ECOLOGY AND BIOLOGY OF REDBAY AMBROSIA BEETLE (*Xyleborus glabratus* EICHHOFF)

By

GURPREET SINGH BRAR

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To my Mom and Dad

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LIST OF ABBREVIATIONS

AC	Acres
ACMF	Austin Cary Memorial Forest
APHIS	Animal and Plant Health Inspection Service
DD	Degree Days
DST	Daylight saving time
H	Hour
HCWMA	Hatchet Creek Wildlife Management Area
NASS	National Agricultural Statistics Service
OSBS	Ordway-Swisher Biological Station
SAS	Statistical Analysis System
USDA	United States Department of Agriculture
Wk	Week

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Gurpreet Singh Brar

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The redbay ambrosia beetle, *Xyleborus glabratus* Eichhoff (Coleoptera: Curculionidae: Scolytinae), is a non-native species transmits the fungus *Raffaelea lauricola* that causes laurel wilt disease in trees of the family Lauraceae. The life cycle and development of *X. glabratus* were studied in logs of three hosts that it colonizes in North America: avocado (*Persea americana*), redbay (*Persea borbonia*) and swampbay (*Persea palustris*) at 25 ± 2°C. Similar developmental patterns were observed in the three hosts. Teneral adults were first encountered on the 31st, 30th, and 26th day after gallery initiation in these hosts, respectively. The life cycle appears to be overlapping. Three larval instars were observed in all three hosts. *Xyleborus glabratus* was successfully reared on soaked swampbay logs and about 2.8 times as many female adults emerged from each log than were inoculated, with emergence continuing for about 240 days and maximum emergence taking place between 120-150 days after gallery initiation. *Xyleborus glabratus* successfully completed its life cycle at 24, 28, 32°C when development and life cycle were studied at temperatures ranging from 12-36°C in avocado logs. Development of egg and pupal stages of *X. glabratus* were studied at temperatures between 12-36°C. Developmental rates of the egg and pupal

stages increased in linear fashion over the range of 16-28°C. Estimates for the lower developmental threshold for egg and pupal stages were estimated to be 10.9 ± 0.5°C and 11.3 ± 0.6°C and the degree-days (DD) for development were 55.3 ± 3.3 DD and 69 ± 4.5 DD respectively. The optimal temperature for life cycle and development of egg and pupal stages was around 28°C. Daylight flight rhythm studies showed that *X. glabratus* flies mostly between 1600 and 1800 h daylight saving time. In a trapping study to determine flight behavior, the largest number of beetles was trapped at heights of 35-100 cm above the ground. Seasonality of *X. glabratus* in north Florida studied from Mar 2010-Dec 2011 showed three peaks of trap catches occurred during Apr 2010, Oct 2010 and Mar 2011. Funnel traps with 8, 12, 16 funnels per trap captured similar numbers of *X. glabratus*, but significantly more than with 4 funnels per trap. New manuka lures trapped significantly more *X. glabratus* than lures aged 2, 4 and 6 wk.

CHAPTER 1
INTRODUCTION

Laurel wilt has caused high mortality of redbay (*Persea borbonia* (L.) Spreng.) in South Carolina, Georgia and Florida (Fraedrich et al. 2008). It is a vascular wilt disease caused by the fungus *Raffaelea lauricola*. Redbay ambrosia beetle (*Xyleborus glabratus* Eichhoff) (Coleoptera: Curculionidae: Scolytinae) is a non-native ambrosia beetle that acts as a vector of this pathogen (Fraedrich et al. 2008, Hanula et al. 2008). *Xyleborus glabratus* was first discovered in the US at 2002 at Port Wentworth near Savannah, Georgia, USA (Rabaglia et al. 2006) mostly infesting redbay *Persea borbonia* (Koch and Smith 2008). *Raffaelea lauricola* has also been reported to be pathogenic to avocado (*Persea americana* Mill), swampbay (*Persea palustris* (Raf.) Sarg.), sassafras (*Sassafras albidum* (Nutt.) Nees), pondspice (*Litsea aestivalis* (L.) Fernald), pondberry (*Lindera melissifolia* (Walter) Blume,), and camphor (*Cinnamomum camphora* (L.) J. Presl), *Umbellularia californica* (Hook. & Arn.) Nutt., (Fraedrich et al. 2008, Smith et al. 2009a, Smith et al. 2009b, Mayfield et al. 2008a. Therefore, *X. glabratus* and *R. lauricola* have become a major problem for the avocado industry and other trees of the family Lauraceae (Crane et al. 2008, Mayfield et al. 2008c, Ploetz and Peña 2007). The avocado industry is the second largest fruit industry in Florida after citrus, with a total acreage of 7,500 acres, with 98 percent in Miami-Dade County. There are also an estimated 250,000 backyard avocado trees in south Florida. If this vector – disease complex establishes in south Florida then the cost of replacement of avocado trees in Miami-Dade, Broward, Palm Beach, and Lee Counties will reach a cost of $ 429 million (Crane et al. 2007, Evans and Crane 2008).

Xyleborus glabratus is not a major pest in its native countries (India, Japan, Myanmar and Taiwan). Therefore, very limited research has been done to study the biology, behavior and the disease vector relationships. Before initiating the behavioral and ecological studies of *X. glabratus,* it is necessary to acquire basic knowledge of its life cycle and development. This should be followed with studies on population dynamics under different temperature regimes and in natural areas containing hosts of the beetle. These three basic elements can be the foundation to develop an effective pest management program. The overall goals of my research were: 1) to develop a rearing method for *X. glabratus* under controlled conditions; 2) to study its life cycle and development under different temperature conditions (12-36°C); 3) to study the population dynamics of *X. glabratus* in the natural areas containing hosts of the beetle using different types of traps.

My central hypothesis was that depending on the temperature regime, *X. glabratus* dwelling in a single gallery system initiated by a female parent develops within 40-60 days, and adults emerge continuously for 20-30 days thereafter. An additional hypothesis was that *X. glabratus* would be active throughout the entire year in Florida, with highest population peaks occurring in the summer months. To test my hypotheses my objectives were:

Objective 1- To study the life cycle and development of *X. glabratus* in logs of different host species under controlled conditions.

Objective 2- To study the temperature-dependent development of *X. glabratus* at constant temperatures.

Objective 3- To study the population dynamics of *X. glabratus* in natural areas with host trees.

CHAPTER 2
LITERATURE REVIEW

Bark Beetles

Bark beetles (Coleoptera, Curculionidae) complete their life cycle inside the wood of a host tree. True bark beetles (many Scolytinae) and ambrosia beetles (all Platypodinae, and many Scolytinae) are. True bark beetles bore inside the host tree, and complete their life cycle by feeding on the nutritive tissues of the phloem (phloeophagous), whereas ambrosia beetles complete their life cycle in the wood by feeding on fungal gardens of ambrosia fungi cultivated by the beetle inside the gallery (xylemycetophagus) (Knizek and Beaver 2004).

The bark beetle life cycle can be divided into three phases: reproduction, development, and maturation and dispersal. The reproductive cycle starts when mature insects arrive on the host tree. After boring inside the host tree they form tunnels in which they oviposit. Different kinds of mating systems are found in bark beetles, including tachygamy, brachygamy, monogamy, inbreeding polygyny, harem polygyny and colonial. In the tribe Xyleborini, the inbreeding polygyny is characterized by. Males are flightless and short lived, and are smaller than the females. The sex ratio is biased and the ratio of males to females is variable. This tribe is characterized by haplodiploidy in their life cycle. For instance, the male: female sex ratio of *X. affinis* is 1: 8.5 (Kirkendall 1983) and *X. ferrugineus* are characterized by a 1: 30 male:female ratio (Norris and Chu 1985) (Sauvard 2004).

Larval developmental behavior varies between true bark beetles and ambrosia beetles. True bark beetles (phloeophagous species) larvae feed on the phloem so they form individual larval galleries radiating out from the main gallery formed by the adult.

Larval galleries may be more or less perpendicular to the main gallery. The pattern of gallery formation is species specific larval molting and pupation takes place in the larval tunnels. In the case of ambrosia beetles (xylemycetophagus species), the larvae remain in the galleries formed usually by the female adult, and feed on the fungus inoculated and gardened by the female adults. Larvae molt and pupate in the maternal galleries (Sauvard 2004).

A teneral adult needs a period for maturation and sclerotization. During maturation, true bark beetles adults feed on the remaining phloem whereas ambrosia beetle adults feed on the symbiotic fungi. After maturation, bark beetles emerge and search for suitable hosts. Sometimes there are two flights following emergence, but the number depends on finding the appropriate host to initiate reproduction (Sauvard 2004).

The association of bark beetles with the host tree and fungus can be classified into primary, secondary and saprophytic (Paine et al. 1997). Primary bark beetles (*Dendroctonus frontalis, D. vitei, D. mexicanus, D. adjunctus, D. ponderosae and Ips typographus*) attack healthy and vigorous trees followed by mass colonization. Due to formation of galleries and feeding, the phloem and xylem vessels are continuously cut and sometimes infected with blue stain fungi, which leads to the starvation of the tree for water and nutrients, and leading to wilt-like symptoms and death.

Other types of primary bark beetles are non-aggregating beetles (*D. micans* and *D. valens*) that rarely kill the tree. They usually bore into the diseased or wounded part of the trees. Boring caused by these beetles can weaken the tree, predisposing it to the attack of other pests. In the case of secondary beetles (*Ips pini* and *Scolytus ventralis*) they colonize the trees already infested by primary beetles. They also colonize fallen or

decaying trees (Paine et al. 1997). These beetles rarely kill the tree, and so are saprophytic. Based on its hosts in the USA, *Xyleborus. glabratus*, can be categorized as a primary beetle because it attacks healthy redbay trees and kills them by vectoring *R. lauricola* (Paine et al. 1997).

Symbiotic Relationships between Bark Beetles and Fungi.

True bark beetles usually feed on phloem tissues, which are comparatively richer in carbohydrates and proteins than the xylem tissue. Bark beetles that feed on phloem tissues deficient in nitrogen can compensate by feeding on large amounts of tissue, as is done by *Ips grandicollis* (Eichhoff), but another strategy is to cultivate mutualistic fungi in the tissues surrounding brood galleries, which helps nitrogen concentration. The growing larvae feed on this phloem fungal complex. *Dendroctonus frontalis* larvae feed on *Ceratocystiopsis ranaculosus* and *Entomocorticium* sp. These are mycangial fungi which grow in the phloem tissues near the brood chamber (Ayers et al. 2000). True bark beetles are also commonly associated with blue stain fungi. Blue stain fungi belong to the ascomycetes genera *Ophiostoma* and *Ceratocystis* and their anamorph (Paine et al. 1997).

In contrast, ambrosia beetles complete their life cycle in the wood (xylem tissue of the host tree). These tissues are rich in lignin, cellulose and hemicelluloses and poor in other nutrients. By differentially feeding on the xylem and phloem, ambrosia and true bark beetles have reduced competition. To cope with the poor nutritional status of the xylem substrate, the ambrosia beetles have obligate ectosymbiosis with an ambrosia fungus. Over time, fungus gardening has evolved seven times in the tribes Xyleborini, Platypodinae, Corthylini, Xyloterini, Scolytoplatypodini and Hyorhynchini (Farrell et al. 2001). This ambrosia fungus concentrates nutrients necessary for survival and

development of the beetle different life stages. Once inoculated, the ambrosia fungus grows in all directions. Part of the nitrogen excreted by the beetle is re-utilized for fungal growth, which in turn is utilized by beetles.

In the ambrosia beetle fungus obligate symbiosis, the insect associate depends primarily on fungus for nutrition. Usually it is a complex of fungi and bacteria with which insect has a mutualistic relationship. For instance, *X. ferrugineus* has three different mutualistic fungi in its oral mycangia (*Fusarium solani*, *Cephalosporium* spp. and *Graphium* spp.), which are inoculated in the xylem tissue of the host plant where it provides symbiotic support (nutritional and developmental) either in combination or as a single component (Baker and Norris 1968). In ambrosia beetle and fungus obligate symbiosis, each species has its own specific fungus. For instance, *Ambrosiella beaveri* spp. nov symbiont occurs in *Xylosandrus mutilatus* (Six et al. 2009). *Dryadomyces amasae* is a symbiont of *Amasa concitatus* and *Amasa aff glaber* (Gebhardt et al. 2005). *Fusarium solani* is the symbiont of *Hypothenemus hampei* (Morales-Ramos et al. 2000). *Raffaelea montetyi* is associated with *Xyleborus monographus* and *Xyleborus dryographus* (Gebhardt et al. 2004). When *X. ferrugineus* was reared to the adult stage on a sterilized artificial meridic diet, adults were not able to reproduce in the absence of mutualistic fungi (*Fusarium solani*) but reproduced when *Fusarium solani* was inoculated (Norris and Baker 1967). However, in the absence of mutualistic fungi, a second generation of beetles was able to pupate when ergosterol was added (Norris et al. 1969). Ergosterol and 7- dehrocholestrol were the sources of sterol that beetles obtain from the fungal symbiont (Kok et al. 1970). Ergosterol was present in the infected coffee beans with *Fusarium solani* and it increased fecundity and survival on

Hypothenemus hampei (Morales – Ramos et al. 2000). Feeding on the ambrosial fungus *Ambrosiella hartiggi* was required for oviposition and larval nutrition of the post diapause females of *Xyleborus dispar* (French and Roeper 1972). Similar association is also found in true bark beetles. Larvae of *Dendroctonus ponderosae* Hopkins and *Dendroctonus rufipennis* Kirbyo obtain ergosterol from its fungal associate *Ophiostoma montium* (Rumfold) von Arx and *Ophiostoma clavigerum* (Robinson-Jeffrey and Davidson) while mining the galleries (Bentz and six 2006).

Bacteria also have symbiotic relationships with ambrosia beetles. Oocytes in the ovarioles of virgin and mated female *X. ferruginesis* are activated by transovarially transmitted bacterial symbionts of the genus *Staphylococcus* (Peleg and Norris 1973, Peleg and Norris 1972). The main benefit provided to fungi by these beetles is transportation, during which it proliferates in the mycangia. The mycangia are invaginated structures of the integument lined with secretory or gland cells that are specialized for the transport and acquisition of fungus (Six 2003).

Ambrosia fungi are pleomorphic, thermophilic, extremely sensitive to drought and lose viability in a short period of time. These fungi, which can proliferate within the mycangia, are inoculated in new galleries during excavation of new tunnels before oviposition by the adult beetles (Batra 1966, Six 2003).

Rearing of *Xyleborus* spp. in Artificial and Semi artificial Media.

Females of *X. ferrugineus* Fabricius were reared through the entire life cycle along with their symbiotic fungus in a medium that contained yeast extract, sucrose, casein, starch, wheat germ, cottonseed oil, salt mixture, agar, cocoa sawdust and cellulose (Saunders and Knoke 1967). However, *X. ferrugineus*, in the absence of mutualistic fungi, was not able to pupate during its second generation, but pupated when ergosterol

or wet fungus was present in the diet (Norris et al. 1969). Aposymbiotic *X. ferrugineus* was reared for three consecutive generations in a holidic diet which contained sucrose, amino acids, inorganic salts, ergosterol, streptomycin, sorbic acid, methyl linolenate, cellulose powder, fibrous cellulose, agar, 95% ethanol, vitamin solution and water (Norris and Chu 1970). Ergosterol and 7- dehydrocholesterol, when used as source of sterol to culture aposymbiotic *X. ferrugineus*, produced several generations of normal adults (Chu et al. 1970).

Similarly, several generations of *X. fornicatus* Eichh were reared on an artificial diet consisting of sucrose, casein, yeast extract, tea bark extract and cellulose powder (Sivapalan and Shivanandarajah 1977). *Xyleborus pfeili* was successfully reared on a semi-artificial diet containing douglas fir sawdust, dried yeast, starch, granulated sugar and water (Mizuno and Kajimura 2002, Mizuno and Kajimura 2009). *Xyleborus affinis, Xylosandrus germanus* and *Xyleborinus saxesenii* were successfully reared for several generations by using a modified medium containing salt mixture, casein, agar, beech tree sawdust, sucrose, peanut oil (Biedermann et al. 2009). Various *Xyleborus* spp. have been reared on media that can support the growth of mutualistic fungi and help initiate boring, gallery construction and oviposition. There has also been some success in rearing *Xyleborus* spp by adding ergosterol as a sole source of sterols.

Life History Studies of *Xyleborus ferrugineus*

Duration of the life cycle of males ranged from 363 – 403 h (15.3 – 16.8 days) and that of females was 360 - 390 h (15 – 16.25 days), with a developmental time for the male and female embryos of 103.5 – 109 h (4.3 – 4.5 days) and 96 - 102 h (4 – 4.25), respectively (Beeman and Norris, 1977). The larval period ranged between 168 – 192 h (3.8 – 4.25 days) and the pupal stage between 92 - 102 h (3.8 – 4.25 days) (Norris and

Chu 1985). There were no differences in the development of male and female larva and pupa, with the pupal stage lasting 4.6 ± 0.6 days, and pupation occurring after 8.1 ± 1.2 days post-eclosion (Kingslover and Norris 1977). Based on head capsule width, there were three larval instars present for both male and female larvae. The mating system of this species is inbreeding polygyny and the ratio of males to females is reported to be small and variable: 1: 35 (Saunders and Knoke 1967) and 1: 30 (Norris and Chu 1985). Female adults create a continuous population in a single gallery system. For instance, eggs, larvae, pupae and adults were observed after at 7, 14, 17 and 22 days respectively, following introduction of the female. After 60 days, the progeny were 96.7 % adults, 1.5% pupae, and 1.8% larvae (Saunders and Knoke 1967).

Life Cycle of *Xyleborus* spp

Review of the studies on the life cycle and development of *Xyleborus* spp. and *Xylosandrus* spp. suggest that there are three larval instars in their life cycle and that the developmental time is species specific, with specific male to female ratios, but in which males are always distinct and rare. Three larval instars were observed in *Xyleborus fornicatus* Eichoff (*Euwallacea fornicatus*), and the larval period averaged 12.4 days (Gadd 1947). The development time (at 25°C) for *X. fornicatus* eggs was 7.3 days and for pupae was 7.5 days (Walgma and Zalucki 2006). In *Xylosandrus germanus*, the total development time from egg to adult averaged 24.9 days, and for larvae and pupae 11.9 and 7.0 days, respectively, with three larval instars during larval development (Weber and McPherson 1983). The pattern of gallery formation is also species specific, with the main entrance tunnel, brood chamber and branch tunnels present in the galleries of *Xylosandrus germanus,* whereas in the galleries of *Xyleborus pfeli* the brood chamber is absent and the main gallery is characterized by branch

tunnels and sometimes with side tunnels. A similar pattern of the main and side galleries structure is present in *Xylosandrus mutilatus* (Kajimura and Hijii 1994).

