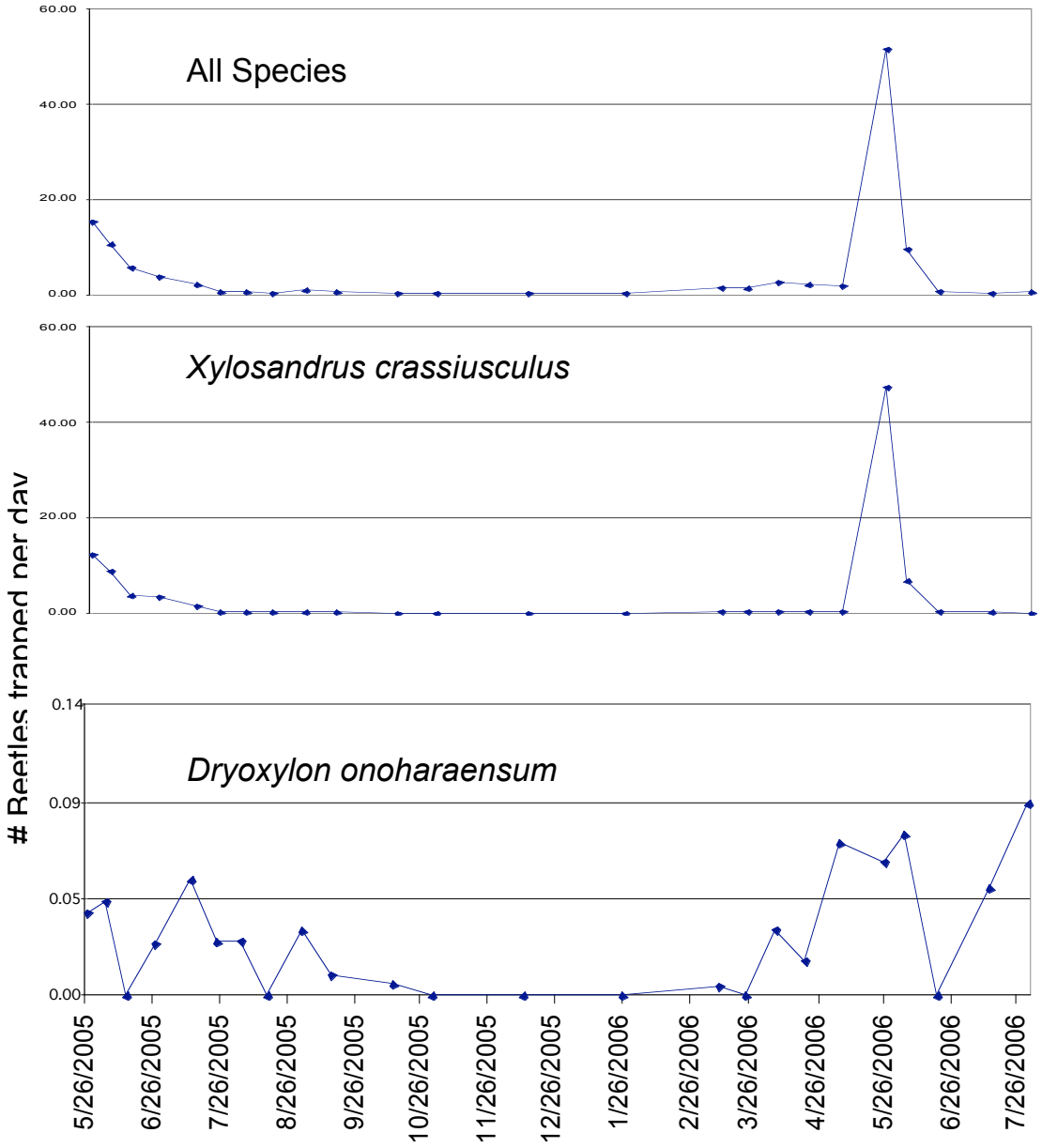

Fig. 2.1c continued. Flight period on Camp Beauregard in 2005-2006 for the most 9 abundant species.

DISCUSSION

Stand Analysis

We were able to use forest stand conditions commonly available to forest managers that will allow increased rapid detection of particular ambrosia beetle species by carefully selecting the stands in which traps are placed.

Xyleborus ferrugineus and the total ambrosia beetle trap catch showed a significant correlation with origin date, revealing larger abundances of ambrosia beetles can be expected in stands that are older. The causes of this could be attributed to stand characteristics such as health, vigor, volume of dead/dying wood, and total biomass. As stands age they are subject to increasing stress from plant competition for light, nutrients and water (Schowalter *et al*. 1986). In some instances, these stressors increase susceptibility to insect damage (Schowalter *et al*. 1986).

The most abundant ambrosia beetle in my study *X. crassiusculus* was found to be significantly correlated to high total volume/hectare and to fewer hardwood trees/hectare. Like *X. saxeseni*, the correlation between high abundance and stands with high total volume/hectare may be attributed the increase in total suitable breeding material. The correlation to less hardwood trees/hectare may be attributed to *X. crassiusculus*' ability to utilize a wide range of host species including pines. Also, the hardwood stands I was working in were dominated by smaller diameter hardwood trees. Finally, it could be attributed to the lack of fire in hardwood dominated stands. The hardwood dominated stands were along river bottoms and no indication of previous fires was observed.

Previous research has shown the affinity of ambrosia beetles, including *X. crassiusculus*, to recently burned areas (Hanula *et al*. 2002, Sullivan *et al.* 2003).

The independent variables had minimal ability to account for the amount of variation in the model as indicated by the low R^2 values. This suggests that the model does not fully explain ambrosia beetle abundances in each stand. It has been shown that fire is responsible for increases in ambrosia beetle abundance (Hanula *et al.* 2002). Unfortunately, the Camp Beauregard fire data were insufficient for use in the model. My results support other studies that suggest ambrosia beetle abundances may be primarily driven by other factors such as temperature, humidity, fire or forest health (Liu and McLean 1993, Coyle *et al* 2005, Mizell and Riddle 2004, Flechtmann *et al.* 2001, Hanula *et al.* 2002).

Species Analysis

Xylosandrus crassisculus flight reached a peak in mid-May with a higher abundance in 2006 than 2005. In Tennessee a study by Oliver and Mannion 2001, *X. crassisculus* flight varied from late April to early May between years with the most tree attacks in early April. Coyle *et al.* (2005) showed the peak flight in early April in coastal South Carolina, although his data suggested yearly variability in flight times. As suggested in previous studies the differences in flight peaks between years and locations could be a result of weather patterns (Coyle *et al*. 2005).

X. saxeseni exhibited a similar flight pattern to that of *X. crassisculus*, peaking in mid-May. These results are consistent with unpublished work of Doerr *et al.* (2003) in Washington state which showed a in *X. saxeseni* flight in

early May, although, their work also showed a even higher peak in late July, which has not been demonstrated before in the Southern United States. Coyle *et al.* (2005) showed the peak flight abundance in early April, but with subsequent equal peaks extending into mid-May. These data suggested variable yearly flight times. Oliver and Mannion (2001) showed the peak flight varied between years from early April to mid-May. My study is consistent with these studies. The peak flight I observed was consistent with the more southerly location.

Xyleborus ferrugineus was the third most prevalent ambrosia beetle in trap catch. Its flight peaked in early June both years. In comparison, *X. impressus* peak flight was in late May. Differences in trap catch numbers also confirm a difference supporting separate species distinctions as described by Rabaglia (2005) and Chamberlain (1939) (Fig 2.2a).

In comparisons to previous works (Atkinson et al 1998, Turnbow and Franklin 1980, Weber and McPherson 1991, Oliver and Mannion 2001, Grant *et al.* 2003) my results showed the highest diversity indices calculated to date for ambrosia beetle surveys. Although, Turnbow and Franklin (1980), Weber and McPherson (1991), Grant *et al.* (2003) used various collecting techniques with no lure, which could account for lower index values. Oliver and Mannion (2001) used ethanol funnel traps in Tennessee resulting in an H' of .72 and an H_{max} of 1.36. By contrast, *Coyle et al*. (2005) calculated the H' at 0.59 and evenness at 0.41. Another contributing factor to our higher diversity indices could be that these previous studies were conducted in higher latitudes where climatic conditions are different and less conducive to higher abundance and diversity of

angiosperm-infesting ambrosia beetles, a predominately tropical and subtropical species. The dominance of *X. crassiusculus* abundance greatly affected the diversity indices over the course of the year, particularly in the spring during *X. crassiusculus* main flight.

This study developed a baseline of diversity data that will be important in future studies that determine the effects of invasive species on forest functions such as nutrient cycling. I was also able to develop a model to help guide forest managers in selecting stands for trap placement to increase monitoring and interception efforts. In addition, my work adds important data on flight times that will be extremely useful for nurseries in determining the timing of appropriate management actions.

CHAPTER III – CHEMICAL ECOLOGY

INTRODUCTION

Forest Management

The success of silvicultural, insecticide, sanitation, semiochemical, and biological control treatments is dependent on accurate detection, monitoring, and interception of ambrosia beetle populations (Stephen and Taha 1976, 1979). Even with these available management strategies, the beetles may still cause significant damage (Waters *et al*. 1985, Preisler and Mitchel 1993, Reynolds and Holsten 1996, Hudson and Mizell 1999). This forest damage is partly due to the difficulty in predicting outbreaks and inability to treat trees undergoing attack. Successful trapping is dependent on selecting an optimum blend of mimicked host volatiles and/or insect-released compounds (semiochemicals) as the bait.

Chemical Ecology and the Role of Ethanol and Turpentine

Ethanol is produced in stressed trees undergoing anaerobic respiration (Mac Donald and Kimmerer and Kozlowski 1982, Kimmerer 1991, Kelsey 1997). Ethanol has been widely used for trapping ambrosia beetles that affect deciduous trees (Schroeder and Lindelöw 1989, Oliver and Mannion 2001, Coyle *et al.* 2005) and, to a lesser extent, conifers. (Klimetzek *et al.* 1986, Schroeder and Lindelöw 1989). Turpentine obtained via distillation of pine resin is composed mainly of the monoterpenes α- and β-pinene, and can be used for trapping ambrosia beetles that infest conifers (Schroeder and Lindelöw 1989). Ethanol, another host volatile, acts synergistically with monoterpenes in attracting some beetle species (Liu 1989). Conversely, α-pinene has been shown to

reduce attraction when released with ethanol for some species (Schroeder and Lindelöw 1989). Recently there has been speculation regarding the seasonal variation in attractiveness of ethanol to ambrosia beetles (Mizell 1994, personal communication J. Labonte and B.T Sullivan 2006). It has been observed that as ambrosia beetle attacks continue throughout the summer and fall, ethanol-baited traps become progressively less effective.

Although ethanol and turpentine have been extensively and successfully used in trapping, specific chemical analysis of beetle attraction has not been conclusive for angiosperm-infesting ambrosia beetles (Phillips et al. 1989). Complete knowledge of the chemical identity of the compounds eliciting responses from beetles could improve population monitoring and trapping efficiency, and thus improve the effectiveness of management strategies. There have not previously been studies applying newer techniques for semiochemical analysis such as gas chromatography-electroantennographic detection (GC-EAD) and gas chromatography-mass spectrometry (GC-MS) to the study of host-derived attractants for ambrosia beetles.

Pheromone production has not been documented in the Xyleborini and it has been suggested that it may not occur in this taxon (Kirkendall *et al*. 1997). However, anectdotal evidence of aggregation in some species has caused speculation about possible pheromone production (Taborsky 2004). Some xyleborine ambrosia beetles secondarily attack trees infested by bark beetles, suggesting that they may respond to bark beetle pheromones. To date, no published studies have attempted to isolate pheromones from angiosperm-

infesting ambrosia beetles. Similarly, very few studies have explored the possibility that angiosperm-infesting ambrosia beetles respond to bark beetle pheromones or host compounds emitted during bark beetle attack.

Objectives

1. Development of an improved trapping bait for ambrosia beetles

My first primary objective was to develop improved trapping methods for the detection and monitoring of populations of native and non-native bark and ambrosia beetles within Camp Beauregard, Louisiana. I focused on bait development for a major ambrosia beetle pest of the Southeast, *Xylosandrus crassiusculus* (motschulsky).

Many species of tree-infesting beetles are attracted to specific volatile compounds emitted from suitable hosts. These compounds can have great value as baits in trapping to monitor and suppress beetle populations. Conversely, unsuitable host trees (inappropriate species or condition) may emit compounds that inhibit attraction or deter beetle attack. Characterization of the behavioral effects of host compounds on ambrosia beetles should lead to the commercial production of trapping baits for luring damaging beetles or tree protectants for repelling them.

2. Development of a novel technique for artificially eliciting host attractiveness.

Research on ambrosia and bark beetles is limited by our ability to consistently predict what hosts the beetles will attack. Development of a technique to stress host trees and reliably stimulate ambrosia beetle attack would

facilitate research on the volatile compounds associated with host susceptibility, the identification of attractants, and the biology of ambrosia beetles.

3. Quantify volatile compounds associated with stressed and unstressed trees.

Advances in chromatography and techniques for on-site sampling of volatile organic compounds allow investigation into tree physiological responses to stress. Quantifying differences between stressed trees displaying attractiveness to ambrosia beetles and unstressed, unattractive trees provides knowledge into the compounds responsible for ambrosia beetle attraction.

