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UNIVERSITY OF CALIFORNIA  
RIVERSIDE

Environmental Effects on Polyphagous Shot Hole Borer

A Dissertation submitted in partial satisfaction  
of the requirements for the degree of

Doctor of Philosophy

in

Entomology

by

Colin Yoneji Umeda

September 2017

Dissertation Committee:

Dr. Timothy Paine, Chairperson

Dr. Richard Stouthamer

Dr. Richard Redak



The Dissertation of Colin Yoneji Umeda is approved:

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Committee Chairperson

University of California, Riverside

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## **Dedications**

To my family

To my friends

And

In loving memory of,

Henry Fong

And

Kiyo Umeda

## ABSTRACT OF THE DISSERTATION

Environmental Effects on Polyphagous Shot Hole Borer

by

Colin Yoneji Umeda

Doctor of Philosophy, Graduate Program in Entomology  
University of California, Riverside, September 2017  
Professor Timothy Paine, Chairperson

Polyphagous Shot Hole Borer (*Euwallacea fornicatus*) (PSHB) is an ambrosia beetle that has invaded southern California and poses a significant threat to many species of trees, both introduced and native. It is associated with three different fungal symbionts which it grows on the walls of the galleries it forms on the inside of its tree hosts. Through a combination of mechanical damage and fungal invasion, PSHB causes dieback and eventual death of its host tree. Very little is known about PSHB as it was originally thought to be a different, congeneric beetle species. The overall goal of this dissertation is to expand the body of knowledge on the life history characteristics of PSHB, especially with regards to development. This research helps define the environmental conditions that are vital to PSHB development and estimates potential areas of risk.

PSHB reared under different temperature conditions exhibited similar developmental responses to its congener the Tea Shot Hole Borer. Its minimum and maximum temperature thresholds were around 13°C and 33°C, respectively. PSHB development rate was calculated to be optimal near 28 °C.



Using the temperature requirements of PSHB it was possible to construct a climate model with MaxEnt and CLIMEX. Using these programs, it is shown that PSHB can spread throughout the southern portion of North America into South America. With respect to California, the climate is suitable enough to allow PSHB to spread all the way through the central valley to northern California.

When comparing attack rates on host trees with different watering regimes, in three different studies, it was found that irrigation did not impact the rate of attack. In these studies, irrigation only affected the level of crown senescence.

When examining the individual effects of PSHB fungal symbionts it was found that only two of the three, *Fusarium euwallaceae* and *Graphium euwallaceae*, were able to provide enough nutrition on their own to allow for larval development to adulthood. Larvae raised on *F. euwallaceae* were larger than those raised on *G. euwallaceae*, but developed slower near optimal temperatures which could indicate a trade-off.

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## **Chapter 1**

### **Polyphagous Shot Hole Borer and *Fusarium* Dieback in California**

## **Abstract**

The Polyphagous Shot Hole Borer (Coleoptera: Curculionidae: Scolytinae), *Euwallacea* sp. near *fornicatus*, is an ambrosia beetle native to Asia that has been introduced into Israel, California, and South Africa. The beetle maintains a symbiotic relationship with three species of fungi that it transmits between host trees by carrying spores within a mandibular mycangium. These ambrosial fungi are inoculated into host trees and are the sole nutritional source for the adults and the larvae. Unfortunately for forest and resource managers, one of the fungi, *Fusarium euwallaceae*, is a moderately virulent pathogen and is responsible for causing a dieback disease in susceptible hosts. High levels of beetle infestation of susceptible host trees have resulted in high levels of mortality. The currently recognized host range for the beetle-fungus complex includes more than 200 tree species that can be attacked by the beetle, more than 100 species that can support growth of the fungus, and 49 species that can be used as a reproductive host by the insect. Many of these reproductive hosts are important agricultural crops, components of the California urban forest, or components of native riparian forest communities. Management is currently focused on monitoring using visual inspections or trapping, sanitation using solarization or chipping, and direct control using contact or systemic insecticides.



## **1. Wood Borers and Ambrosia Beetles**

As international trade and movement of people increases, there are increased movements of insects to new environments. This has become a particular problem with wood borers and ambrosia beetles (Haack 2006). The emerald ash borer, a buprestid native to Asia, has killed hundreds of thousands of trees in central North America where it has been accidentally introduced and threatens to invade the Mediterranean forests of the west (Herms and McCullough 2014). The redbay ambrosia beetle was also introduced into southeastern North America where, in combination with a highly pathogenic fungus, is killing large numbers of native trees (Mayfield et al. 2013).

Scolytinae bark beetles have a wide spectrum of symbiotic associations with fungi (Paine et al. 1997, Six and Wingfield 2011). At one end of the spectrum are species associated with fungi that contaminate the external surface of the body or with phoretic mites. At the other end of the continuum are beetles whose sole source of nutrition are the fungi transmitted into new host trees. The ambrosia beetles may be associated with one or more symbiotic fungi which are cultivated on the walls of the beetle's galleries and serve as their source of nutrition (Batra 1985, Farrell et al. 2001; Hulcr et al. 2007; Klepzig and Six 2004). These fungi allow the beetles to survive in a habitat that is low in nutritional value and provides vital components, such as proteins, for development (Beaver 1989; Kok 1979). In return the fungi are provided transportation to new hosts and purposefully propagated by the beetle. Fungi are carried between hosts in special ectodermal pouches

known as mycangia that protect and culture the fungi (Batra 1963). The ambrosia beetles may colonize a range of host species and may also be associated with both living and dead or dying trees.

## **2. Polyphagous Shot Hole Borer Biology**

The Polyphagous Shot Hole Borer (Scolytinae: *Euwallacea* sp.) (PSHB) is an invasive ambrosia beetle that has recently been found spreading throughout southern California (Eskalen et al. 2012). Based on morphological characteristics, the PSHB was initially identified as the Tea Shot Hole Borer (Scolytinae: *Euwallacea fornicatus*) (TSHB). However, Mendel et al. (2012) noted that *E. fornicatus* established in Israel exhibited previously undocumented host preferences. The two were distinguished as separate species based on DNA samples collected in California compared to those collected from tea plantations in Sri Lanka. There were significant differences in nuclear and mitochondrial DNA sequences which distinguished PSHB as a cryptic species (Stouthamer et al. 2017). Until a full taxonomic evaluation and navigation of the numerous revisions that have occurred in the genus *Euwallacea* is complete, the common name of Polyphagous Shot Hole Borer has been adopted by Eskalen et al. (2013) and referred to as *Euwallacea* sp. #1 by O'Donnell et al. (2015) and Cooperband et al. (2016). The appellation “Polyphagous” refers to the broad range of trees attacked by the beetle rather than the different kinds of fungi eaten.

The first documentation of PSHB in California was in 2003 on black locust (*Robinia pseudoacacia*) (Rabaglia et al. 2006). However, no fungal damage was recorded at the time. It was not until 2012 that nine avocado trees exhibiting branch dieback were observed in Los Angeles County (Eskalen et al. 2012). The cultivars that were attacked consisted of Hass, Bacon, Fuerte, and Nabal (Eskalen et al. 2012). Branch dieback is due to the disease *Fusarium* dieback which is caused by fungal invasion of the vascular elements of the tree, which prevents transport of water and nutrients to the branches (Eskalen et al. 2012, Freeman et al. 2013). Before this the only prior reports of damage caused by PSHB were on avocado in Israel (Mendel et al. 2012). Trees in Israel started showing symptoms of beetle attack in 2005 which includes a characteristic accumulation of white exudate around penetration site (Mendel et al. 2012). Because ambrosia beetles do not feed on the wood itself, the sawdust that is created during tunneling is cleaned out by the mother (Batra 1985) and often accumulates around the entrance to the gallery. Active galleries created by PSHB can often be found by the accumulation of sawdust on the bark of the tree along with discoloration around the gallery entrance.

The mycangia of PSHB is located in a cuticular invagination associated with the mandibles (Freeman et al. 2015). The beetle maintains a symbiotic relationship with three different fungal species that are carried within the fungus-bearing structure. The first is *Fusarium euwallaceae* which is the causal agent of *Fusarium* dieback (Freeman et al. 2013). The other two are *Paracremonium pembeum* and *Graphium euwallaceae* (Lynch

et al. 2016). Sequences obtained from fungal isolates were deposited in Genbank under accession numbers: JQ723753, JQ723760, JQ723756, and JQ723763 (Eskalen et al. 2012) and TreeBASE (submission IDs 17955, 17954) (Lynch et al. 2016). The fungi are grown on the walls of the galleries the beetle bores into its host plant and establishment of fungal growth precedes oviposition.

Female polyphagous shot hole borers are 1.8-2.5mm in length and range from brown to black in color. Males are 1.5-1.67mm in length, light brown to black in color, and lack wings. Long distance dispersal and gallery formation is strictly limited to females. Males are only able to disperse by walking to adjacent galleries on the same host. Males are less common than females and galleries will usually only contain one to two males. The exception to this is in galleries created by unmated females. The beetles have a haplo-diploid sex determination system; diploid fertilized eggs give rise to females and haploid unfertilized eggs give rise to males (Peer & Taborsky 2005). Consequently, galleries created by unmated females will consist of only male progeny (Cooperband et al. 2016).

Galleries are only about as wide as the female beetle and are initially dug to a depth of about 15 mm into the tree. As the initial gallery is formed, the fungal symbionts are established on the walls using spores carried in the mycangia. If fungal growth is sufficient, the female will lay approximately five eggs at the end of the initial gallery before starting to expand the gallery further. Eggs and larvae are usually found in clusters part way through the gallery where they are laid before the gallery is fully extended as

well as at the end of the completed gallery. The growing fungal mats are grazed upon by both larvae and adults throughout their entire occupation of the gallery. Larvae usually remain in the area where they hatched and stay in a clumped distribution throughout the gallery, even when pupating. After pupation, both sexes reach a teneral adult stage that is characterized by lack of fully developed wings and lighter coloration.

While in the gallery, the progeny beetles will mate with their siblings and continue to feed on the fungi growing on the walls. During this time, the mycangia of the females will be filled with fungal spores that will be transported to the next parental gallery. Adult beetles may remain within their natal gallery past maturation and disperse at irregular intervals. Dispersing females may either fly from their natal host to search for a new suitable host tree or they may recolonize the same host tree. The stimuli associated with the decision to disperse or recolonize are currently very poorly understood but may possibly be associated with densities of occupied galleries and condition of the natal host. Beetle emergence is usually found to coincide with periods of warmer weather.

Damage to host trees is characterized by different symptoms, depending on the species attacked. The tree may exhibit signs of *Fusarium* dieback in the branches. Dieback symptoms include wilting branches, discolored leaves, and breaking of heavy branches (Mendel et al. 2012). These symptoms will be typically observed on primary branches (Eskalen et al. 2013). However, PSHB can start galleries in branches as small as 2 cm in diameter of almost any age (Mendel et al. 2012). On the trunk of the tree the most

common symptom of beetle invasion is the presence of staining or discoloration of the bark around the entry hole due to necrosis of the tissue (Eskalen et al. 2013). Some tree species will also respond to attack with gum deposition around the attack site. Infested avocado exhibits a unique response where large deposits of white and powdery exudate composed of the sugar perseitol form around the entry hole (Mendel et al. 2012).

Infested trees have been located in Los Angeles, San Diego, San Bernardino, Riverside, Ventura, and Orange County in southern California. Records of the beetles' movement are tracked using traps baited with an attractant lure (quercivorol) set up along the borders of the known infested areas. Known host species are also visually inspected for signs of attack. The earliest mapped infestations in 2012 covered an area that included Los Angeles, Pasadena, Pomona, and Seal Beach (Eskalen et al, <http://eskalenlab.ucr.edu/distribution.html>). The following year, in 2013, the infestations had expanded its borders in each direction to include Brentwood, Altadena, Ontario, and Laguna Beach. During 2014 PSHB expanded its range northwest to Sunland above the Verdugo Mountains and east into Corona. This trend has continued in 2015 where the beetle has been found in Ventura County near Oak View and Santa Paula. 2015 saw an expansion southward into Temecula as well. No northern or southern expansion in PSHB's range was found during 2016, however the beetle moved further inland and was found infesting trees in the city of Riverside. As of 2017 no dramatic expansion of its current range has occurred.

Another congeneric species to PSHB was discovered in San Diego. The two types are distinguished by having different fungal complements and different mitochondrial DNA sequences (Stouthamer et al. 2017). The species found in California and Israel has kept the common name Polyphagous Shot Hole Borer and is referred to as *Euwallacea* sp. #1 by O'Donnell et al. (2015). The second species in California has been named Kuroshio Shot Hole Borer (KSHB), *Euwallacea* sp. #5 (O'Donnell et al. 2015). The KSHB has been originally located around the cities of Escondido and San Diego in 2014. In 2015 KSHB expanded outwards to the Santa Ana mountains and into the Tijuana River Valley where it caused significant damage to the riparian habitat (Boland 2016). The KSHB continued expand its presence in San Diego county in 2016 and two instances of KSHB were documented far north in Santa Barbara and San Luis Obispo where none of the *Euwallacea* complex had been found previously. *Euwallacea* sp. #2 is attributed to the TSHB population found invading Florida and *Euwallacea* sp. #3 and #4 are attributed to the genetically different TSHB found in Australia and Sri Lanka, respectively (O'Donnell et al. 2015)

The infested areas of Los Angeles County include the Los Angeles Arboretum and the Huntington Library and Botanical Gardens. These botanical gardens contain a wide variety of identified tree species and cultivars. Trees in these collections were surveyed in order to estimate the range of possible hosts within California. Of the 335 tree species in 85 families that were examined 207 of them showed symptoms consistent with attack (Eskalen et al. 2013). More than 100 of the tree species were susceptible to colonization

by *F. euwallaceae* (Eskalen et al. 2013), which indicates that even if the beetle doesn't reproduce inside that tree that it may still suffer the effects of *Fusarium* dieback. The number of tree species that supported reproductive success for PSHB was 19 species (Eskalen et al. 2013). That number has expanded to a list of 49 species in at least 16 families (Eskalen, <http://eskalenlab.ucr.edu/shotholeborerhosts.html>). California native forest species that are threatened include box elder (*Acer negundo*), big leaf maple (*Acer macrophyllum*), California sycamore (*Platanus racemosa*), Gooding's black willow (*Salix goodingii*), red willow (*Salix laevigata*), white alder (*Alnus rhombifolia*), cottonwood (*Populus fremontii*), black cottonwood (*Populus trichocarpa*), Engelmann oak (*Quercus engelmannii*), valley oak (*Quercus lobata*), and coast live oak (*Quercus agrifolia*) (Eskalen et al. 2013). Of these trees, PSHB has a strong preference for box elder and California sycamore.

To estimate the potential impact PSHB could have on an urban forest, a comparison was made between a list of common street trees and the list of trees that support either or both fungus and beetle growth. It was determined that 48% of street trees belong to a species that are suitable for fungal colonization and that 26% of street trees are a suitable host for beetle development (Eskalen et al. 2013). Urban tree species on this list include Japanese maple, English oak, mimosa, mesquite, and camellia (Eskalen et al. 2013). Once again the PSHB shows preference for trees in the genus *Acer*.



A similar comparison was made to estimate the threat of PSHB to agricultural crops. The fungus *F. euwallacea* was able to grow on avocado, eastern mulberry, olive, macadamia, Turkish hazelnut, loquat, peach, and grapevine (Eskalen et al. 2013). Of these crops only avocado supported beetle development (Eskalen et al. 2013). Trees of agricultural significance that were not attacked by PSHB include date palm, pomegranate, fig, apple, and citrus (Eskalen et al. 2013). Agricultural trees that PSHB attacked but did not support fungal growth were cassava, Japanese persimmon, Japanese crabapple, and eastern black walnut (Eskalen et al. 2013).