Field Ecological Studies

For monitoring beetle populations, different types of commercially available traps and synthetic attractants have been used. The basic concept for trap design is that it could trap the maximum number of target insects, and beetles should be kept trapped until being monitored. Synthetic attractants should attract target insects with maximum specificity. Lindgren multi-funnel traps baited with synthetic attractants are one of the most widely used traps for monitoring bark beetles and ambrosia beetles. These traps are used in the Cooperative Agricultural Pest Survey (CAPS) and the Early Detection and Rapid Response program (EDRR) for detecting exotic species (USDA APHIS 2007; Rabagalia et al. 2008). Funnel traps (Lindgren 12 unit) and modified panel traps caught same number of wood boring insects in the Black Hills of South Dakota (Costello et al. 2008). *Xyleborus affinis* Eichhoff were trapped more in slot traps as compared with multi-funnel, ESALQ – 84 and drain pipe traps in Brazil (Fletchtmann et al. 2000). Sticky screen traps (10 × 0.5 m) caught more adults of *Platypus quercivorus* as compared with smaller sticky screen traps (Igeta et al. 2004). *Xyleborus* spp. were caught more frequently in 16 unit funnel traps as compared with 8 unit funnel traps in Olustee, Florida (Miller and Crowe 2009) Hanula and Sullivan (2008) found that manuka oil and phoebe oil are the best attractive baits for trapping *Xyleborus glabratu*s.

Redbay Ambrosia Beetle *(Xyleborus glabratus)*

Xyleborus glabratus (Coleoptera: Curculionidae: Scolytinae : Xyleborini) is a non-native ambrosia beetle that was introduced in United states, possibly in solid wooden packing material. It has been reported in India, Japan, Myanmar, and Taiwan (Haack

2003). Females are 2.1-2.4 mm in length, three times as long as wide and dark brown to black in color. Males are smaller and rare, 1.8 mm in length, and 2.5 times as long as wide (Rabagalia et al. 2006). *Xyleborus glabratus* is the twelfth species of non-native ambrosia beetle that has established in the United States since 1990 (Haack 2003). So far, it has been recorded in South Carolina, North Carolina Georgia, Florida, and Mississippi. In its area of origin, host plants are in the families Lauraceae (*Lindera latifolia* Hook. f., *Litsaea elongata* (Nees) Benth. Et Hook. f. and *Phoebe lanceolata* (Wall. ex Nees) Nees); Dipterocarpaceae (*Shorea robusta* C. F. Gaertn), Fagaceae (*Lithocarpus edulis* (Makino) Nakai), and Fabaceae (*Leucaena glauca* (L.) Benth.) (Rabaglia et al. 2006). In the USA, it has been found to attack only plants in the family Lauraceae.

Xyleborus glabratus actively carries in its mycangia *R. lauricola*, *R. arxii*, and four new fungal species: *R. subalba*, *R. ellipticospora*, *R. fusca* and *R. subfusca.* *Raffaelea lauricola* has been found to cause laurel wilt in the plants of the family Lauraceae (Harrington et al. 2008, Harrington et al. 2010) in the US, mortality up to 90% has been recorded in redbay, and the disease also has been found infecting yard and experimental avocado trees. This vascular wilt disease leads to the death of the entire host plant. Laurel wilt has been reported in Florida, Georgia, Mississippi, North Carolina, Alabama, and South Carolina. So far, laurel wilt has caused mortality to redbay (*Persea borbonia* (L.) Spreng.), avocado (*Persea americana* Mill), swamp bay (*Persea palustris* (Raf.) Sarg.), sassafras (*Sassafras albidum* (Nutt.) Nees), pondspice (*Litsea aestivalis* (L.) Fernald), pondberry (*Lindera melissifolia* (Walter) Blume,), and camphor (*Cinnamomum camphora* (L.) J. Presl) (Fraedrich et al. 2008, Smith et al. 2009a, Smith

et al. 2009b, Mayfield et al. 2008a). Limited control of *X. glabratus* has been observed using contact and systemic insecticides. (Pena et al. 2011) Similarly microinfusion of fungicides in the host tree give short term control of the pathogenic fungus *Raffaelea lauricola* (Mayfield et al. 2008c, Ploetz et al. 2011b)

Ecological and Economic Impact of the Disease

Redbay is important to wildlife because its fruit, seed and/or foliage are eaten by several species of songbirds, wild turkeys, quail, deer, and black bear (Brendemuehl 1990). Larvae of the Palamedes swallowtail (*Papilio palamedes* (Drury) feed on *Persea* spp. *X. glabratus* will affect their ecology negatively by causing mortality of this tree species. Moreover, laurel wilt has become an imminent threat to the avocado industry in south Florida.

Avocado

Avocado (*Persea americana* Miller) belongs to family Lauraceae. It is one of the important fruit crops of tropical America. Commercially, it is grown in Brazil, Chile, Dominican Republic, Australia, Israel, Mexico, tropical Africa, Spain, and Indonesia and in other countries with tropical and subtropical climates. In the USA, avocados are grown commercially in California, Florida, Puerto Rico, Hawaii and Texas. There are three groups of avocado varieties: West Indian, Guatemalan and Mexican (Crane et al. 2007). The Florida avocado industry ranks as the second largest fruit industry after citrus, with an estimated value of $30 million at the wholesale level (Evans and Crane 2008) and $12 to 14 million a year at the farm gate (USDA/NASS 2008).

CHAPTER 3
LIFE CYCLE, DEVELOPMENT, AND CULTURE OF *XYLEBORUS GLABRATUS* (COLEOPTERA: CURCULIONIDAE: SCOLYTINAE)

The redbay ambrosia beetle, *Xyleborus glabratus* Eichhoff (Coleoptera: Curculionidae: Scolytinae) transmits the fungus *Raffaelea lauricola* that causes laurel wilt in trees of the family Lauraceae. The life cycle and development of *X. glabratus* were studied in logs of three natural hosts: avocado (*Persea americana*), redbay (*Persea borbonia*) and swampbay (*Persea palustris*) at 25±2° C. Similar developmental patterns were observed in the three hosts. Eggs were first encountered on the 7th, 11th, and 10th day after gallery initiation and the larval stage was first observed on the 14th, 20th and 14th day after gallery initiation in avocado, redbay and swampbay, respectively. Pupae were first encountered on the 24th, 26th, and 26th day and teneral adults on 31st, 30th, and 26th day after gallery initiation in the same hosts, respectively. The adult females excavate galleries perpendicular to the tree trunk; galleries are characterized by a main tunnel, branching into secondary tunnels that in turn branch into tertiary tunnels. The life cycle appears to be overlapping. All developmental stages can be observed in the gallery one month after gallery initiation by a beetle, and continuously thereafter. Three larval instars were observed in all three hosts, with head capsule widths of 0.20-0.22, 0.25-0.27, 0.35-0.40 mm, respectively, for instars 1-3. *Xyleborus glabratus* was successfully reared on soaked swampbay logs and about 2.8 times as many female adults emerged from each log than were inoculated, with emergence continuing for about 240 days and maximum emergence taking place between 120-150 days after gallery initiation.

Background

The redbay ambrosia beetle *Xyleborus glabratus* Eichoff (Coleoptera: Curculionidae: Scolytinae) is an Asian species recently introduced into North America. It was first discovered at Port Wentworth near Savannah, Georgia in 2002. It is a minute beetle with females 2.1-2.4 mm in length, three times as long as wide and dark brown to black in color. Males are smaller, rare, and flightless, 1.8 mm in length, and 2.5 times as long as wide (Rabagalia et al. 2006). This insect was likely introduced to the United States in solid wood packing material (though this is an unproven hypothesis), and is considered as the twelfth species of non-native ambrosia beetle that has established in the United States since 1990 (Haack 2003). So far, it has been reported in North and South Carolina, Georgia, Florida, Alabama and Mississippi. The beetle is native to India, Japan, Myanmar, and Taiwan (Haack 2003). In its area of origin, the beetle is probably a generalist, since it has been recorded from a variety of plant families: Lauraceae (*Lindera latifolia* Hook. f., *Litsaea elongata* (Nees) Benth. Et Hook. f. and *Phoebe lanceolata* (Wall. ex Nees) Nees); Dipterocarpaceae (*Shorea robusta* C. F. Gaertn); Fagaceae (*Lithocarpus edulis* (Makino) Nakai); and Fabaceae (*Leucaena glauca* (L.) Benth.) (Rabaglia et al. 2006).

Xyleborus glabratus acts as a vector of the fungus *Raffaelea lauricola* that causes the disease laurel wilt (Fraedrich et al. 2008, Hanula et al. 2008). *Raffaelea lauricola* has been recovered in the USA from redbay (*Persea borbonia* (L.) Spreng.), avocado (*Persea americana* Mill), swampbay (*Persea palustris* (Raf.) Sarg.), sassafras (*Sassafras albidum* (Nutt.) Nees), pondspice (*Litsea aestivalis* (L.) Fernald), pondberry (*Lindera melissifolia* (Walter) Blume,), and camphor (*Cinnamomum camphora* (L.) J. Presl) (Fraedrich et al. 2008, Smith et al. 2009a, Smith et al. 2009b, Mayfield et al.

2008a). Laurel wilt disease is associated with high mortality of redbay trees and swampbay trees in the southeastern United States and is responsible for the death of backyard, commercial and experimental avocados in Florida (Mayfield et al. 2008). Consequently, the beetle and its fungal complex are considered a threat to the commercial avocado production in Florida (Crane et al. 2008, Ploetz and Peña 2007, Ploetz et al. 2011).

The ambrosia beetle-fungus obligate symbiosis is characterized by the nutritional dependence of the insect associate on the fungus and/or a mutualistic relationship between the beetle and a complex of fungi and bacteria. In the first case, each ambrosia beetle species has its own specific fungus. For instance, *Ambrosiella beaveri* sp. nov. symbiont occurs in *Xylosandrus mutilatus* (Six et al. 2009). *Dryadomyces amasae* is a symbiont of *Amasa concitatus* and *Amasa aff. glaber* (Gebhardt et al. 2005). *Raffaelea montetyi* is associated with *Xyleborus monographus* and *Xyleborus dryographus* (Gebhardt et al. 2004). An example of second mutualistic relationship is shown by *Xyleborus ferrugineus* with three different mutualistic fungi in its oral mycangia (*Fusarium solani, Cephalosporium* spp. and *Graphium* spp.), which are inoculated in the xylem tissue of the host plant where they provide (nutritional and developmental support either in combination or alone (Baker and Norris 1968). *Xyleborus glabratus* actively carries *R. lauricola* in its paired mycangia near the mandibles, along with other fungal species (i.e., *R. arxii*, *R. subalba*, *R. ellipticospora*, *R. fusca* and *R. subfusca*) (Harrington et al. 2008, Harrington et al. 2010). However, so far it has only been assumed, not tested, that these fungal species serve as actual symbionts and food for the larvae of this species.

Xyleborus glabratus has a different pattern of host selection and flight behavior in North America, where it was recently introduced, than other ambrosia beetles. The beetle attacks healthy trees of family Lauraceae (other species colonize only dead or moribund trees), is not attracted to ethanol (Hanula et al. 2008) (most other ambrosia beetles are), and is attracted to host sesquiterpenes ((Hanula and Sullivan 2008; Kendra et al. 2011, Kendra et al. 2012b) and it has a unimodal flight peak between 1600 – 1900 hr (Brar et al. 2012, Kendra et al. 2012a, Kendra et al. 2012c). *Xyleborus glabratus* is not an economic pest in its native areas and no reports of its life cycle and development are available from its reported original occurrence areas in Asia. To better understand the behavior, host-pathogen interaction, and beetle symbiosis, and to plan better management strategies, knowledge of the *X. glabratus* life cycle and development is required. Here we report for the first time the life cycle, and development of this species using redbay, swampbay and avocado as hosts, and we describe the characteristics of the gallery pattern. We also present the rearing methodology of the beetle on logs.

Material and Methods

Beetle Source

Redbay trees with high infestations of *X. glabratus* were scouted at Austin Cary Memorial Forest (ACMF), Alachua County, FL and Ordway-Swisher Biological Station (OSBS), Putnam County, FL. The main trunks of infested trees were cut at the baseline and sectioned into 40-45 cm logs. Logs were immediately transferred to the laboratory and 4-6 logs were placed in a beetle-emergence container (Figure.3-8). The beetle-emergence container consisted of a 32-gallon Rubbermaid roughneck refuse container, with a collection cup attached to the side of container near the neck. The collection cup

was partitioned into two compartments using plankton netting (150 micron, Bioquip products). One manuka lure (Semiochemicals Corp., BC, Canada) was placed in the lower compartment of the collection cup and replaced every 14 days. Adult beetles were collected in the upper compartment of the collection cup that had a moist paper towel to maintain high humidity. The permeable partition allowed the movement of attractant volatiles released from the manuka lures, but prevented the beetles from directly accessing the lure (Figure.3-6). The containers were placed on wire shelf (Perfect home commercial grade, The Home Depot) with their sides parallel to the ground. A total of 20 beetle-collecting containers were maintained in two rearing rooms at the Entomology and Nematology Department, Gainesville, FL. Rearing rooms were maintained at 25±2^{o} C in complete dark conditions. The beetles were collected daily, with fully sclerotized *X. glabratus* females sorted and used for the different developmental studies.

Life Cycle and Development of *X. glabratus*

Life cycle and developmental studies of *X. glabratus* were studied in artificially infested logs of redbay (*Persea borbonia* (L.) Spreng.), avocado (*Persea americana* Mill) and swampbay (*Persea palustris* (Raf.) Sarg.) trees. Avocado cv. 'Booth 7' logs were procured from the Tropical Research and Education Center, Homestead, FL. A certified municipal arborist in Volusia County, FL, provided redbay and swampbay trees and the tree species identity was confirmed at the Department of Plant Industry, Gainesville, FL. All the logs were cut from healthy trees with no symptoms of Laurel wilt or with visible signs of *X. glabratus* attack. Developmental studies in avocado were conducted in Sep-Oct 2010, for redbay during Mar-Apr 2011 and for swampbay during May-Jun 2011. All developmental studies were conducted in the laboratory of the University of Florida, Entomology and Nematology Department, Gainesville, Florida.

Logs of 4.5-6.5 cm diam. were cut into 8-10 cm lengths and then soaked in tap water for 48 h. For each host, 150 logs were used. To preserve internal humidity, each log was kept standing in a 946 ml clear plastic container (American Plastics, Gainesville, FL) throughout the experiment; the exposed water surface covered with Plankton netting (150 micron, Bioquip products). To inoculate logs with a controlled number of founding females, twenty fully sclerotized female adult beetles (dark brown to black in color) were placed on the bark of each log, and allowed to bore. Logs were kept in an incubator (Precision® illuminated incubator) at 25 ± 2^{o} C in complete darkness. Each day, three logs were randomly selected and were split into small longitudinal pieces. Each beetle gallery was thoroughly searched for the developmental stages. The duration of each study was ca. 40 days or until the teneral adult stage was observed. Observations recorded for each log were: number of successful borings and gallery pattern, and number of different developmental stages encountered for each log. Boring was considered successful if, after removing the outer bark, the gallery reached the inner bark (phloem). Based on the development of *X. glabratus* in avocado, redbay, and swampbay, a generalized life cycle of the beetle was constructed.

Description of Life Stages

Egg, larval, and pupal stages were described in this study. During the life cycle and development study of *X. glabratus* in redbay, swampbay, and avocado, different stages encountered were collected and preserved in 70% ethyl alcohol. The egg and pupal stages were studied and the number of larval instars determined by measuring the head capsule under a binocular microscope with an ocular micrometer. To ascertain the head capsule width of the first instar larvae, *X. glabratus* eggs were reared to first instar stage. Using the same methodology of the life cycle and development study, *X.*

glabratus female adults were reared for 20 days in six avocado logs. Logs were split and eggs recovered from the galleries. Individual eggs were placed in a petri dish (BD Falcon Petri Dishes, 50x9 mm) on moist paper towel. Head capsule width of the emerging larva was recorded.

Gallery Pattern

Redbay logs (5-6 cm dia.) with a high infestation of *X. glabratus* were brought from Ordway-Swisher Biological Reserve, Putnam County, FL. *Xyleborus glabratus* entry holes were identified and marked based on the size of entry hole (0.8 mm in diameter) (Hanula et al. 2008, Mayfield and Hanula 2012). Marked entry holes were further chipped to expose the boring *X. glabratus* female. Exposed entry holes with a female *X. glabratus* were horizontally dissected using a miter saw to expose the gallery system. The exposed galleries were traced on transparency sheets and the structure and pattern of the galleries was described.

***Xyleborus glabratus* Culture on Swampbay Logs**

For the rearing study, 34 swampbay logs with no infection of laurel wilt and no infestation of *X. glabratus* were used. Log length and diameter (mean ± SE) was 9.5 ± 1.6 and 5.9 ± 0.2 cm respectively. Logs were soaked in tap water for 48 hours, removed, and after removing the excess of water, labeled and placed in a 946 ml clear plastic container (American Plastics, Gainesville, FL). Each plastic cup was then covered with Plankton netting (150 micron, BioQuip products). One hundred ml of water were maintained in the containers throughout the experiment to keep the logs moist, with a cut end in the water. Twenty fully sclerotized female adult beetles were placed on the bark of each log and were allowed to bore. Logs were kept in an incubator (Precision® illuminated incubator) at 25 ± 2^{o} C in complete darkness. The numbers of

female adults emerging from each log were counted at intervals of 7-14 d until 240 d after gallery initiation. The rearing study was carried out during Apr-Dec 2011.

Statistical Analysis

Analysis of variance for the successful borings by the beetles in three different hosts was conducted using Proc GLIMMAX (SAS Institute 2004). Monthly emergence of beetle from the swampbay logs for the rearing study was analyzed using Proc GLM in SAS. Regression analysis was conducted to find the relationship between instars and head capsule width using SAS (SAS Institute 2004).

Results

Life Cycle and Development of *X. glabratus*

In avocado, eggs were first encountered on the 7th day, larval stage on the 14th day, pupal stage on the 24th day, and the teneral adult on the 31st day after the initial boring (Figure 3-1). In redbay logs, eggs were first encountered on the 11th day, larval stages on the 20th day, pupal stages on the 26th day, and teneral adult on the 30th day after initial boring (Figure.3-2). In swampbay logs, eggs, larva, pupal and adult stage were encountered on 10th, 14th, 26th and 26th day after initial boring, respectively (Figure 3-3). There were significant differences in successful boring by the beetle in the three hosts ($F_{2, 185} = 10.90$; $P < 0.0073$). Highest successful borings by the beetles was observed in avocado logs followed by swampbay and redbay logs (Table 2-1). Data from the three hosts were combined to construct the life cycle of the beetle. Mean ± SD for duration of the egg, larval, and pupal stage was 6.6 ± 2.5, 9.3 ± 3.0, and 5.0 ±1.4 d, respectively. Mean ± SD for pre-oviposition period was 9.3 ± 2.0 d. Highest numbers of egg, larval, pupal and teneral adult stages were encountered in swampbay logs as compared to redbay and avocado logs (Table 2-1).