4. Begin semiochemical exploration of ambrosia beetle responses to bark beetle aggregation pheromones and host volatiles from bark beetle-initiated attacks.

Ambrosia beetles often occur in trees experiencing bark beetle attack. However, little research has been published on ambrosia beetles cueing into bark beetle pheromones or host tree associated compounds. The discovery of this phenomenon could result in dramatic strides in bait development and management of angiosperm-infesting ambrosia beetles.

METHODS

Site

All trapping experiments were performed and live beetles were obtained at the LSU Agcenter facilities in Baton Rouge, LA (Latitude = 30.3691N, Longitude = -91.1828W), the USDA Forest Service Southern Forest Research Station in Pineville, LA (Latitude = 31.4275N, Longitude = -92.4747W), or the LSU

Agcenter Idlewild Research station (Latitude = 30.8123N, Longitude = -90.9687W).

Insects

All insects used in the following experiments were lab reared in artificial (sawdust-agar; Peer and Taborsky 2004) or natural media (wood bolts; Katajima and Hijii 2004).

Porapak Q Columns – Construction, Conditioning, and Extraction

Porapak Q (Millipore Inc., Billerieca, MA) a porous polymer with a high affinity and adsorbent capacity for a wide variety of volatile organic compounds at room temperature, was used to sample host volatiles. Adsorbent columns consisted of a 2 mm i.d. Teflon® pipe filled with 0.1 g of 50/80-mesh Porapak Q (Millipore, Inc.). Prior to use, each column was sequentially rinsed with 1 ml each of chromatography grade acetone and methylene chloride, followed by 2 ml redistilled pentane. Pressure from a tank of ultra-pure nitrogen maintained a constant flow of 1-2 drops per second of conditioning solvents through the columns, helped prevent oxidation, and forcefully expelled the remaining liquid solvent after the final rinse. Nitrogen flow was then maintained for 5 min while the columns were heated to 100º C to purge residual solvent adsorbed onto the Porapak. The columns were allowed to cool for 1 min before disconnecting the nitrogen flow. The conditioned columns were handled with Kimwipes (Kimberly-Clark Corp. Roswell, GA) and immediately placed into screw-cap culture tubes with Teflon-taped threads. The columns were then used immediately or stored under refrigeration for less than 1 week before use.

Sampled volatiles adsorbed onto Porapak Q columns were extracted by allowing 1.5 ml of redistilled pentane to percolate through the column for approximately 6 min. A low pressure stream of nitrogen gas was applied to the column to force any remaining solvent from the column. All extractions were collected into glass vials, labeled, and immediately transferred to ultra cold storage.

Host Volatile Sampling

In an initial attempt to stress host trees and stimulate host attractiveness by flooding of the roots, two white oak (*Quercus alba*) saplings (6.35 to 7.62 cm diam. at root collar) with 18.92 L, burlap-enclosed root balls were placed in plastic tubs of water to above the top of the root ball. This method was chosen because it is known that oxygen-deprived tree tissues produce ethanol (Kimmerer and Kozlowski 1982; MacDonald and Kimmerer 1991), the most commonly used trapping bait for ambrosia beetles. Tree flooding stimulated attacks by *X. crassiusculus* and other ambrosia beetle species within 3-7 days. Two white oak trees whose root balls were watered regularly but allowed to drain were kept as controls. After moderate numbers (approximately seven per tree) of ambrosia beetle frass tubes appeared on the flooded trees, the saplings were severed at the base and sectioned into 25 cm-long pieces and placed into large glass desiccators. Air purified by an activated charcoal filter was passed through the host material and then through a 0.5 g Porapak column for 12 hrs at room temperature. Extractions of the columns were performed as described above

and used in subsequent analyses by gas chromatography-electroantennographic detection (GC-EAD) and gas chromatography-mass spectrometry (GC-MS).

In a subsequent experiment at Idlewild Research Station and the Forest Service Research Station, we simultaneously compared the attractiveness and determined the composition of associated volatiles from oak saplings either subjected to root-drowning or with drained roots (control). The stems of half of the saplings in each treatment were wrapped in fine screen to prevent ambrosia beetle attacks. Screening treatments were intended to allow us to determine if the exclusion of ambrosia beetle attacks 1) slowed mortality of flooded trees, 2) altered attractiveness to ambrosia beetles, and/or 3) altered the profile of volatiles arising from saplings. White oak saplings (n=32) were arranged in a randomized complete block design with four treatments per block (Fig. 3.1). Treatments included: 1) Flooded – not screened 2) Flooded – screened 3) Not flooded – not screened 4) Not flooded – screened. Flooded trees were placed in plastic tubs filled with water that covered the root ball as described above. For screened treatments, plastic screening (80 mesh; Chicopee Manufacturing Co., Cornelia, GA) was wrapped securely around the stem from the soil line up to 3 m. Each block consisted of four saplings from each of the four treatments separated by a minimum of 1.5 m. Blocks were separated by a minimum of 10 m, and tree position within each block was re-randomized every three days. Two sticky traps were wrapped around the bole on every tree at the root collar and 1.5 m above the root collar. Sticky traps were made from 22 X 28 cm sheets of

overhead projection transparency film coated with Stikem Special (Seabright Laboratories, Emeryville, CA).

Complete experimental blocks were established at three different times/locations. Four blocks (16 trees) were established on May 28, 2006 at the LSU Aquaculture facilities in Baton Rouge, LA, within the margin of an open field and 9 m from a large hardwood lot. Two blocks were established each on June 23, 2006 and July 9, 2006 at the USDA Forest Service Southern Forest Research Station in Pineville, LA, in an open grass area 3m from the edge of a large mixed hardwood-pine stand. The numbers and species of trapped ambrosia beetles, the numbers of visible attacks on unscreened trees, and the percentage of green leaves remaining were recorded daily.

Collection of Volatiles

Volatiles were collected from saplings on the day ambrosia beetles were first observed in sticky traps and every 2 days thereafter. Teflon bags (.005cm thick, 30.5 x 63.5cm; Welch Fluorocarbon, Dover, NH) were used to make headspace enclosures around either a portion of the lower bole or a single small branch and its associated foliage. Care was taken not to puncture the bags when placing them around the foliage. A Porapak column with a length of flexible Teflon tubing attached at one end was placed inside the enclosure such that the opposite end of the tubing extended outside. The open ends of the headspace enclosures (the points where the tree branch/bole entered the enclosures) were sealed to allow air movement into the headspace but prevent the incursion of outside volatiles.

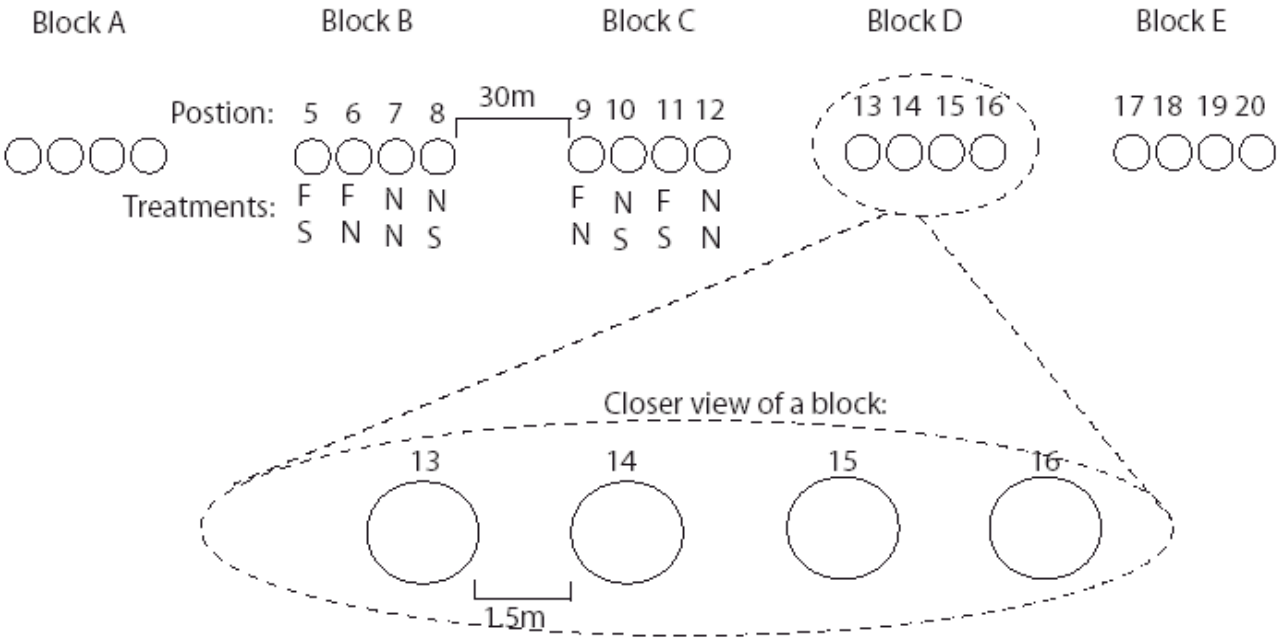

Fig. 3.1. Effects of flooding on attractiveness of host trees - experimental layout. Each tree received the same treatment throughout the length of the experiment, and their position within the block was randomized every two days. Treatment key: F=Flooded, E=Exclusion, N= No treatment. The first row refers to the flooding treatment. The second row refers to the exclusion treatment. Example: F, N - refers to a flooded treatment but no exclusion treatment.

Sealing was accomplished by wrapping several layers of (5-cm wide) activated charcoal filter mesh around the bole or branch and the Teflon tubing at the point where it exited the enclosure, and then securing the mouths of the Teflon bag enclosures tightly against this belt of charcoal mesh. Air in the bag was drawn through the Porapak Q column at a rate of 150ml/min for 2 hours by a Gillian 3500 Live Flow® air sampling pump attached to the extruded end of the Teflon tubing. Following termination of sampling, columns were sealed in clean screw-cap vials for transportation to the lab where the trapped volatiles were immediately extracted. Volatiles were desorbed from the Porapak columns at room temperature as described above, and an internal standard of 5 µl of a 1/1000 dilution of heptyl acetate in hexane was added to each of the samples. An approximate 1 ml aliquot of each sample was concentrated ten-fold by allowing solvent to evaporate from an open vial for 45 min, and the concentrated sample was then transferred to a 150 µl-volume insert of a GC autosampler vial. Pentane was added to the vial, outside the insert tube, to prevent the evaporation of the sample. Between each sampling, the Teflon headspace enclosures and tubing were washed with water and Alconox powdered soap (Alconox, Inc. White Plains, NY), thoroughly rinsed with ultra-pure water, and dried in an oven for at least 5 hours.

Chemical Analysis

The samples were analyzed on a Hewlett-Packard 6890-5973 coupled gas chromatograph/mass spectral detector (GC-MSD) employing helium as the carrier gas. Two microliters of concentrated sample were injected splitless and

analyzed with a semi-polar phase capillary GC column (INNOWax; 60 m x 0.25 mm x 0.25 µm film; Agilent Technologies, Wilmington, Delaware). The oven program was 40° C for 1 min, 16° C/min to 80° C, then 7° C/min to 230° C and held 10 minutes. Compounds in the samples were identified by mass spectral and retention time matches with known standards. Quantities of identified compounds in each sample were determined relative to the internal standard, heptyl acetate. All results were imported into SAS Analyst (SAS Institute Inc., Cary, NC) for further statistical analysis.

Electrophysiological Studies of *X. crassiusculus* Antennae

GC-EAD analyses were performed to identify olfactory stimulants for *X. crassiusculus* present in volatiles collected from root-drowned, attractive host trees. Procedures and equipment were largely identical to those described in Asaro *et al.* (2004) and Sullivan (2005). Electrical contact was made with each assayed antenna by inserting the glass-pipette Ag/AgCl reference electrode into the beetle's excised head and inserting the tip of a similarly-constructed recording electrode into the antennal club in the center of the distal patch of olfactory sensillae. Antennae from twelve apparently undamaged, recently-emerged females were assayed. Only female antennae were examined since this is the sex that disperses to new hosts. Concentrated extract (1 µl) from a Porapak Q aeration of pieces from a white oak undergoing attack by *X. crassiusculus* was injected splitless onto the GC and provided the olfactory stimulus. The GC column was the same as for the GC/MS analyses described

above, and the oven temperature program was 40° C for 0.5 minutes, then ramped 6° C/min to 230° C and held constant 5 minutes.

Sensitivity of *X. crassiusculus* to Compounds Not Present in Hosts

A mixture of host compounds and pheromones of the southern pine beetle, *Dendroctonus frontalis* Zimmermann (SPB), were assayed both by electroantennogram technique (EAG) and GC-EAD in preliminary trials aimed at developing antennal preparation methodology for *X. crassiusculus*. Compounds present in the SPB-associated odor mixture included α-pinene, β-pinene, limonene, γ-terpinene, ρ-cymene, dimethylstyrene, camphor, *p*-cymen-8-ol, isopinocamphone, fenchone, terpinen-4-ol, myrtenal, *(E)*-pinocarveol, 4-allylanisole, α-terpineol, borneol, myrtenol, *endo*-brevicomin, verbenone, and frontalin. All were diluted to approximately 100 PPM in solvent. In one EAG run, air (30 ml/min for 2 sec) was puffed onto the antennal preparation from a glass pipette containing a filter paper strip to which had been applied 10 µl of either the SPB semiochemical mix (dissolved in mineral oil) or 50% ethanol (Fig. 3.6). A second EAG run compared antennal responses to puffs from pipettes containing either the SPB mix, odor from five frass "toothpicks" placed directly into the pipette, or nothing (blank) (Fig 3.7). The frass "toothpicks" consisted of 1 day-old extruded frass from *X. crassiusculus* galleries initiated two weeks earlier in the stem of a beech tree (*Fagus grandifolia*). Finally, a GC-EAD run of the SPB semiochemical mixture using procedures described previously determined *X. crassiusculus* antennal responses to the 20 compounds in the mixture (Fig. 3.8).