Two reproductive hosts of exotic origin are of particular note. The first is castor bean (*Ricinus communis*). Castor bean is a favored reproductive host of the PSHB (Eskalen et al. 2013) and is considered an obnoxious weed because of the toxic seeds and aggressive growth characteristics. The second is Tree of Heaven (*Alianthus altissima*). It is also considered an aggressive weedy tree species that will establish thick stands in disturbed habitats. Due to the widespread nature of these two weedy species, they may function as potential alternate hosts of PSHB that can facilitate reestablishment of the beetle in managed areas or allow for a population of beetles to move between areas if castor bean patches or *Alianthus* stands exist in between and act as corridors for invasion across the interfaces between urban, agricultural, and native forest communities.

### **3. Approaches for Management**

The biology of the polyphagous shot hole borer limits potential options for management of the insect to reduce the number of affected trees. Females emerge from the natal galleries already inseminated, so there is no known sex pheromone for the insect. There does not appear to be an aggregation pheromone as has been identified for many other species of bark beetles. Consequently, survey and detection has been limited to visual inspections until very recently. The discovery of the response to quercivorol has made it possible to trap beetles in the landscape. This discovery should make it possible to both delimit infestations and to monitor the movement of individuals. Because of the high risk of tree mortality, quarantine, detection, and restriction of movement of infested or potentially infested host material is critical to limiting tree loss.

Disposal of infested trees and wood residues provides a challenge for forest managers. Sanitation is critical to reduce the population of beetles within an area, eliminate human-aided dispersal of the insects, and to prevent on-site emergence and reinfestation of trees. It is possible to chip or grind infested material to kill the beetles in the wood. Material chipped to sizes 2.5 cm or less prevented any emergence of adults (Eatough-Jones and Paine 2015). Chipping material to approximately 5 cm in size did not completely prevent emergence of the adults, but the population was reduced by more than 90% (Eatough-Jones and Paine 2015). An alternative approach to chipping for sanitation is to use solarization. Covering infested log sections with 0.0508 mm (= 2 mil) clear plastic in the

summer months in southern California raised the temperatures within the log pile to more than 60°C when the daily ambient temperatures had an average maximum of 32°C and eliminated any signs of beetle activity within six weeks (Eatough-Jones and Paine 2015). Unfortunately, solarization in the cooler fall and winter seasons has not proven to be effective (Eatough-Jones and Paine 2015).

It may be possible to use direct control with insecticides to reduce the risk of infestation of individual high value trees. Synthetic pyrethroid contact insecticides have been demonstrated to be effective at establishing a barrier to beetles attempting to penetrate through the bark during colonization. There are systemic insecticides that have proven effective against other wood borers (McCullough et al. 2015), but these could be acting either on adults when they feed on foliage or on adults and larvae tunneling in the wood. Unlike the many other wood borers, the ambrosia beetles do not feed on the wood; rather, they only feed on the fungi. Consequently, it remains unclear whether they would consume the systemic insecticides in a way to acquire a toxic dose. However, their absolute reliance on fungi for nutrition may provide another opportunity for chemical control. It may be possible to apply a fungicide either systemically or in conjunction with a bark penetrant to kill the fungal symbionts and deprive the beetles of their source of nutrition. Pesticides applied to the surface of the tree would only be useful at killing beetles during their dispersal from their galleries or during seeking new hosts. Among the multiple insecticides tested on cut branches, the contact pesticide bifenthrin is the most effective at killing PSHB (Eatough Jones and Paine 2017b). Trials conducted at the

University of California Irvine demonstrate that a combination of systemic and contact insecticides work better in combination than individually to reduce populations of PSHB (Eatough Jones et al. 2017).

The identified list of susceptible or suitable hosts continues to change as the beetles encounter new potential host species. Although the range of potential hosts does change, it is clear that there are some species that are regularly infested (e.g., box elder) and others that are not (e.g., conifers). Amongst varieties of avocado Fuerte, Gwen, and Bacon are the least susceptible to beetle attack while Zutano is the most susceptible (Eatough Jones and Paine. 2017a) Thus, it is possible to generate a series of recommendations to landscape and forest managers of high risk tree species to either carefully monitor in established landscapes or to avoid when replanting. Careful host selection should be practiced to limit future risk from the ambrosia beetle.

The other option for long term management of the beetle is establishing biological control. Investigators have collected a number of candidate fungi, nematode, and arthropod natural enemies in preliminary trips and these opportunities will be pursued in the future. Currently 13 types of bacteria isolated from PSHB infested trees are undergoing testing and is showing some ability to inhibit *Fusarium* growth (Na et al. 2014). Comparable historical data for the natural enemies is essentially nonexistent. Biological control remains an important option, but will not be established in a short time frame.

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## **Chapter 2**

### **Effects of Temperature on Polyphagous Shot Hole Borer Development**



## Introduction

Temperature plays a major role in the development of insects (Wagner et al. 1991; Regniere et al. 2012; Stinner et al. 1975; Sharpe et al. 1977). As ectothermic organisms, insect metabolism is heavily affected by ambient temperatures. Over a set range of temperatures, depending on the insect, insect development is proportional to their environment's temperature with deviations occurring at the more extreme temperatures due to inactivation of control enzymes (Sharpe and DeMichele. 1977; Sharpe et al. 1977). Temperature can affect emergence synchronization and voltinism based on temperature thresholds required to move on to the next life stage (Bentz et al. 2010; Hansen et al. 2001). Reliance on temperature limits dispersal and potential invasive range due to the inability to develop in areas that become either too cold or hot (Ungerer et al. 1999). An insect's relatively consistent response to temperature can be used to predict life history characteristics such as developmental rate and potential distribution.

Developmental rate is the speed at which an insect will progress through its different life stages at a given temperature, from egg to larvae or larvae to adulthood for example. The greater the developmental rate is the less time it will take for that insect to complete that life stage. Developmental rate is calculated as the reciprocal of the amount of time it takes to transition from at least one life stage to another at a particular temperature (Campbell et al. 1974; Sharpe et al. 1977; Regniere et al. 2012). When plotted against temperature, insect development rates are generally linear over a middle range of

temperatures and start to curve to form a sigmoid shape at the more extreme temperatures (Campbell et al. 1974; McCambridge 1974; Ratte 1985; Wagner et al. 1991; Gilbert and Raworth 1996; Wermelinger and Seifert 1998; Yang et al. 2010). These middle range temperatures are considered the ecologically relevant temperatures where predictable, non-zero increases in development occur (Liu et al. 1995). At extreme temperatures development starts to be negatively affected and stress accumulates (Sharpe et al. 1977). The linear model is useful for predicting the time it takes for an insect to complete development from egg to adult (Campbell et al. 1947) due to its simplicity and ability to predict the thermal constant. However, constant exposure to temperatures near the limits of the insect's thermal tolerances will add bias to the linear model. Due to the change in development to form a curve in the rate function at extreme temperatures, development times at lower temperatures will tend to be underestimated and development times at higher temperatures will tend to be overestimated by the linear model (Wagner et al. 1991). Consequently, non-linear models, such as the Briere and Lactin models (Briere et al. 1999; Lactin et al. 1995), may be more appropriate to determine the upper and lower thresholds of development at temperatures approaching the insect's thermal tolerances.

The Polyphagous Shot Hole Borer (PSHB) *Euwallacea nr. fornicatus* (Coleoptera: Curculionidae; Scolytinae) is an ambrosia beetle that has invaded southern California (Eskalen et al. 2012). The beetle is believed to be from southeast Asia where it was collected in Thailand, Vietnam, China, Taiwan, and Japan (Stouthamer et al. 2017). PSHB has been collected in Israel and South Africa as well (Stouthamer et al. 2017;

Mendel et al. 2012). It has been collected from numerous different host plants, including multiple species of oaks and maples, sycamore, and different varieties of avocado (Eskalen et al. 2013). Based on phylogenetic analysis of COI the species complex hiding behind the morphological type *Euwallacea fornicatus*, consists of two additional species: the Tea Shot Hole Borer (TSHB) and the Kuroshio Shot Hole (KSHB). PSHB and KSHB are thought to have a more northern distribution than TSHB's distribution, which includes India, Thailand, Singapore, and Sri Lanka (Stouthamer et al. 2017). TSHB was reported to have a life cycle that takes approximately 45 days to complete (Gadd 1941). Unfortunately, Gadd (1941) only used the temperatures 20, 25, and 28 °C which covered a portion of the insect's linear development, but did not address its limits.

Danthanarayana (2003) used the data from Gadd 1941 to calculate the lower development threshold and the thermal constant (=the number of degree days required for complete development). This work on TSHB was pursued further by Walgama and Zalucki (2006). They expanded the range of tested temperatures to include 15, 18, 22, 30, and 32 °C and found that the Briere and Lactin models were the only ones able to calculate critical temperatures  $t_{min}$  (lower developmental threshold temperature),  $t_{opt}$  (temperature at which optimum development occurs) and  $t_{max}$  (higher developmental threshold temperature) for TSHB. Walgama and Zalucki (2007) found that the number of degree days required for development from egg to egg-laying adult was approximately 373. Development times were averaged together based on the findings by Gadd (1941) that indicated that eggs were laid at a rate of approximately one per day and would not be all similar ages.

Here we determine the critical temperatures for PSHB  $t_{\min}$ ,  $t_{\text{opt}}$  and  $t_{\max}$  in order to determine the beetle's potential invasive range. The range of temperatures tested was based on previous work done on the closely related TSHB (Walgama and Zalucki 2006; Walgama and Zalucki 2007) and included temperatures between 15 and 32 °C.

## Materials and Methods

### Insect specimens

Insects were obtained from colonies maintained in the University of California Riverside (UCR) Insectary and Quarantine facility. Colonies were established from PSHB adults collected from infested wood at the Huntington Gardens in San Marino. Infested logs were split open to obtain live adult females which were then transported to UCR to be raised in quarantine. Beetles were raised on a semi-artificial diet based on the modified recipe of Biedermann et al. (2009) inside modified Falcon (Corning Inc, New York) centrifuge tubes. A single adult female was placed within each tube that was sealed with cotton. Beetles were allowed to form galleries within the media at 25 °C. Colony tubes were harvested for adults every six weeks by removing the media from the tube and dissecting it.

### Semi-artificial media

The diet was composed of 236 g castor bean sawdust, 35 g agar, 6 g sucrose, 6 g potato starch, 12 g casein, 6 g yeast extract, 1.5 g Wesson's salt, and 685 mL water. Ingredients

were mixed together inside a plastic tub before being packed into 15 mL Falcon centrifuge tubes. Tubes containing the diet were autoclaved at 120 °C for 40 minutes and then allowed to sit for 2 days before use to allow the evaporation of excess moisture.

### Temperature study

Adult female PSHB were placed individually in tubes containing semi-artificial media and incubated at one of six temperatures in a temperature cabinet set to an 8L:16D light cycle. The temperatures used were 15, 18, 20, 25, 30, 32 °C with a minimum of 80 tubes per temperature. A subset of tubes at 15, 18, and 20 °C were dissected every three weeks and all life stages were recorded. It was necessary to dissect tubes, rather than monitor for emergence, due to the habit of adult beetles remaining inside their natal galleries or emerging then reinfesting the semi-artificial media. As late instar larvae and pupae started to be recorded, the tubes were dissected every week. At 25, 30, and 32 °C a subset of tubes was dissected every other week until late instar larvae and pupae were recorded, after which a set tubes were dissected every week to record life stages. When teneral adult stages were recorded a set of tubes was dissected every day until fully developed female adults were recorded. The teneral adult stage occurs just after pupation and is not fully mature. Teneral adults can be distinguished from fully developed adults by coloration. A teneral adult's carapace is not fully sclerotized which results in a light yellow-brown color that shifts to a shiny black or dark brown color when it has fully matured. During each dissection the number of eggs, larvae, pupae, teneral adults, and full adults was recorded. Fecundity was analyzed using only the offspring present in

tubes that were dissected after the first generation of new adults had been discovered. This was done to reduce the variation generated from offspring counts in early dissected tubes and to allow females to generate as many offspring as possible before the next generation could potentially contribute. Development time was recorded for each adult as the number of days elapsed from the start of the trial to the time the adult was discovered and rate was calculated as  $1/\text{development time}$ . The mean number of offspring per female was divided by the amount of time the beetles had been developing to account for differences in the amount of time between daily tube dissections. Tubes were opened in similar subsets following the first adult recorded every following day until no tubes remained. To account for the possibility that adults opened in later tubes had been present earlier, but were not discovered until later due to chance, the number of adults used to calculate development time were adjusted. The number of adults, and their associated development time, used from tubes dissected after the first set of adults was found were the number of adults from later tubes minus the number of adults that had been found the day prior. For example, if a set of tubes was dissected after 25 days and found to contain 12 adults and the day prior there were only 5 adults found, then 7 adults with a developmental time of 25 days would be recorded.

### Statistical methods

Development was analyzed using PROC GLM in SAS (SAS Institute, 1995). Regression analysis was performed in SAS ( $\alpha = 0.05$ ) to verify that there was an interaction between temperature and development time. Fecundity was analyzed using PROC ANOVA in

SAS (SAS Institute, 1995) to determine if temperature affected the number of offspring. For determining the thermal constant, which is the number of degree days required to complete development, and the lower development threshold  $t_{min}$  the linear model was used. Linear development rate, set as 1/days required to complete development, was regressed against temperature (Campbell et al. 1974). From the equation of the line created, the thermal constant was calculated as the inverse of the slope,  $1/b$ , and the lower development threshold was calculated as  $-a/b$ , where  $a$  is the x-intercept and  $b$  is the slope of the line. The standard errors for the thermal constant and lower development threshold were calculated using the methods of Campbell et al. (1974). Among nonlinear models the Briere (Briere et al. 1999) and Lactin (Lactin et al. 1995) model had the capability to calculate  $t_{opt}$  and  $t_{max}$ . The values of  $t_{min}$  and  $t_{max}$  represent the lower and higher temperature thresholds, respectively, for PSHB where no development will occur. The  $t_{opt}$  represents the value at which the development rate is expected to be the greatest. Both the Briere and Lactin models were tested in order to determine which was the most suitable for calculating  $t_{opt}$  and  $t_{max}$ .  $T_{opt}$  was calculated using the equation 3 from Briere et al. (1999).

## Results

### Temperature study

At 15 °C beetles would occasionally lay eggs. However, none of the progeny completed development and reached adulthood. Adult parental beetles had extended longevity at 15 °C and survived within the semi-artificial media for over 200 days. There was a linear

increase in development rate from 18 °C to 30 °C. Development was fastest at 30 °C, averaging only  $23.84 \pm 0.19$  days from the point of initial tube infestation to the formation of mature adult offspring (Table 2.1). Past 30 °C development rate sharply decreased and overall development was negatively impacted. At 32 °C the development rate dropped to about the same rate as at 20 °C (Table 2.1). Fecundity increased with temperature until 25 °C after which it declines significantly ( $n=150$ ,  $\alpha = 0.05$ ,  $p = 0.0189$ ). As temperature increased from 15°C observation of life stages occurred earlier in the trials until 32°C was reached (Fig. 2.1). Life stages were seen to develop weeks earlier with a change in 5°C. The relationship between temperature and developmental rate was confirmed with linear regression analysis ( $n=174$ ,  $\alpha = 0.05$ ,  $p < 0.0001$ ) ( $r\text{-square}=0.946$ ). Temperatures above 30°C were not used due to their non-linearity. Temperatures 15°C and 30°C were included due to their close proximity to the linear portion of the development curve and because their inclusion helped to fit the slope of the line to the data. The equation of the line for PSHB development was found to be  $y = -0.03349 + 0.00251x$  which corresponds to the following formula,  $y = a + bx$ , where  $y$  is the developmental rate,  $a$  is the  $x$  intercept,  $b$  is the slope of the line, and  $x$  is the temperature. Based on the slope, the thermal constant for PSHB was calculated to be  $398.41 \pm 52.23$  degree days (DD). This means that PSHB requires the accumulation of  $398.41 \pm 52.23$  DD over 15 °C to complete its development from the egg stage to adult stage, not including the preoviposition period.