Developmental Stages

Egg: White, translucent and ovoid in shape. Means ± SE for length and breadth are 0.63±0.004 and 0.27±0.003 (n=44). Larva: Legless, whitish in color with head capsule white in color. Head capsule measurement of larvae for three hosts showed three peaks. The range of head capsule widths for 1^{st}, 2^{nd}, 3^{rd} instars were 0.20-0.22, 0.25-0.27, 0.35-0.40 respectively, based on the frequency distribution in the three hosts (Figures 3-4) (Table 3-2). The mean ± SE for the head capsule width for first larval instar reared from eggs at 25°C was 0.22 ± 0.001 mm (n = 25) which was similar to the head capsule widths of larval stages collected from galleries. The best-fit linear function for the three-instar model raised on avocado was Y = 0.1267 + 0.075 X (R^2 = 0.972) (X= instar, Y= head capsule width and for both redbay and swampbay was Y = 0.1067 + 0.085 X (R^2 = 0.964) (Figure 3-6). Pupal stage: white, exarate pupa, typical scolytine pupa

Gallery Pattern

Females of *X. glabratus* initiate the gallery by pushing the wood tissue out, which appears as sawdust noodles. A gallery system created by female *X. glabratus* consists of a primary tunnel that branches into 2-5 secondary tunnels, with each secondary tunnel branching into 0-3 tertiary tunnels (Figure.3-7). In redbay logs with a diameter of 5-6 cm, the mean ± SE for primary tunnel length was 8.52 ± 0.8 mm (n =24). The total gallery system length and width recorded was 32.1± 2.0 and 28.0 ± 2.1 cm (mean ± SE) (n =24), respectively. Eggs were observed usually at the distal ends of secondary and tertiary tunnels, and usually in groups of 1-8. The gallery system is perpendicular to the

trunk of tree. One month after gallery initiation, all the developmental stages (egg, larva, pupa and teneral adult) were present in the gallery system of each initial beetle.

Xyleborus glabratus Culture on Swampbay Log

Xyleborus glabratus was successfully cultured on the soaked swampbay logs. Over the period of study, 1947 beetles emerged from 34 logs. (mean = 57.3 *X. glabratus*/log, S.E = 5.7, n = 34). Beetle emergence started around the 60th day after gallery initiation and the highest number of beetles emerged between 120-150 days after gallery initiation. Emergence of beetles lasted until 240 days after gallery initiation (Figure. 3-5). There were total of 2.86 emergent beetles/initial females placed.

Discussion

Redbay and swampbay trees are the two ecologically important trees of the family Lauraceae that have been severely affected by laurel wilt disease, with high mortality recorded in the areas of its spread (Fraedrich et al. 2008). Laurel wilt disease has infected yard and experimental avocado trees, and poses an imminent threat to commercial avocado groves (Ploetz and Peña 2007, Ploetz et al. 2011). To date, *Xyleborus glabratus* is the only known vector of this disease. In our studies, we investigated and compared the development of *X. glabratus* in swampbay, redbay, and avocado tree logs. Based on the time of development, a similar pattern of development of *X. glabratus* was observed in the logs of all three hosts. This suggests that the beetle successfully complete its life cycle in the three hosts in about same period.

Hanula et al. (2008) reported similar attraction of *X. glabratus* to swampbay and avocado wood. Bolts of avocado were more attractive than unbaited traps in field studies (Kendra et al. 2011). This would suggest that under field conditions suitably attractive avocado trees could be subject to attack from *X. glabratus.* Currently,

however, a good estimate of the number of progeny that would be produced from a newly colonized live tree is not available. Mayfield et al. (2008) reported that *X. glabratus* will successfully bore into healthy avocado potted plants in no-choice tests, and five *X. glabratus* were able to transmit laurel wilt in the Simmonds avocado cultivar. However, it remains to be seen how avocado trees grown under field conditions will act as reservoirs for beetles as compared to redbay and swampbay trees.

Developmental time for *X. glabratus* eggs, larvae, and pupae averaged 6.6, 9.3, and 5.0 days, respectively, in the logs of avocado, swampbay and redbay trees at 25±2 °C. Similarly, development time for eggs, larvae, and pupae of *Xyleborus ferrugineus*, when reared on artificial diet, was 4.52, 8.1, and 4.6 days, respectively (Kingsolver and Norris 1977 a, b). *Xyleborus fornicatus* Eichoff larval and pupal developmental periods were 12.4 and 5.3 days, respectively, when reared at 28°C (Gadd 1947). *Xyleborus pfeili* eggs, larvae, pupae, and adults reared on artificial diet required a minimum of 4, 10, 18, and 22 days to develop (Mizuno and Kajimura 2002). Development of *Xylosandrus germanus* larvae and pupae averaged 11.9 and 7.0 days, and egg to teneral adult averaging 24.9 days at 24 °C in artificial diet (Weber and McPherson 1983). *Xylosandrus compactus* egg, larval, pupal and adult maturation times at room temperature in twigs of *Cornus florida* L. were 5, 7.5, 7.5 and 8.5, days, respectively, at room temperature (Ngoan et al. 1976). Total development time of egg to teneral adult of *X. glabratus* averaged 29 ± 2.6 days at 25 ± 2 °C. Development of *Xyleborus celsus* from egg to adult required approximately 35 days in its natural host *Carya texana* (Gagne and Kearby 1979). Similarly, egg to adult development of *Ganthotrichus*

retusus took a minimum 40 days in the galleries of Douglas fir logs (Liu and Mclean 1993). Thus, *X. glabratus* displayed development rates similar to its congeners.

Three larval instars of *X. glabratus* were observed. Average head capsule width of the 1st, 2nd and 3rd larval instars was 0.21, 0.26, and 0.37 mm, respectively. Three larval instars have also been reported in diploid larvae of *X. ferrugineus, with* head capsule breaths of 0.24, 0.34, and 0.46 mm for 1st, 2nd, and 3rd larval instars (Norris and Chu. 1985). Similarly, head capsule ranges of *Xyleborus celsus* three larval instars were 0.28-0.34, 0.35-0.54 and 0.57-0.69 mm, respectively (Gagne and Kearby 1979). *Xylosandrus germanus* had three larval instars with mean head capsule width of 0.25, 0.33, and 0.47 respectively (Weber and McPherson 1983). Dyar's rule of geometric progression of widths of head capsule in successive instars can be expressed by the linear as regression model Log Y= a + bX where Y = head capsule width and X = instar (Dyar 1890, Gaines and Campbell 1935, Klingenberg and Zimmerman 1992). To find the closeness of fit of the head capsule width with three instars of *X. glabratus,* we used this regression model, which produced a good fit: R^2 = 0.964 for larvae collected from avocado and R^2 = 0.972 for larvae collected from redbay and swampbay. The closeness of our three-instar model with a straight line indicates constant incremental growth of head capsule breadth of larval stages, suggesting that there are three larval instars in the life cycle of beetle.

The size of the gallery formed by ambrosia beetles is important in their biology. *Xylosandrus mutilatus* gallery length and the number of offspring per gallery system were positively correlated at every growth stage of brood development (Kajimura and Hijii 1994). A similar positive correlation was observed between gallery length and

number of offspring per tube for *Xyleborus pfeili* reared on artificial diet (Mijunoa and Kajimura 2002). In both the aforementioned studies, gallery length was used as an indicator of the amount of fungal resource available for the beetle and its brood development. Delayed oviposition and delayed gallery formation were observed in *Xyleborus ferrugineus* when reared in artificial diet containing no fungus, as compared to diet containing symbiotic fungus. This suggests that there is a positive correlation between amounts of symbiotic fungus present in the total gallery length with the ovpositional behavior of the beetle (Kingsolver and Norris 1977a). Brood production with successful brood development by ambrosia beetles is directly related to the amount and quality of symbiotic fungus available in the galleries. In conclusion, the amount of symbiotic fungus directly relates to the expanse of gallery system in the tree and the community of fungus growing in the galleries.

The fungal mycelium consumed by ambrosia beetles derives nutrition from materials stored within cell cavities (Panshin and De –Zeeuw 1977, McIntosh 1994). The fungus use sugars, starch along with other nutritional substances present in the lumen of host cells for its growth (Chapman et al. 1963, McIntosh 1994). Adequate growth of ambrosia fungus will depend on favorable temperature, oxygen, adequate moisture, and nutritive resources (Panchin and De-Zeww 1977, Rudinsky 1962, McIntosh 1994). The quality of the wood as a substrate likely also depends on the tree host species and its physiological state. In our study, logs from three hosts were given the same treatment of moisture and a constant amount of water was maintained in the containers, and the physiological state of host was dead (because logs were used). Therefore, from our results, it appears that the tritropic interaction between host-fungi-

beetle might have led to fewer numbers of eggs, larvae, pupae and teneral adults in avocado as compared to swampbay. Because of destructive sampling and unavailability of sufficient large diameter live trees, we were unable to study the tritropic interaction in living plant hosts. To better understand the tritropic interaction, follow-up studies should be conducted in live host trees, concentrating on the development of beetle and its relation to development of fungus/fungi in the beetle galleries during the complete life cycle of beetle.

The gallery systems of *X. glabratus* resemble a tree-like branching pattern. Female adults of *X. glabratus* initiate the gallery system perpendicular to the trunk of trees, with an entrance hole of about 0.8 mm diameter (Hanula et al. 2008, Mayfield and Hanula 2012), which is extended to form a primary tunnel that branches to form secondary tunnels, which in turn branch into tertiary tunnels. Tertiary tunnels usually extend to the xylem vessels of the tree. A similar gallery pattern had been reported for *Xyleborus pfeili*, with the gallery system having a main gallery branching into branch tunnels, which in turn branch into side tunnels, when reared on semi-artificial diet (Kajimura and Hiji 1994). In contrast, *Xyleborus ferrugineus* reared on artificial diet had a gallery pattern of a main gallery with branch galleries having branch cells commonly containing eggs (Kingsolver and Norris 1977a). *Xyleborus celsus* constructed a gallery pattern horizontal to the tree trunk, with the main gallery having 0-6 branch galleries per gallery system in *Caraya texana* trees (Gagne and Kearby 1979). The *Xylosandrus germanus* gallery system in black walnut, tulip, sweetgum, and oak trees consisted of a horizontal entrance tunnel extending up to 2-3 mm that widened to form vertical brood chamber of 7-12 mm (Weber and McPherson 1983). The gallery pattern of *Xylosandrus*

mutilatus was characterized by horizontal main gallery with several vertical branch tunnels where larval and pupal developmental took place (Kajimura and Hiji 1994). The *Xyleborus glabratus* gallery pattern, as compared with other *Xyleborus* spp., was similarly branched in a horizontal plane in the tree, extending to the pith, with no specific larval or pupal chamber observed. Eggs were laid at the distal ends of primary and secondary tunnels and larval and pupal development took place in the tunnel. Larval stages pupated in primary or secondary tunnels.

Overlapping generations are observed in ambrosia beetles, due to differential gallery extension time and eggs deposition time in the galleries. *Xyleborus pfeili*, when reared on artificial diet, constructed a vertical gallery that was further extended to form branch tunnels on the 4^{th}, 6^{th}, 12^{th} and 18^{th} day. Eggs were laid in each tunnel as they were extended. The beetle determined the number of eggs in response to the amount of ambrosia fungus available in the galleries. Due to differential gallery extension time and eggs deposition time in the galleries, overlapping generations were observed in the gallery system of one female adult (Mijunoa and Kajimura 2002). Similarly, *Xylosandrus mutilatus* laid eggs in branch tunnels as soon as they are constructed, which leads to overlapping generations (Kajimura and Hiji 1994). In *X. glabratus* after about a month of gallery initiation, all the developmental stages are present, so we postulate that *X. glabratus* lays eggs in the secondary and tertiary tunnels as they are constructed.

Xyleborus glabratus is a recently introduced ambrosia beetle in the United States, so there has been no available methodology to efficiently culture the beetle on a semi-artificial diet. Based on the results of X*. glabratus* development in three hosts, we used swampbay logs to develop a rearing methodology of beetle under controlled

conditions. *Xyleborus glabratus* was successfully cultured on moist swampbay logs. Over a period 240 days, we recovered approximately 2.86 adult female X*. glabratus* for every female adult used to infest each bolt initially. Successful boring could not be accurately assessed, as logs were not split and bark not stripped. Therefore, to estimate the number of female adults emerging per successful gallery, we used the successful boring data from the aforementioned swampbay life cycle and development study as a basis for comparison. We estimate that an average of 6.36 female adults emerged per successful boring in the log. Similarly, *Platypus quercivorus* was reared using similar technique on soaked logs of *Quercus serrata* Thunb. Ex Murry and 0.7 – 3.9 times as many beetles emerged as were released on the logs (Kitajima and Goto 2004).

In conclusion*, Xyleborus glabratus* female adult beetles bore inside the logs of redbay, swampbay and avocado trees and initiate the main tunnel, followed by primary and then secondary branches. The eggs are laid at the end of primary or secondary branches of tunnel about 7-10 days after gallery initiation. Development from egg to teneral adult occurs over a period of 30 days, with larval and pupal time averaging about 10 and 5 days, respectively. New adult females emerge in approximately 60 days *Xyleborus glabratus* was successfully cultured on water-soaked swampbay logs.

Table 3-1. Development of *X. glabratus* in the logs of different species under controlled conditions for 40 days. (N = 120).

Host	Successful boring (Mean ± SE)	No. of each developmental stage collected			
		Egg	Larva	Pupa	Adult
Avocado	10.1±0.3a	56	144	23	7
Redbay	8.5±0.3b	64	135	34	33
Swampbay	9.0±0.4ab	158	306	127	106

Means followed with same letter are not significantly different based on Tukey - Kramer test for difference of means ($P < 0.05$).

Table 3-2. Head capsule widths of three instar classes of *X. glabratus* in Redbay, Swampbay and Avocado.

Host	Class of larvae		Width of head capsule (mm)			Xn+1/Xn
	Instar no	No. of larvae	Mean (X)	SE	Range of size	
Redbay						
	I	13	0.21	0.003	0.20 - 0.22	1.30
	II	34	0.26	0.002	0.25 - 0.27	1.42
	III	48	0.37	0.002	0.35 - 0.40	
Swampbay						
	I	36	0.21	0.001	0.20 - 0.22	1.30
	II	52	0.26	0.002	0.25 - 0.27	1.42
	III	69	0.37	0.001	0.35 - 0.40	
Avocado						
	I	101	0.21	0.001	0.20 - 0.22	1.24
	II	78	0.26	0.001	0.25 - 0.27	1.40
	III	66	0.36	0.001	0.35 - 0.40	

X_{n+1}/X_n = Mean Of Subsequent Larval Stage/Mean of Previous Larval Stage

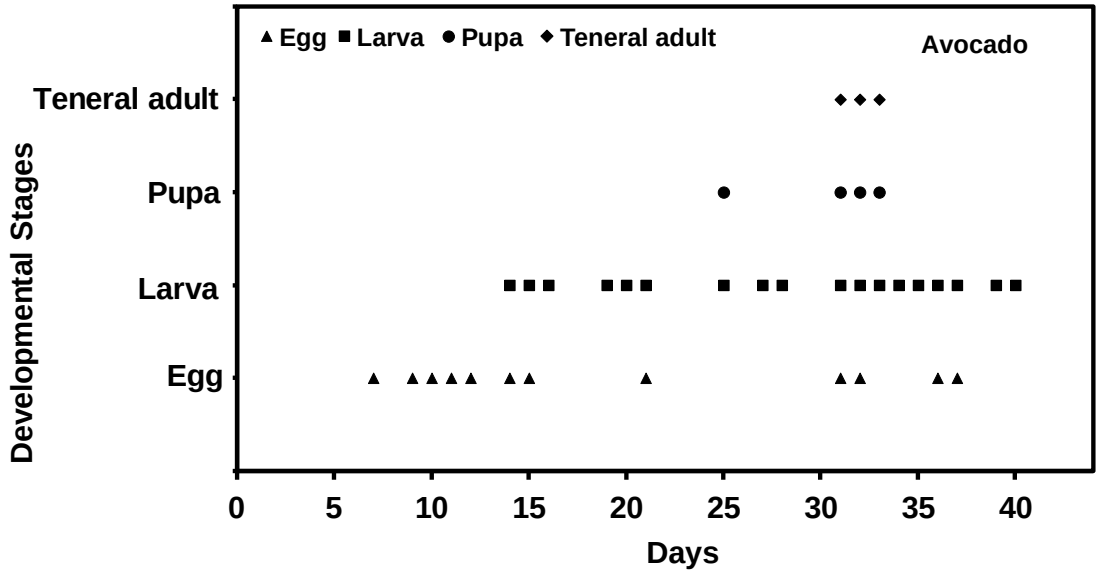


Figure 3-1. Development of *Xyleborus glabratus* in the logs of avocado (*Persea americana* Mill) based on the encounter of different developmental each day for 40 days.

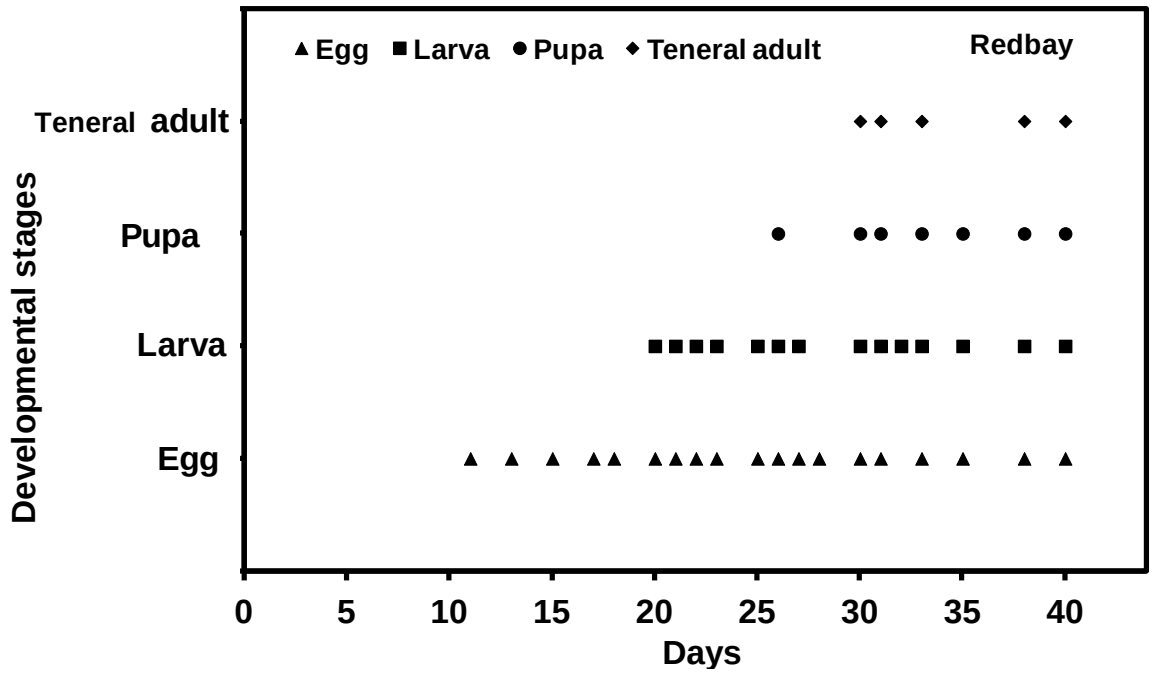


Figure 3-2. Development of *Xyleborus glabratus* in the logs of redbay (*Persea borbonia* (L.) Spreng.), based on the encounter of different developmental stages each day for 40 days.