Field Trapping Assays with Candidate Attractants

Compounds identified in attractive host tissue that were both electrophysiologically active with *X. crassiusculus* antennae and commercially available were subsequently tested in the field, both in combination and individually, for attractiveness to ambrosia beetles. In the first experiment, we assayed a bait composed of all 14 compounds identified in attractive host tissue. These compounds were combined in equal proportions by volume: hexenal, *trans*-2-hexenal, 3-hydroxy-2-butanone, 6-methyl-5-hepten-2-one, 1-hexanol, *(E)*-3-hexen-1-ol, nonanal, *(Z)*-2-hexen-1-ol, *(Z)*-2-hexen-1-ol, benzaldehyde, 6-methyl-3,5-heptadiene-2-one, methyl salicylate, ethyl salicylate, guaiacol, and eugenol. A randomized complete block design experiment (8 blocks) compared *X. crassiusculus* responses to 12-unit Lindgren multiple-funnel traps baited with one of four different bait combinations: control (unbaited trap), the bait mixture, ethanol, and the bait mixture plus ethanol. Each block was replicated 4 times spatially and 2 times temporally, and trapping occurred from March 31, 2006 to Apr. 18, 2006. Traps within blocks were spaced by 30 m. Blocks were separated by at least 90 m.

A second set of experiments was performed that were identical to the aforementioned four-treatment experiment except that a single compound was used in place of the mixture. I selected the most antennally active compounds for individual evaluation (2-hexen-1-ol, 6-methyl-5-hepten-2-one, ethyl salicylate, nonanal, eugenol, guaiacol, 1-hexanol). Antennally active compounds were chosen by calculating the ratio of EAD response amplitude to quantity of

compound present. Compounds for which low quantities produced high EAD responses were presumed to have a greater probability of behavioral activity. We tested four complete blocks for all but two of these compounds (eugenol, 17 blocks; guaiacol, 8 blocks). Eugenol and guaiacol received additional replication because they produced mean increases in catch in the initial four blocks. Ethanol was eluted from a .5 L bottle with a 1.3 cm length of cotton wick (1 cm diam.) extending through the cap. Test compounds were released from an open 20-ml scintillation vial containing 3 ml of bait. The vial mouth was protected from rain and attached at the third funnel from the top of the trap. Field testing of individual compounds occurred from Apr. 18, 2006 to Aug. 11, 2006.

Data Analysis

For each ambrosia beetle species, the difference in mean catch between the upper and lower traps was analyzed by a paired t-test. The differences in trap catch between flooding and screening treatments were analyzed using a 2-way factorial ANOVA with SAS software.

Trap catch of *X. crassiusculus* was $\log_{10}(X+1)$ transformed to reduce heteroscedasticity in the data, and results were then analyzed using a 2-way factorial ANOVA employing bait and block as factors. Comparisons between baits and individual compounds were made using SNK-pairwise comparisons.

RESULTS

Host Volatile Sampling

A variety of compounds was detected in the control and flooded trees with the glass desiccator-Porapak Q sampling method. Green leaf volatiles and

monoterpenes composed a large portion of the total volatiles. Numerous compounds elicited responses from the antennae of *X. crassiusculus* (Table 3.1). Time was not taken in this preliminary experiment to identify every compound in every sample.

The same compounds were present in both flooded and non-flooded treatments, however, all but α- and β-pinene, camphene, limonene, 3-carene, anisole, and eugenol were produced in higher mean amounts by the flooded, than the non-flooded tees (Table 3.2). Only hexanal, benzaldehyde, *(E)*-3-hexen-1-ol, and *(Z)*-2-hexen-1-ol were significantly different (P=0.0479, 0.0467, 0.0318, 0.0475, respectively; t-test).

The same compounds were detected from both leaf and bole aerations. Compounds α- and β-pinene, camphene, limonene and guaiacol were present in higher mean amounts in the leaf aerations than the bole aerations (Table 3.2). Only α- pinene, camphene, 3-carene, anisole and *(E)*-3-hexen-1-ol were significantly different using a paired t-test of means (P= 0.0006, 0.0038, 0.0118, 0.0348, 0.0273, 0.006, respectively). The same compounds were detected from both the screened and unscreened treatments and they did not differ significantly in quantity.

Ambrosia Beetle Attraction to Flooded Trees

Flooded and non-flooded trees differed significantly in attractiveness to ambrosia beetles. In addition to *Xylobiops basilaris,* .six species of ambrosia beetles were trapped on flooded trees (in order of abundance): *X. crassiusculus*, *X. saxeseni , X. basilaris, X. ferrigeneous, X. impressus, X. compactus and*

Hypothenemus sp. Ambrosia beetle arrival began one day after flooding and continued until the termination of the study on day nine (Fig. 3.2). The three most abundant species trapped (*X. crassiusculus, X. saxeseni, X. basilaris*) accounted for 91% of trap catch. The highest diversity of ambrosia beetles was trapped on day five (six species).

The mean catch per tree of ambrosia beetles on sticky traps placed 1.5 m above the root collar (1.53), was significantly less than catch at the root collar (2.69 p=0.0479, paired t-test; Fig 3.3). The trap height effect was most apparent in *X. saxeseni*, which had a mean of 0.7 beetles in the upper traps and 2.1 in the beetles in the lower traps, although this was not significantly different (P=0.0959; Fig 3.4). The mean catch per tree of *X. crassiusculus* was 0.53 in the upper traps and 0.75 in the lower traps, although this difference was not significant (P=0.4893).

Ambrosia beetle catch also did not differ significantly between screened and unscreened trees (P=0.8569). A mean of 1.7 ambrosia beetles were trapped on the screened trees and 1.6 on non-screened trees. Time to tree death after flooding was not affected by screening.

Electrophysiological Studies of *X. crassiusculus* Antennae

Xylosandrus crassiusculus antennae responded to 29 compounds found in samples from attractive hosts (Table 3.2). A composite GC-EAD trace of runs of 6 female *X. crassiusculus* exposed to Porapak Q-collected volatiles from attractive hosts is shown in figure 3.5.

Table 3.1. Summarized results from the flooding and screening analysis. Compounds that varied significantly (t<.05) from the control are highlighted in yellow. For each compound the treatment with a higher mean value is highlighted in red. P-values are from paired t-tests of means between the flooded and non-flooded trees or leaf and bole. Heptyl acetate was used as the internal standard in all samples hence the unavailable P-values.

			FLOODED		CONTROL			LEAF			BOLE	
Host Volatile	**retention time**	**t-value**	**mean amount**	**Standard error**	**mean amount**	**Standard error**	**t-value**	**mean amount**	**Standard error**		**mean amount**	**Standard error**
.alpha.-Pinene	7.90	0.831	0.0881	0.0286	0.1004	0.0419	0.001	0.0081	0.0022		0.1841	0.0436
Hexanal	8.97	0.048	0.0072	0.0031	0.0006	0.0005	0.23	0.0059	0.0141		0.0020	0.0028
.beta.-Pinene	9.41	0.743	0.0201	0.0076	0.0255	0.0133	0.004	0.0019	0.0007		0.0466	0.0137
3-Methyl-1-Butanol	11.19	0.093	0.0023	0.0013	0.0000	0.0000	0.09	0.0023	0.0013		0.0000	0.0000
trans-2-hexenal	11.72	0.078	0.0114	0.0061	0.0000	0.0000	0.08	0.0114	0.0061		0.0000	0.0000
2-Butanone, 3-hydroxy-	13.04	0.166	0.0025	0.0018	0.0000	0.0000	0.17	0.0025	0.0018		0.0000	0.0000
1-Hexanol	14.10	0.233	0.0413	0.0333	0.0002	0.0002	0.09	0.0076	0.0040		0.0006	0.0004
Heptyl Acetate	14.65	N/A	0.3010	0.0000	0.3010	0.0000	N/A	0.3010	0.0000		0.3010	0.0000
3-octanol	14.88	0.186	0.0006	0.0005	0.0000	0.0000	0.33	0.0002	0.0002		0.0000	0.0000
cis-2-Hexen-1-ol	15.34	0.163	0.0004	0.0003	0.0000	0.0000	0.16	0.0004	0.0003		0.0000	0.0000
Benzaldehyde	17.80	0.047	0.0053	0.0022	0.0006	0.0004	0.26	0.0043	0.0022		0.0016	0.0006
ethyl salicylate	22.70	0.186	0.0006	0.0005	0.0000	0.0000	0.33	0.0004	0.0004		0.0000	0.0000
benzyl alcohol	23.47	0.081	0.0074	0.0040	0.0000	0.0000	0.13	0.0063	0.0040		0.0000	0.0000
Camphene	8.60	0.489	0.0057	0.0023	0.0105	0.0059	0.01	0.0000	0.0000		0.0161	0.0058
3-Pentanol	9.15	0.165	0.0069	0.0043	0.0006	0.0004	0.15	0.0069	0.0043		0.0004	0.0003
Heptanal	10.98	0.577	0.0004	0.0003	0.0002	0.0002	0.58	0.0004	0.0013		0.0002	0.0010
Limonene	11.24	0.834	0.0017	0.0006	0.0019	0.0010	0.19	0.0013	0.0005		0.0026	0.0010
3-Carene	12.23	0.142	0.0182	0.0072	0.0472	0.0174	0.03	0.0538	0.0171		0.0111	0.0061
5-Hepten-2-one, 6-methyl-	14.02	0.120	0.0024	0.0011	0.0006	0.0004	0.16	0.0026	0.0011		0.0009	0.0004
Anisole	14.23	0.569	0.1138	0.0613	0.1502	0.0708	0.03	0.0450	0.0450		0.1854	0.0743
cis-3-Hexen-1-ol	14.78	0.032	0.0139	0.0051	0.0021	0.0010	0.01	0.0156	0.0052		0.0004	0.0004
trans-2-Hexen-1-ol	15.16	0.048	0.0082	0.0037	0.0002	0.0002	0.07	0.0076	0.0037		0.0008	0.0006
1-heptanol	16.07	0.163	0.0013	0.0006	0.0004	0.0003	0.43	0.0009	0.0004		0.0004	0.0003
1-octanol	17.96	0.268	0.0011	0.0005	0.0004	0.0003	0.75	0.0009	0.0005		0.0006	0.0004
Methyl Salicylate	22.17	0.121	0.0133	0.0077	0.0006	0.0005	0.08	0.0142	0.0077		0.0000	0.0000
guaiacol	23.28	0.233	0.0011	0.0009	0.0000	0.0000	0.33	0.0000	0.0000		0.0002	0.0002
Eugenol	27.17	0.302	0.0000	0.0000	0.0038	0.0036	0.33	0.0002	0.0002		0.0000	0.0000

Table 3.2. Compounds present in Porapak Q aerations of attractive white oak saplings.that elicited antennal responses from *X. crassiusculus* A relative EAD response value (*=weak antennal response, **=intermediate antennal response ***=strong antennal response) was assigned based on the ratio of antennal response amplitude to stimulus concentration. *(E-)* and *(Z-)* linaloxide could not be distinguished by their mass spectra alone and we lacked an analytical standard for these compounds.

compound name	other name	CAS #	retention time	EAD response
hexenal				*
(Z)-2-hexenal		505-57-7	12.57	**
3-hydroxy-2-butanone	acetoin	513-86-0	14.23	***
6-methyl-5-hepten-2-one	sulcatone	110-93-0	15.5	**
1-hexanol		111-27-3	15.63	**
(E)-3-hexen-1-ol		923-96-1	16.49	*
nonanal		124-19-6	16.88	***
(E)-2-hexen-1-ol		928-95-0	16.98	**
(*Z)*-2-hexen-1-ol		928-95-0	16.98	**
linaloxide ? (*E*?)		5989-38-8	18.02	***
linaloxide ? (*Z*?)		34995-77-2	18.7	***
benzaldehyde		100-52-7	20.25	**
6-methyl-3,5-heptadiene-2-one		1604-28-0	21.63	**
methyl salicylate		119-36-8	25.8	**
ethyl salicylate		118-61-6	26.48	***
guaiacol	2-methoxyphenol	90-05-1	27.26	***
eugenol		97-53-0	32.9	***