Table 2.1. The mean values for development time, development rate, and offspring/time per foundress at each test temperature (SE given as  $\pm$  of the mean).

Temperature (C)	Development Time	Development Rate	Offspring/Time
15	X	X	X
18	76.80 $\pm$ 0.49	0.0130 $\pm$ 0.0001	0.1206 $\pm$ 0.0288
20	50.02 $\pm$ 0.14	0.0200 $\pm$ 0.0001	0.2157 $\pm$ 0.0282
25	38.86 $\pm$ 0.26	0.0258 $\pm$ 0.0001	0.7345 $\pm$ 0.1237
30	23.84 $\pm$ 0.19	0.0421 $\pm$ 0.0003	0.7172 $\pm$ 0.0634
32	48.5 $\pm$ 0.67	0.0206 $\pm$ 0.0003	0.2274 $\pm$ 0.0697

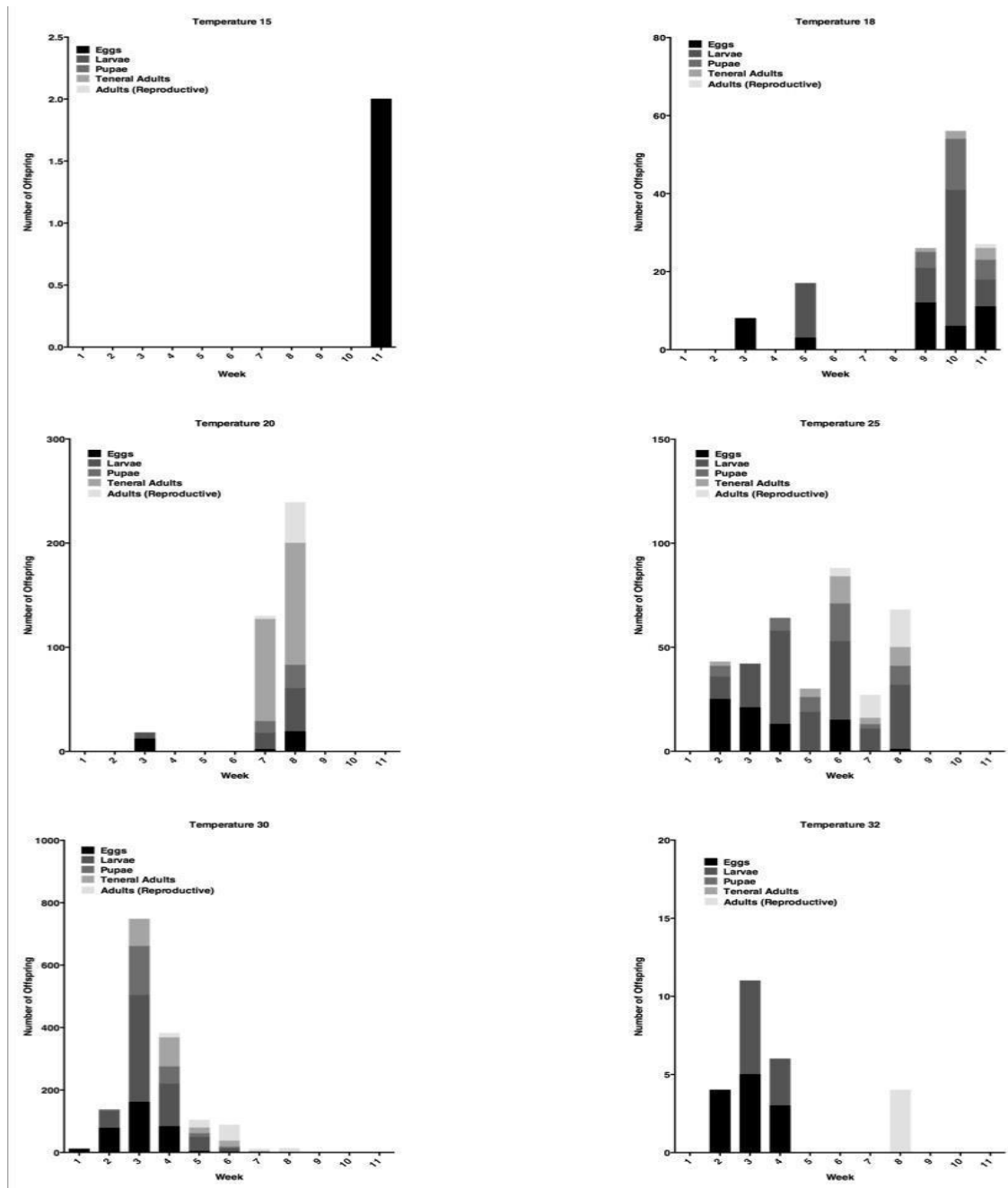


Figure 2.1. The number of offspring at different life stages found during tube dissections.

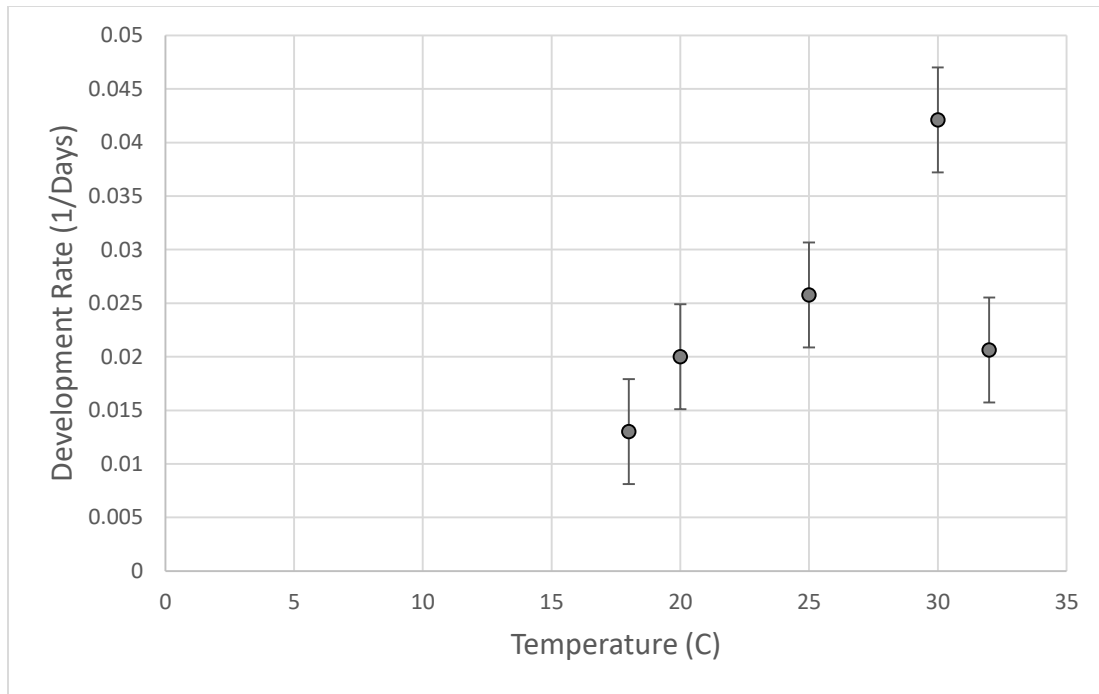


Figure 2.2. Non-zero developmental rates (1/days to adulthood) for PSHB at each tested temperature (°C).

The Lactin model was unable to fit to the shape of the data and the convergence criterion could not be met. The Briere model was able to fit to the shape of the data and was able to determine the values for  $t_{opt}$  and  $t_{max}$  (Table 2.2). Parameters  $a$  and  $m$  were constants that help the shape of the graph fit better with the data (Briere et al. 1999; Koda and Nakamura 2012). The lower threshold temperature was calculated to be 13.34°C, and the upper threshold temperature was just above the highest temperature tested at 33.08 °C. The optimal temperature for development was 27.58 °C.

Table 2.2. Temperature parameters for PSHB development derived from the Briere model.

Parameter	Value
$t_{\min}$	$13.34 \pm 0.10$ °C
$t_{\max}$	$33.08 \pm 0.10$ °C
a	$0.000053 \pm 0.00000074$
m	2
$t_{\text{opt}}$	27.51 °C

## Discussion

Temperatures were kept constant during the tests as Campbell et. al. (1974) found that development did not significantly differ between insects at fluctuating temperatures and constant temperatures. Furthermore, ambrosia beetles are sheltered within the host plant and are not as subject to environmental extremes as other insects that feed on the outside of their hosts. The range of temperatures that PSHB can develop in stretches between 13.34 to 33.08 °C. These values are similar to the range of 15 to 32 °C set by Walgama and Zalucki (2007) and Gadd (1947) for its congeneric species TSHB's developmental range. Even though their ranges appear similar, and they do both occur in south eastern

Asia, PSHB was shown to have a more northern distribution than TSHB in areas like China and Japan (Stouthamer et al. 2017). The optimum temperature was calculated to be between the tested temperatures of 24 and 30 °C which means that at 30 °C the development rate was already beginning a downward trend. Based on these results, it would be expected that the PSHB will be able to complete its developmental cycle within three weeks at the optimum temperature. As temperatures increased it was easy to see the earlier development of different life stages (Fig. 2.1). While these findings are only an approximation of when the life stages may occur, as tubes were not dissected every day, it could be useful when making general predictions about the amount of time until the next round of adult emergence from wood samples containing specific life stages or for choosing when to enact countermeasures that affect specific life stages. It appears that an additional round of eggs will be laid around the time the first generation is reaching adulthood. These eggs are unlikely to be from the first generation as they are occurring when the first generation is in their teneral adult phase and not reproductively mature. Even though PSHB was unable to have any offspring develop into adults at 15 °C, it is important to note that the adults used to infest the semi-artificial media were able to survive for over 200 days at that temperature. It is possible that the beetles would have been able to survive much longer, but a malfunction with the temperature cabinet caused the experiment to be terminated earlier than expected. PSHB larvae were also able to survive for at least two weeks at 15 °C as well. PSHB was able to persist at lower temperatures so it is likely that they will be able to spread into areas with mild temperatures as long as the winter isn't cold enough to kill the beetle and the warmer

periods of the month generate enough degree days to complete a generation. The adult beetles will be able to wait out the colder months. PSHB lives within sheltered galleries, often deep within the wood, and are partially insulated from extreme temperatures. In colder weather, cambial temperatures have been shown to lag behind the surface temperatures of the tree by 3-4 °C (Harvey 1923) and are often degrees warmer than the external air temperature (Keena and Moore 2010; Logan and Powell 2001). During warmer months, this has been shown to provide some insulation. The internal temperatures of a tree can be warmer than the air temperature at night and about 1 °C cooler during the day (Powell 1967) depending on which side of the tree is being sampled. The northern side of the tree generally is cooler than the air temperature during the day and the southern side can exceed air temperature by multiple degrees (Powell 1967). Building galleries on the northern side of trees can prevent the beetles from being exposed to a lethal amount of heat during the summer months. Applying sublethal heat stress to insects can induce heat stress proteins which make them more resistant to control by high temperatures (Thomas and Shellie 2000). This buffer provided by the host plant could provide the insect with more extreme heat tolerance. By building galleries on the northern side of the tree beetles could potentially survive in warmer climates and galleries on the southern sides of trees could provide additional heat during winter months, allowing the beetles to expand into cooler areas.

Since the beetles do not seem to be negatively impacted at 15°C, the cause for a lack of development could be attributed to lack of fungal resources. At 15°C the fungal

symbionts of PSHB may not be able to grow fast enough to support the development of the beetle and its offspring, but just fast enough to support a single adult, hence the persistence of the adults at 15°C. Fungal growth speed may also play an important role in brood size. Even though beetles developed faster at 30°C than at 25°C the average number of offspring was significantly lower (Table 2.1). In dissections of media all life stages were present, but the overall average amount of offspring in trials conducted at 30°C were lower than those conducted at 25 °C. At 30°C the beetles may be developing faster than the fungal symbionts and, as a result, the mother generates fewer successful offspring due to resource constraints. This could be related to how foundresses in 15°C trials expanded their galleries but generated few, if any, offspring as conditions inside the gallery were either not providing enough nutrition for the adult to lay eggs or the growth of the fungi were not satisfactory to encourage oviposition by the adult due to the lack of resources that would be available to hatching larvae.

The PSHB has a temperature range similar to its congeneric species TSHB between 15 and 33.08 C. It is able to persist around its minimum temperature for extended periods of time which suggests that the temperature may be affecting the development of its fungal symbionts rather than the beetle itself. It is possible that the upper and lower limits of its temperature thresholds can be stretched based on its host's ability to buffer it from unsuitable weather conditions, such as utilizing the southern sides of trees to persist in cooler climates. Additional information on its tolerances and interaction with its fungal symbionts will help to further define the limits of its invasive range.

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## **Chapter 3**

### **Climate Modeling the Potential Range of PSHB with Vegetation Data**

## Introduction

For invasive organisms it is not always clear what their potential for dispersal may be within novel habitats. Often little is known as to which areas are potentially threatened by a new invasive species and whether or not that species will be able to expand and establish beyond its point of introduction. Climate-based distribution modelling or climate matching attempts to identify areas that are potentially suitable for species development based on available species data. With insects, whose distribution is heavily influenced by seasonal temperatures, climate matching can be a useful tool in predicting potential range and identifying native ranges. CLIMEX is a program that has been used to estimate the range of numerous invasive species, including wood-boring insects (Hoddle 2004; Kumar et al. 2014; Macleod et al. 2002; Lozier and Mills 2011; Vanhanen et al. 2008). CLIMEX uses aspects of the organism's biology, such as development data under different temperatures, along with existing records of where the organism has or has not successfully established to assign different regions an ecoclimatic index (EI) The ecoclimatic index is an estimate of overall climatic suitability for the organism of interest (Sutherst and Maywald 1985).

Polyphagous Shot Hole Borer (PSHB) *Euwallacea nr. fornicatus* (Coleoptera: Curculionidae: Scolytinae) is an invasive ambrosia beetle that has established and spread throughout southern California (Eskalen et al. 2012). Based on findings from Stouthamer et al. (2017), the native range of the beetle is thought to be southeastern Asia and forms a

cryptic species complex with the Tea Shot Hole Borer (TSHB) and the Kuroshio Shot Hole Borer (KSHB). The threat that PSHB poses to forested areas in California is due to the symbiotic plant pathogenic fungus it carries and transmits. When PSHB establishes a gallery inside a host tree it cultivates its fungi along the gallery walls. The fungus, *Fusarium euwallacea*, subsequently grows and blocks the vascular elements of the plant. This blockage, along with the structural damage created during gallery formation in the wood causes what is referred to as *Fusarium* dieback (Eskalen et al. 2012, Freeman et al. 2013). *Fusarium* dieback causes a variety of symptoms in the tree and eventually results in tree mortality (Mendel et al. 2012). When the beetle was first studied by Eskalen et al. (2013), it was reported to have 19 different reproductive hosts (host trees in which the beetle could produce offspring) in California. The list has been updated to include a total of 49 different reproductive hosts (Eskalen, <http://eskalenlab.ucr.edu/shotholeborerhosts.html>). The areas of infestation have also spread from Los Angeles county to currently include, Orange, Riverside, San Bernardino, and Ventura counties (Eskalen et al. <http://eskalenlab.ucr.edu/distribution.html>).

The potential range of PSHB in California is of interest due to the threat that it poses to many native and urban trees. As there is no established way to prophylactically control the beetle in areas that have been infested, in most cases the only recourse is expensive and labor intensive removal of infested trees. To reduce the risk of spreading PSHB, monitoring is vital. Efforts to produce a map of potentially threatened areas may be a valuable contribution to the monitoring program.

## Materials and Methods

### Climate modeling

The climate modeling program, CLIMEX, was used to model potential habitat and climatic suitability across the world with a focus on the United States of America and California. CLIMEX uses a set of development parameters, derived from data gathered in Chapter 2, and fitted parameters, based on known distribution data, to estimate potential growth in different environments. Distribution data was based on beetle collection location information provided by the Eskalen laboratory PSHB distribution map (Eskalen et al. <http://eskalenlab.ucr.edu/distribution.html>) and from Stouthamer et al. (2017).