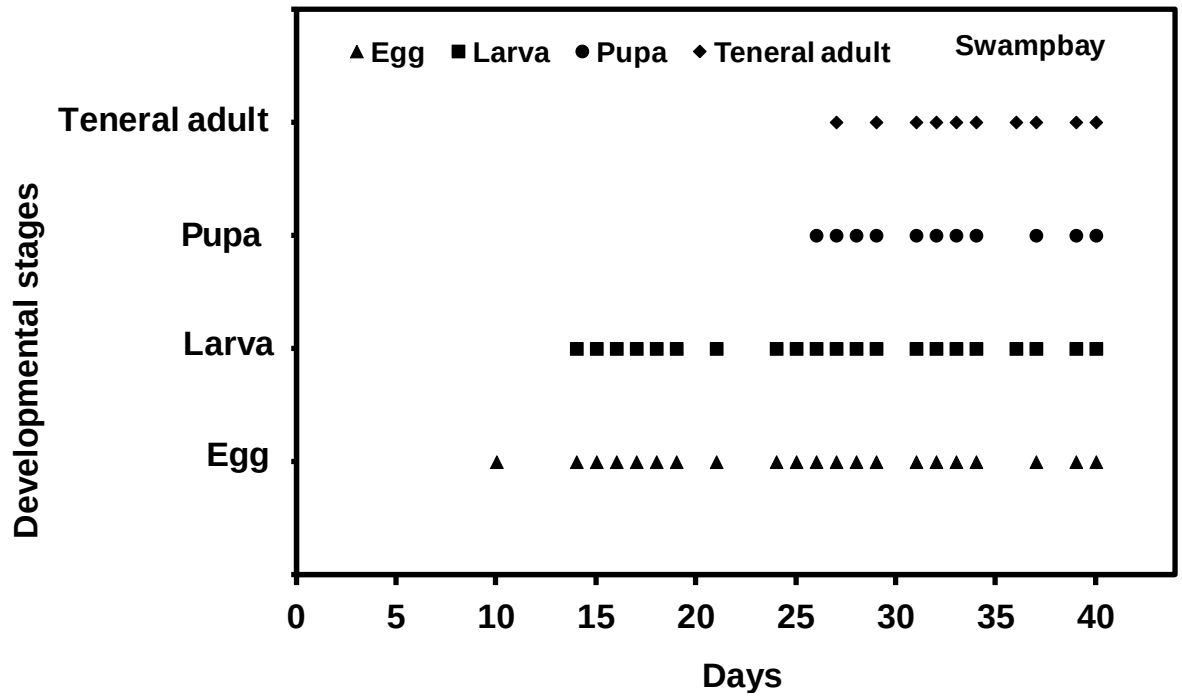


Figure 3-3. Development of *Xyleborus glabratus* in the logs of swampbay (*Persea palustris* (Raf.) Sarg.), based on the encounter of different developmental stages each day for 40 days.

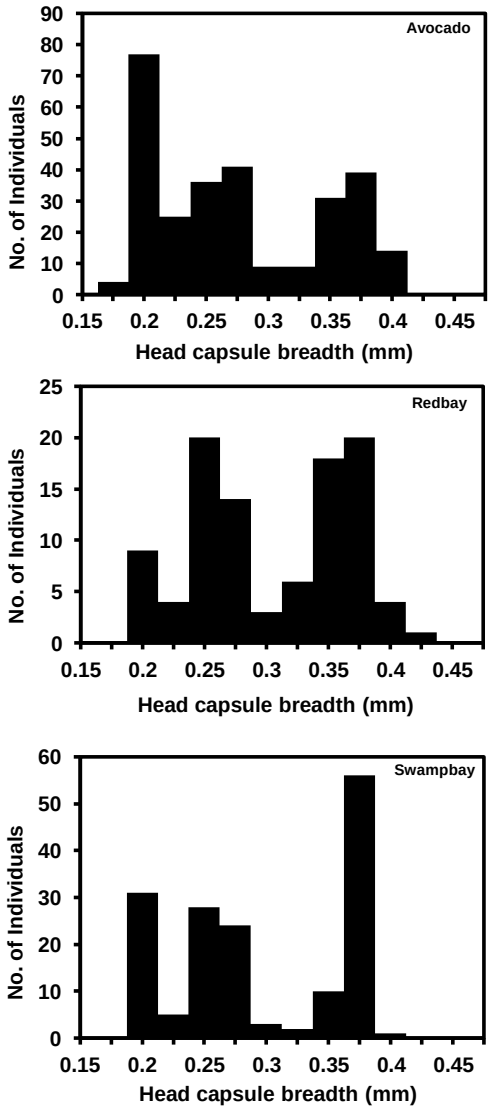


Figure 3-4. Frequency distributions of head capsule widths of *X. glabratus* larvae. reared in avocado (*Persea americana* Mill), redbay (*Persea borbonia* (L.) Spreng.), swampbay (*Persea palustris* (Raf.) Sarg.), at 25±2°C (n= 157).

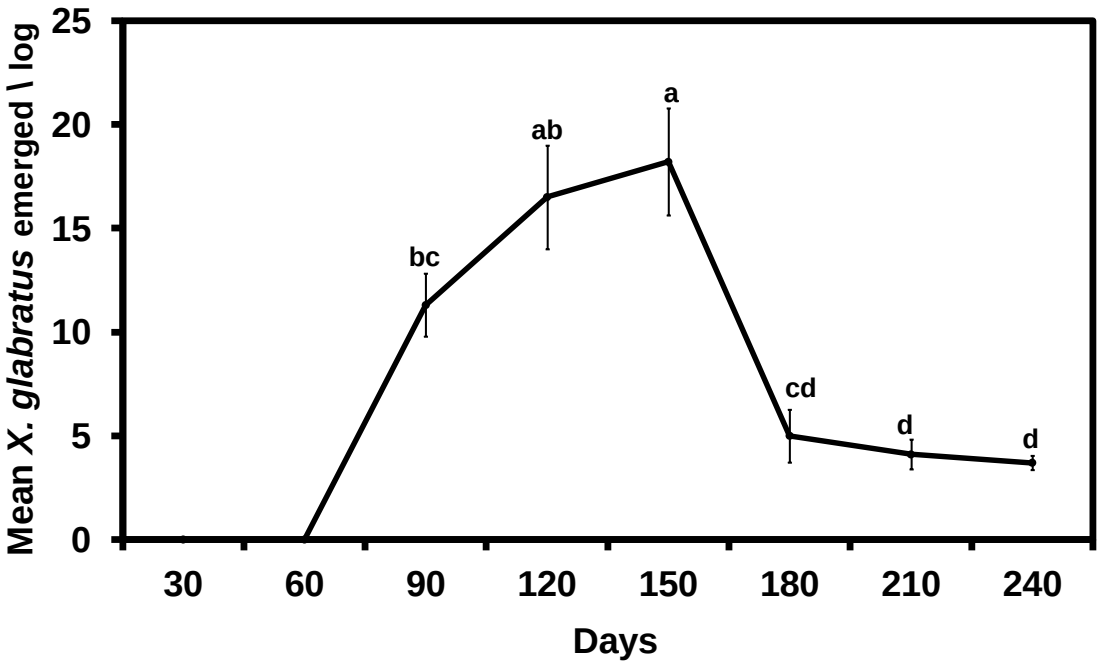


Figure 3-5. Mean ± SE of emergence of *X. glabratus* / log / month from swampbay logs at 25±2°C at 24 hrs dark conditions over a period of 240 days (n= 34). The study was conducted during April – December 2011. Means followed by the same letter are not significantly different based on Tukey - Kramer test for difference of means ($P < 0.05$) ($F_{5, 165} = 19.26$; $P < 0.0001$).

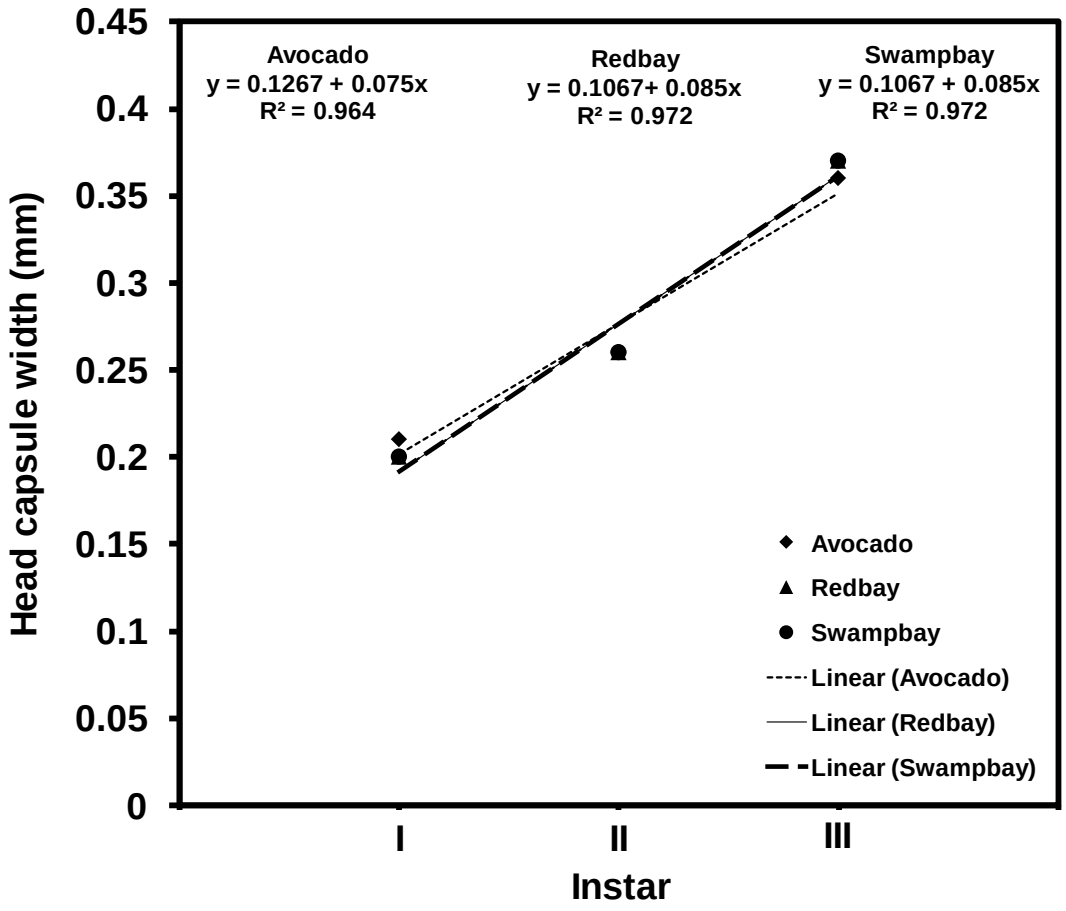
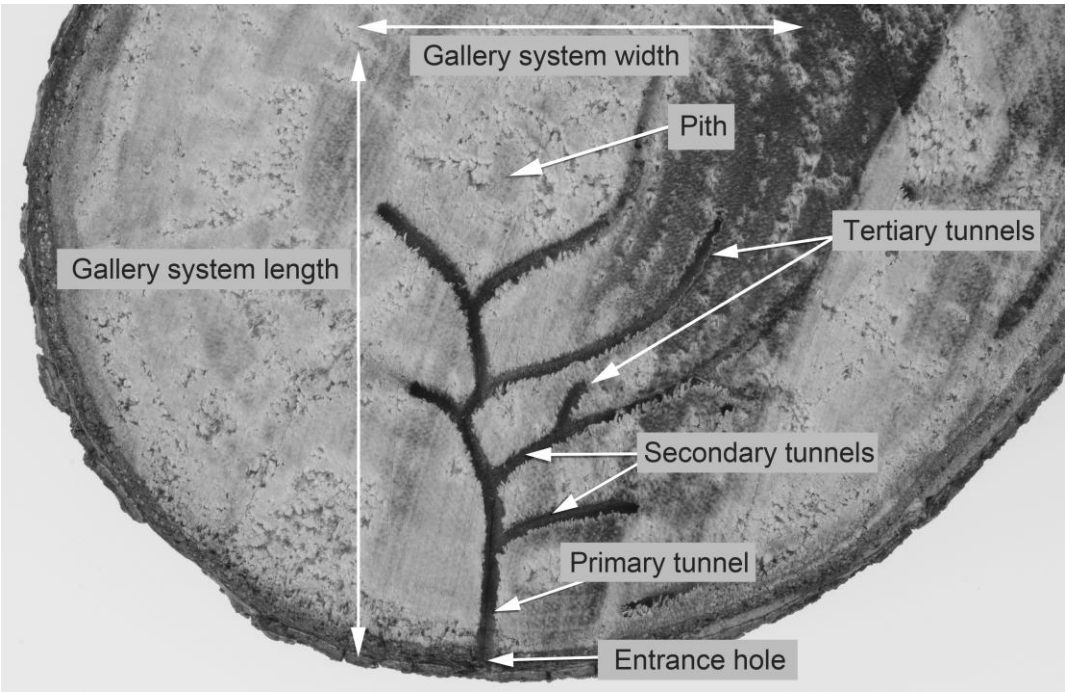


Figure 3-6. Closeness of fit of the mean head capsule width to three instar model, using linear progression model (y = a + bx).



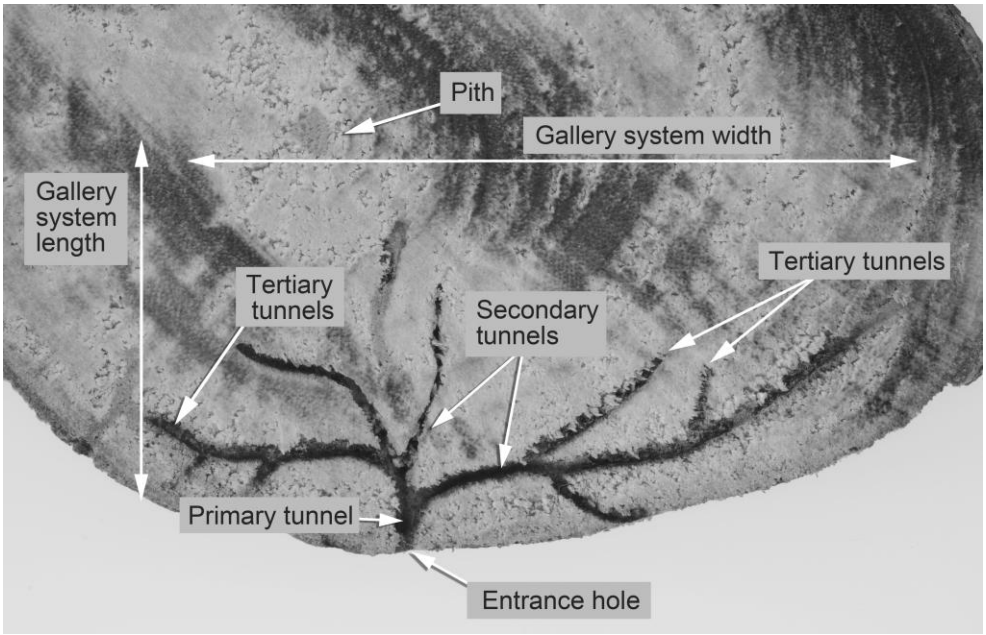


Figure 3-7. Gallery pattern of *Xyleborus glabratus* in the redbay trees

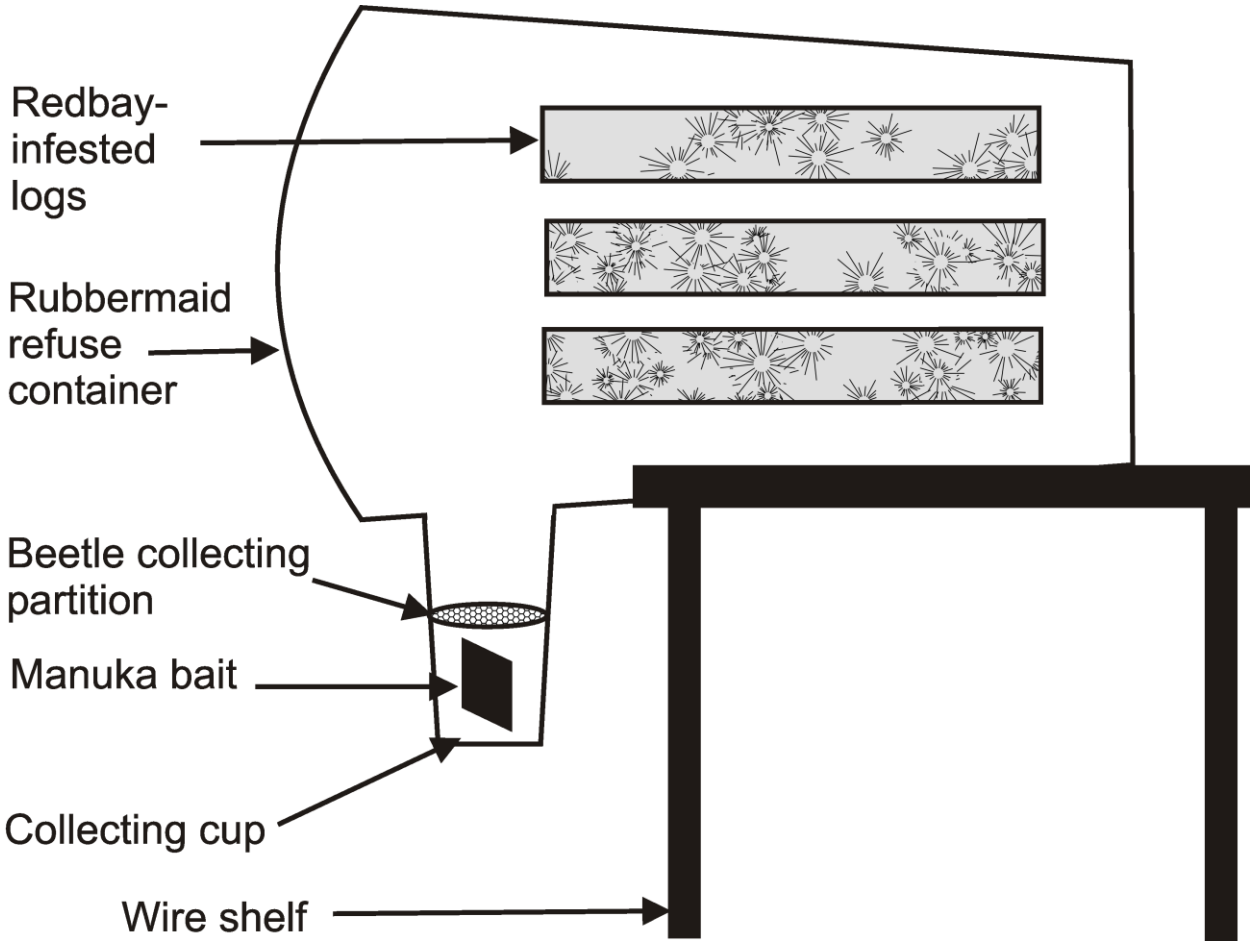


Figure 3-8. Schematic diagram of *Xyleborus glabratus* collecting apparatus

CHAPTER 4
TEMPERATURE-DEPENDENT DEVELOPMENT OF REDBAY AMBROSIA BEETLE *XYLEBORUS GLABRATUS* (COLEOPTERA: CURCULIONIDAE: SCOLYTINAE)

Redbay ambrosia beetle, *Xyleborus glabratus* Eichhoff (Coleoptera: Curculionidae: Scolytinae) is a nonnative pest that vectors the pathogenic fungus *Raffaelea lauricola* which causes a vascular wilt disease known as laurel wilt in redbay (*Persea borbonia*) and swampbay (*Persea palustris*) two ecologically important trees in the family Lauraceae. Laurel wilt disease has also infected yard and experimental avocado trees (*Persea americana*) and poses an imminent threat to commercial avocados groves in Florida, California and Mexico. The life cycle and development of *X. glabratus* were studied in avocado logs kept at 12, 16, 20, 24, 28, 32 and 36°C. *Xyleborus glabratus* successfully completed its life cycle at 24, 28, 32°C. There were no developmental stages encountered at 12, 16, 36°C. Highest numbers of egg and larval stages were encountered in the logs placed at 28°C followed by the logs placed at 24 and 32°C. The optimal temperature for the beetle was around 28°C. Development of egg and pupal stages of *X. glabratus* were studied at the same temperatures. Developmental rates of egg and pupal stages increased in linear fashion over the range of 16-28°C. Estimates for lower developmental threshold for egg and pupal stages were estimated to be 10.9±0.5°C and 11.3±0.6°C and the degree-days for development were 55.3±3.3 DD and 69±4.5 DD, respectively. Our results suggest that temperature will play an important role in the spread and successful establishment of the beetle based on the latitudinal distribution of its host plants.