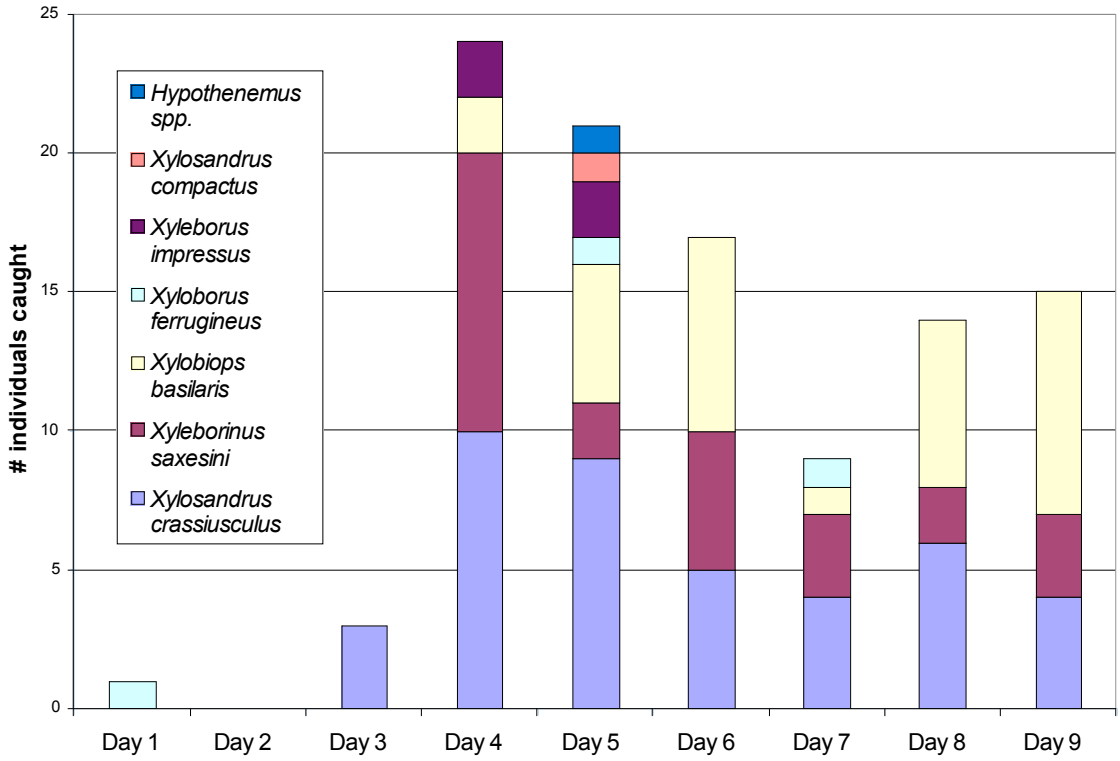

Fig. 3.2. Summed species contribution and arrival time to 35 flooded white oak trees over 9 days.

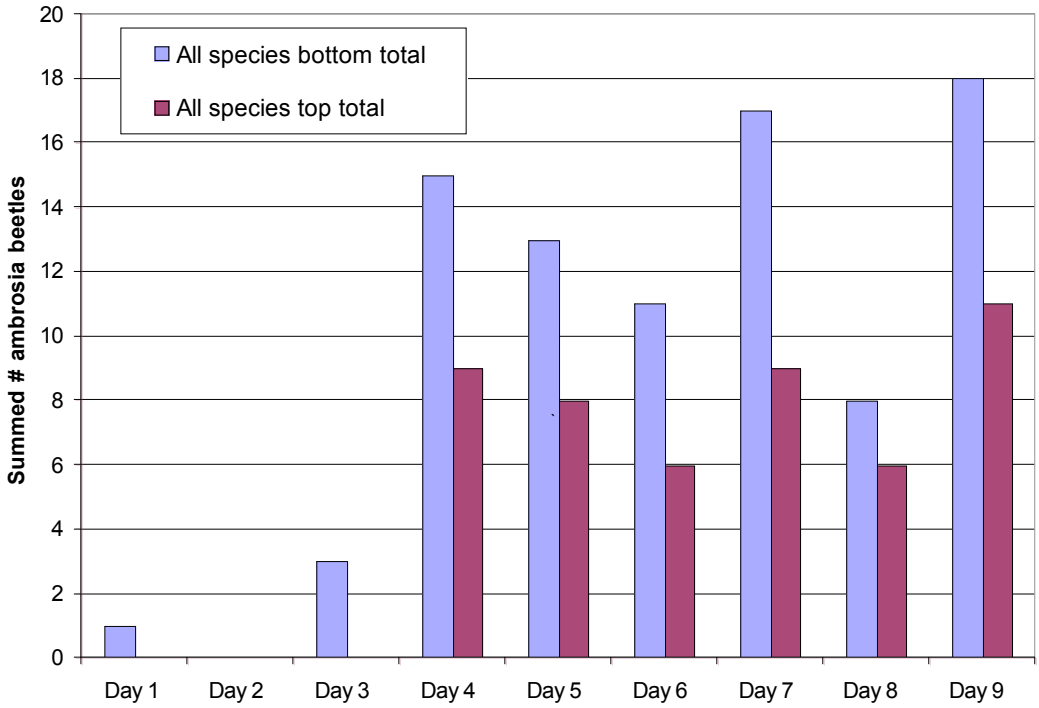

Fig. 3.3. Summed sticky trap catch (all species) comparing top (1.52m above the root bole) and bottom (0m above the root bole) trap catch on 35 flooded white oak trees.

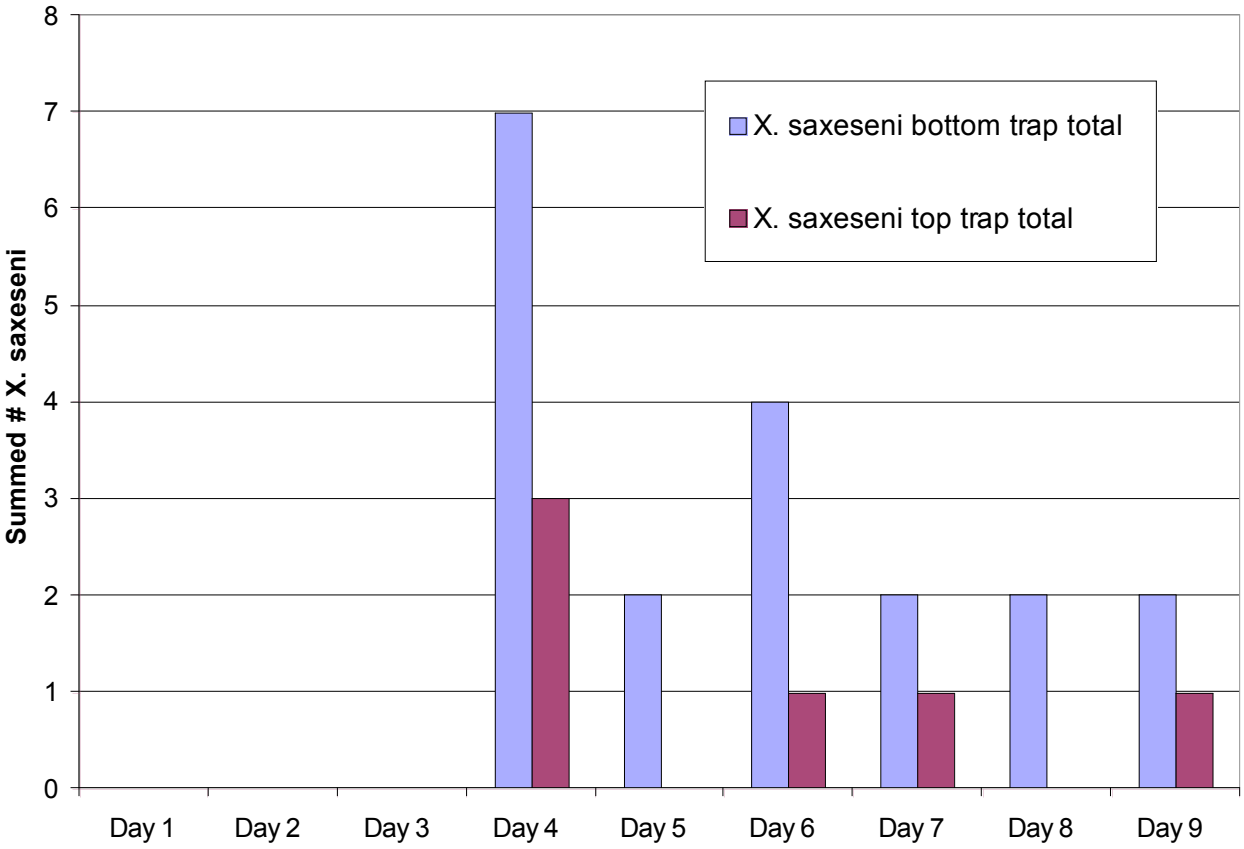

Fig 3.4. Summed *Xyleborinus saxeseni* sticky trap catch. Comparing top (1.52m above the root bole) and bottom (0m above the root bole) traps on 35 flooded white oak trees. *X. saxeseni* had the most apparent difference between trap catch of top and bottom traps.

Sensitivity of *X. crassiusculus* to Compounds Not Present in Hosts

Xylosandrus crassiusculus responded to multiple volatiles associated with SPB attacks and a mixture of these volatiles presented as an EAG stimulus elicited a stronger response than ethanol (Fig. 3.6).

A second EAG tested the SPB mix, odor from *X. crassiusculus* frass "toothpicks" and a blank (Fig. 3.7). The frass toothpick odor was derived from five, one day-old extruded frass "toothpicks," from 2 wk-old *X. crassiusculus* galleries. Frass "toothpicks" are exuded from the gallery system that tends to clump together as it's being expelled, forming a "toothpick-like" structure. A GC-EAD run of the SPB semiochemical mixture showed antennal responses to 12 of 20 compounds (Fig. 3.8). Based upon the strong antennal responses to frontalin *endo*-brevicomin and verbenone, these three compounds appear to be potentially biologically important.

Field Trapping Assays with Candidate Attractants

A bait composed of all EAD-active compounds in attractive host tissue failed to attract ambrosia beetles or increase attraction to traps baited with ethanol (Fig. 3.9). Additionally, when presented singly, no individual compound of the bait mixture was attractive to ambrosia beetles or significantly improved the performance of ethanol baits (Table 3.3). However, eugenol and ethanol offered promise as an improved trap bait over the traditionally-employed ethanol ($p=0.0736$; Fig. 3.10). High variation in captures probably accounts for the high P-value. Factorial analysis showed no significant blocking influence. Extremely

low trap catch, most likely due to season and weather, may have limited statistical power to detect effective compounds.

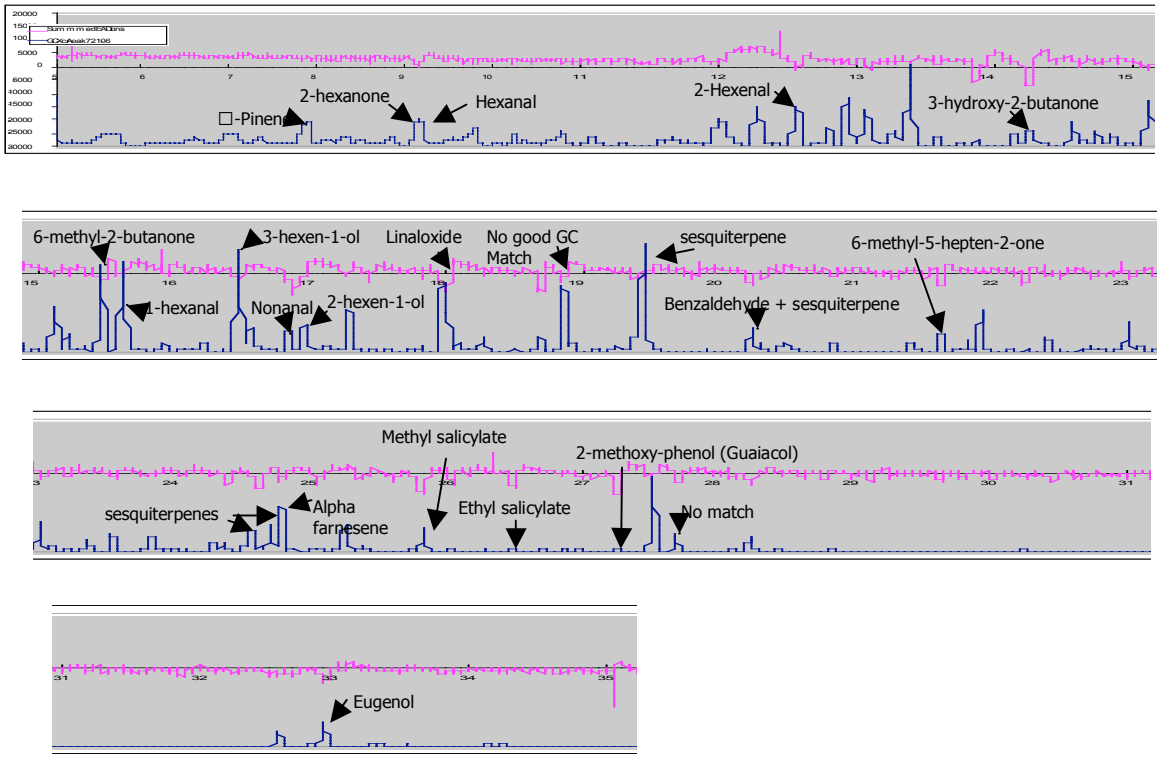

Fig. 3.5. Summed Coupled Gas Chromatographic-Electroantennographic Detection trace results of six female *X. crassiusculus* exposed to Porapak Q-collected volatiles from attractive hosts. The EAD trace (pink) is on top, the GC trace (blue) is on bottom.