CLIMEX prediction of the suitable climatic range was done with gridded data at 0.5° or 30 arc-minutes. The 0.5° gridded data is formed from weather station and world climate data interpolated to form data cells at approximately 36 km intervals. The gridded data was obtained from the CLiMond dataset (Kriticos et al. 2012). Along with the 0.5° gridded data, CLIMEX uses temperature, moisture, and diapause parameters to generate measures of weekly growth and stress. Stress indices are heat, cold, wet, and dry stress. These indices were fitted to recover the known range of PSHB and used to create a map that showed the climatic suitability or EI of each of the data cells that formed the gridded dataset. EI values were set such that a value of 0 meant that that habitat was unsuitable for development, 1 to 5 was marginally suitable, 6 to 15 was suitable, 16 to 25 was favorable, and above 25 was very favorable. Within each cell CLIMEX also calculated

the number of potential generations per year based on average yearly climate conditions so that each cell contained both an EI and generations per year value. Initial temperature parameters were set based on the results of the temperature study. Moisture and diapause parameters were not included due to the beetle's habit of attacking trees in agricultural and urban settings where irrigation is common and the beetle is not known to enter diapause. Parameters were modified based on the range of PSHB identified by Stouthamer et al. (2017).

We also focus on the state of California. The focus reflected the urgency for quickly determining areas of potential spread in California before the beetle further expands its range. For refining CLIMEX results with vegetation data CLIMEX gridded data at 0.5° was used. The basemap for California and its representative counties was obtained from ArcGIS online (ESRI, Redlands CA). The California basemap was used to help align and confirm that vegetation data was in the correct projection. Only CLIMEX data with points containing EI's greater than 0 were plotted, rather than all locations, as the purpose of this study was to determine if climatically suitable areas would contain the hosts suitable to actually support PSHB development and spread.

#### Vegetation data

A list of 49 reproductive species was obtained from publically available data (Eskalen et al. <http://eskalenlab.ucr.edu/shotholeborerhosts.html>) (Table 3.1) to determine which plants would be potential targets for attack in regions with suitable climates.



Table 3.1. List of reproductive hosts and their scientific names

Reproductive hosts	Scientific name	CA Native?
Acacia	<i>Acacia spp.</i>	No
Trident maple	<i>Acer buergerianum</i>	No
Big leaf maple	<i>Acer macrophyllum</i>	Yes
Box Elder	<i>Acer negundo</i>	Yes
Japanese maple	<i>Acer palmatum</i>	No
Evergreen Maple	<i>Acer paxii</i>	No
California buckeye	<i>Aesculus californica</i>	Yes
Tree of heaven	<i>Ailanthus altissima</i>	No
Mimosa	<i>Albizia julibrissin</i>	No
Titoki	<i>Alectryon excelsus</i>	No
White Alder	<i>Alnus rhombifolia</i>	Yes
King Palm	<i>Archontophoenix cunninghamiana</i>	No
Mule Fat	<i>Baccharis salicifolia</i>	Yes
Kurrajong	<i>Brachychiton populneus</i>	No
Camelia	<i>Camellia semiserrata</i>	No
Moreton Bay Chestnut	<i>Castanospermum australe</i>	No
Blue palo verde	<i>Cercidium floridum</i>	Yes
Brea	<i>Cercidium sonora</i>	No
Carrotwood	<i>Cupaniopsis anacardioides</i>	No
Coral tree	<i>Erythrina corallodendron</i>	No
Red Flowering Gum	<i>Eucalyptus ficifolia</i>	No
Japanese beech	<i>Fagus crenata</i>	No
Black mission fig	<i>Ficus carica</i>	No
Kentia Palm	<i>Howea forsteriana</i>	No
Chinese holly	<i>Ilex cornuta</i>	No
American sweetgum/Liquidambar	<i>Liquidambar styraciflua</i>	No
Palo verde	<i>Parkinsonia aculeata</i>	Yes
Avocado	<i>Persea americana</i>	No

Mexican sycamore	<i>Platanus mexicana</i>	No
California Sycamore	<i>Platanus racemosa</i>	Yes
London plane	<i>Platanus x acerifolia</i>	No
Cottonwood	<i>Populus fremontii</i>	Yes
Black Poplar	<i>Populus nigra</i>	Yes
Black cottonwood	<i>Populus trichocarpa</i>	Yes
Mesquite	<i>Prosopis articulata</i>	Yes
Coast live oak	<i>Quercus agrifolia</i>	Yes
Canyon Live oak	<i>Quercus chrysolepis</i>	Yes
Engelmann Oak	<i>Quercus engelmannii</i>	Yes
Valley oak	<i>Quercus lobata</i>	Yes
English Oak	<i>Quercus robur</i>	No
Cork Oak	<i>Quercus suber</i>	No
Castorbean	<i>Ricinus communis</i>	No
Weeping willow	<i>Salix babylonica</i>	No
Black willow	<i>Salix gooddingii</i>	No
Red Willow	<i>Salix laevigata</i>	Yes
Arroyo willow	<i>Salix lasolepis</i>	Yes
Tamarix	<i>Tamarix ramosissima</i>	No
Japanese wisteria	<i>Wisteria floribunda</i>	No
Dense logwood	<i>Xylosma congestum</i>	No

Individual vegetation maps for 14 reproductive host species were obtained through the USGS Geosciences and Environmental Change Science Center's digitizing of Elbert Little's compiled tree range maps (Little Jr. <https://esp.cr.usgs.gov/data/little/>). Each host species was assigned an abbreviated name (Table 3.2) and their data was extracted in ArcMap. Extracted data were mapped to California along with the CLIMEX gridded data in order to determine if climatically suitable areas overlapped with known ranges of host

trees that would allow the beetle to reproduce. Both sets of data were used as the two different datasets contain information in one that was absent in the other. Multiple of the plant species in the plant community datasets were not represented amongst the 14 available tree species maps created from Little's tree range maps and species range found in some of Little's tree maps have greater coverage than the plant community maps.

Table 3.2. Map IDs and names of 14 reproductive hosts with vegetation map data.

Map ID	Full Name	Common Name
AcermacrCA	<i>Acer macrophyllum</i>	Bigleaf Maple
AcerneguCA	<i>Acer negundo</i>	Boxelder
AesccaliCA	<i>Aesculus californica</i>	California Buckeye
AlnurhomCA	<i>Alnus rhombifolia</i>	White Alder
CerflorCA	<i>Cercidium floridum</i>	Blue Paloverde
PlatraceCA	<i>Platanus racemosa</i>	California Sycamore
PopufremCA	<i>Populus fremontii</i>	Cottonwood
PoputricCA	<i>Populus trichocarpa</i>	Black Cottonwood
QueragriCA	<i>Quercus agrifolia</i>	Coast Live Oak
QuerchryCA	<i>Quercus chrysolepis</i>	Canyon Live Oak
QuerengeCA	<i>Quercus engelmannii</i>	Engelmann Oak
QuerlobaCA	<i>Quercus lobata</i>	California White Oak
SalilaevCA	<i>Salix laevigata</i>	Red willow
SalilasoCA	<i>Salix lasiolepis</i>	Arroyo willow

## Results

### Climate matching

The CLIMEX model conformed to the known distribution in Asia (Fig. 3.1). The CLIMEX model predicted that the inner areas of India would be unsuitable for development due to excessive heat stress. The model identified northern California as the extent of its spread, with further spread restricted by cold stress. In California the range of EIs varied between 1 to 27. The highest EIs were found in Los Angeles and San Diego areas (Fig. 3.3). Using data from CLIMEX, weather stations with EIs  $>0$  were plotted on a map of California containing average temperature data from 2013 to 2016 (Fig. 3.4) obtained from the California Climate Data Archive (CalClim, <http://www.calclim.dri.edu/>). Weather stations had to be manually plotted due to the map from CalClim being in a format that was not supported by ArcGIS.

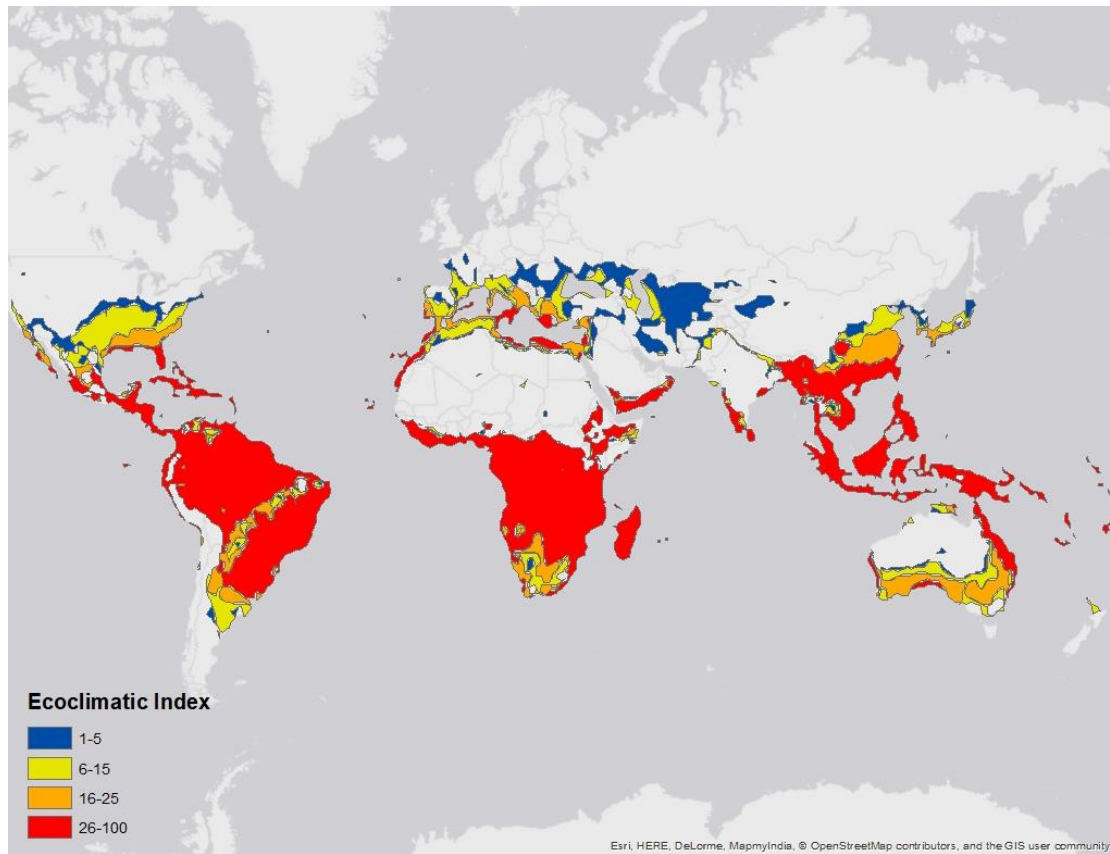


Figure 3.1. The predicted range of PSHB using CLIMEX. Warmer colors indicate areas with a more suitable climate and no color indicates an EI less than 1 or missing data.

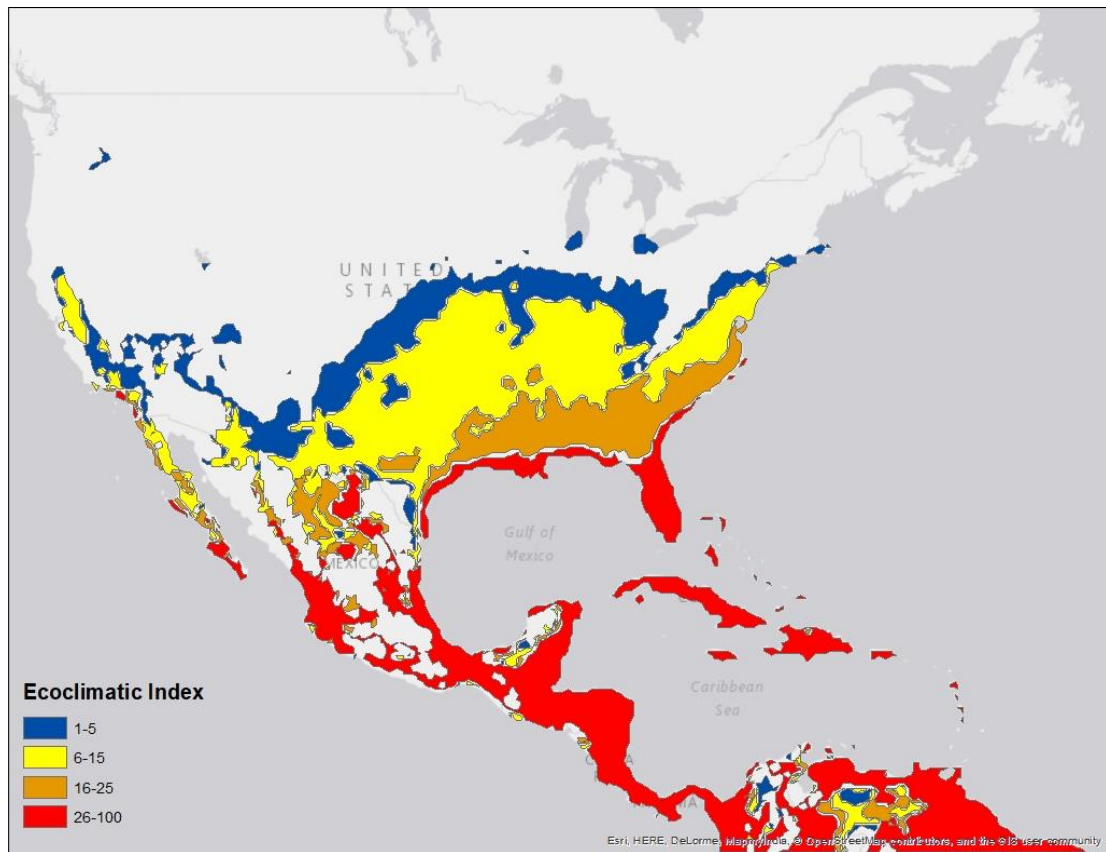


Figure 3.2. Enlarged view of CLIMEX results regarding the range of PSHB in North and Central America.

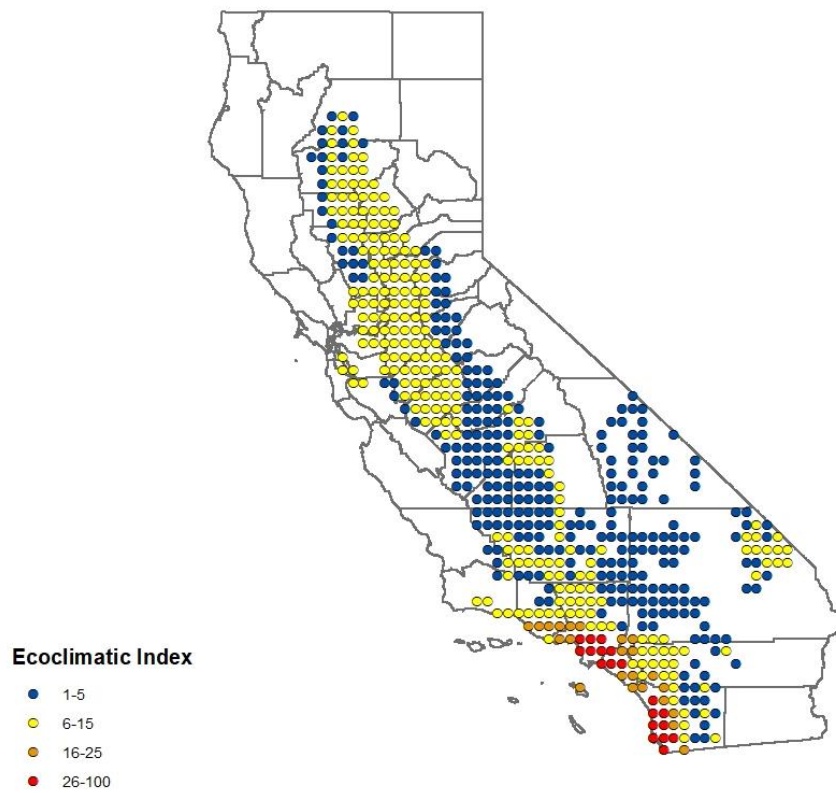


Figure 3.3. California map with CLIMEX grid data indicating suitable ecoclimatic indices (EI) for development of PSHB. Larger EI values indicate more suitable climate.