Background

The exotic redbay ambrosia beetle *Xyleborus glabratus* Eichoff (Coleoptera: Curculionidae: Scolytinae) has established as a serious pest of trees of the family

Lauraceae in the United States. The beetle is native to Southeast Asia and was accidentally introduced in the southeastern United States around 2002 (Rabagalia et al. 2006, Haack 2003). The redbay ambrosia beetle acts as a vector of *Raffaelea lauricola* (Fraedrich et al. 2008, Hanula et al. 2008) that causes laurel wilt in infested trees.. Since the introduction of the beetle, the laurel wilt pathogen has been detected in redbay (*Persea borbonia* (L.) Spreng.), avocado (*Persea americana* Mill), swampbay (*Persea palustris* (Raf.) Sarg.), sassafras (*Sassafras albidum* (Nutt.) Nees), pondspice (*Litsea aestivalis* (L.) Fernald), pondberry (*Lindera melissifolia* (Walter) Blume,), and camphor (*Cinnamomum camphora* (L.) J. Presl) (Fraedrich et al. 2008, Smith et al. 2009a, Smith et al. 2009b, Mayfield et al. 2008) sometimes causing causing mortality of 90 percent of infested trees. The disease has also been reported from backyard and experimental avocado trees in Florida (Crane et al. 2008, Mayfield et al. 2008, Ploetz and Peña 2007, Ploetz et al. 2011).

Since its introduction, the beetle has been reported in North Carolina, South Carolina, Georgia, Florida, Alabama and Mississippi and has expanded more quickly than predicted by Koch and Smith (2008). The climate and host plant distribution have favored its expansion and establishment in the southeastern United States. Beetle population dynamics studies in South Carolina and Georgia have shown that adult beetles were active throughout the year, with high activity in the month of Sep as compared to Jan and Feb winter months (Hanula et al. 2008, Hanula et al. 2011). In Florida, high activity of the beetles was recorded in the months of Apr 2010, Oct 2010 and Mar 2011 while low activity was recorded in the months of Nov 2010, Dec 2010, and Jan 2011 (Brar et al. 2012a). This suggests that variation in the temperature played

an important role in the beetle population dynamics. At 25±2°C, egg, larval, pupal and teneral adult stages were first encountered on the 7th, 14th, 24th and 31st day, respectively, after gallery initiation in infested avocado (*Persea americana*) logs (Brar et al. 2012b). Similarly, in laboratory-infested swampbay logs placed at 25±2°C, teneral adults were observed after the 26th day of gallery initiation and emergence of beetles started around the 60th day after gallery initiation.

Temperature is the main abiotic factor that influences insect biology and population dynamics (Walgama and Zalucki 2006). Then, information on beetle development in relation with temperature is required to interpret its population dynamics and to create phenological predictive models. The development rate resulting from plotting development time against temperature is a sigmoid curve that is linear over the middle range of temperature. Below the middle range of temperature, there is a temperature threshold where no development takes place. Similarly, at the upper temperature threshold the developmental rate decreases and the insect dies (Walgama and Zalucki 2006, Campbell et al. 1974). The linear model (Campbell et al. 1974) is simple and sufficient to both predict the lower development threshold and the thermal constant within limited ranges of temperature. Given the potential impact of the beetle-fungus complex on the avocado industry of Florida and California, and its potential threat to other Lauraceous plants of North America (Graming et al. 2010), it is desirable to develop phenological and population dynamics models to help predict pest infestations, and to initiate control measures. The objective of my research was to describe how the development of *X. glabratus* egg and pupal stages depends on temperature. We used seven levels of constant temperature, ranging between 12-36°C.

We also studied the duration of life cycle and development of the beetle in avocado logs at constant temperatures between 12-36°C.

Materials and Methods

Beetle Source

Redbay and swampbay trees with high infestations of *X. glabratus* were scouted at three locations in Florida: Austin Cary Memorial Forest (Alachua County), Ordway-Swisher Biological Station (Putnam County) and Hammock dunes club golf course Palm Bay (Brevard County). Infested logs were collected, and a beetle colony was maintained based on the methodology explained in chapter 3.

Rearing of Redbay Ambrosia Beetle for Developmental Stages

Avocado cv. 'Booth 7' 4.5-6.5 cm diam. and 8-10 cm length logs were procured from the Tropical Research and Education Center, Homestead (Miami Dade County) FL. and soaked in tap water for 48 hours, removed, and individually placed in a 946 ml clear plastic container (American Plastics, Gainesville, FL). Each container was covered with Plankton netting (150 micron, BioQuip products). To keep the logs moist, one hundred ml of water was maintained in the containers throughout the experiment. Twenty fully sclerotized female adult beetles were placed directly on the bark of each log and were allowed to bore. Logs were kept in an incubator (Precision® illuminated incubator) at 25±2°C in complete darkness. Based on the life cycle of the beetle on avocado logs (Brar et al. 2012b), the logs were split on the 10-13th day after gallery initiation and eggs were carefully extracted using sterilized needles. Similarly, the logs were split on the 30-33rd day (Brar et al. 2012b) after gallery initiation, and the pupal stages were extracted carefully using sterilized needles.

Development of Egg and Pupal stages at Constant Temperatures

The duration of development of egg to larval stage and pupal stage to adult stage were studied at constant temperatures. Eggs extracted from avocado logs were placed in petri dishes (BD Falcon 50×9mm) on moist paper towel. The petri dishes were then placed at in incubators (Precision® illuminated incubator) held at 12, 16, 20, 24, 28, 32 and 36°C (± 0.05°C) and kept under constant darkness. For each temperature, observations were recorded every day for development of the egg to larval stage. The number of eggs used for each temperature study was dependent on numbers available after splitting the logs. Similarly, the pupae extracted from avocado logs were placed in petri dishes (BD Falcon 50×9mm) following the same methodology used for eggs development. Observations were recorded daily for the number of days required for development of egg stage to larval stage and pupal stage to the adult stage for all the temperatures.. Paper towels were kept moist all the time to prevent the desiccation of developmental stages. The study was conducted between Mar–Jun 2012.

Life cycle and Development of Beetle in the Avocado Logs at Different Temperatures

The duration of life cycle and development of *X. glabratus* in avocado logs were studied at constant temperatures in two independent studies. The first study was conducted during Sept-Dec 2011. In first study, the development of beetle was studied at five constant temperatures 16, 20, 24, 28, and 32°C (± 0.05°C). Similar methodology and similar dimensions of avocado logs were used in this study as mentioned in the first study in chapter 3. One hundred twenty logs were used for each temperature. Five newly sclerotized adult female beetles were placed on the avocado logs and allowed to bore. Twenty four h after, the infested logs were placed at different temperatures in the

incubators (Precision® illuminated incubator) and kept in complete darkness. Every other day, three logs were randomly removed from the incubators. The logs were split and the presence and the number of each developmental stage recorded. The second life cycle and development study was conducted during Jan–May 2012 at seven different temperatures 12, 16, 20, 24, 28, 32 and 36°C (± 0.05°C). Similar methodology and similar dimensions of avocado logs were used in this study as mentioned in the first study. For the second study, twenty sclerotized adult beetles were placed on each avocado log and allowed to bore. After 24 hours, the infested logs were placed at different temperatures in the incubators (Precision® illuminated incubator) which were kept in complete darkness. The logs were split and observations were recorded for the presence and the number of each developmental stage for five galleries. The same observations were recorded for each temperature.

Statistical Analysis

ANOVA and Tukey-Kramer tests were conducted using SAS to determine differences between the duration of development of egg to larval stage and pupal stage to adult at constant temperature. .The developmental rate of each stage at 16, 20, 24 and 28°C was regressed against these temperatures using SAS to estimate a linear regression. The linear model $y = a + bx$ (Campbell et al. 1974) was used to estimate development threshold ($t_{min} = -a/b$), and thermal constant ($K=1/b$). The S.E. for the development threshold (t_{min}) thermal constant (K) was calculated using Campbell et al.(1974).

Results

Egg Development *in vitro*

Temperature had a significant effect on the egg development period (F 5, 186 = 192.3.90; P < 0.0001) (Table 4-1). No development of eggs took place at 12°C. Development periods for the egg stage decreased significantly from 16 to 28°C and then increased from 28 to 36°C. The fastest rate of development took place at 28°C (Table 4-1). This indicates that the optimum temperature for egg development is about 28°C. Mean egg development time ranged from a mean of 21.1±0.4 d at 16°C to 10.9±0.54 d at 36°C. Linear regression (Y = 0.0187T - 0.2625, R2 =0.67) of temperatures ranging from 16–28°C yielded a lower temperature threshold of 10.9±0.5 days, requiring 55.5±3.3 DD above the developmental threshold to develop to the larval stage (Table 4-2) (Figure 4-1).

Pupal Development *in vitro*

Temperature had a significant effect on the development of the pupal stage (F 4, 144 = 97.5; P < 0.0001) (Table 4-1). No pupal development was observed at 12°C. Mean pupal development ranged from a mean of 6.4±0.2 d at 32°C to 12.9±0.5 d at 16°C (Table 4-1). Development time for the pupal stage decreased from 16-28°C and then increased to 32°C. Optimum development of the pupal stage took place at 28°C. In the temperature ranges of 16-28°C, linear regression was represented by the equation (y = 0.0143T - 0.1583, R2 = 0.64). This yielded the minimum development time 11.3±0.6 days, with the pupal stage requiring 69 ± 4.5 DD above the developmental threshold to develop to adult stage (Table 4-2) (Figure 4-2).

Life Cycle and Development of Beetle in logs at Constant Temperatures:

In first study, there were no egg, larval, pupal and adult developmental stages encountered in the logs held at 16°C. Eggs were first encountered on an average of 22.0, 17.0,14. 0 and 18.0 days, respectively, after gallery initiation in the logs held at 20, 24, 28 and 32°C. Larval stages were first observed on an average of 24.0, 18.0, 16.0 and 18.0 days after gallery initiation in the logs held at temperatures 20, 24, 28 and 32°C, respectively. Similarly, pupal stages were encountered first on an average of 40.0, 27.0, 25.0 and 38.0 days respectively, at 20, 24, 28 and 32°C. Teneral adults were first encountered on an average of 36.0, 26.7 and 40.0 days, respectively, at temperatures of 24, 28 and 32°C. Teneral adults were not observed in the logs placed at 20°C (Table 4-3).

In second study there were no developmental stages observed at temperatures of 12, 16, 20 and 36°C. The eggs were first encountered on an average of 13.3, 10.7, 15.3 days after gallery initiation at temperatures 24, 28 and 32°C. The larval stage was first encountered on an average of 22.0, 16.7 and 19.3 days respectively at 24, 28 and 32°C. Pupal stages were first encountered on an average of 26.0, 24.7 and 30.0 days after gallery initiation when held at 24, 28 and 32°C respectively. Teneral adults were first encountered on an average of 31.3, 27.3 and 35.3 days after gallery initiation at temperatures 24, 28 and 32°C respectively (Table 4-3).

Based on the cumulative data of two studies, highest oviposition per log was observed at 28 followed by 24, 32 and 20°C. Similarly highest number of larval and teneral adults were encountered at 28, followed by 24, 32 and 20°C. Highest number of pupal stages was observed at 24°C followed by 28, 32 and 20°C. Similarly highest number of larval and teneral adults were encountered at 28, followed by 24, 32 and

20°C. These suggest that the optimum temperature for development of beetle is around 28°C (Table 4-4) (Figures.4-3, 4-4, 4-5, 4-6).

Discussion

Temperature influences the development of immature insects. In nature, insects are not normally exposed to constant temperatures, but the controlled study of insect development at constant temperatures provides an important knowledge of insect development and dynamics.

I exposed egg and pupal stages of redbay ambrosia beetle to constant temperatures ranging from 12-36°C. There was no development of egg or pupal stages at 12°C. Development of egg stage was observed between 16-36°C, and development of the pupal stage between 16-32°C. In similar studies with related species there was no development reported from the egg and pupal stages of *Xyleborus fornicatus* held at 15°C. Development of *X. fornicatus* egg stage occurred over the temperature range of 18-32°C when constant temperatures ranging from 15-32°C were tested. Similarly, pupal development temperature occurred from 18-32°C, when constant temperatures ranging from 5-32°C were tested (Walgama and Zalucki 2007, Gadd 1949). Development temperature of eggs of *Ips calligraphus* was observed from 12.5-35.0°C, and for pupae at 12.5-37.0°C when constant temperatures between 10°C and 37.5°C were tested (Wagner et al.1987, Wagner et al. 1988). *Ips avulsus* egg and pupal stages developed at temperatures ranging between 15-35°C when tested at seven constant temperatures between 10-35°C (Wagner et al. 1988). Thus, redbay ambrosia beetle egg and pupal development range temperatures were similar as other bark beetles.

In *Xyleborus glabratus*, the shortest development time for egg and pupal stages was observed at 28°C based on the experimental values, suggesting optimum

temperature near 28°C. The development of egg and pupal stages was linearly related between 16-28°C. *Xyleborus glabratus* estimated egg and pupal development thresholds were 10.9 and 11.3°C, which are slightly lower than the experimental threshold of 12°C. Pupal development threshold was slightly higher than the egg development threshold. The estimated development thresholds for egg and pupal stages of *Xyleborus fornicatus* were 15.7 ± 0.5°C and 14.3 ± 1.4°C (Walgama and Zalucki 2007). Similarly, Danthanarayana (2003) calculated lower development thresholds of egg and pupal stages of *Xyleborus fornicatus* to be 15 and 14 days, respectively. The estimated lower development threshold calculated for *Ips typographus* was 10.6°C and 9.9°C for egg and pupal stages, respectively (Wermelinger and Seifert 1998). Our estimated thresholds were below the lowest temperature tested experimentally where no growth took place. This discrepancy might be due to non-linear relation between temperature and development rate near the threshold temperatures (Wagner et al. 1991).

In two independent studies, we studied the life cycle and development of the beetle in the avocado logs. Similar pattern of development were observed in both studies with no development at 12, 16 and 36°C and incomplete development at 20°C. This suggests that beetle optimal temperature for development between 24-32°C indicating temperature conditions of Florida to be suitable for the optimal development of beetle.

In similar studies conducted with *Ips typographus* development time from egg to, adult stage averaged 48.9, 29.1, 20.1, 17.3, 13.2 days at temperatures of 15, 20, 25, 30 and 33°C, respectively (Wermelinger and Seifert 1998). Generation time for *Ips*

typographus averaged 50.2±2.05, 32.1±1.33, and 30.7±0.87 days at 20, 25 and 30°C, respectively (Wermelinger and Seifert 1999). *Dendroctonus ponderosae*, when reared in the bolts of lodgepole pine (*Pinus contorota* var *latifolia* Engelmann) and in axenic media, displayed arrested development at larval stage at 10°C and 15°C but complete development to adult at 24°C and 28°C. Egg to adult development took 30.2 days at 24 (Safranyik and Whitney 1980). Similarly, Whitney and Spanier (1982) reported egg to adult development of *Dendroctonus ponderosa* beetles in 31 days. The development and emergence of *Ips avulsus* from laboratory-infested loblolly pine logs (*Pinus taedea* L.) required 2 months at 20°C and 2 weeks at 35°C (Wagner et al. 1988).

The redbay ambrosia beetle required a month for development to teneral adults and about two months to emerge from the swampbay logs from the laboratory-infested logs (Brar et al. 2012b). Similarly, we obtained complete development of the beetle at 24, 28 and 32°C, with teneral adults observed after about 34, 27 and 37 days after initial infestation, respectively. It appears that *Ips avulus* and *Ips calligraphus* are comparatively more heat tolerant than redbay ambrosia beetle based shorter developmental times for egg to adult stage at higher temperatures.

In conclusion, temperature had significant effect on the development of egg and pupal stages of redbay ambrosia beetle. The exact length of development of the larval stage could not be assessed because of unavailability of diet on which to rear it. The temperature had significant effects on the duration of the life cycle in the avocado logs. We hypothesize that redbay ambrosia beetle will have more generations per year in lower latitudes as compared to higher latitudes. The redbay ambrosia beetle is expanding its range at pace more rapidly than expected by earlier models (Koch and

Smith 2008). It may be important to use non-linear models to describe development at temperature extremes. Further investigations needs to be conducted on the larval development and the development of beetle along with its obligate symbionts. This information will help in predicting the distribution of beetle and the predicting the establishment of beetle-fungus complex in host distribution in different latitudes in North America.

Table 4-1. Mean ± S.E of developmental periods of eggs and pupae at constant temperatures.