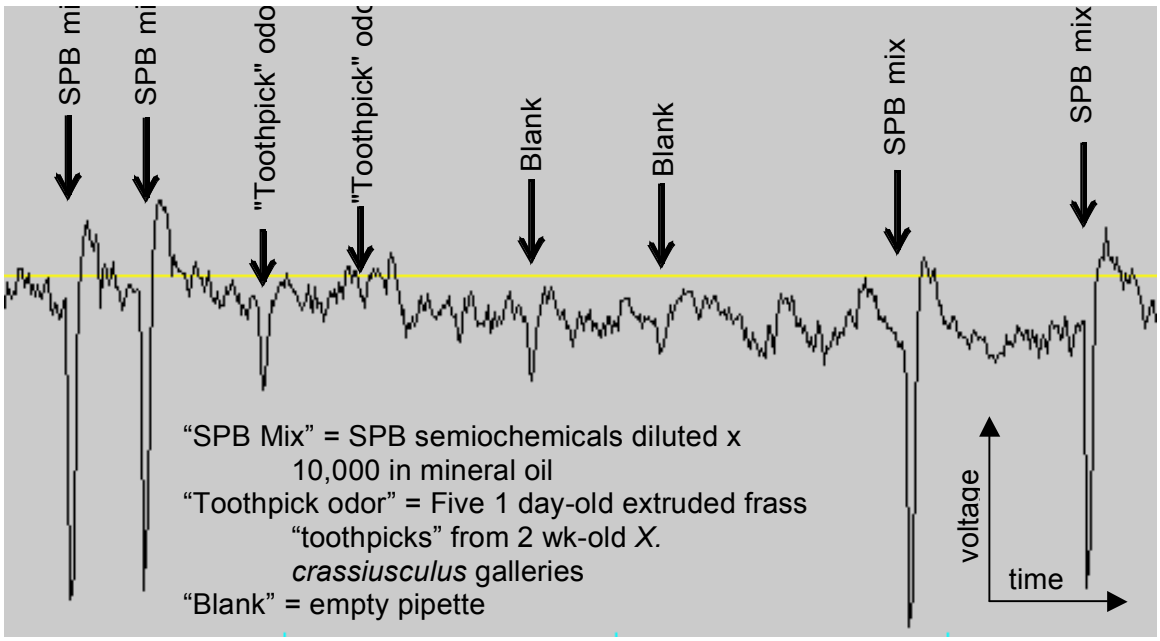

Fig. 3.6. EAG tests. Test odors in pipette "puffed" over *X. crassiusculus* antennae.

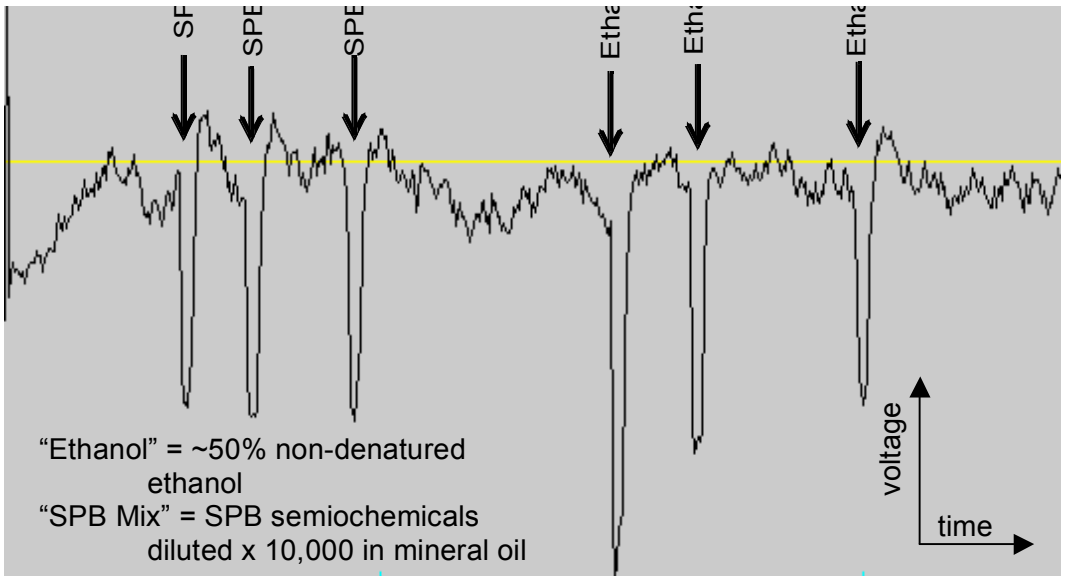

Fig. 3.7. EAD test. Antennal responses of *X. crassiusculus* to compounds associated with SPB-infested trees.

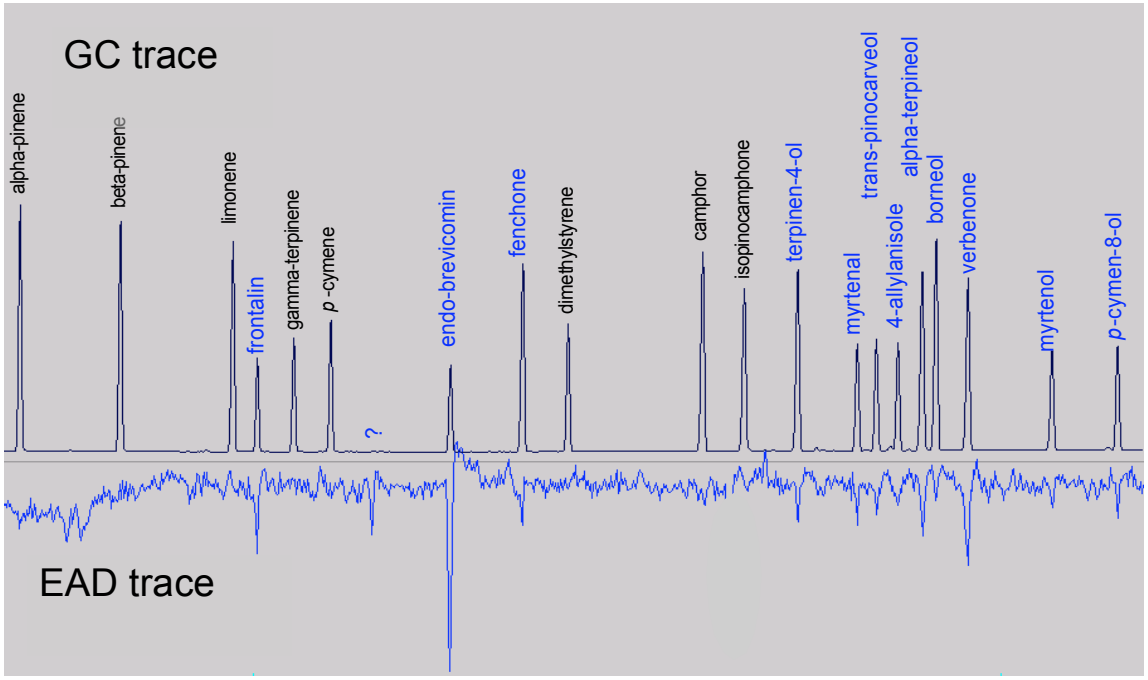

Fig. 3.8. Antennal responses of *X. crassiusculus* to compounds associated with SPB-infested trees.

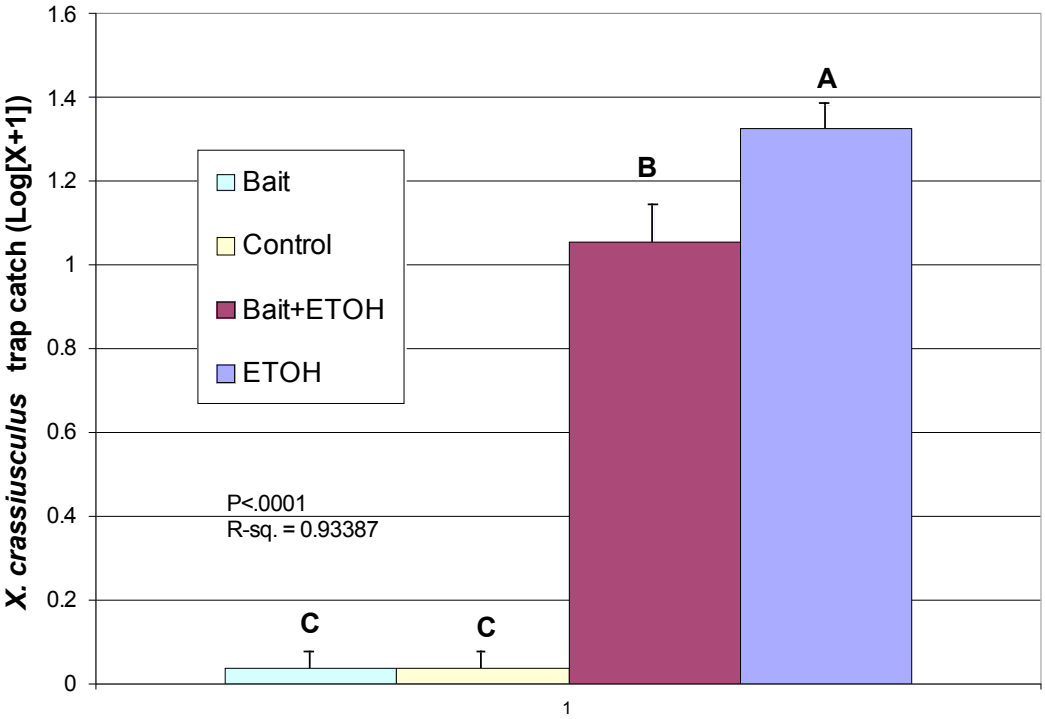

Fig. 3.9. Field evaluation of a bait mixture, bait + ETOH and control treatments against ETOH. Values are mean log transformed trap catch for each treatment. ANOVA SNK comparisons, P-value and R^2 are given.

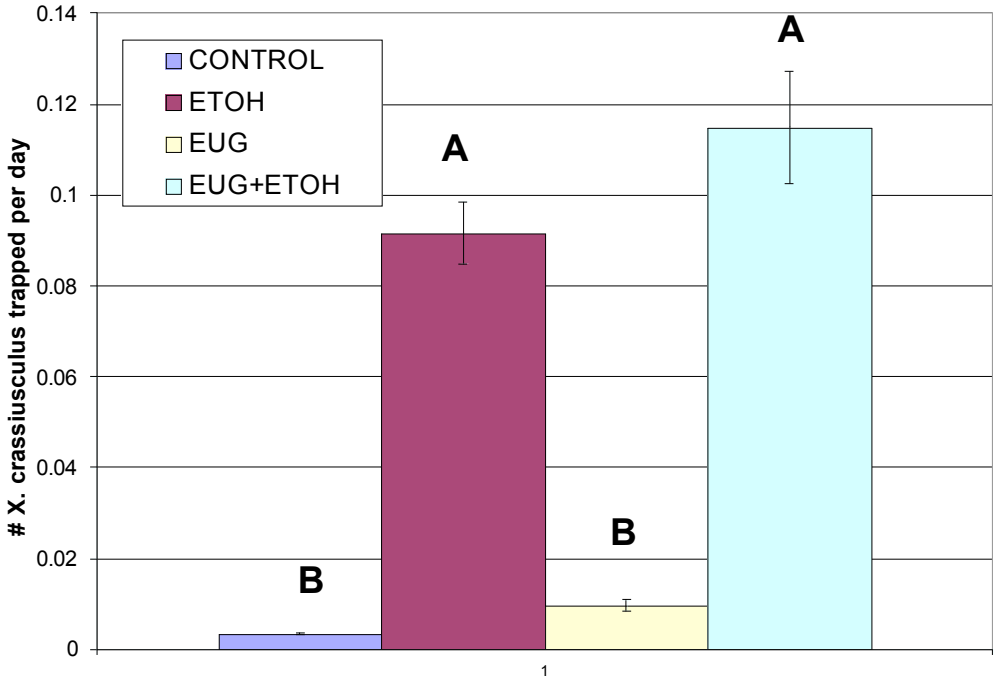

Fig. 3.10. Field evaluation of Eugenol, Eugenol + ETOH and control treatments against ETOH. ANOVA SNK comparisons are shown for each treatment.

Table 3.3. Log transformed mean trap catch of *X. crassiusculus* for each compound tested. Each compound was blocked with four treatments; alone, ETOH alone, compound + ETOH and a control.

		Means			
Compound of interest	**# of replications**	**Compound alone**	**ETOH**	**Compound + ETOH**	**Standard error**
2-hexen-1-ol	4	0.000	0.2222	0.0000	0.0097
6 methyl-5-hepten-2-one	4	0.000	0.1111	0.0000	0.0051
ethyl salicylate	4	0.000	0.0833	0.0833	0.0062
nonanal	4	0.000	0.0335	0.0417	0.0061
eugenol	17	0.024	0.1311	0.2239	0.0226
guiacol	8	0.016	0.2090	0.1683	0.0336
1-hexanol	4	0.000	0.0333	0.0167	0.0028

DISCUSSION

Volatile monoterpenes and green leaf volatiles have a well-documented capacity for influencing scolytine behavior (Bedard *et al*. 1969; Rudinsky *et al*. 1972; Werner 1972). Often these compounds have been associated with tree stress (Ebel *et al*. 1995). It has been demonstrated that stressed trees produce different amounts of volatile compounds than healthy trees (Ebel *et al* 1995, Fan *et al* 2000, Byers *et al* 2000). It would be expected that stressed trees would also have higher amounts of 'stress-related,' attractive compounds. It should also be noted that ethanol, a product of anaerobic metabolism in trees, was not found in our samples. However, this is most likely due to Porapak Q's inability to absorb extremely polar compounds.