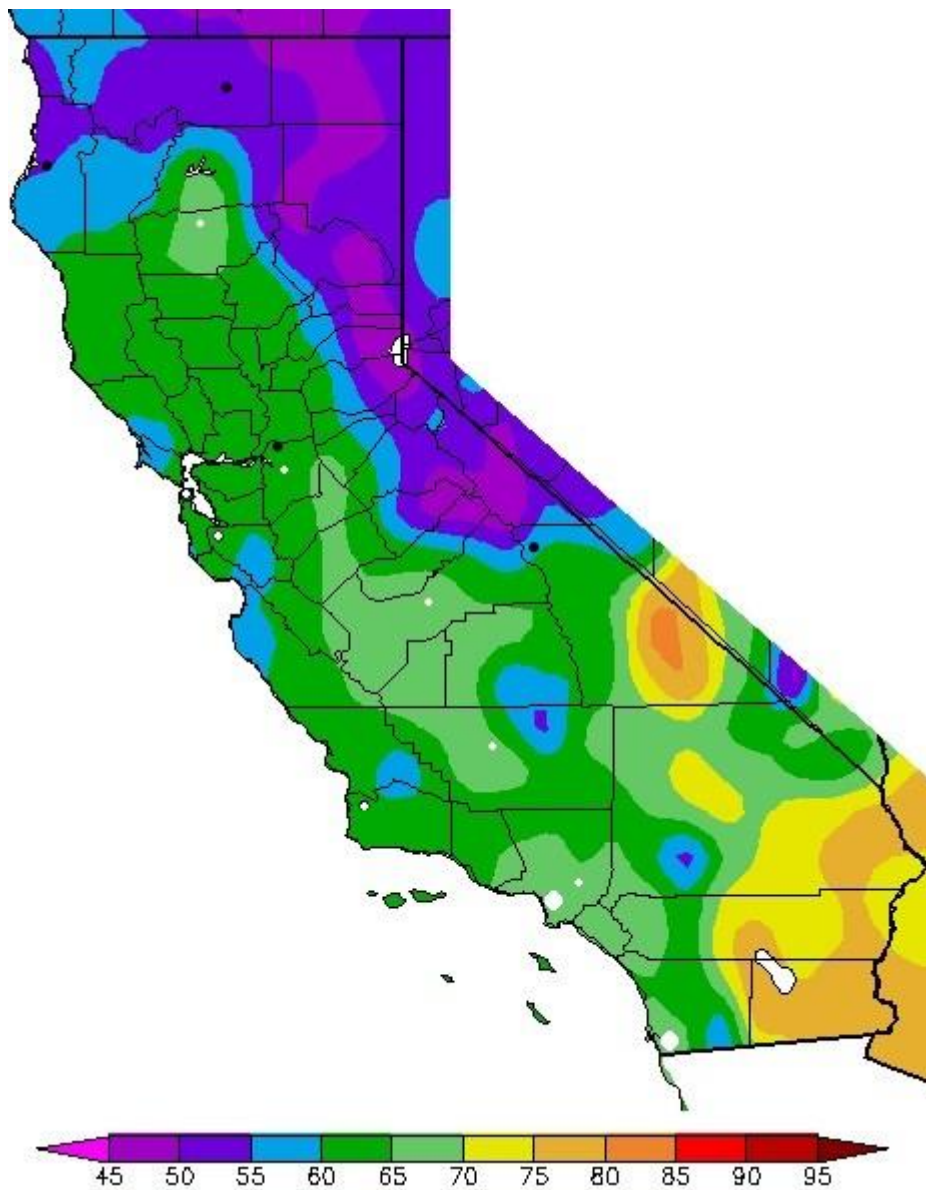


Figure 3.4. CLIMEX findings plotted on an average temperature map obtained from California Climate Data Archive (<http://www.calclim.dri.edu/>). Color of areas corresponds to average temperature (in Fahrenheit). White circles indicate weather stations designated by CLIMEX as having suitable climatic conditions for PSHB with larger white circles being more suitable for development. Black circles indicate weather stations where climatic conditions were unsuitable for development.



Data obtained from CLIMEX was used to map the number of generations of PSHB per year based on the developmental data from Chapter 2 and climate data contained within CLIMEX. The number of generations per year in areas with EIs 1 and greater ranged from 1 to above 9 in certain areas (Fig. 3.5). The higher numbers of generations per year coincided with areas with high EI and areas with greater than 8 generations per year only occurred close to the tropics (Fig. 3.5). In the United States, the majority of suitable climates allowed for the growth of 1 to 6 generations per year and only exceeding that in the southern parts of Florida and Texas (Fig. 3.6). Mexico appears to be particularly suitable as the majority of predicted suitable areas will produce over 4 generations per year and even exceeding 8 in some areas (Fig. 3.6). Californian populations of beetles will generally be able to complete between 3 to 6 generations per year and perform well through the Central Valley (Fig. 3.7).

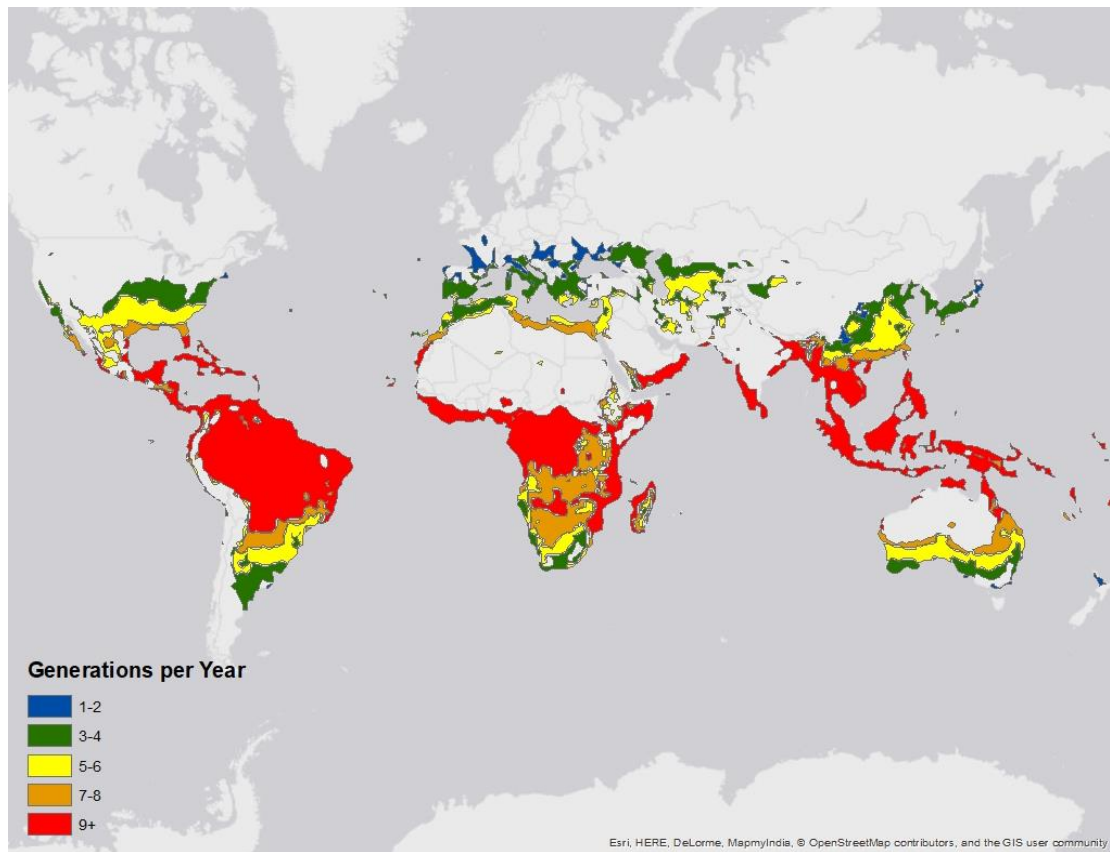


Figure 3.5. World view of the number of PSHB generations per year in different areas calculated by CLIMEX in zones with an EI of at least 1. Europe and south eastern Asia are incomplete due to the absence of gridded data for those areas.

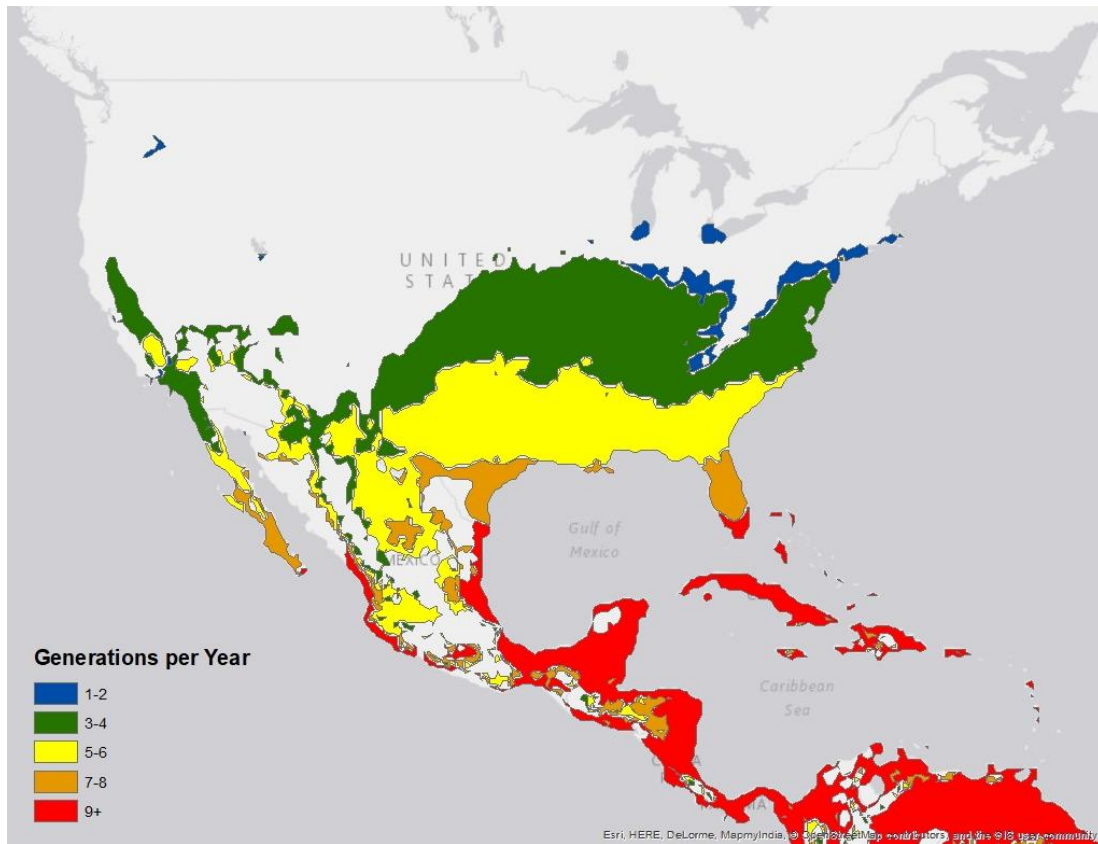


Figure 3.6. View of North and Central America and the number of PSHB generations per year in different areas calculated by CLIMEX in zones with an EI of at least 1.

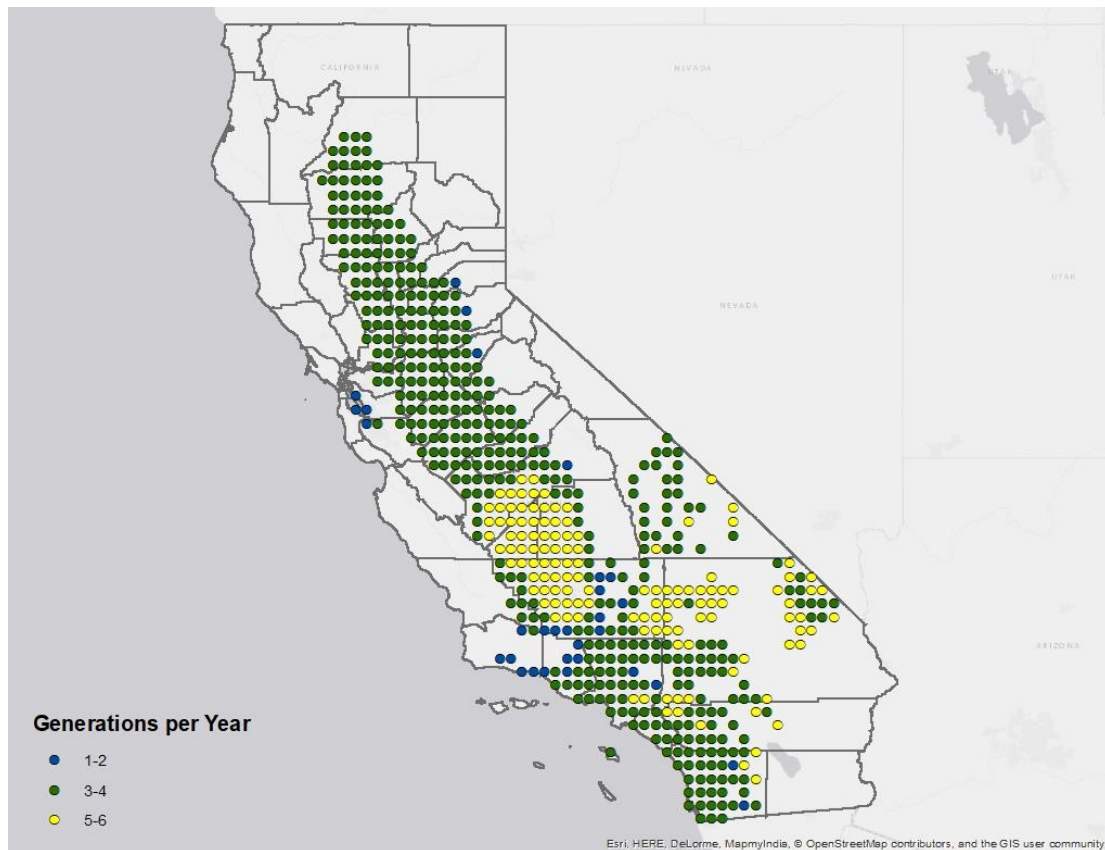


Figure 3.7. The number of PSHB generations per year in California with an EI of at least 1 generated using CLIMEX gridded data.

### Vegetation data

When comparing the recorded ranges of reproductive hosts many of them overlapped (Fig. 3.8). Many of the climatically suitable zones identified with CLIMEX overlapped with at least one of the reproductive hosts (Fig. 3.8) and only lacked hosts in the desert and areas where there is significant urbanization, such as the inner part of the central valley.