Temperature (°C)	Developmental period in days			
	Eggs		Pupae	
	N	Mean ± S.E	N	Mean ± S.E
12	30	no egg hatch	24	no egg hatch
16	30	21.1±0.75a	38	12.9±0.51a
20	56	9.5±0.21b	34	9.1±0.31b
24	36	6.6±0.30c	26	5.8±0.20c
28	41	3.9±0.17d	26	4.3±0.22c
32	24	7.3±0.51c	28	6.4±0.26d
36	20	10.9±0.75b		

Means followed with same letter are not significantly different based on Tukey - Kramer test for difference of means ($P < 0.05$).

Table 4-2. Linear regression parameters of development rate of eggs and pupae of *Xyleborus glabratus*.

Linear regression parameters	Life stage	
	Eggs	Pupae
Intercept ± SE	-0.262±0.024	-0.158±0.02
Slope ± SE	0.0187±0.001	0.0143±.001
R^2	0.664	0.635
[a] *p* value	<0.0001	<0.0001
[b] t min ± SE	10.9±0.55	11.3±0.65
[c] K ± SE	55.5±3.3	69±4.5

[a] P value test of significance of the regression coefficient.

[b] Tmin = intercept/slope; t represents the lower temperature threshold expressed in °C.

[c] K= 1/ Slope; K represents the thermal constant expressed in degree days.

Table 4-3. Development of *Xyleborus glabratus* in the logs of avocado tree (*Persea americana Mill*) based on the encounter of different developmental stages every other day.

		Developmental period in days							
	Temperature (°C)	Eggs	S.D	Larvae	S.D	Pupae	S.D	Teneral adults	S.D
Study 1									
	16								
	20	22		24		40			
	24	17	1.4	18	2	27	4.2	36	2.9
	28	14	2	16	2	25	4.2	26.7	1.2
	32	18	2.9	18		38		40	
Study 2									
	12								
	16								
	20								
	24	13.3	1.2	22	2	26		31.3	2.3
	28	10.7	2.3	16.7	3.1	24.7	2.3	27.3	1.2
	32	15.3	1.2	19.3	2.3	30	5.3	35.3	2.3
	36								

No development was observed at temperatures where there are no values

Table 4-4. Number of different developmental stages encountered during the development of beetle in the avocado logs

Temperature (°C)	No of different developmental stages			
	Egg	Larva	Pupa	Teneral adult
20	17	20	3	------
24	80	106	62	11
28	121	251	55	33
32	50	84	15	17

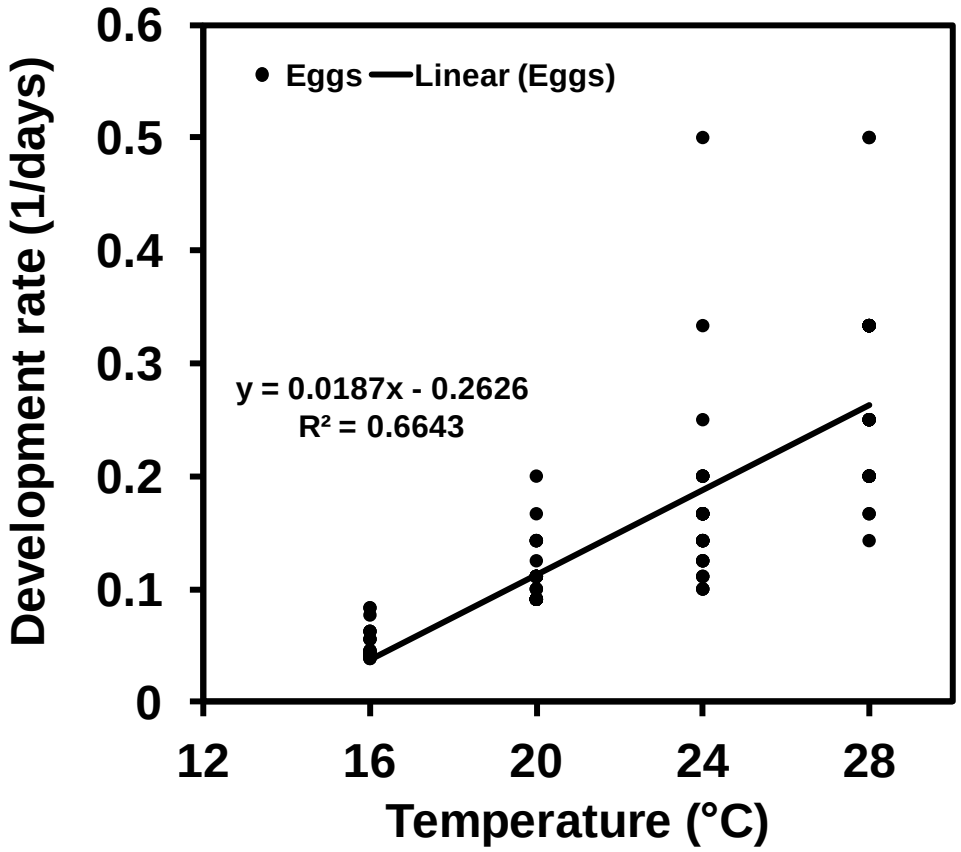


Figure 4-1. Linear regression of the development rates of eggs of *Xyleborus glabratus*

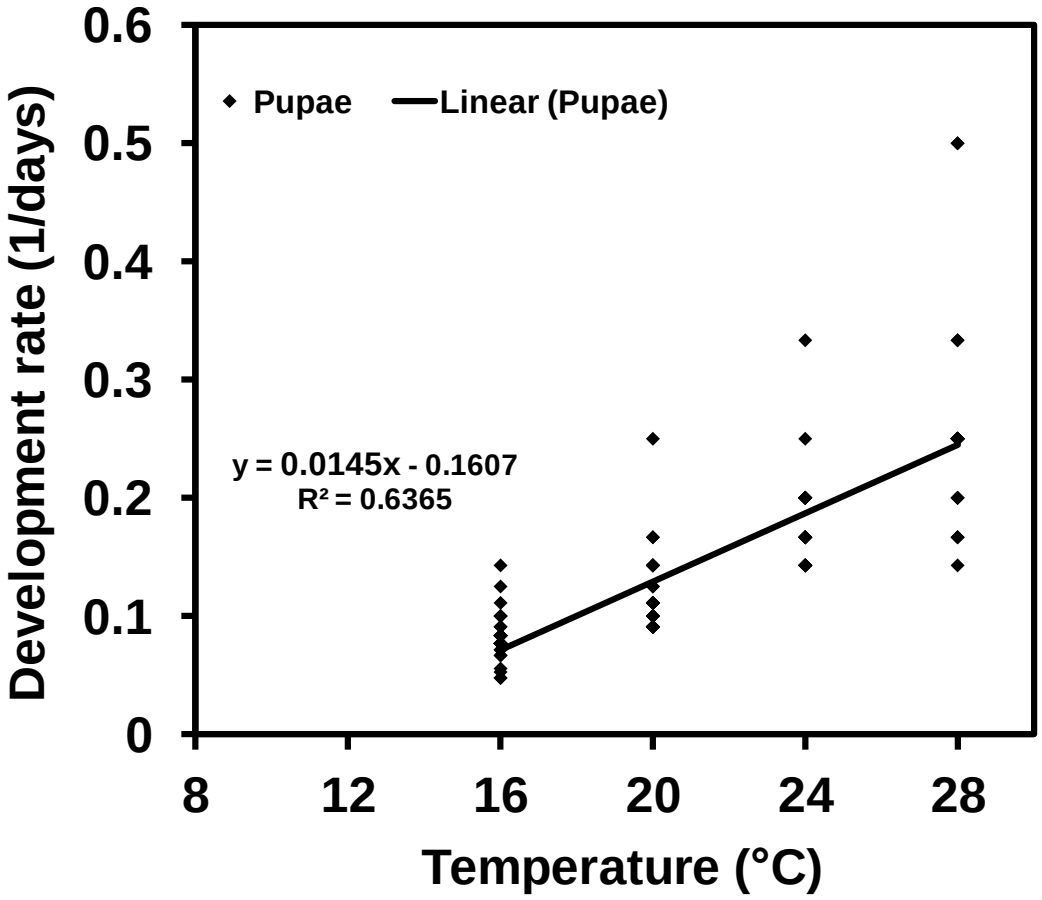


Figure 4-2. Linear regression of the development rates of pupae of *Xyleborus glabratus*

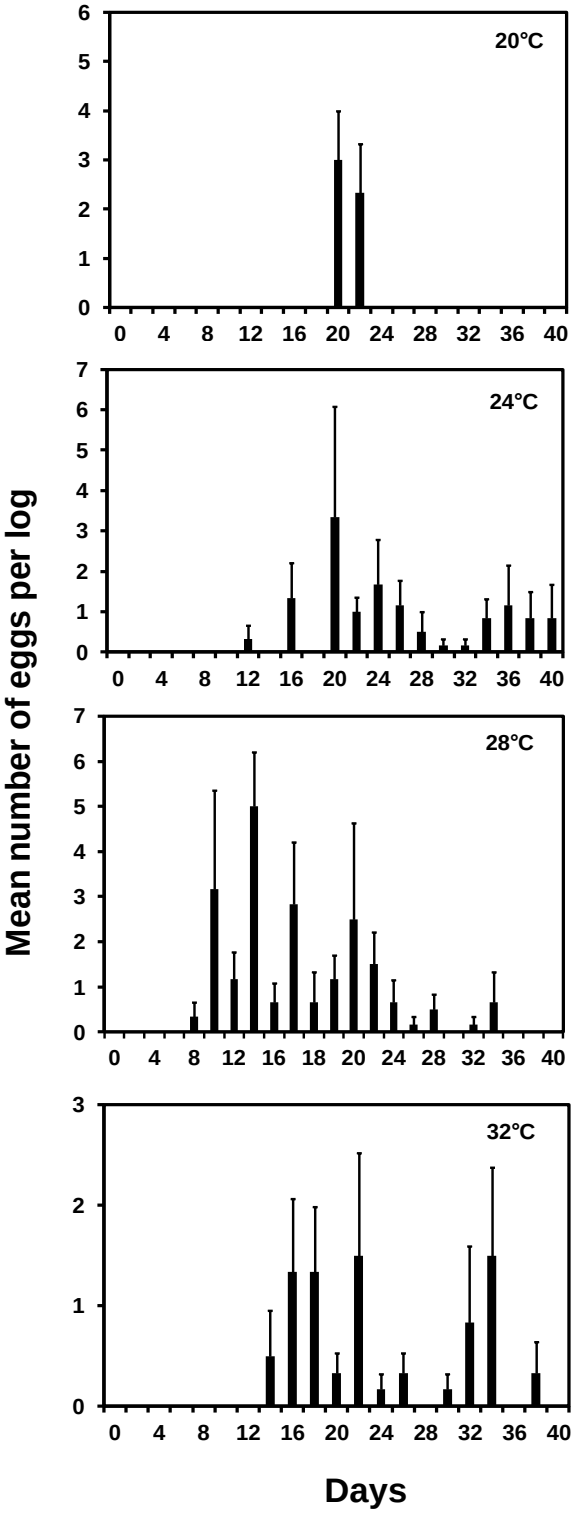


Figure 4-3. Mean ± SE of number of egg stages encountered in the avocado logs at constant temperatures.

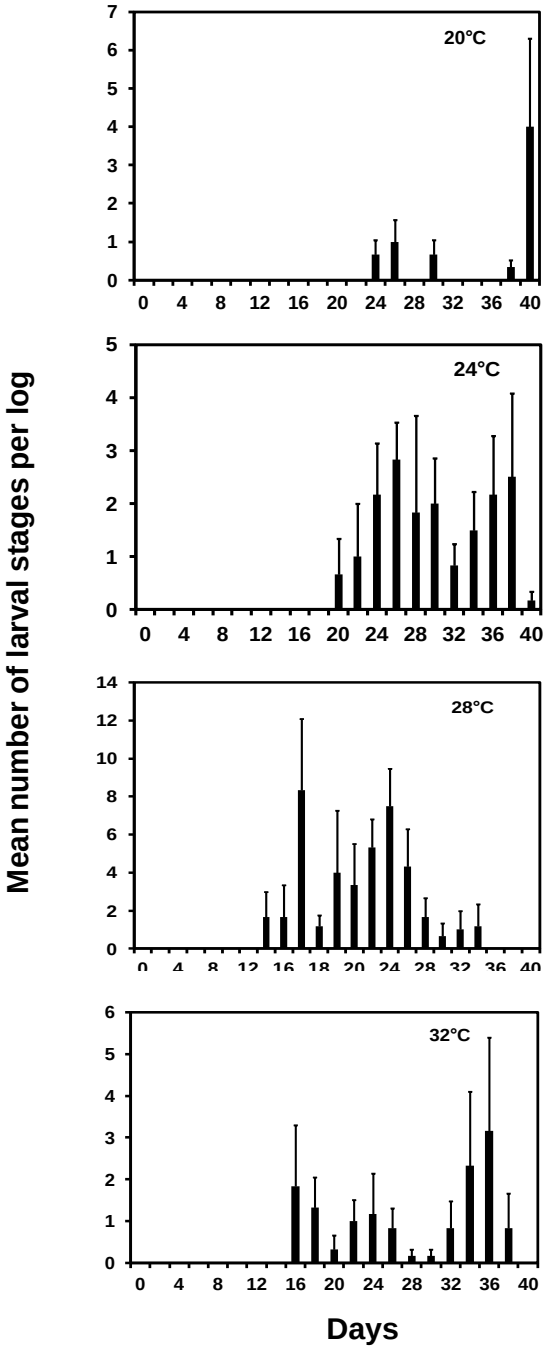


Figure 4-4. Mean ± SE of number of larval stages encountered in the avocado logs at constant temperatures.

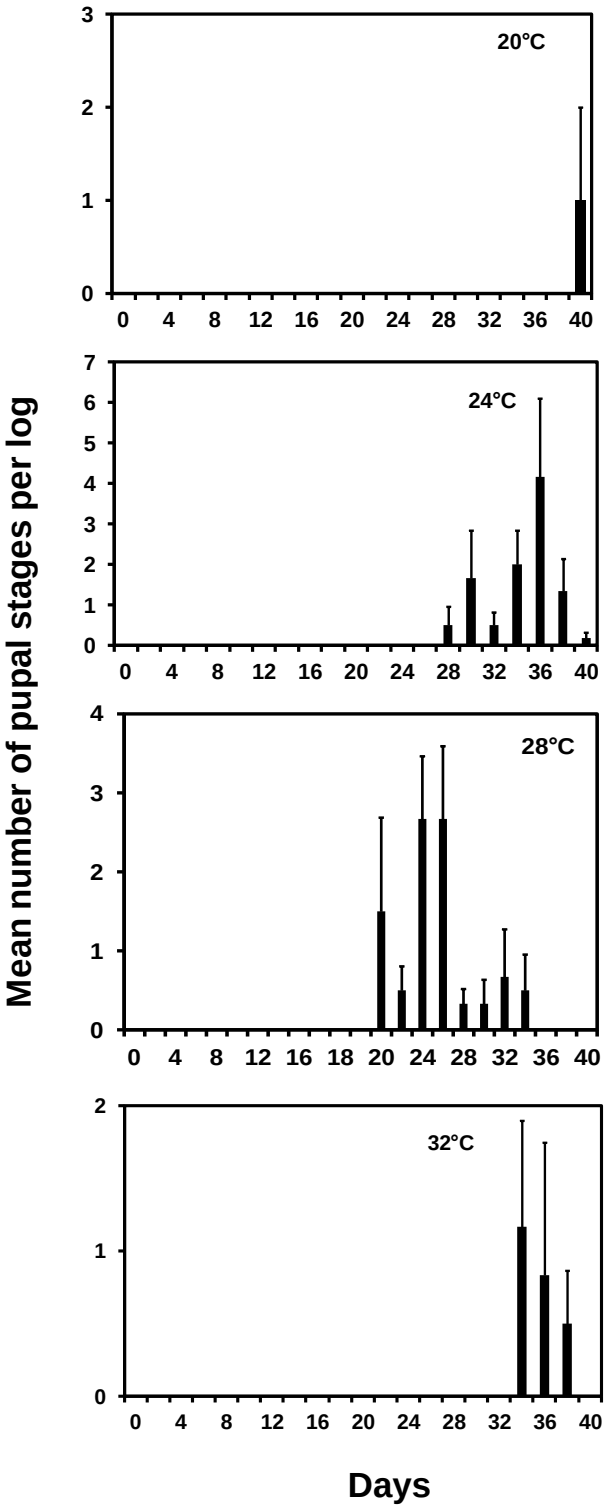


Figure 4-5. Mean ± SE of number of pupal stages encountered in the avocado logs at constant temperatures.

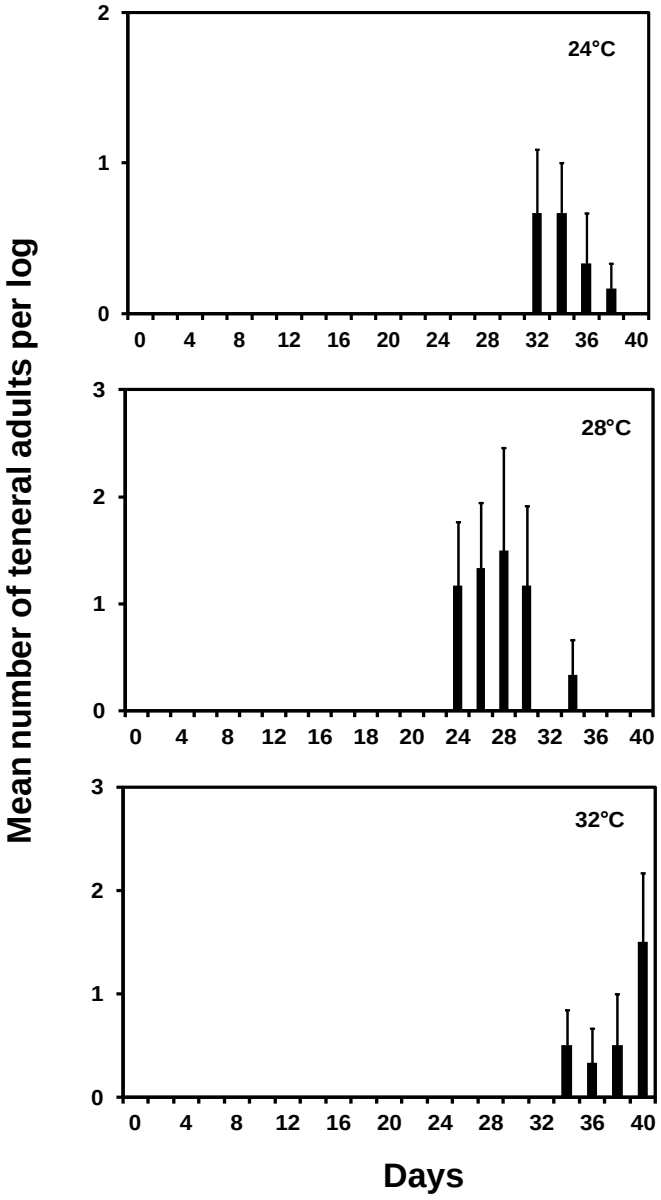


Figure 4-6. Mean ± SE of teneral adults encountered in the avocado logs at constant temperatures.