Interestingly, the consistency of compounds being released from leaf and bole samples has not been observed previously (Byers 2000). Whereas, β-pinene, camphene and limonene were higher in the leaf than the bole of *Betula pendula* (Byers 2000), I observed the opposite trend in white oak. This could be a result the different methods of sampling and tree species used. Byers' study used healthy, chipped *Betula pendula*. My study utilized stressed trees and healthy trees sampled with no damage to the sampled material. I did find higher levels of *(E)*-2-hexen-1-ol and 1-hexanol in the stressed trees which is consistent with Ebel (2005), who showed elevated levels of these two compounds in stressed apple trees.

Screened and non-screened trees showed no differences in chemical composition, indicating that tree-colonizing beetles did not significantly affect the

profile of compounds being emitted by the tree. This could, in part, be explained by the minimal damage to the physiologically active portions of the tree caused by ambrosia beetles and associated fungi.

The greater ambrosia beetle catch by traps on the lower bole does not necessarily suggest that these parts are producing more attractive volatiles. Gallery location choice is completely unstudied in ambrosia beetles. In the bark beetles, compounds responsible for host location may have different effects on beetle movement after landing (Wallin and Raffa 2000) suggesting that preference for a particular portion of the bole may have no relationship to volatiles emitted from that portion. My results suggest that ambrosia beetles tend to approach trees near to the ground. Upon landing, these beetles may then crawl to find a suitable location for gallery initiation. Additionally, it was observed that the vast majority of ambrosia beetle attacks also occurred below 1.5m. My results are congruent with the widely-employed procedure of trapping ambrosia beetles using Lindgren funnel traps hung close to the ground.

The 14 compounds combined in my bait mixture significantly reduced *X. crassiusculus* trap catch. It thus seems likely that at least one compound included in the mixture was a deterrent. My testing of 7 individual compounds from attractive hosts yielded suggestive but not significant results. Ethanol released in tandem with eugenol, yielded a higher mean log transformed trap catch (0.23) than ethanol alone (0.13). Although promising, it was not a statistically significant difference.

Eugenol is a allylbenzene and associated with the incomplete combustion of lignin (Bernd and Simoneir 2002). It is also known as an attractant for *Xylosandrus morigerus* (Nakayama and Terra 1986). Elevated ambrosia beetle trap catch has been associated with recently burned forests (Hanula *et al.* 2002, Sullivan et al. 2003, Bauman 2003), which supports the possibility of eugenol as a potential compound in improving trap baits

Guaiacol, 2-methoxyphenol, a natural organic compound also showed promise for attracting *X. crassiusculus* and *X. saxeseni*. Guaiacol is also a product of pyrolysis of lignin (Bernd and Simoneir 2002).

As opportunistic generalists, it is possible that many ambrosia beetles rely upon a multitude of volatile compounds during host location (Kuhnholtz 2001). Zhang and Schlyter (2004) proposed that bark beetles have the capacity to detect a large number of both host and nonhost volatiles. Furthermore, the chemical bouquet from a single tree is extremely complex, and both attractive and deterrent compounds can elicit synergistic and antagonistic effects upon each other depending upon their identity and concentration.

Trap catch in the flooded treatments offered an interesting comparison to the ambrosia beetle survey. Most results mirrored those from the trapping survey which used ethanol baited Lindgren funnel traps. Of particular interest is that *X. crassiusculus* had higher numbers (64% in trap survey, 40% in flooding experiment) responding than *X. saxeseni* (11% in trap survey, 24% in flooding experiment) in both of our experiments. Coyle *et al.* 2005 found that *X. saxeseni* composed 64% of the total trap catch using ethanol baited Lindgren funnel traps.

Other studies in southeastern US have also found *X. saxeseni* to be the most prevalent ambrosia beetle (Roling and Kearby 1975, Flechtmann *et al.* 1999, Hanula *et al.* 2002) suggesting that there is a difference in species composition in central Louisiana. Also noteworthy is that in comparison to our trapping survey, *X. ferrugineus* (3% of total) composed a lower percentage of total trap catch in the flooding experiment (9% of total). Surprisingly, *Xylobiops basilaris* responded quickly to flooded trees. To our knowledge this is the first documented experiment of *X. basilaris* attacking living, stressed trees. Our current understanding of *X. basilaris* biology is that it breeds in dead wood in trees and on the forests floor. My data suggest it may at times take on a more "aggressive" habit, attacking living, stressed trees.

Hardwood-infesting ambrosia beetles have an extremely wide host range, do not appear mass-attack trees as do aggressive bark beetles, and have no known pheromones. To date we have recorded 41 compounds eliciting antennal responses from *X. crassiusculus*. The diversity of olfactory stimulants makes the development of baits difficult, as many combinations of different compounds may need to be tested.

The chemical ecology of "aggressive" bark beetles has been relatively well studied when compared to the limited and rudimentary studies concerning ambrosia beetle chemical ecology. Because of the lack of knowledge on ambrosia beetle chemical ecology, it is helpful to compare the two systems for further insight into the subject. Bark beetles have a small host range and employ pheromones, hence the range of compounds to which they respond tends to be

limited. In contrast, ambrosia beetles are thought to be generalists, as indicated by their wide host ranges. This generalist approach makes isolation of one or two compounds that greatly effect behavior less likely as this compound would have to be present in many trees under many environmental conditions. Also, because of their inbred nature, it has been theorized they would have very little need for a pheromone (Kirkendall *et al.* 1997). Pheromones probably are more necessary for coordinating mass attacks needed by bark beetles to overwhelm a host's defenses. Because of the resin defenses of conifers, many bark beetles cue into aggregation and repellant kairomones and pheromones to coordinate mass attack. Exotic angiosperm-infesting ambrosia beetles in the United States have been documented attacking stressed broadleaf trees or dying pines, where overcoming host defenses is of relatively minor importance. Broadleaf plants produce lower quantities of volatiles compared to the conifers (Byers 2000) making quantification and experimentation more difficult.

Hylesinus pruinosus (Eichhoff) produces both *exo*- and *endo*-brevicomin (B. Sullivan personal communication), and both the genus *Hylesinus* and *X. crassiusculus* are native to Asia (S.L. Wood 1982). It is possible that *X. crassiusculus* evolved electrophysiological sensitivity to *endo*-brevicomin as a means of locating hosts previously colonized by hardwood-infesting bark beetles such as *H. pruinosus*.

The strong antennal response to the semiochemical mixture is encouraging because it opens up the possibility that *X. crassiusculus* responds to heterospecific compounds. However, the possible permutations are poorly

studied and complex. For example, the compound verbenone is produced by plants, animals and fungi and can act as a repellent or attractant depending on the bark beetle species and context. A repellant, 4-allylanisole, is a host produced compound (Hayes *et al*. 1994). Finding an antennal response to these SPB-associated compounds is interesting, but unfortunately, not of practical management implications.

Interestingly, there was a strong antennal responses to ethanol and very small responses to the *X. crassiusculus* frass. The strong antennal response to ethanol, a byproduct of anaerobic tree metabolism, was expected. The low response to frass may strengthen the argument against the existence of a *X. crassiusculus* produced pheromone.

CHAPTER IV - FUNGAL INTERACTIONS

INTRODUCTION

In 2002, as part of the Early Detection Rapid Response program, a Lindgren funnel survey trap baited with ethanol near Port Wentworth, Georgia detected the first *Xyleborus glabratus* in the U.S. (Rabaglia 2005). Substantial redbay (*Persea borbonia* (L.) Spreng.) Eichhoff mortality was also observed in the same area. By 2005 the wilt and had spread to coastal Florida and South Carolina and was affecting sassafras [*Sassafras albidum* (Nutt.) Nees] with an estimated rate of spread of 32.1869 Km/year. Current knowledge of the system indicates that *X. glabratus* introduces an unspecified vascular fungus (*Rafaella sp*.) into its host (Fraedrich 2005), causing infected redbays to wilt and die within a few weeks or months. The symptoms include extensive vascular streaking that is usually associated with as few as 1 or 2 *X. glabratus* galleries. *Rafaella sp*. is thought to be the primary mycangial associate of *X. glabratus* (Harrington and Fraedrich personal communication). Since initial detection no peer reviewed publications have addressed the issue. Research is, however is continuing among cooperating agencies including the South Carolina, Florida and Georgia DNR's, USDA Forest Service and university personal from around the country.

During investigation of the wilt, several ambrosia beetles (*X. crassiuculus* and *X. compactus*), including *X. glabratus* have been seen tunneling in the same host tree and even within the same gallery. This close association between ubiquitous, ambrosia beetles with large host ranges and a highly virulent fungus raised the concern that these other ambrosia beetles (*X. crassiuculus* and *X.*

compactus) could serve as secondary vectors exacerbating the problem. Also of concern was the possibility that the prevalence of *Rafaella sp*. in suitable host material could serve as an additional nutrient source for *X. crassiuculus* and *X. compactus*, confounding their effects. As seen in leaf-cutter ants (Mehdiabadi et al. 2005) and ambrosia beetle introductions into new environments (Batra 1963) there is some precedence for the possibility of symbiont switching.

The objectives of this study were to determine if *X. crassiusculus* could mycangially or phoretically vector *Rafaella sp*. and to determine if *Rafaella sp.* has any effects upon *X. crassiusculus* fitness.

METHODS

Fungal cultures

All *Rafaella sp.* cultures were obtained from S. Fraedrich (USDA Forest Service, Southern Research Station) who aseptically isolated from galleries in vascular tissue of infected red bay (*Persea borbonia* (L.) Spreng.) on Hilton Head Island, GA, USA in March 2006. Cultures were allowed to grow for 1 week and a sample of hyphal growth of each isolate was aseptically transferred onto fresh MEA plates.

Cultures of *A. xylebori* were obtained via mesonotal mycangial isolations from live beetles collected in ethanol baited Lindgren funnel traps placed at the USDA Forest Service Southern Research Station facility in Pineville, LA from Aug. 20 to 25, 2005. All beetles were surface sterilized via agitation for 25 seconds in 95% ethanol, followed by a 15 second rinse in sterile water. Beetles which died within 30 minutes after the sterilization were disposed of. The

remaining beetles were then used in mycangial isolations. Fungal identification were conducted by Dr. Diana Six through molecular techniques at the University of Montana – Missoula.

Mycangial isolations

The mycangial isolation technique for *X. crassiusculus* was adapted from existing techniques (Kajimura *et al*. 1992). A sterilized beetle was held firmly against solid paraffin wax in a petri dish via a sterile insect pin inserted through the top of the head. Two sterile insect pins, one on each side of the head-abdomen connection, were used to slightly pry apart the head from the abdomen and expose the mesonotal mycangium (taking care not to rip connective tissues). The mycangium was evident from the visible fungal spores dorsally exposed between the head and abdomen. Another sterile insect pin was used to extract the mycangial contents which were then streaked onto MEA. Four isolations were plated on the same dish in opposing corners. All isolations were examined daily and resulting fungal species isolated and plated onto fresh plates. *Ambrosiella xylebori* (as identified by D. Six, University of Montana) was consistently recovered from all *X. crassiusculus* mycangial isolations.

Vectoring

Disposable16 X 125mm, test tubes (CMS Vineland, NJ) of sawdust-agar based rearing medium were used to study *X. crassiusculus* gallery initiation, construction, egg laying, brood care and brood development. Test tubes of the rearing medium were constructed using a technique described by Taborsky and Peer (2004). A plug of *Rafaella* sp. and a foundress beetle were aseptically

added at different dates in relation to each other in five treatments (Table 4.1; n=52). A control treatment included adding *X. crassiusculus*, without its *Rafaella* sp. symbiont. To simulate the beginning of gallery construction (when the *Rafaella* sp. is most likely to grow within the gallery in natural conditions) a small (1cm deep by .25 cm wide) artificial gallery was constructed. Being careful to not touch the plug to any other surface, a single 0.5 cm diameter plug of *Rafaella* sp. was inserted into the artificial gallery. After inoculation and addition of beetles, the experiment was monitored daily for evidence of fungal contamination, mutualistic fungal growth, and beetle activity. Gallery initiation, total offspring, larvae left in tube, number of emerged adults, number of males, and fungal species present (mycangial and phoretic) were recorded for each treatment.

Fungal Competition - Spatial Separation – Primary resource capture

Rafaella sp. and *A. xylebori* were pitted against each other in spatial competition laboratory experiments (n=10). A single plug of each fungus was placed on opposite sides of a MEA petri dish. The petri dishes were stored upside down for 24 days at 20º C in the dark and sealed with Parafilm© (SPI, West Chester, PA). After 3 days, the furthest extent of hyphal growth for each fungus was traced on the petri dish every 2 days. After the termination of the experiment on day 25, the total surface area for each fungus was recorded using a digital planimeter (Lasico Los Angeles, CA). The area of resource captured (cm^2) was recorded for each fungus.

Fungal Competition - Differential Resource competition – Primary resource capture.