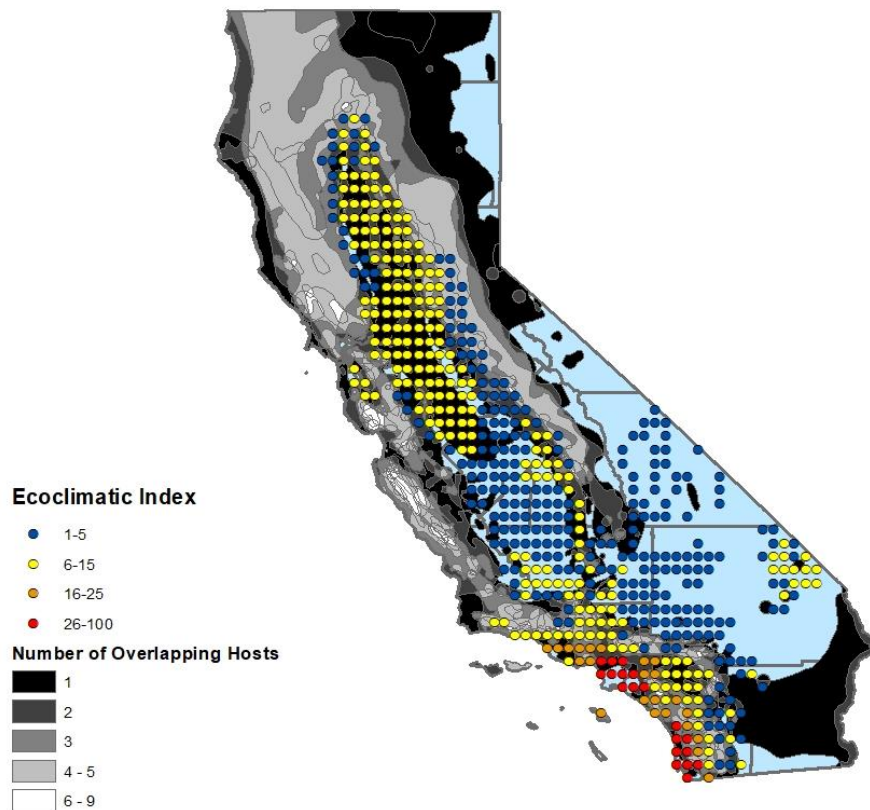


Figure 3.8. California map with CLIMEX gridded data indicating suitable EI's for development of PSHB. Greyscale areas indicate the number of overlapping reproductive hosts indicated by the color. A light blue background was used to help differentiate areas with overlapping hosts and EI values.

Discussion:

Climate modeling

MaxEnt performed well with an AUC value above 0.9. It showed high suitability in the PSHB's native range in southeast Asia. MaxEnt shows a greater range of low EI values than CLIMEX, showing values that include those between 0 and 1 rather than 1 and

above. Predictions in east Asia are conservative and place low climatic suitability on locations where beetles have been collected in China, Taiwan, and Japan (Stouthamer et al. 2017). This is may be due to overly conservative estimates on the model side or the available temperature data are inadequate.

CLIMEX matched the known Asian range of PSHB better than MaxEnt and assigned a higher level of climatic suitability. Aside from the edges of the northern range both MaxEnt and CLIMEX models are in good agreement. Areas where the beetle is thought to have originated have high EIs  $> 50$  which indicates extremely high suitability and adds credibility to the models. Both models conform to key areas in Israel and California where beetles have been invaded. Unlike MaxEnt, CLIMEX shows that the central parts of India and Mexico, along with Arizona and New Mexico are unlikely to experience established invasion due to high heat stress on the beetle.

Of particular interest to this study was the potential range of invasion of PSHB in California. Both MaxEnt and CLIMEX predict that the range of suitable environments extends to northern California. Past that point, the beetles are limited due to the accumulation of cold stress. The most suitable areas were predicted to be around Los Angeles and San Diego which also happen to be the warmest areas near the coast and contain major ports of entry for invasive species. The warmer temperatures combined with the regulating effect of the ocean on temperature could be major factors in increasing the suitability of the climate around Los Angeles and San Diego compared to

other locations in California. It is important to note that infestations in San Diego are currently the congeneric Kuroshio Shot Hole Borer species and not PSHB. However, it makes sense that San Diego should be recovered as a climatically suitable site since the two species share partially overlapping native ranges and hosts. Ventura county and Santa Barbara county were not included as having a positive EI despite having relatively moderate temperatures. KSHB has been collected in Santa Barbara, while numerous finds of PSHB are recorded from Ventura county. Possible explanations for this could be that, based on Figure 5, the models are somewhat off, or there is too much of a climatic gradient over these counties that was not captured by the available climate data, or that those areas are just cold enough compared to areas like San Diego that CLIMEX registers enough accumulated cold stress to prevent development, or beetles are colonizing their hosts in a manner that provides sufficient buffer from colder weather. Multiple studies have shown that the cambial temperature of trees can be multiple degrees centigrade different from air temperature, especially on the southern side of the tree (Harvey 1923; Keena and Moore 2010; Logan and Powell 2001; Powell 1967).

When the CLIMEX results are plotted on a climate map of California, predicted suitable climates follow the light green climate zone representing average temperatures between 65 and 70 F or 18.3 and 21.1 C which stays above the lower limits of linear growth described in the temperature study (Chapter 2) (Fig. 3.3). Based on the results it shows that the PSHB could potentially spread through the Central Valley all the way up to

Tehama County. Due to the number of important agricultural tree crops in the Central Valley the spread of PSHB north could pose a serious problem to growers. PSHB has a large host range and more potential hosts are being discovered which could include new crops that it has not come into previous contact. The climate zones help to explain the presence of suitable climates identified by CLIMEX along the eastern border of southern California near Nevada as the daytime extreme temperatures drop to suitable levels in a pocket around that area.

The number of generations per year generally followed the same spatial trend as EI. Areas with high EI produced more beetles per year, as was to be expected. EIs did not translate directly into a number of generations per year as can be seen in differences between Figures 3.1 and 3.5. Having an EI between 15-25 did not always translate into 5 to 6 generations per year and EIs above 25 did not necessarily predict a number of generations above 6. Even in California where the highest EIs were found in southern California around Los Angeles and San Diego (Fig. 3.3) the greatest number of generations was predicted to occur more in the Central Valley (Fig. 3.7). The slight mismatch may be due to the number of degree days that are calculated in the different areas which are separate from the amount of stress factors such as cold and heat that are used to generate the EI. Based on the number of generations possible, Florida and Mexico are potentially at high risk as both can support the development of at least 5 generations of PSHB per year.



## Vegetation data

The range of PSHB has increased tremendously over the years since its first discovery in 2003 (Eskalen et al. 2012). With a reproductive host list that has more than doubled and an expansion into four new counties in the past four years, PSHB poses a serious invasive threat. With many of the reproductive hosts it is difficult to set a range as their presence is due to human introduction and cultivation or their recorded range is too broadly defined. Hosts used as ornamental landscape trees, such as liquidambar (*Liquidambar styraciflua*) or Japanese maple (*Acer palmatum*), are found throughout California's urban forests and no complete records exist of their distribution in California. We focused on the reproductive hosts whose data was available in order to develop at least conservative estimates as to the possibility of spread of PSHB and to verify prior CLIMEX predictions.

This is due to the large scale urbanization and cultivation of land for agricultural purposes. While there are parts of central California unaccounted for, Figure 3.8 shows that there is an avenue of suitable hosts that spans from southern to northern California. Furthermore, since the plant communities include hosts, such as Mule Fat and Mesquite, that are not represented amongst Little's 14 reproductive tree species maps it serves to increase our confidence in the presence of suitable hosts throughout California.

There is a clear trail of hosts and suitable climatic conditions that the PSHB could use to spread from southern to northern California (Fig. 3.8). Zones showing a high number of

overlapping hosts can indicate which areas may be the most suitable for beetle development. The presence of multiple different hosts will increase the chances of the beetle encountering a preferred host tree and establishing due to the existence of multiple ranges it could spread through. Southern California appears to be the most suitable area for PSHB as EIs are higher and numerous hosts overlap their ranges. Some areas with EIs greater than one still do not appear to contain suitable hosts, but it is important to note that the absence of host records does not necessarily indicate that these areas will not support PSHB establishment. More than likely, these empty areas are highly urbanized. Due to host trees existing in urban forests as street trees (Eskalen et al. 2013; McPherson et al. 2016) and even more unaccounted for due to their location on private property there are likely numerous trees within urban environments that could allow for PSHB development. Though the trail of known reproductive hosts leading from southern California to northern California is not large, it does present an opportunity for the beetle to spread where its northern range of suitable climatic conditions all overlap with at least one host over their entire range. This is also not taking into account the presence of urban hosts through the central valley and human transportation which can be a major mode of rapid, long distance spread of invasive insects and can bypass large areas of land necessary to limit the spread of wood-inhabiting insects (Herbert and Cristescu. 2002; Muirhead et al. 2006; U. S. Department of Agriculture, Animal and Plant Health Inspection Service [USDA APHIS] 2010).

Having at least one reproductive host along the length of California supports CLIMEX's findings that PSHB could potentially spread all the way to northern California. A major concern with the CLIMEX model output was the assumption that only climate dictated the potential range of the beetle. If other factors prevent establishment, then CLIMEX's predictions would be too liberal. As shown here, the widespread range of reproductive hosts across California indicates that host availability is generally not a limiting factor. There are multiple hosts spanning the entirety of California and overlapping the CLIMEX gridded data with positive EIs. Furthermore, predictions based on available hosts through which PSHB could move are conservative because many of the reproductive hosts are found in urban areas and are used as street trees across California. At least 26% of trees found in urban forests are reproductive hosts (Eskalen et al. 2013). The reproductive hosts London plane (*Platanus x acerifolia* var. *hispanica*) and American sweetgum (*Liquidambar styraciflua*) are common across California and make up 10.5% and 3.4% of the relative species abundance of urban street trees, respectively (McPherson et al. 2016). In the inland valley portion of California, where multiple generations of beetles can develop in a single year, at least 14.3% of urban trees are a reproductive host (McPherson et al. 2016). These values do not account for the number of reproductive hosts found on private property. As PSHB has the potential to spread north through the center of California, it is vital that steps be taken to ensure that the spread of the beetle is not facilitated. Current methods for treating infestations of PSHB are expensive and difficult to implement; consequently, monitoring its current range and preventing infested material from being moved into uninfested areas are top priorities.

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## **Chapter 4**

### **Irrigation and its Effect on Polyphagous Shot Hole Borer Attack Rate**



## Introduction

During times of water stress numerous physiological changes occur within the host plant. In certain cases, water stress may cause changes in plant development which can lead to increases in features such as cell wall thickness, fiber content, and secondary compounds (Herms and Mattson 1992; Mattson and Haack 1987). A reduction in water can cause the concentration of vital nutrients that are otherwise diluted by high water content (Mattson and Haack 1987). An increase in nutritional content would be advantageous as the wood of trees is generally poor as a food source. Insects, especially wood-boring insects, are frequently noted as benefiting by water stress in their hosts (Bauerfeind and Fischer 2013; Mattson and Haack 1987; Waring and Cobb 1992). Stress due to lack of water can lower host defenses by diminishing sap pressure or lowering the amount of defensive compounds present due to growth versus defense trade-offs (Herms and Mattson 1992; Mattson and Haack 1987). Volatile compounds that can serve as attractants are found in greater concentrations in stressed trees (Hodges and Lorio 1975; Millar et al. 1986; Ranger et al. 2010).

The performance of bark beetles and the fungi that are often associated with them are known to be affected by the water stress of their host trees (Paine et al. 1997). Primary and especially secondary bark beetles prefer to attack weakened trees (Paine et al. 1997). In some trees, water stress needs to be above certain threshold levels for invading beetles to bypass the host's defenses (Ferrell 1978). Water stress concentrates chemicals inside

the tree, such as monoterpenes and alcohols, that could promote beetle virulence and lowers the concentrations of resin acids which reduces the viscosity of the resin response (Hodges and Lorio. 1975; Ranger et al. 2010). High levels of water stress increase a tree's susceptibility to fungal inoculation (Croise et al. 2001; Encina et al. 2012) which plays a role in tree mortality (Paine 1997).

For bark beetle associated fungi, in some cases mild water stress has been shown to make the host tree more resistant to fungal infection (Salle et al. 2008). Wet environments and heavily watered trees can promote the growth of bark beetle associated fungi (Lorio and Hodges 1968; Salle et al. 2008). In other cases, pathogen growth has been noted to not always be affected by water stress status of the host tree (Croise et al. 1998; Croise et al. 2001).

While much work has been done on the relationship between water stress and bark beetle attacks, little is known about how water stress affects the attractiveness of trees to ambrosia beetles. Ambrosia beetles, unlike other bark beetles, form an obligate symbiotic relationship with one or more fungal species. Reliance on a fungal symbiont may change host requirements. Depending on the fungal species a wetter environment or a tree with less water stress may be more preferable.

Polyphagous Shot Hole Borer (PSHB) *Euwallacea nr. fornicatus* (Coleoptera: Scolytidae) is an ambrosia beetle that has invaded and established itself in southern

California (Eskalen et al. 2012). It has a symbiotic relationship with three different fungal species; *Fusarium euwallaceae*, *Graphium euwallaceae* and *Paracremonium pembeum* (Lynch et al. 2016). *Fusarium euwallaceae* is the causal agent of *Fusarium* dieback whose spread has resulted in extensive tree mortality (Freeman et al. 2013). It is uncertain whether PSHB would prefer water stressed trees, as many bark beetles do, or whether its close association with its fungal symbionts would cause it to attack trees with a higher level of moisture available.

The purpose of this study was to determine if water stress and irrigation status of the host tree affects its likelihood of being attacked by PSHB. This study was carried out in parts:

- Determine if beetle attacks were different on irrigated trees versus trees whose irrigation had recently ceased.
- Determine if beetle attacks were different on trees that were irrigated, never irrigated, or whose irrigation had recently ceased.
- Determine if beetle attacks were different on potted trees with different water potentials.

Using this information, we hope to recommend cultural control methods to reduce the risk of attack by PSHB. Furthermore, by determining the host tree preference we seek to gain a better understanding of the relationship between PSHB and its symbiotic complex.

## Materials and Methods

University of California Irvine: Influence of irrigation on infestation in Sycamore trees

To evaluate the impact of decreasing irrigation for trees naturally infested with PSHB, attacks on sycamore trees with and without irrigation were monitored. Monitored trees were located throughout the University of California Irvine (UCI) campus. All trees were planted in grass landscaping, and had been irrigated. On May 5, 2015, irrigation was shut off to an area approximately 4500 m<sup>2</sup>. All sycamore within this area were tagged with metal number tags to allow for identification of each tree. A total of 19 trees were tagged in the unirrigated area. Infestation levels were assessed as light, moderate, or heavy (categories based on a visual assessment as: light < 10 attacks, moderate 20-75 attacks, or heavy over 100 attacks in approximately 1.5 meter of trunk length centered at breast height). The number of trees in each category were recorded. Twenty sycamore trees were selected in the surrounding irrigated landscape. Selected sycamore trees had similar diameters at breast height (DBH) ( $F = 0.41$ ,  $p = 0.53$ ), with mean DBH of irrigated trees at  $0.33 \pm 0.02$  m and unirrigated trees at  $0.35 \pm 0.02$ , and initial infestation level (attacks/m<sup>2</sup>) ( $F = 0.66$ ,  $p = 0.42$ , d.f. = 1,37), with mean initial infestation of irrigated trees at  $38.68 \pm 18.49$  attacks/m<sup>2</sup> and unirrigated at  $21.40 \pm 9.71$  attacks/m<sup>2</sup>. All irrigated trees were planted in within 200 m of the area where irrigation was eliminated.

For all trees selected for the study all PSHB entrance holes were counted around the entire circumference of the main trunk. The search area was equal to the surface area of a

cylinder ( $2\pi rh + 2\pi r^2$ ) with  $h = 1.5$  meters, located approximately 0.3 meters from the ground to 1.8 meters trunk height, and  $r = \frac{1}{2}DBH$ . Attacks density was recorded as attacks/  $m^2$  to account for different shapes and sizes of the trees. Each hole was marked with a small dot to the side of the hole using a paint marker, and counts were tallied with a hand-held counter. Entrance holes were determined by their shape and size, a round hole approximately the size of the tip of a ballpoint pen (0.85mm diameter). If there was an accumulation of sap or boring dust, but it was unclear if there was a hole underneath, the area was cleared to verify the presence of a hole. Initial attacks for all trees were surveyed in May 2015, shortly after water was shut off. Trees were surveyed again in June, July and September 2015, and in January and April 2016. For each survey period, we calculated the number of new attacks, as (attacks for current sampling period – initial attacks for May 2015).