CHAPTER 5
EFFECT OF TRAP SIZE AND HEIGHT AND AGE OF LURE ON SAMPLING OF *XYLEBORUS GLABRATUS* (COLEOPTERA: CURCULIONIDAE: SCOLYTINAE), AND ITS FLIGHT PERIODICITY AND SEASONALITY

Xyleborus glabratus (Coleoptera: Curculionidae: Scolytinae) is a non-native pest in the US that transmits the causal pathogen of laurel wilt disease to plants belonging to the Lauraceae. To improve the current monitoring and survey techniques of *X. glabratus,* various traps were tested and flight behaviors studied in natural areas with host species in Alachua County, Florida. Daylight flight rhythm was studied at Austin Cary Memorial Forest twice in Sep 2010 using sticky traps baited with manuka lures showed that *X. glabratus* flies mostly between 1600 and 1800 h daylight saving time. Flight height of the beetle was determined in a trapping study using ladder-like traps. The largest number of beetles was trapped at heights of 35-100 cm above the ground. Seasonality of *X. glabratus* was studied in Florida from Mar 2010-Dec 2011. Three peaks of trap catches occurred during Apr 2010, Oct 2010 and Mar 2011. To find the optimal Lindgren funnel trap design for *X. glabratus*, a study was conducted using 4, 8, 12 and 16 funnels per trap. Funnel traps with 8, 12, 16 funnels per trap captured similar numbers of *X. glabratus,* but significantly more than with 4 funnels per trap. The effect of aging of manuka lures was studied at 2 different sites in Alachua County, Florida. New manuka lures trapped significantly more *X. glabratus* than lures aged 2, 4 and 6 wk. Trap color, whether black, white, blue, yellow, red or transparent, had no significant influence on the number of *X. glabratus* trapped.

Background

The redbay ambrosia beetle (*Xyleborus glabratus* Eichhoff) (Coleoptera: Curculionidae: Scolytinae) is a non-native ambrosia beetle that acts as a vector of the pathogenic fungus, *Raffaelea lauricola* (Fraedrich et al. 2008; Hanula et al. 2008). It causes laurel wilt in species of the family Lauraceae, including avocado (*Persea americana* Mill), redbay (*Persea barbonia* (L.) Spreng.), swampbay (*Persea palustris* (Raf.) Sarg.), sassafras (*Sassafras albidum* (Nutt.) Nees), pondspice (*Litsea aestivalis* (L.) Fernald), pondberry (*Lindera melissifolia* (Walter) Blume,), and camphor (*Cinnamomum camphora* (L.) Sieb). *Xyleborus glabratus* was first discovered at Port Wentworth near Savannah, Georgia in 2002 (Rabaglia et al. 2006) and has since established in South Carolina, Georgia, Florida, Alabama and Mississippi. *Xyleborus glabratus* is a small cylindrical beetle about 2 mm in size. Males are flightless and smaller than females. The beetle actively carries the fungus *Raffaelea lauricola* in its mycangia along with *R. arxii*, *R. subalba*, *R. ellipticospora*, *R. fusca* and *R. subfusca* (Harrington et al. 2008; Harrington et al. 2010). Both the larvae and adults of the beetle are thought to feed on the fungal complex that grows inside the galleries formed by the adult females. The beetle inoculates the tree with *R. lauricola* while excavating the galleries and, subsequently, infects the host systemically, causing a vascular wilt and tree death within a few weeks to months of infection. Laurel wilt has caused mortality of yard and experimental avocado trees (Mayfield et al. 2008) and now threatens the commercial avocado production in Florida (Ploetz and Peña 2007; Crane et al. 2008; Ploetz et al. 2011).

In order to develop efficient management and monitoring strategies, a greater understanding of the life history and flight dynamics of the beetle is required.

Preliminary studies have shown that adult beetles were active throughout the year with high activity in Sep in South Carolina and Georgia (Hanula et al. 2008; Hanula et al. 2011).

Manuka oil and phoebe oil can be used as attractive baits for trapping *X. glabratus* (Hanula and Sullivan 2008). However, Kendra et al. (2011b) demonstrated that phoebe lures attracted more *X. glabratus* than manuka lures. *Xylebrous glabratus* was significantly more attractive to scents from *Raffaelea lauricola* as compared with scents from non-symbiotic fungi *Trichoderma* and ethanol (Hulcr et al. 2011). In the field tests conducted in Florida, the *X. glabratus* was attracted to Manuka lures, phoebe lures, lychee wood and avocado wood. Lychee wood a presumed non-host attracted more *X. glabratus* adult beetles as compared to avocado wood in two choice laboratory assays. The emissions of four sesquiterpenes, a-copaene, b-caryophyllene, a-humulene and cadiene from host trees of the family Lauraceae, lychee wood, manuka lures, phobe lures were identified as potential host based attractants with a-coapene as the primary kairomone for dispersing females. (Niogret et al. 2011, Kendra et al. 2011a, Kendra et al. 2011b Kendra et al. 2012)

Monitoring and survey of invasive bark beetles and ambrosia beetles are generally conducted using Lindgren multifunnel traps (Lindgren 1983; Miller and Duerr 2008; Kendra et al. 2011). Hanula et al. (2011) recommended using funnel traps baited with a single manuka lure for trapping *X. glabratus*. We therefore designed tests to compare the 4-, 8-, 12-, 16-funnel Lindgren traps to find the optimal length of funnel trap for trapping *X. glabratus*. We investigated the effectiveness of the manuka bait as it ages in the hot and humid climate of Florida during field tests. In the previous studies, it was

reported that 85% of *X. glabratus* were trapped at 1.5 m above the ground when flight height was investigated between 1-15 m above the ground (Hanula et al. 2011). We further investigated the flight height of the *X. glabratus* for a height of 0-3.45 m above the ground, to determine the height of the trap having the highest probability of trapping the beetle. We also investigated the flight periodicity of *X. glabratus* and the seasonality of beetle. This research also included evaluation of trap color.

Materials and Methods

Daylight Flight Periodicity

To study the daylight flight periodicity of *X. glabratus* flight activity, 2 independent studies were conducted from 17-21 Sep and 23-30 Sep 2010 at the Austin Cary Memorial Forest (ACMF), Alachua County, Florida (N 29° 45.084′ W 082°12.875′). ACMF has approximately 800 ac of planted pine, primarily slash pine, 900 ac of 60-80 yr old naturally regenerated pine (predominately longleaf pine), 35 ac of bottomland hardwood, including native Lauraceae, (i.e., *Persea borbonia*), 265 ac of cypress ponds and cypress or hardwood drains, and 40 ac of non-timbered land. Transparent plexiglass panels 20 cm wide and 41 cm high were used as traps. Transparent films (3M™ Write On transparency film, AF4300) smeared with Tree-Tangle foot® (Tree Tanglefoot Company, Grand Rapids, Michigan) were clipped on both sides of the plexiglass with the help of small binder clips (1.9 cm, Office Depot). A manuka lure (Synergy Semiochemicals Corp., British Columbia, Canada) was used as an attractant and was tied at the top of the plexiglass panel. Hourly environmental data for Alachua County, Florida (fawn.ifas.ufl.edu) were used to assess the correlation of hourly trapping of *X. glabratus* with solar radiation. Solar radiation was recorded at the Florida

Automated Weather Network site at Department of Agronomy Forage Reseach Unit, Alachua County (N 29° 48.160' W 082° 24.649'). The distance between the two sites is about 20 km.

The traps were hung between 2 non-host (*Pinus* spp.) trees at an average height of 0.5 m above the ground. Each trap was ca. 5 m away from a host tree, i.e., *Persea borbonia.* Eight traps were used, with each trap 10 m apart. The numbers of *X. glabratus* trapped each h were counted using a 10X hand lens and removed after each observation. During the first study, daylight observations were recorded from 700-1900 h each d while during the second study daylight observations were recorded from 1200-2000 h. Observations were taken using daylight saving time (DST).

Trap Height

The effect of trap height on catch of *X. glabratus* was studied at ACMF from 5-13 Oct 2010. Ten transparent plexiglass panels panel (20 × 30 cm) were joined lengthwise with 5 cm distance between each plexiglass (ladder-like trap). Each panel was numbered, with the top panel numbered trap 10 and lowest panel (touching the ground) numbered trap 1. A manuka lure was tied between each plexiglass panel. A transparent film was clipped on both the sides of plexiglass as described above and Tree-Tanglefoot was smeared on the transparent film. The total trap height extended 3.45 m above the ground. Traps were hung at 5 different locations within the forest. Traps were set 10 m from each other and at a distance ca. 5 m away from the host trees. Traps were hung between 2 non-host trees (*Pinus* spp.). Transparent films were removed each d and transferred to the laboratory where *X. glabratus* beetles were counted using a 10X hand lens.

Trap Design

The optimal trap design for trapping *X. glabratus* was studied using 4- , 8- , 12- and 16-funnel Lindgren multifunnel traps. Traps were hung in 5 blocks (locations). Each block was supplied with 1 funnel trap of each design. The various traps were hung 50 m apart from each other in the 3 blocks at Austin Cary Memorial Forest (ACMF), in 1 block at Ordway Swisher Biological Station (OSBS), Alachua County, Florida (N 29°41.040′ W 082°22.109′) and in 1 block at Hatchet Creek Wildlife Management Area (HCWMA), Alachua County, Florida (N 29°42.509′ W082°12.502′). OSBS is characterized by a mosaic of wetlands and uplands that include sandhills, xeric hammock, and upland mixed forest that includes *P. borbonia*, swamps, marshes, clastic upland lakes, sandhill upland lakes, and marsh lakes. HCWMA comprises 1,932 ha of mixed canopy of hardwoods that include *Persea pallustris*, and *P. borbonia* with cypress as well as stands of slash and loblolly pine, *Pinus taeda* L.; Pinales: Pinaceae. In each of the above mentioned 5 blocks, the traps were spaced at least 10 m apart. Each trap was suspended at 0.5 m above the ground from a rope tied between 2 non-host trees. Each trap was ca. 5 m away from each of the trees. Manuka lure was used as an attractant. The manuka bait was tied half way between the lower and the top funnel. Manuka lures were replaced monthly from Mar-Oct 2010 and biweekly from Oct 2010-Dec 2011. A wet collecting cup (Synergy Semiochemicals Corp., British Columbia, Canada) was placed in the lower funnel and filled with antifreeze (Prestone prediluted anti-freeze, Prestone Corp. Danbury, Connecticut). The contents of the cup were collected every 14 d and brought to laboratory where X. glabratus specimens were recorded using a microscope. This study was conducted from Mar 2010-Dec 2011.

Seasonality

Annual changes in abundance of *X. glabratus* in 3 different areas in Alachua County, Florida were determined based on the mean number of *X. glabratus* trapped every 2 wk for each kind of trap design. These bimonthly trap catches were plotted and their trends compared from Mar 2010-Dec 2011.

Trap Color

Black, red, yellow, blue, white and transparent colors were tested for influence on *X. glabratus* trap catch. Plywood panels were cut to 20 × 41 cm and painted with 5 colors, i.e., black, red, yellow, blue, and white (Rust - Oleum® Gloss protective enamel spray paint). The colors of the traps were described by analyzing JPEG images of different colored traps using Java software described by Byers 2006 (Byers 2006). The color attributes measured were RGB (red, green, blue) values, trichometric percentages, and HSL (hue, saturation, luminosity) values (Table 1). The different colored traps were photographed 1600 h in the sunlight on 3 Jan 2012 using a Canon Power Shot SD 880 IS digital camera at 2048 × 3648 pixel resolution. Transparent plexiglass was also cut at the same length as plywood panels (20 × 41 cm). Transparent film was attached to each trap with Tree-Tanglefoot smeared on it as described in the flight periodicity procedure. Five blocks were set up at 5 different locations. One block was at OSBS and another at HCWMA plus 3 blocks at ACMF (blocks separated by at least 50 m). Each of these 5 blocks had a different color trap and a transparent plexiglass trap. Within each block, traps were at least 10 m apart. The traps were hung between 2 non-host trees about 0.5 m above the ground. The study was conducted from 16 Aug 2010-8 Oct 2010. The transparent films were brought to the laboratory every other wk and *X. glabratus* were counted using a 10X hand lens.

Manuka Lure Aging and Effectiveness of Aged Lures

Manuka lures (P385-Lure M, Synergy Semiochemicals Corp., British Columbia, Canada) were aged 2, 4 and 6 wk by hanging them on non-host trees in the field (ACMF). After aging, manuka lures were placed halfway between the top and lower funnel of each 4-funnel Lindgren multifunnel trap. A wet collecting cup was fixed below the bottom funnel and filled with antifreeze. The contents of each collecting cup were removed every 14 d and bought to the laboratory where *X. glabratus* specimens were recorded using a microscope. Four blocks of traps were placed in the field (3 at ACMF and one at the OSBS). Each block had traps with different aged manuka lures. Traps were spaced approximately 10 m. The control treatment was a new manuka lure (no aging). This study was conducted from 16 Aug 2010-8 Oct 2010.

Statistical Analysis

Data were analyzed using SAS (SAS Institute 2004). The data from the diurnal flight periodicity studies were analyzed by repeated measure analysis of variance using Proc GLIMMAX with an individual trap as the replicate. Flight periodicity data for both the studies between 1200h-1900h was combined and correlated with the solar radiation for same times using PROC CORR (Pearson correlation coefficient) in SAS. Trap catch data at each height were analyzed after log-transformation using the repeated measure of analysis of variance using Proc GLIMMAX with an individual trap as the replicate. Proc GLIMMAX was used for repeated measure analysis of variance of trap design, trap color and manuka lures studies with individual blocks as a replicate. Means were separated using the Tukey-Kramer multiple comparisons test.

Results

Daylight Flight Periodicity

There was a significant effect the time (h) of day on the mean number of *X. glabratus* captured per trap per h in the first study (F = 7.47; df = 11, 464; $P < 0.0001$). During daylight hours, there was only one peak flight of the beetle, which occurred between 1700–1900 h (Figure.5-1). A similar flight trend was observed during the second study. There was a significant effect of time of d (h) on the mean X. *glabratus* numbers/trap/hr during the study (F = 11.72; df = 7, 49; P < 0.0001) with one flight peak between 1600-1800 h. The least number of beetles were trapped between 1900-2000 hr. (Figure 5-2). For both the studies mean *X. glabratus* numbers/trap/h between 1200h-1900h were significantly negatively correlated with solar radiation (r = -0.36, P < 0.0001, N= 616). The sunset time averaged 19.30 h for first study and 19.14 h for second study (Edwards 2012)

Trap Height

Height of the trap above the ground had significant effects on the number of *X. glabratus* trapped (F = 36.30; df = 9, 36; $P < 0.0001$). The highest numbers of *X. glabratus* were trapped on panels 35-100 cm above the ground. The fewest *X. glabratus* were trapped on the panels 315-345 cm above the ground. Therefore, the number of beetles trapped decreased with increasing height (Figure.5-3).

Trap Design

There was a significant effect of trap design on the trap catch of *X. glabratus* (F = 5.24; df = 3, 426; P = 0.0015). Four-funnel traps captured the least number of beetles. There was no significant difference between captures of beetles in 8- , 12- and 16-funnel traps (Table 5-2). There was a significant difference in capture per funnel for

each trap design (F = 11.52; df = 3, 83; P < 0.0001). Most beetles per funnel were trapped in the 4-funnel (mean = 1.1 per funnel per 2 wk, SE = 0.18) and 8-funnel trap (mean = 1.0 per funnel per 2 wk, SE ± 0.18) and the fewest were captured in the 12-funnel trap (mean = 0.59 per funnel per 2 wk, SE ± 0.18) and 16-funnel trap (mean = 0.46 per funnel per 2 wk, SE ± 0.18) (Table 5-2).

Seasonality

Xyleborus glabratus were trapped throughout the period of the study from Mar 2010 - Dec 2011. During the winter months of Nov, Dec and Jan very few beetles were trapped with trap catches ranging from 0.5-3.3 per trap per 2 wk. The greatest numbers of beetles were trapped in early Apr 2010 and in early Mar 2011 with trap catches ranging from 42.9 to 49.9 per trap per 2 wk, respectively. During the period of the study, 3 peaks of trap catches were observed, i.e., in Apr 2010, Oct 2010 and Mar 2011. Trap catches of beetles declined from Mar 2011 to Dec 2011 (Figure.5-4).

Manuka Lure Aging

Age of the lure had a significant effect on the numbers of *X. glabratus* trapped (F =17.34; df = 3, 11; P = 0.0002). The highest numbers of beetles were caught when fresh lures were used (mean = 9.5 per trap per 2 wk, SE = 2.7). There were no significant differences between the traps catches when the lures were 2, 4 and 6 wk old (Table 5-3).

Trap Color

Color had no significant effect on catch per trap of *X. glabratus* (F = 0.87; df = 5, 29; P = 0.5153). Nevertheless the black colored traps caught the most beetles (mean = 1.1 per trap per 2 wk, SE ± 0.5), and the transparent traps caught the least (mean = 0.5 per trap per 2 wk, SE ± 0.2).

Discussion

Daylight flight of *X. glabratus* started in the late afternoon for a period of 3 h from about 1600-1900 h, ending at sunset. A similar pattern of unimodal behavior in midday was observed in the bark beetle *Ips typographus L.* and *Pityogenes chalcographus* L. in Sweden (Byers 1983). In contrast, bimodal flight with peaks in early morning and soon after dusk have been observed for *Orthotomicus erosus* and *Pityogeges calcaratus* (Mendel et al. 1991). High flight activity in low light was recorded for *Gnathotrichus retusus* (Lee) (Liu and Mclean 1993) and *G. sulcatus* (Lee) (Rudinsky and Schneider 1969). This suggests that flight pattern is species specific for the Scolytinae. It is probably also a function of interaction of 3 environmental cues: light intensity, temperature and humidity (Rudinsky and Schneider 1969; Liu and Mclean 1993). The unimodal flight behavior of *X. glabratus* may be considered a species-specific phototactic response to decreasing average solar radiation and decreasing average temperatures in the late afternoon. Whether solar radiation and temperature are the sole or primary factors that influence the flight behavior of *X. glabratus* would require additional data from repeated experiments during a wider range of environmental conditions.

Hanula and Sullivan (2011) reported that 85% of *X. glabratus* were trapped at a height of 1.5 m above the ground using sticky traps. We found similar results using a different type of trap (ladder- like trap) and experimental design. The maximum numbers of *X. glabratus* were caught at a height 35-100 cm above the ground, with beetle captures decreasing as the trapping height increased. We suggest that traps for *X. glabratus* should be placed between 35 cm and 100 cm from the ground in order to optimize trapping results. *Xylosandrus crassiusculus* were caught more in traps at 0.5 m

than at 1.7 and 3.0 m, whereas more *Xylosandrus germanus* were caught in traps at height 0.5 m and 1.7 m than at 3.0 m (Reding et al. 2010). *Ips duplicatus* (Sahlberg) were trapped significantly more in window-slot traps at a 1.5 m above the ground compared with traps at ground level and at 3.5 m above the ground (Chen et al. 2010). More *Ips typhographus* were trapped at 0.7 m than at heights ranging from 1.5 to 11.5 m using semiochemical lures (Byers et al. 1989). Thus, it appears that many Scolytinae fly relatively close to the ground.