Twenty plugs of *Rafaella* sp. and *A. xylebori* were placed onto a MEA petri dish at 5 varying proportions (0, .25, .5, .75, 1) on a 4 by 5 cm grid. The petri dishes were stored upside down for 24 days at 20 °C in the dark and sealed with Parafilm©. After 3 days (and every other day thereafter), the furthest extent of hyphal growth for each fungus was traced on the bottom of each petri dish. Each dish was monitored daily for fungus growth and any evidence of antibiosis. After the termination of the experiment on day 24, the total surface area for each fungus was recorded using a digital planimeter. The area captured by each fungus was recorded and analyzed as a function of each fungus' competitive ability (n=25) (Klepzig and Wilkens 1997). A deviation from linearity in the relationship between population size and inoculum proportion was taken to indicate differential competition (Wilson *et al* 1994).

Fungal Competition Studies - Secondary Resource Capture

Rafaella sp. and *A. xylebori* were pitted against each other in a secondary resource competition laboratory experiment. A single plug of each fungus was placed on in the center of a 9 cm plate of MEA (n=10). The dishes were stored upside down for the duration of the 24 day experiment at 20° C in the dark and sealed with Parafilm©. After 7 days, a plug of the competing fungus was placed at the leading edge of the original hyphal growth and in the center of the dish where the media was already colonized. The furthest extent of hyphal growth for each fungus was traced on the petri dish every 2 days. After the termination of the experiment on day 24, the total surface area for each fungus was recorded

using a digital planimeter. Area of resource capture was recorded as a function of direct competitive ability. The mean colony size at the beginning and end of the experiment for each fungus in each treatment was calculated and compared by the least-squares means procedure in ANOVA using SAS (SAS Institute 2003.)

RESULTS

Vectoring

The earlier the *Rafaella* fungus was introduced into media, the fewer ambrosia beetle offspring ultimately emerged (Table 4.1). The control treatment had the highest mean number of offspring emerging (35.667) while treatment '*Rafaella sp.* before Beetle' had the lowest (18.44). Fewer than 1% of all beetles sampled (n=467) incorporated the *Rafaella sp*. into their mycangium. Of the beetles sampled (n=467) 95, 99, 100, 100, and 99% incorporated *A. xylebori* into their mycangium in treatments '*Rafaella sp.* before Beetle', 'Simultaneous', '*Rafaella sp*. day 12', '*Rafaella sp*. day 24', '*No Rafaella sp*.' respectively. There was no significant incorporation of *Rafaella* sp. into mycangia by *X. crassiusculus*. Of beetles sampled in treatments 'Simultaneous' and '*Rafaella sp*. day 24' fewer than 1% incorporated *Rafaella sp*. into their mycangia vs. 0% in all other treatments. In contrast, 98, 98, 100, 100, and 0% of beetles in treatments '*Rafaella sp.* before Beetle', 'Simultaneous', '*Rafaella sp*. day 12', '*Rafaella sp*. day 24', '*No Rafaella sp*.' respectively, carried *Rafaella sp.* phoretically.

The percentage of beetles constructing galleries for treatments 'Beetle before *Rafaella sp*.', 'Simultaneous', '*Rafaella sp*. day 12', '*Rafaella sp*. day 24', '*No Rafaella sp*.' were 30, 30, 90, 100, and 100%, respectively (Table 4.1; Fig. 4.1). Treatments '*Rafaella sp.* before Beetle', 'Simultaneous', '*Rafaella sp*. day 12' demonstrated a significant difference ($Z<.0001$, $Z<.0001$, $Z=.0042$, respectively) from the control treatment '*No Rafaella sp*.' in the percentage of beetles constructing galleries (one sample hypothesis test of proportions for each comparison). Treatment '*Rafaella sp*. day 24' did not demonstrate a significant difference ($Z=1.00$), indicating a decreased likelihood of gallery construction with the earlier addition of *Rafaella* sp. into the tubes.

The percentage of beetle galleries overtaken by *Rafaella sp*. for treatments '*Rafaella sp.* before Beetle', 'Simultaneous', '*Rafaella sp*. day 12', '*Rafaella sp*. day 24', '*No Rafaella sp*.' were 10, 10, 70, 10, and 10% respectively (Fig. 4.2). Treatments '*Rafaella sp.* before Beetle', 'Simultaneous', '*Rafaella sp*. day 24' did not significantly differ ($Z>1.00$ for all) in the percentage of beetle galleries overtaken by *Rafaella sp*. from the control treatment (one sample hypothesis test of proportions). Treatment '*Rafaella sp*. day 12' was significantly higher ($Z<.0001$) from the control in the percentage of beetle galleries overtaken by *Rafaella sp*.

Fungal Competition - Spatial Separation – Primary resource capture.

Ambrosiella xylebori had a significantly higher ability to capture primary resource when spatially separated from *Rafaella* sp. ($p<.0001$). The mean areas colonized after 25 days for *A. xylebori* and *Rafaella sp*. were 44.45 and 14.80cm^2

respectively (Fig. 4.3). *A. xylebori* and *Rafaella sp.* first came into contact during day 11. At this time antibiosis was observed and the fungi continued to colonize free resources but avoided each other. This pattern was observed in the other experiments as well. *A. xylebori* and *Rafaella sp*. increased to their highest mean colony diameter by day 19. At this time mean colony growth rates were greatly slowing as unutilized resources were limited.

Fungal Competition - Differential Resource competition – Primary resource capture.

There was an indication of differential competition between *A. xylebori* and *Rafaella* sp. (Fig. 4.4, Table 4.2). *A. xylebori* had a slight, but significantly higher ability to capture resources in the differential resource competition than *Rafaella* sp. ($p<.0001$). The point at which the fungi had colonized equal areas was near the 65% *Rafaella* sp. inoculum proportion level.

Fungal Competition Studies - Secondary Resource Capture.

Neither fungus exhibited the ability to effectively secure areas of substrate already colonized by the other. Upon introduction onto utilized substrate, neither fungus exhibited significant resource acquisition as the competing fungus with a head start was able to grow around the introduced fungus in 6 days. The fungus being tested for secondary resource capture spread very little as the primary colonizer quickly surrounded it.

Table 4.1. Summary of *Rafaella sp*. fitness and vectoring-related capabilities of *X. crassiusculus* in a laboratory experiment. * indicates a contamination problem where the invading fungi overtaking the galleries was not *Rafaella* sp.

Treatment	Day Beetles Added	Day *Rafaella sp.* Added	% Constructing Galleries	Average # Offspring	Average # Emerging Adults	% Galleries Overtaken by *Rafaella sp.*	% emerging beetles with *A. xylebori.* Into mycangia	% Beetles with phoretic *Rafaella sp.* spores
Rafaella sp. before beetle	6	0	30	18	18.44	10	95.42	98.78
Simultaneous	0	0	30	31	35.38	10	99.16	98.68
Rafaella sp. added day 12	0	12	90	36	29.00	70*	100	100
*Rafaella sp.*added day 24	0	24	100	20	35.10	10	100	100
No *Rafaella sp.* (control)	0	NONE	100	35	35.67	10	99.35	0

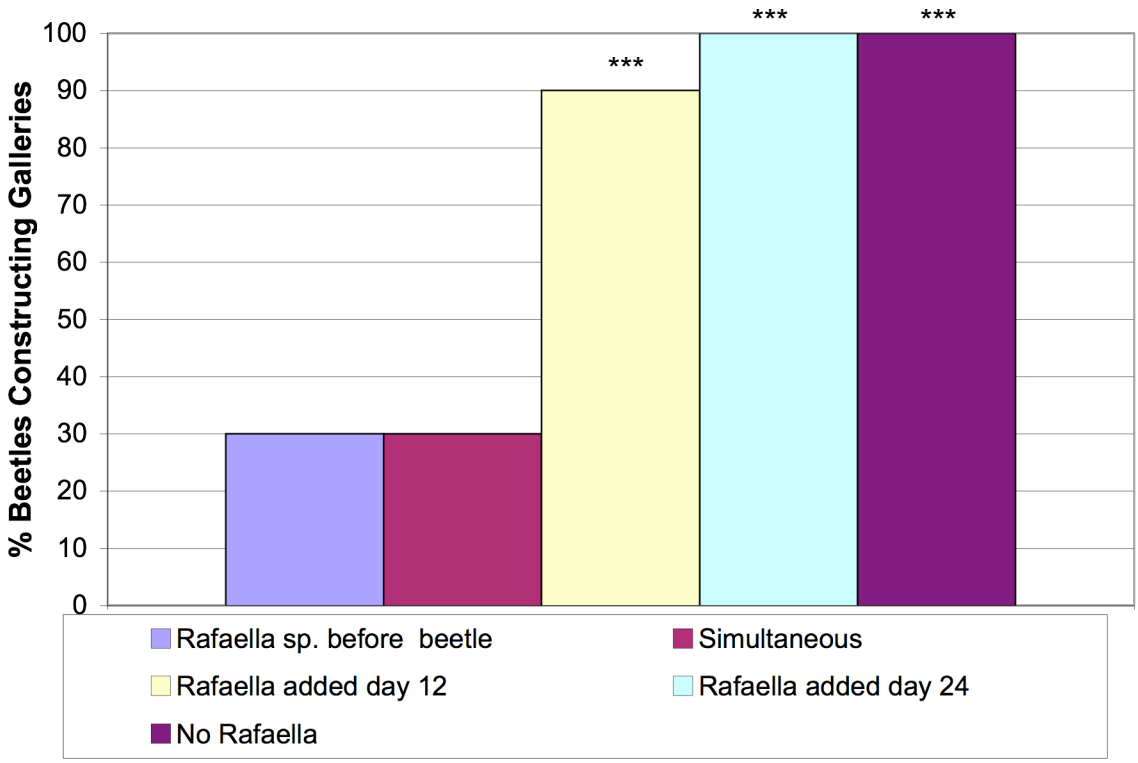

Fig. 4.1. Percentage of *X. crassiusculus* constructing galleries once introduced into artificial rearing tubes for the various treatments. *** indicates a significant ($p<0.05$) difference from the control treatment E (no *Rafaella sp*. added) tested via a one sample hypothesis test of proportions for each comparison.

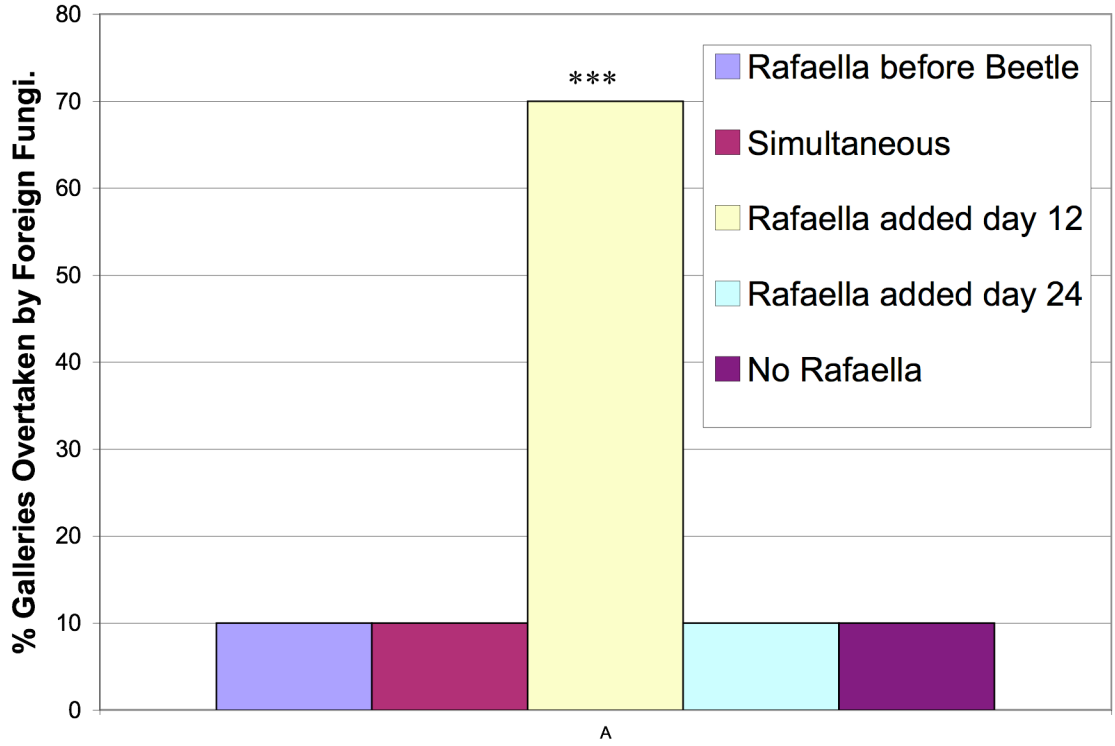

Fig. 4.2. Percentage of *X. crassiusculus* galleries by treatment, overtaken by *Rafaella sp*. *** indicates a significant ($p<0.05$) difference from the control treatment E (no *Rafaella sp*. added) tested via a one sample hypothesis test of proportions for each comparison.