Crown condition was surveyed qualitatively in June, July, September and April. Crown condition was not surveyed in January since all trees lacked leaves. Trees were given a categorical rank for crown condition based on the approximate proportion of branches showing signs of leaf senescence. Categories for crown condition were 0 = little to no senescence, 25 = less than half, 50 = half, 75 = more than half, and 100 = bare.

As newly emerging PSHB are likely to initiate attacks on their natal host tree, rather than migrating to a new host the number of new attacks recorded for each tree was expected to be correlated with the total number of attacks already present on the tree. Most trees

selected for this study were lightly attacked, with fewer than 10 attacks on the main trunk. However, 25% of trees selected in both irrigated and unwatered treatments were more heavily attacked. To account for the wide variation in initial attacks for both treatments, we used logistic regression (PROC GLM, SAS Institute Inc 2010) to assess the strength of the association of initial attacks and irrigation treatment to increased attacks over time. A separate logistic regression was performed for each sampling period. Factors included in the regression to predict number of new attacks were irrigation level (0 = no irrigation, 1 = irrigation on), and the initial number of attacks on each tree. Significant effects were evaluated at  $\alpha = 0.05$ . Logistic regression was used to assess factors that were associated with crown condition (5 qualitative categories). Data from all four periods when crown condition was assessed were included in a single regression. Factors included for predicting crown condition were days since water was turned off, initial attacks, current attacks, tree size (DBH), and irrigation treatment (0 or 1). Significant effects were evaluated at  $\alpha = 0.05$ .

Huntington Botanical Gardens: Influence of irrigation on infestation in Liquidambar trees

In July 2013 a field survey of PSHB attacks on preexisting liquidambar trees (*Liquidambar styraciflua*) was conducted around the Huntington Botanical Gardens in San Marino, CA. The trees had been maintained in one of three ways: never irrigated, irrigation had been discontinued within the past year, and continuously irrigated. A total of 57 trees were surveyed across the 3 irrigation types with 22 receiving no water, 15 with discontinued irrigation, and 20 with continuous irrigation. The number of beetle

attacks were counted in an area of trunk that spanned its circumference between 0.3 m and 1.8 m trunk height. The number of attacks were counted 4 times and the values averaged to account for variability between recorders. Attacks were recorded as attacks/m<sup>2</sup> to account for different trunk shapes and sizes. All trees were lightly attacked and the number of attacks per tree never exceeded 40. Attacks were identified by small circular holes approximately 0.85mm in diameter, sawdust plugs, and occasional necrotic staining. In addition to attacks, the crown health was recorded by the visually assessing the proportion of unhealthy or dead branches and assigned a health category the same as in the UCI study (0 = no unhealthy or dead branches, 25 = less than half, 50 = half, 75 = more than half, and 100 = all dead or dying).

Logistic regression (PROC GLM, SAS Institute Inc 2010), with significance set at  $\alpha = 0.05$ , was to determine if irrigation and DBH had an effect on the number of attacks per tree. All 3 irrigations types were kept separate for this test to determine if any differences in watering could promote PSHB attack. A separate logistic regression was used was used to assess whether tree health was associated with attacks, irrigation, or DBH. Data for all three factors were included in a single regression and significance was set at  $\alpha = 0.05$ . For this analysis the irrigation factor combined the two groups that had been irrigated at least once into one group forming an irrigated versus nonirrigated group.

Huntington Botanical Gardens: Influence of irrigation box elder

In May 2015 space to conduct the water stress experiment was obtained at the Huntington

Botanical Gardens. The location of the experimental plot was located behind The Garden of Flowing Fragrance on a plot of cleared land that stood between already infested trees and stands of castor bean. The site was chosen both for its access to a water line for irrigation and its proximity to already infested wood on multiple sides. Experimental trees consisted of 64 young box elder (*Acer negundo*) trees all of the same age and grown together in a greenhouse at UCR Agricultural Operations since Fall 2014. Box elder was chosen based on its status as one of the most preferred host species for PSHB at the time of purchase (Eskalen et al. 2013). Trees were kept in 5 gallon pots and subjected to one of four different watering regimes (Table 4.1) for 1 year prior to the experiment. Irrigation was applied twice a week for 10 minutes at a time and the amount of water received was based on emitter (Table 4.1). Water was temporarily increased during July 2016 to restore vigor to trees that had become too water stressed due to a malfunction with the water line. Pressure bomb readings were taken with a PMS Company Pump-Up Chamber (<http://pmsinstrument.com/>) on cut leaf stems, approximately 1.5 mm in diameter and 50 mm in length, from 11:00 AM to 5:30 PM every half hour to determine the time at which trees are maximally stressed.



Table 4.1. Color of the irrigation nozzle, how much water the nozzle dispenses, and how much water each treatment received weekly.

Color Irrigation Nozzle	Liters per minute (lpm)	Liters per week (LPW)
Black	0.79	15.9
Blue	0.57	11.4
Green	0.34	6.8
Grey	0.15	3.0

Two pressure bomb readings from each watering treatment from random blocks were taken twice a month for 4 months to monitor water stress in the different treatments. Approximately 30 hours elapsed between watering and pressure bomb readings. Pressure bomb readings were used to quantify the amount of water stress through measuring stem water potential (SWP) (Shackel 2007). Hacke and Sperry (2003) demonstrated that water stressed box elder was more likely to experience embolism that will reduce the suitability of the internal environment for the fungi due to less water flow within the tree. Each tree was infested with 16 adult female PSHB resulting in 16 beetles per each of the 64 trees as no PSHB attacks occurred naturally during the summer. Beetles were placed on trees approximately 30 hours after the last watering. Individual PSHB were placed inside a 0.5 mL centrifuge tube (Eppendorf, Germany) containing a small piece of potato dextrose agar (PDA) with viable *F. euwallaceae* to provide food and shelter. Beetles were obtained 24 hours before, from a combination of centrifuge tubes containing semi-artificial media and as they emerged from infested wood that was collected previously

from the Huntington Botanical Gardens. Beetles were placed in a petri dish containing PDA and *F. euwallaceae* and stored at 17 °C until the following day. Centrifuge tubes were loosely attached to the trunk of the tree in a ring using marking tape. This method was used in order to place an equal number of beetles in close proximity to each of the test trees and to allow beetles to disperse after being placed on the tree. Beetles were placed on trees during Fall at around 4:00 PM. Tubes were attached simultaneously with the assistance of multiple other people at approximately the same height midway on each tree. Degree days were monitored using weather data ([www.wunderground.com/weather/us/ca/san-marino](http://www.wunderground.com/weather/us/ca/san-marino)). The trees were harvested 400 degree-days after infestation. This allowed an adequate period of development such that beetles could complete one generation. Trees were subsequently harvested by cutting the trunk just above the roots. Harvested trees were transported back to the laboratory at the University of California Riverside (UCR) where they were destructively sampled to determine the number of PSHB holes and galleries present on each tree. A gallery was differentiated from a hole if the length of the tunnel created was greater than 1 cm.

To determine if there was a significant difference in the negative water potential between treatments at the Huntington Botanical Gardens ANOVA and Tukey's pairwise comparison tests were conducted at  $\alpha = 0.05$  (PROC GLM, SAS Institute Inc 2010). Logistic regression (PROC GLM, SAS Institute Inc 2010) at  $\alpha = 0.05$  was to determine if the number of attacks and galleries being formed was affected by irrigation treatment.

## Results

University of California Irvine: Influence of irrigation on infestation in Sycamore trees

Irrigation treatment was never significantly associated with the number of new attacks for trees in any time period (Table 4.2). The number of attacks initially recorded on each tree was always significantly associated with the number of new attacks, with more heavily attacked trees having a higher rate of new attacks. Crown condition was significantly associated with irrigation treatment (Table 4.3). The crown condition survey indicated more senescence for trees where irrigation had been shut off compared to irrigated trees. Crown condition was not significantly associated with the number of attacks on the tree or the length of time the tree had gone without irrigation.

Table 4.2. Logistic regression assessing initial attack density and treatment (irrigated, no water) as predictors for the number of new attacks within each survey period. Significant effects were assessed at  $\alpha = 0.05$ .

Period	N	Initial attacks		Irrigation on or off	
		Wald's $\chi^2$	P	Wald's $\chi^2$	p
June 2015	39	5.30	0.02	1.64	0.20
July 2015	39	16.68	<0.001	1.18	0.28
September 2015	38	7.88	0.005	3.11	0.08
January 2016	38	3.94	0.05	2.18	0.14
April 2016	37	3.71	0.05	1.10	0.29

Table 4.3. Logistic regression assessing factors for predicting crown condition. Significant effects were assessed at  $\alpha = 0.05$ .

Parameter	Estimate	S.E.	Wald's $\chi^2$	p
Initial attacks	-0.005	0.006	0.73	0.39
Current attacks	0.002	0.005	0.15	0.70
Days without water	0.0001	0.002	0.001	0.97
DBH	2.803	1.809	2.40	0.12
Irrigation on or off	1.219	0.370	10.84	0.001

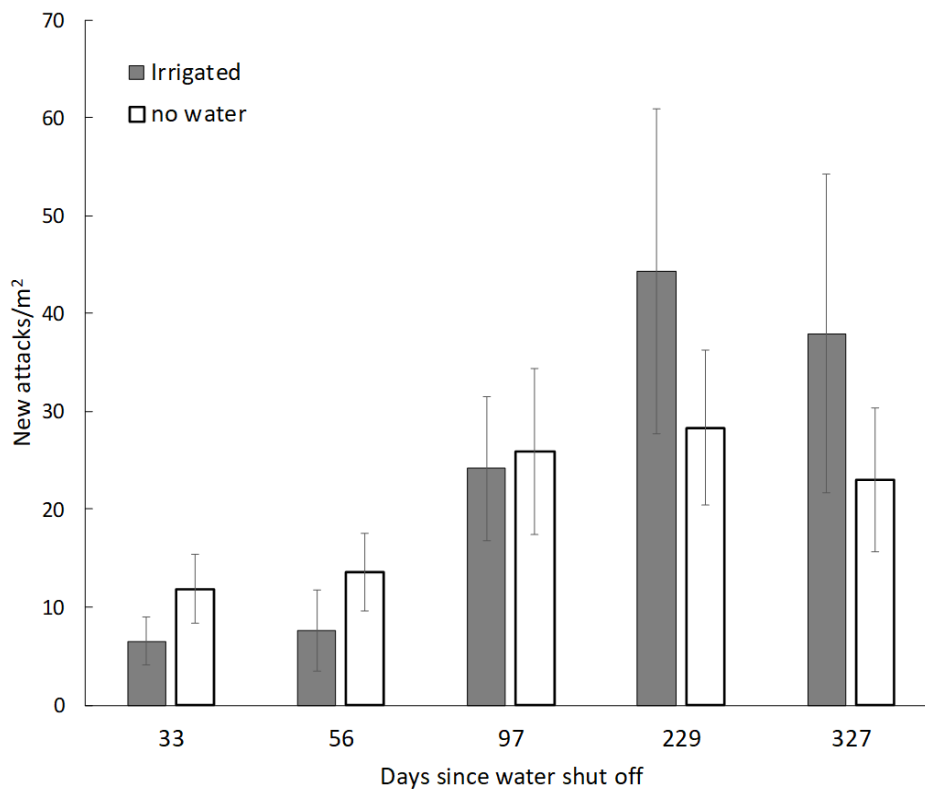


Figure 4.1. New attacks/m<sup>2</sup> (mean and S.E.) for irrigated and unwatered trees for attacks at each time interval compared to initial attacks. Within each survey period, there was no significant effect of irrigation treatment on attack rates.

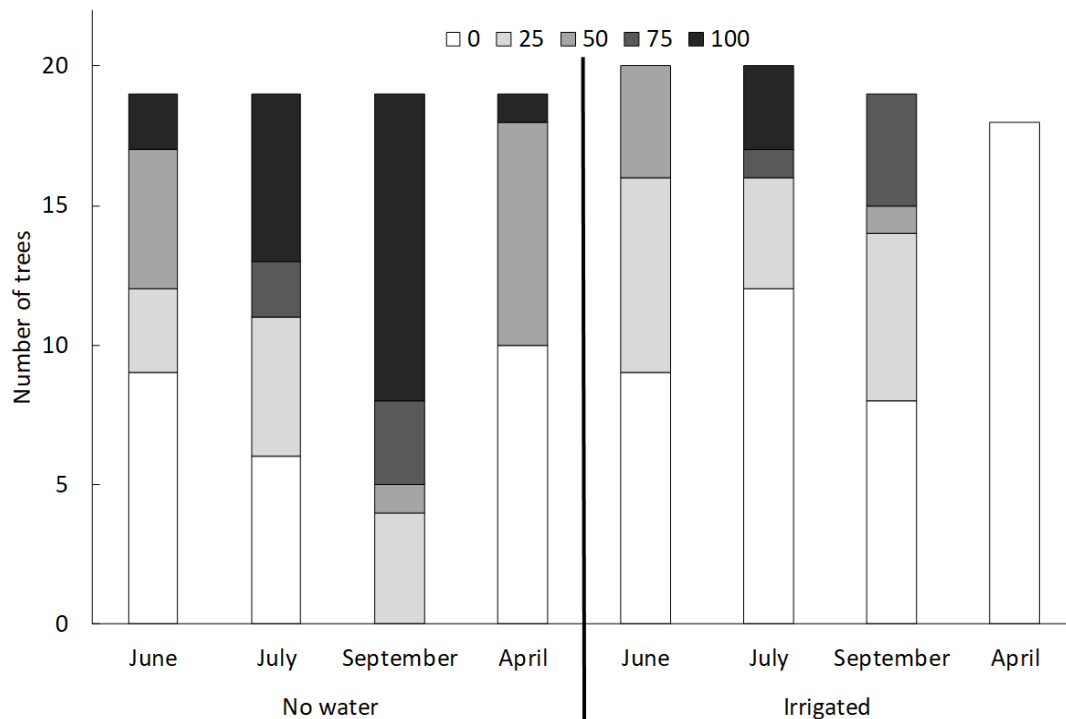


Figure 4.2. Number of trees in each category for crown condition surveys. Logistic regression showed a significant association between irrigation treatment and crown condition.

#### Huntington Botanical Gardens: Influence of irrigation on infestation in Liquidambar trees

Irrigation level and DBH did not have a significant effect on PSHB attack rate (Table 4.4). The average number of PSHB attacks on liquidambar were similar between all three irrigation treatments (Fig. 4.3). Crown health was only significantly affected by the presence or absence of irrigation and the number of PSHB attacks were not significantly associated with poor crown health (Table 4.5). Unirrigated trees had a higher average level of canopy senescence than irrigated trees (Fig. 4.4).

Table 4.4 Logistic regression assessing factors for predicting PSHB attack. Significant effects were assessed at  $\alpha = 0.05$ .

Parameter	Estimate	S.E.	Wald's $\chi^2$	p
Irrigation	1.2708	0.4978	0.0963	0.9530
DBH	0.00404	0.00796	0.1099	0.7403

Table 4.5. Logistic regression assessing factors for predicting crown health. Significant effects were assessed at  $\alpha = 0.05$ .