In South Carolina, Hanula et al. (2008) and in Georgia, Hanula et al. (2011) detected peak activities of *X. glabratus* during Sep, with low activity in Jan and Feb. In Florida, peaks in beetle catches were observed in Mar-Apr 2010, Sep-Oct 2010 and Feb-Apr 2011, suggesting 2 major peaks of trap catches in a year. Small peaks in beetle catches were observed from May-Aug 2010. However, it is suggested that decrease in beetle catches might be a function of combination of several factors, i.e., age of the manuka lure, temperature, rainfall frequency and scarcity/absence of hosts in the study areas. For instance, baits were changed every 4 wk before Oct 2010, after which baits were changed every 2 wk, based on the results of the manuka lure aging experiment. The low trap catches of *X. glabratus* observed in the cold months of Dec and Jan can be related to low temperatures. It is possible that the second peak in trap catches during 2011 was not observed because most redbay and swampbay trees in the areas of study had already perished. For instance, during 2009 at the Ordway Swisher site, *X. glabratus* densities built up when there was a mixture of laurel wilt-symptomatic and asymptomatic trees. By 2010, all of the trees were wilted and were in decaying condition. *Xyleborus glabratus* trap averaged 0.82/trap/2 wk over the total

period of study (Jul 2010-Dec 2011). Trap catches decreased at all study sites from 2010 to 2011, due to exhaustion of host trees.

Higher numbers of *Xyleborus glabratus* were trapped in 8- , 12- and 16- funnel traps as compared with 4-funnel traps. In a similar comparison of 8- and 16- funnel trap baited with ethanol and (-) - α - pinene, greater numbers of *Xyleborus* spp. were trapped in the 8-funnel trap, whereas more *Ips grandicollis* and *Xyleborinus saxesenii* were trapped in the 16-funnel trap (Miller et al. 2009). Trap catches of *Trypodendron lineatum* increased with an increase in number of funnels per trap with 16 funnels trapping more than 12- , 8- and 4- funnel traps (Hoover et al. 2000). Results from these studies indicate that there is a relationship between funnel length and trap catches of various Scolytinae species. The number of *X. glabratus* trapped per funnel was highest in 4-funnel and 8-funnel traps. Based on the economics of using funnel traps, the 4-funnel traps are the most economical (Table 5-2) However, the optimal trap design may mainly depend on our goals (e.g., for population dynamics, 4-funnel traps may be the best, but for monitoring the appearance of *X. glabratus* in new areas, the 8-funnel might be the best).

Trap color had no significant effect on capture of *X. glabratus* in nonbaited traps. This result agrees with the results of Hanula et al. (2011) in South Carolina and Georgia. In contrast, trap color impacted the capture of other scolytinae. In Florida, multifunnel traps colored black, blue, brown, gray, green, and red trapped more *Dendroctonus frontalis* Zimmermann than white and yellow traps (Strom and Goyer 2001). Significantly, higher numbers of *Ips typographus* and *Trypodendron lineatum* were trapped in pheromone -baited flight barrier traps than were transparent, black,

green, grey or red brown as compared to white (Dubbel et al. 1985). More *Ips duplicatus* (Sahlberg) were trapped in window slot traps colored black or red compared to white or yellow (Chen et al. 2010).

Attractiveness of manuka bait decreases quickly with time in Florida, probably due to high temperatures and relative humidities. The temperature over the period of investigation from 16 Aug 2010-8 Oct 2010 averaged 24.6 ^{o}C, with maximum and minimum temperatures of 36.5 ^{o}C and 5.5 ^{o}C, respectively and with relative humidity averaging 83% (fawn.ifas.ufl.edu). In our study, we found that manuka bait loses its attraction in 2 wk. Kendra et al. (2012b) concluded that due to reduced emissions of sesquiterpene a-copaene, a-humulene, and cadinene from manuka lure the maximum field life of manuka lures is 2-3 wk Therefore, these lures should be replaced every 2 wk for optimal beetle catch.

To conclude, our studies suggest that for monitoring the spread of *X. glabratus* into new areas, the 8-funnel Lindgren funnel trap is optimal. Traps should be set at 35-100 cm above the ground for maximum probability of trapping *X. glabratus*. Manuka bait should be changed every other week, and the trap color does not influence the catch

Table 5-1. Red, green, blue values (Mean ± SE) and trichromatic percentages from areas of digital photos of colored traps analyzed by the java software from byers (2006).

Trap color	Pixels[a]	Red Mean ± SE	%	Green Mean ± SE	%	Hue Mean ± SE	%	HSL Hue	Saturation	Luminosity
Black	1223694	50±22	44	47±19	41	45±18	41	0.06	0.05	0.18
Blue	1194164	30±5	16	125±6	5	226±3	1	0.91	0.62	0.50
Yellow	1022250	175±11	6	132 ±8	6	1±3	183	0.12	0.98	0.34
Red	1228437	231±3	1	30±5	17	34±5	15	0.99	0.65	0.51
White	872410	142±11	8	143±10	7	143±10	7	0.94	0.01	0.56

[a]Areas analyzed in pixels

Table 5-2. Numbers of *Xyleborus glabratus* trapped in lindgren traps with different numbers of funnels in Alachua county, Florida from Mar 2010-Nov 2011.

Trap design	N	Mean ± SE of *X. glabratus*/trap/2 wk	Mean ± SE of *X. glabratus*/funnel/2 wk[a]	Cost of complete trap with wet cup[b] (US $)
4 Funnels	4	4.1 ± 2.1b	1.1 ± 0.18a	32.40
8 Funnels	4	7.0 ± 3.4a	1.0 ± 0.18a	43.28
12 Funnels	4	6.0 ± 3.2a	0.59 ± 0.18b	52.37
16 Funnels	4	6.3 ± 3.3a	0.46 ± 0.18b	60.40

[a]Analyses were conducted on square roots of transformed data.

[b]Costs are based on prices of Synergy Semiochemicals Corp., British Columbia, Canada.

Means followed with same letter are not significantly different based on the Tukey-Kramer test for separating means ($P < 0.05$).

Table 5-3. Numbers of *Xyleborus glabratus* trapped using manuka lures of different ages.

Aging interval in weeks	N	Mean ± SE of *X .glabratus*/trap/2 wk
0-2	4	9.5 ± 2.3a
2-4	4	1.8 ± 0.8b
4-6	4	1.5 ± 0.6b
6-8	4	0.6 ± 0.4b

Means followed by the same letter are not significantly different based on Tukey- Kramer test for difference of means ($P < 0.05$). Numbers of *Xyleborus glabratus* (mean ± SE) trapped per trap per hour over five days at Austin Cary memorial forest, Alachua County, Florida (17-21 Sep 2010) ($N = 8$). Bars with same letter are not significantly different according to the Tukey-Kramer test for difference of means ($P < 0.05$)

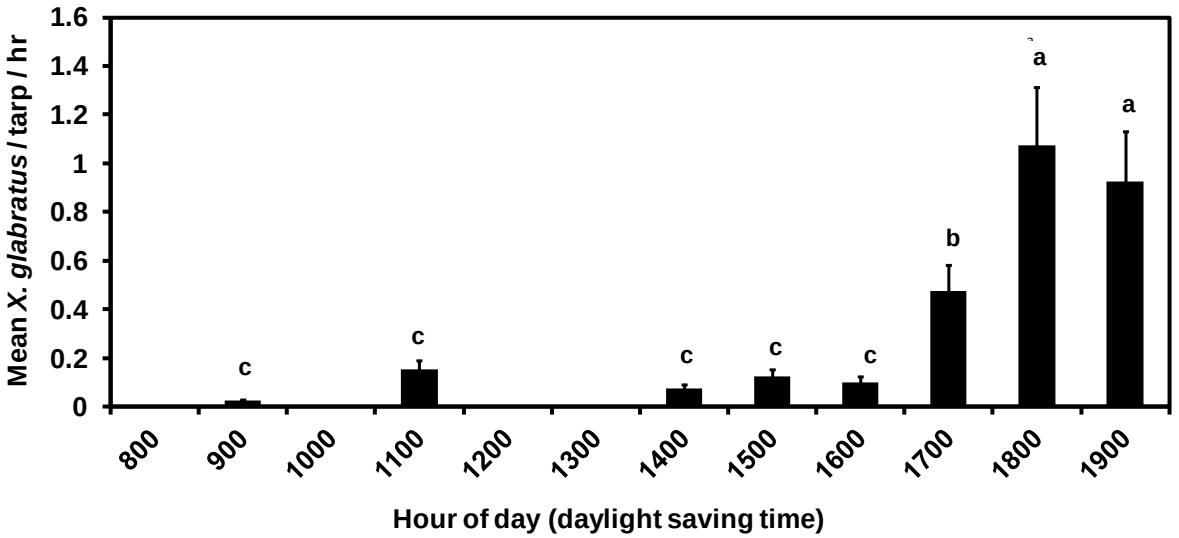


Figure.5-1. Numbers of *Xyleborus glabratus* (mean ± SE) trapped per trap per hour over five days at Austin Cary memorial forest, Alachua County, Florida (17-21 Sep 2010) (*N* = 8). Bars with same letter are not significantly different according to the Tukey-Kramer test for difference of means ($P < 0.05$)

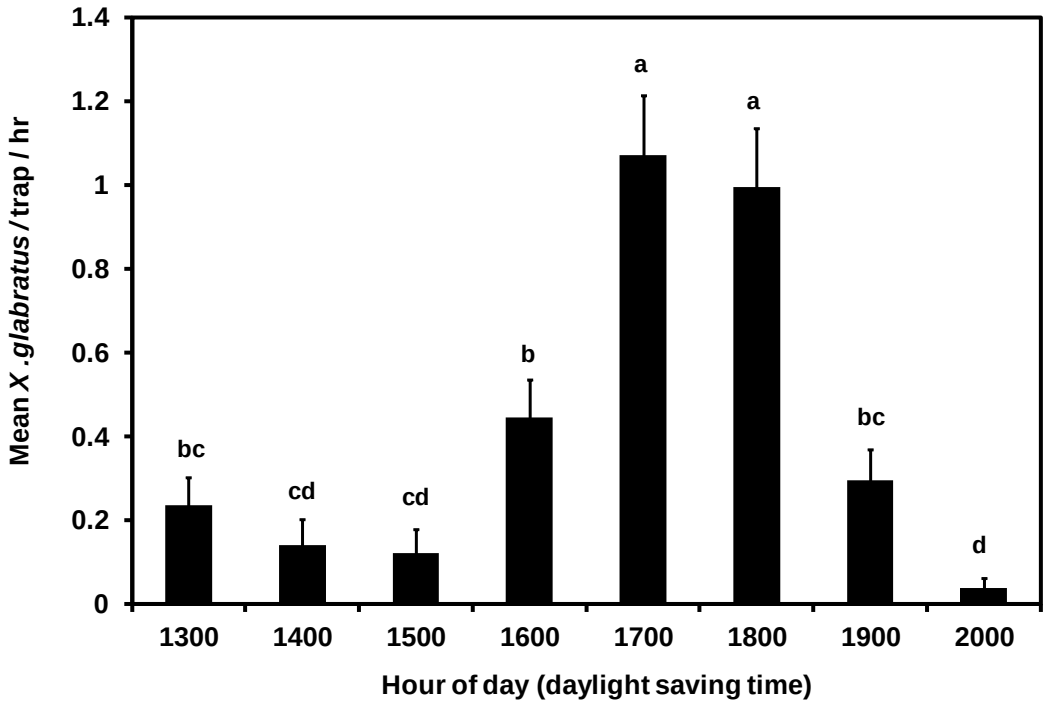


Figure 5-2. Numbers of *Xyleborus glabratus* (mean ± SE) trapped per trap per hour over eight days (23-30 Sep 2010) conducted at Austin Cary Memorial Forest, Alachua County, Florida (N = 8). Bars with same letter are not significantly different according to the Tukey- Kramer test for separating means ($P < 0.05$).

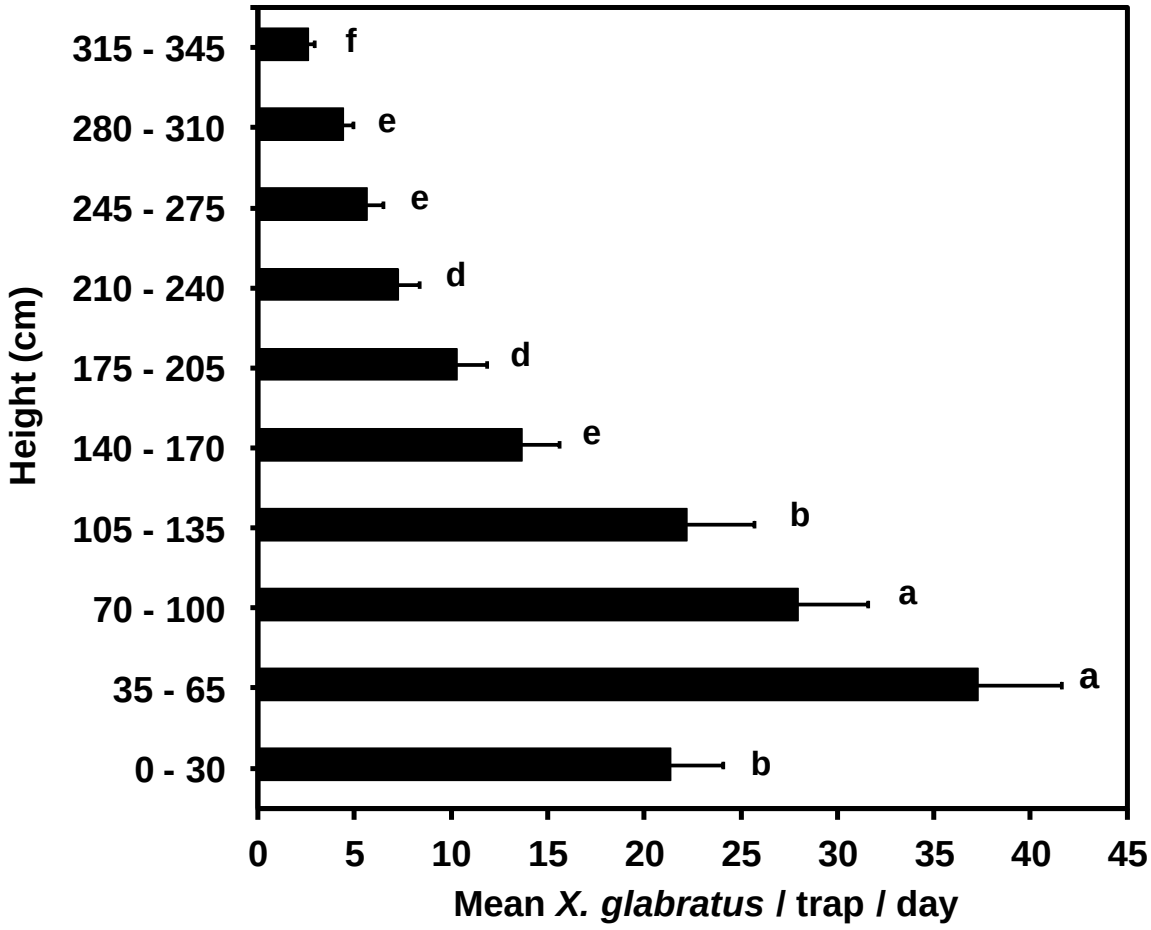


Figure 5-3. Effect of height of the trap on numbers of *Xyleborus glabratus* trapped. Bars are numbers (mean ± SE) of *X. glabratus*/trap/day in a study at Austin Cary Memorial Forest, Alachua County, Florida during (5-13 Oct 2010). Analysis was conducted on log-transformed data. Bars with same letter are not significantly different according to the Tukey-Kramer test for separating means ($P < 0.05$)

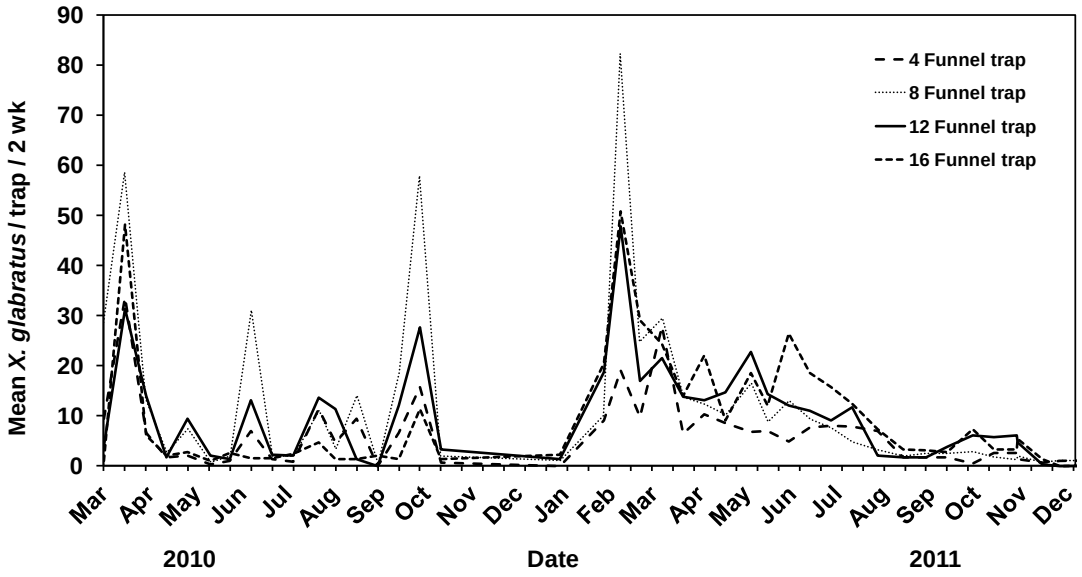


Figure.5-4. Seasonality of *Xyleborus glabratus* in Florida. Mean *X. glabratus* numbers/trap/2 wk in 4 different kinds of trap, using manuka lure. Manuka baits were replaced monthly from Mar to Oct 2010 and biweekly from Oct 2010 to Dec 2011. Study was conducted at 4 different sites near Alachua County, Florida during Mar 2010-Dec 2011. Traps were serviced every 2 wk.

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BIOGRAPHICAL SKETCH

Gurpreet Singh Brar was born in 1982 in Punjab, India. He attended school at Shahid Ganj Public School Mudki. He earned a Bachelor of Science with Honors in agriculture from Punjab Agricultural University, India and a Master of Science in plant pathology from Punjab Agricultural University, India. In 2009, he joined the University of Florida for his doctorate degree. He received his Ph.D. from the University of Florida in the fall of 2012.