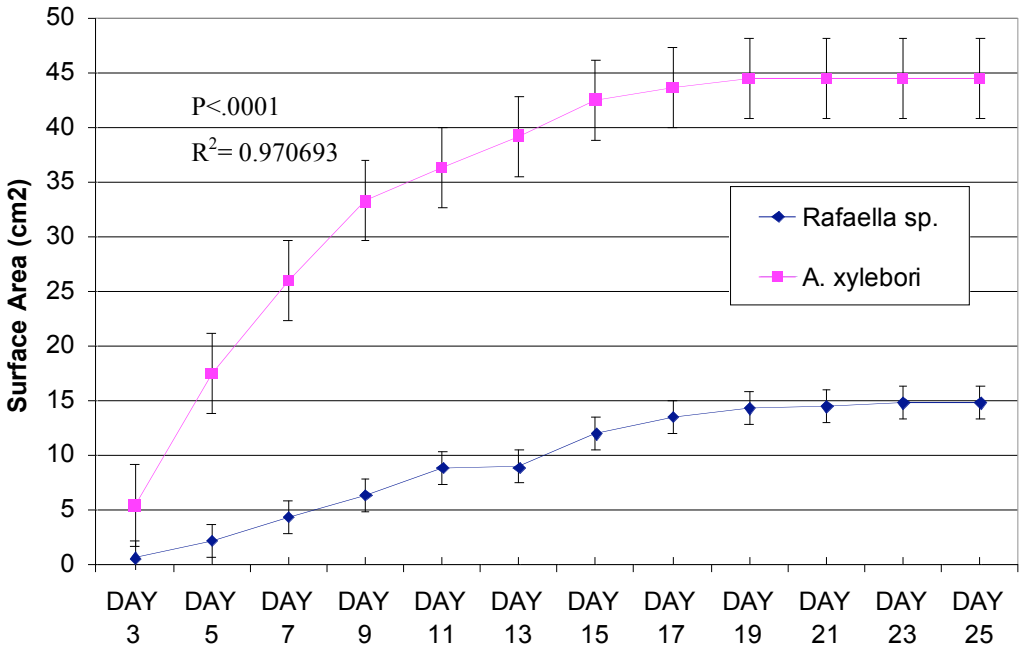

Fig. 4.3. Primary resource capture with spatial separation. Values are surface area (cm^2) occupied by each fungus after 25 days since initial inoculation. A single plug of each fungus was placed on each side of a Petri dish and growth recorded in a two-way competition between *A. xylebori* and *Rafaella sp*. A significant difference in resource capture was present (P<.0001).

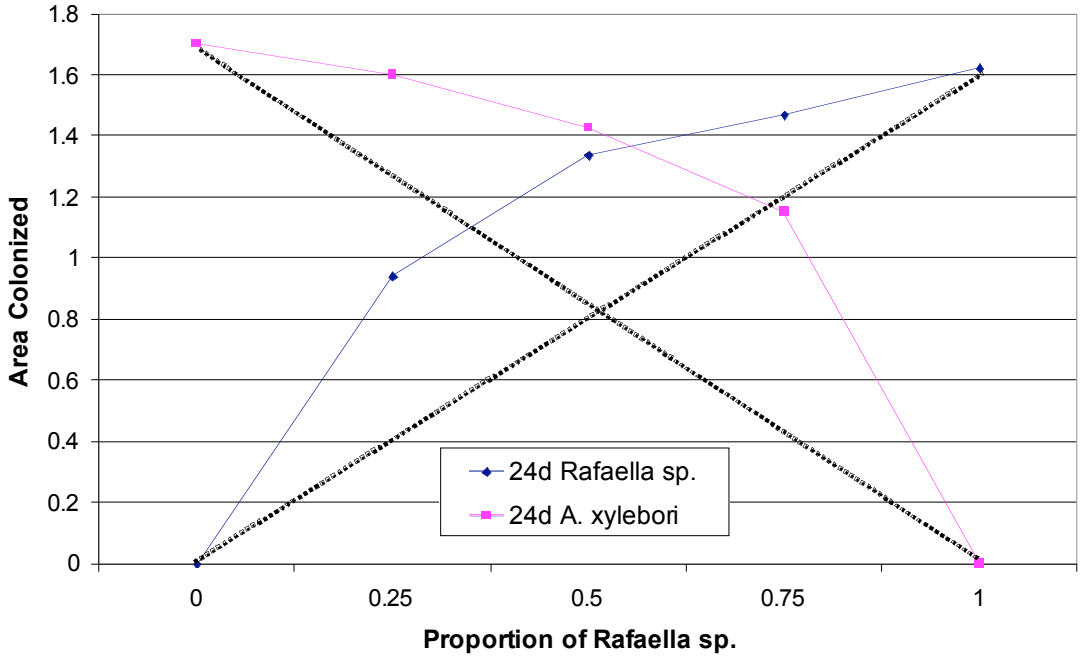

Fig. 4.4. Differential resource competition between *Rafaella sp.* and *A. xylebori*. Values are mean total areas colonized by each fungus versus proportion of *Rafaella sp.* 25 days after inoculation. A significant deviation from linearity indicated a significant difference in competitive ability (p<.0001).

Table 4.2. ANOVA results performed on adjusted areas [log(area occupied by fungus + 0.5) 2 log(initial inoculum proportion + 0.5)] to test for differential competition. A significant *P* value (P<.05) indicates significant differences in the adjusted means and a significant deviation from linearity.

Comparison and value group	Source	df	Sum of squares	Mean square	*F*	*P*
Rafaella sp. vs *A. xylebori*						
Area occupied by *Rafaella sp.*	Proportion	4	7.18	1.796	278.19	<.0001
	Residual	19	0.12	0.006		
Area occupied by *A. xylebori*	Proportion	4	8.27	2.068	134.22	<.0001
	Residual	19	0.29	0.015		

DISCUSSION

It has been previously shown that the competitive interactions between fungi within a gallery system can have implications to the biology of developing beetles (Klepzig and Wilkens 1997, Klepzig *et al.* 2001, Lombardero *et al*. 2003). Despite this, investigations into the competitive interactions between co-occurring, beetle-associated fungi are rare (Barras 1970, Klepzig and Wilkens 1997, Klepzig 1998). This is the first study, to my knowledge, that shows fungal competition affecting a beetle's fungal-transmission capabilities.

There is increasing world-wide movement of bark beetles (Haack 2001, Rabaglia 2004). Beetle-associated tree pathogen introductions likely will also increase, although hard data on these potentialities are lacking (Wingfield 2001). In this study we began exploring competition between two exotic fungi and their effects upon one exotic ambrosia beetle. We addressed relatively unstudied topics such as fungal competition affecting beetle fitness, mycangial intake and nutritional benefits of the *Rafaella sp*. to *X. crassiusculus*.

Ambrosia beetle galleries are subject to a succession of many fungi (Kajimura 1997) often making quantitative measurements difficult. With our laboratory based, closed system, introductions of fungi were easily manipulated and recorded. Through differential competition studies I was able to quantify the effects of the fungal competition on *X. crassiusculus* fitness.

I provide evidence that *Rafaella sp*. provides significantly less nutritional benefit to *X. crassiusculus* than its known primary mycangial symbiotic fungus *A. xylebori*. The farther in advance *Rafaella sp*. was introduced into media, the

fewer offspring ultimately emerged. The fungal competition experiments support the hypothesis that the two fungi are actually competing for spatial and/or nutritional resources, ultimately lowering the foundress' fitness. This hypothesis was further supported by *A. xylebori's* superior ability to secure and hold resources in the differential and spatial separation experiments. However, my study did not address related issues such as host responses to fungi, naturally invading fungi, or ambrosia beetle behavior affecting fungal growth, which could affect competitive outcomes.

My study demonstrated a decreasing likelihood of gallery construction with the earlier addition of *Rafaella* sp. into the tubes suggesting the beetles could detect the presence of *Rafaella* sp. in the rearing media. Raffa *et al*. (2004) found similar results in *Ips pini* when preinoculating pines with the fungal pathogen *Ophiostoma ips* (Rumbold). Our results indicate that *Rafaella sp*. is detrimental to *X. crassiusculus* fitness and therefore avoided by the beetle if possible. This result, although extremely important, needs to be considered in the context of the limitations of this study. My study did not take into account host tree and environmental influences on the fungal associates, which in the bark beetles can greatly influence fungal growth (Paine *et al.* 1997). However, the impacts of the tree defenses on ambrosia beetle fungi are relatively unknown. Further replication of the study conducted in the natural system would help account for these variables.

The significant increase of galleries overtaken in '*Rafaella sp*. day 12' was most likely a contamination problem. The contaminant fungus growth observed

after gallery initiation was extremely aggressive possessing different behavioral growth traits from *Rafaella sp.* The data is included to show the corresponding significant decrease in average number of emerging adults (Table 1) further suggesting the deleterious effects of fungal competition on the number of emerging *X. crassiusculus*.

There was no significant measured incorporation of *Rafaella sp*. into mycangia by *X. crassiusculus*. Once inside the mycangium, fungi are subject to selection (Schneider and Rudinsky 1969; Happ et al. 1971; Barras and Perry 1972). This further strengthens the argument that *Rafaella sp*. is not normally associated with *X. crassiusculus* and will not be vectored mycangially.

Phoretic transmission of the *Rafaella sp*. seems possible as all *Rafaella sp.* addition treatments had 98% or more individuals with *Rafaella sp*. isolated by plate rolling. This ability to phoretically carry fungi, does not mean that the beetle will transmit the fungi to other trees. Our experiment did not control for many factors that would greatly contribute to vectoring capabilities such as; environmental conditions, time outside galleries, and host tree defenses.

Rafaella sp. host range (limited to Lauraceae) is rather narrow when compared to the extremely broad host range of *X. crassiusculus*. Fungal host limitations, lack of mycangial intake and superior competitive abilities of *A. xylebori* may act as a filter, preventing continual association between *X. crassiusculus* and *Rafaella sp*. Further evidence suggesting *Rafaella sp*. is acting as an antagonist of *X. crassiusculus* is shown by *Rafaella sp*. limiting gallery initiation and emerging offspring. This negative interaction would tend to

serve as a destabilizing force (Poulsen *et al.* 2003), selecting against any extended association between the two organisms.

CHAPTER V - SUMMARY

This series of experiments was conducted to provide information necessary to detect, monitor, and manage several species of invasive ambrosia beetles. These experiments provided data on stand characteristics that affect beetle abundance, periods of ambrosia beetle flight activity, effects of host stress on production of chemicals that may attract beetles, and aspects of fungal transmission and competition that may affect beetle reproduction.

FOREST STAND CORRELATIONS

During a trapping survey, I determined flight periods and biodiversity indices from data collected on 37 species of ambrosia beetles. Over the course of this survey, 9,775 ambrosia beetle specimens were tallied using ethanol-baited Lindgren funnel traps. We caught two *Xyleborous viduus* a new species record for Louisiana. The three most prevalent species (and their total percentage of trap catch) were *X. crassisculus* (65%), *X. saxesini* (11%), and *X. ferrugineus* (9%). The prevalence of *X. crassisculus* over *X. saxesini,* has not been reported in other ambrosia beetle surveys throughout the southeast, suggesting Louisiana's ambrosia beetle species abundances are unique. Flight peaks for the majority of the ambrosia beetle species occurred between Apr. 26, and June 4, 2006. Correlations between four species of ambrosia beetles and forest stand characteristics revealed that total volume/ha is a useful stand characteristic in predicting abundance of the ambrosia beetles, *X. crassiusculus*, and *X. saxesini* on Camp Beauregard, LA.

CHEMICAL ECOLOGY

Our study of the chemical ecology of these ambrosia beetles yielded novel techniques and observations. We developed a new, extremely effective method of attracting ambrosia beetles by flooding potted trees. Using this technique we were also able to identify compounds being emitted at significantly higher levels in the flooded treatment: hexanal, benzaldehyde, cis-3-hexen-1-ol, and (*E*)-2-hexen-1-ol. Comparisons of differences in leaf and bole samples showed a statistical difference between α-pinene (higher in bole), camphene (higher in bole), 3-carene (higher in leaf), 4-allyl-anisole (higher in leaf) and (*Z*)-3-hexen-1-ol (higher in leaf). GC-EAD analysis of *X. crassiuculus* using volatile samples of attractive hosts and southern pine beetle-associated semiochemicals revealed antennal responses to 41 compounds. Field testing seven of these compounds in combination with ethanol revealed no improvement in attractiveness over ethanol alone.

FUNGAL INTERACTIONS

We provide evidence that *Rafaella sp*. does not provide significant nutritional benefits to *X. crassiusculus.* The farther in advance of beetle introduction *Rafaella sp*. was inoculated into artificial medium, the fewer offspring ultimately emerged. Similarly, *A. xylebori* demonstrated superior ability to secure and hold resources in differential competition and spatial separation colonization experiments. Our study demonstrated a decreased likelihood of gallery construction with earlier addition of *Rafaella* sp. into tubes. This suggests that beetles can detect the presence of *Rafaella* sp. in rearing media. These three

experiments support the hypothesis that the two fungi are actually competing for spatial and/or nutritional resources, ultimately lowering the foundress fitness. There was no significant incorporation of *Rafaella sp*. into mycangia by *X. crassiusculus*.

Faced with todays highly connected world trade, future ambrosia beetle introductions seem highly probable. This research uncovers a complex host recognition system, offering the possiblity of novel (and improved) monitoring bait development. Recording baseline diversity and forest stand preference data may also allow resource managers to more effectively predict effects of current and future ambrosia beetle introductions. Also of importance to resource managers is the discovery of flight periods for these beetles, allowing the potential for accurate timing of appropriate treatments. Finally, my work also used novel laboratory assays to quantify phoretic and mycangial transmission capabilites of an ambrosia beetle and a newly introduced wilt fungus.

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VITA

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In the fall of 2004, Eric enrolled in the Louisiana State University Department of Entomology to pursue a master of science degree. He received a Research Assistantship through the United States Department of Agriculture Forest Service and Louisiana National Guard to work through his education. He completed requirements and graduated with a master of science degree in December 2007.