Parameter	Estimate	S.E.	Wald's $\chi^2$	p
DBH	0.00404	0.00796	0.2574	0.6119
Attacks	-0.0352	0.0475	0.5486	0.4589
Irrigation on or off	1.2708	0.4978	6.5171	0.0107

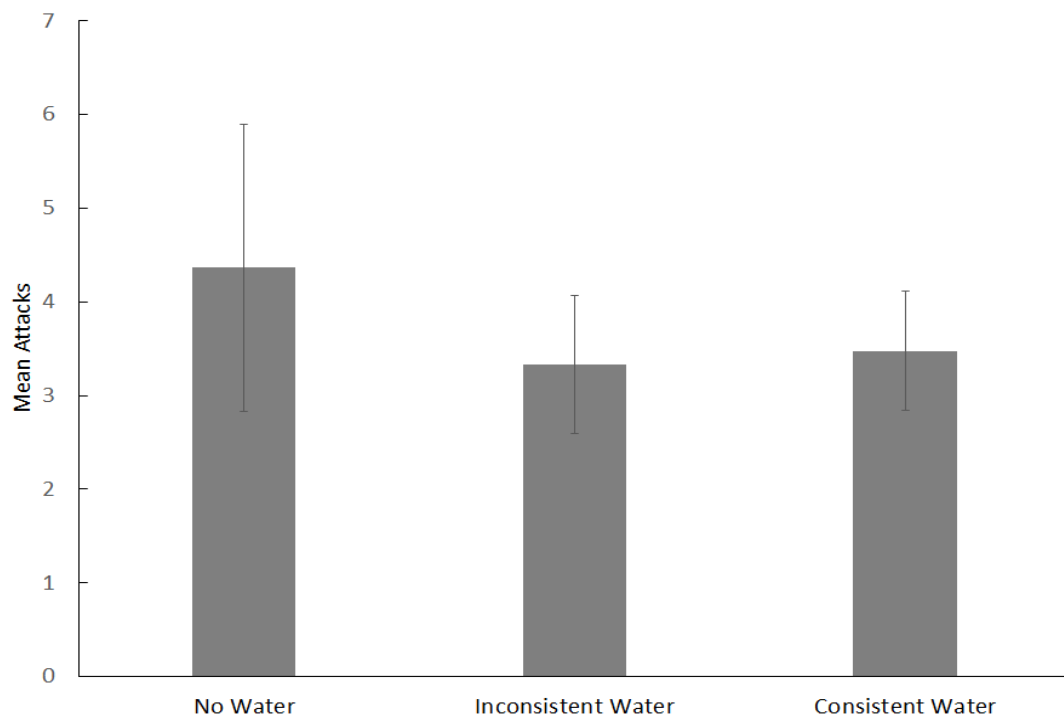


Figure 4.3. The mean number of attacks on liquidambar trees at the Huntington Botanical Gardens in areas with one of three different watering levels.

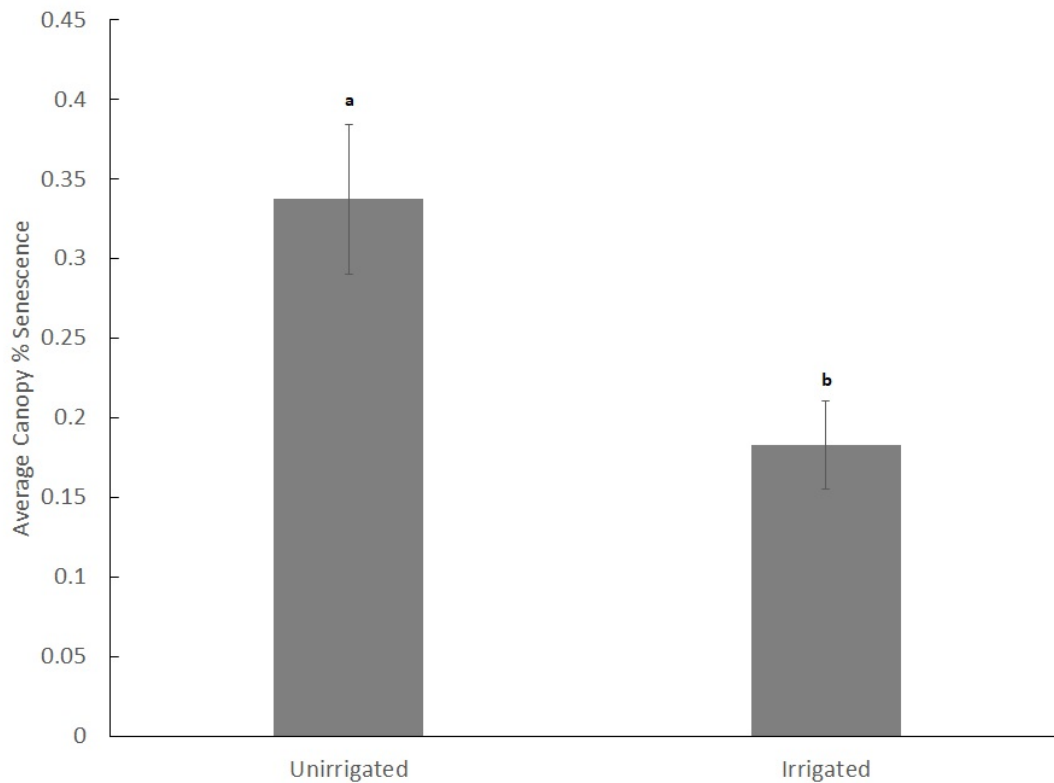


Figure 4.4. The average percentage of unhealthy branches for trees in unirrigated and irrigated sections of the Huntington Botanical Gardens. Different letters indicate significantly different values.

#### Huntington Botanical Gardens: Influence of irrigation box elder

There was a significant difference in negative water potential between the different irrigation treatments (ANOVA  $df=3$ ,  $F = 60.58$   $P = <0.0001$ ) (Figure 4.5). Tukey's pairwise comparison test determined that each of the irrigation treatments, with the exception of 11.4 LPW to 6.8 LPW, were significantly different. Based on this, 11.4 LPW and 6.8 LPW irrigation treatments were combined and the three remaining treatments were compared as High (3 LPW), Middle (11.4 LPW and 6.8 LPW), and Low (15.9) water stress for subsequent analysis.



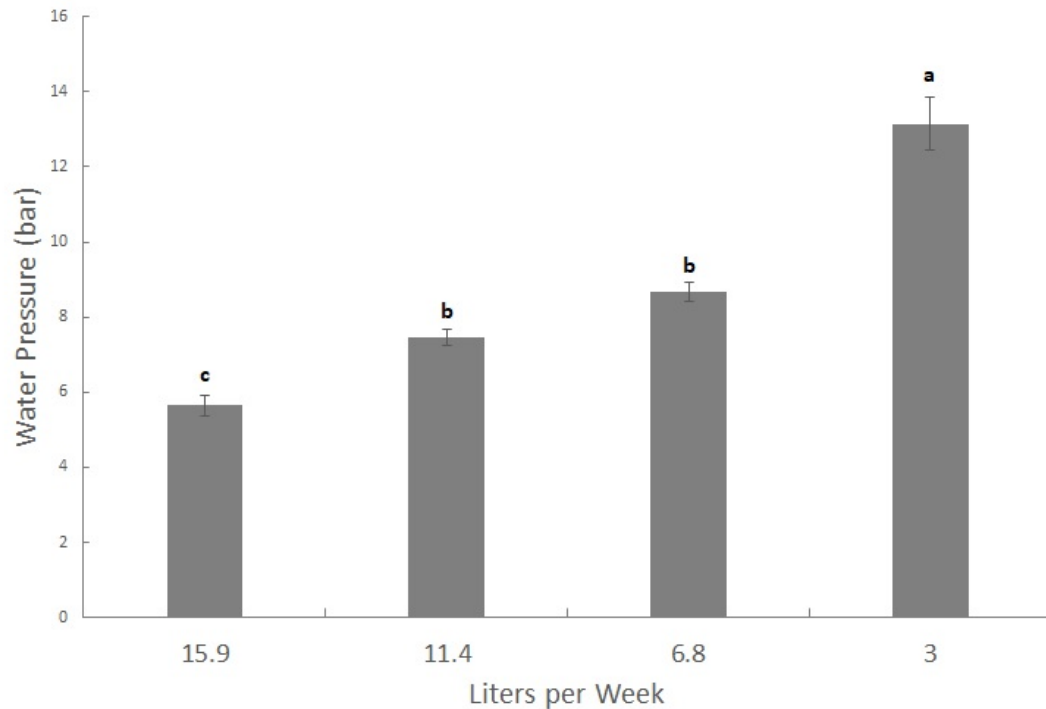


Figure 4.5. The mean pressure values per irrigation treatment. Higher levels of pressure indicate the amount of pressure need to be applied to match the negative water pressure inside the leaf. Different letters indicate significant differences.

The mean number of attacks per tree was variable. Of the 16 PSHB used to infest each of the trees, only a small number of beetles created a hole or gallery in the trunk (Table 4.6).

The mean number of attacks per tree was below 3 for all treatments (Figure 4.6). A logistic regression of the three different irrigation treatments showed that there was no significant difference in the number of attacks ( $df = 2$ ,  $F=0.94$ ,  $P=0.3971$ ). Trees were equally likely to be attacked despite their level of water stress. Likewise, the number of galleries formed in attacked trees was not significantly affected by irrigation treatment ( $df = 2$ ,  $F=1.57$ ,  $P=0.2158$ ). For trees that were attacked it was equally likely among trees

with different levels of water stress that the beetle would create a gallery after the initial hole was formed by the beetle (Fig. 4.7). Overall, the likelihood of PSHB attack on host trees was unaffected by irrigation status of the host.

Table 4.6. Mean attacks and galleries formed for each irrigation treatment. Standard errors recorded as  $\pm$ .

Water Stress	Mean Attacks	Mean Galleries
Low	$1.625 \pm 0.576$	$1.375 \pm 0.554$
Medium	$1.344 \pm 0.248$	$0.813 \pm 0.198$
High	$2.063 \pm 0.403$	$1.625 \pm 0.427$

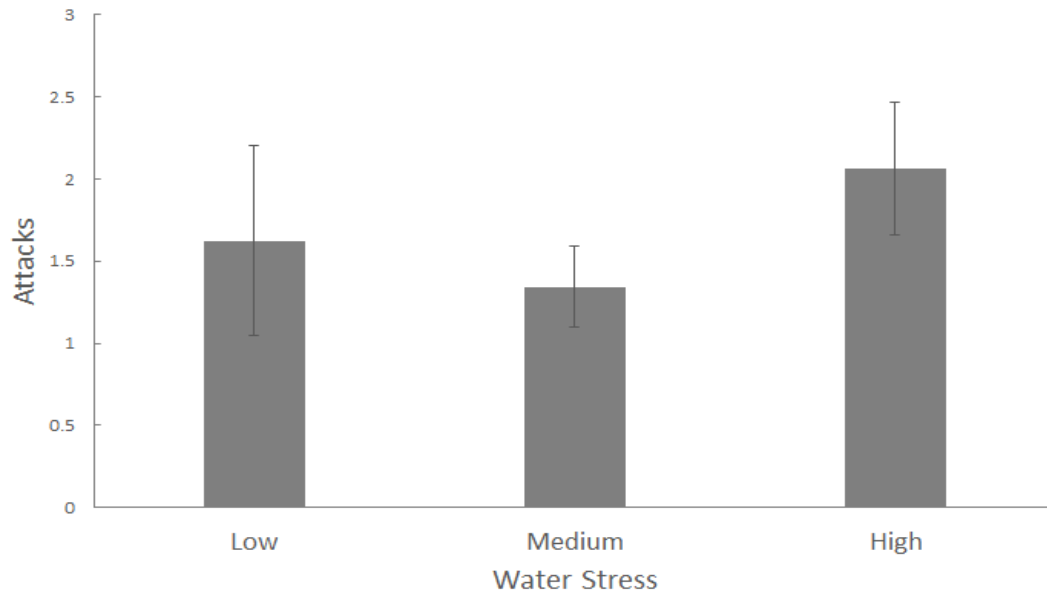


Figure 4.6. The mean number of PSHB attacks per tree with either high, low, or medium levels of water stress.

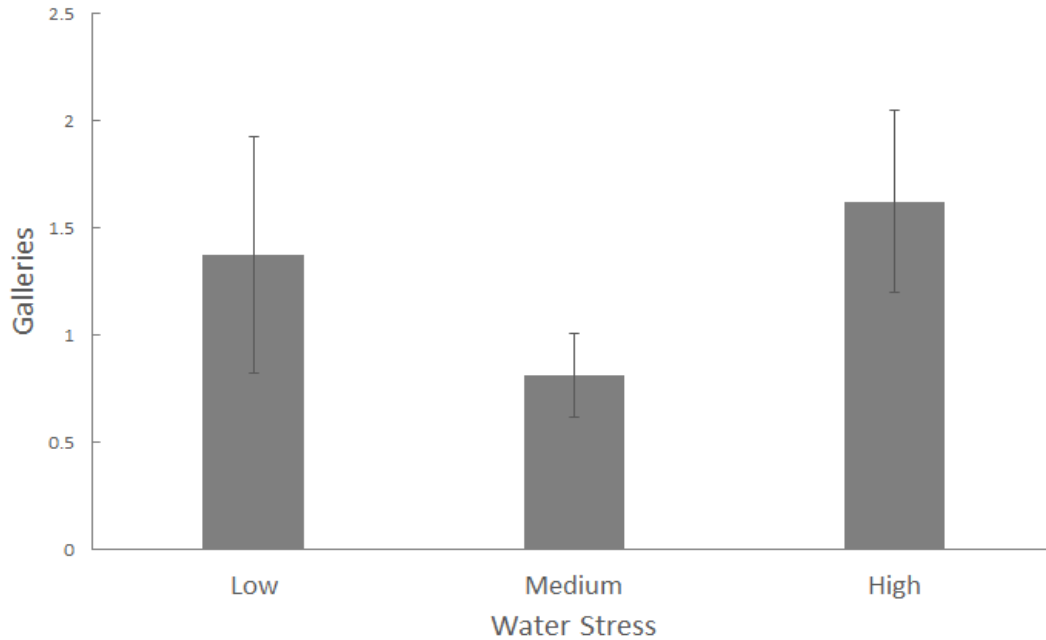


Figure 4.7. The mean number of PSHB galleries formed per tree with either high (Grey irrigation emitter), low (Black irrigation emitter), or medium (Blue and Green irrigation combined) levels of water stress.

## Discussion

For studies conducted at UCI irrigation status did not significantly alter the number of new attacks on infested sycamore trees. Unlike many wood-boring beetles (Mattson and Haack 1987; Waring and Cobb 1992), PSHB do not appear to favor water stressed trees for attack. The only factor that was significantly associated with the number of new attacks was the number of initial beetles infesting the host tree. These increases in attacks were unsurprising as PSHB will often reinfest the same host after it reaches maturity.

The crown condition of trees was not significantly affected by the initial number of initial beetle attacks or the subsequent resulting beetle attacks that occurred. Trees that were continuously watered exhibited less senescence in their crowns than trees whose watering had been halted. Despite beetle attacks being associated with damage such as gallery formation in the cambium and fungal inoculation it did not appear that the number of attacks was indicative of how much senescence the tree exhibited. How long it had been since the tree was last watered also had no effect on the level of senescence in their crown. It is possible that since beetle attacks can negatively affect tree health due to the interference of the flow of water and nutrients (Eskalen et al. 2012; Mendel et al. 2012) that the effects of beetle attacks are mitigated by having steady irrigation as there is enough water being absorbed to not express any noticeable stress or fungal levels did not reach high enough concentrations to noticeably affect tree health. With non-irrigated trees it is possible that attack levels did not significantly change senescence levels as the trees were already deprived of water, regardless of internal damage.

For the liquidambar survey, the only factor that affected visible tree health was the level of irrigation (Table 4.3). As long as the trees had been irrigated they were more likely to show lower levels of crown senescence. Here also PSHB attacks did not occur more often on water stressed trees (Fig. 4.3). Problems that arose with this study were the fact that there was not much known about the trees life histories beforehand and that data on the exact levels of irrigation for each plot were unavailable. Due to this it is possible that there were other factors involved which resulted in the low beetle attack rates. In order to

further confirm the lack of preference between trees with irrigation and without the study involving box elders was conducted in order to use hosts whose characters could be kept more similar.

The second study at the Huntington Botanical Gardens, featuring box elders, found that irrigation affected the mean water potential, which can provide an indication on the level of water stress (Shackel 2007). Trees were originally placed between sites of infested wood and wood refuse in order to make them readily accessible to dispersing beetles. However, no natural attacks occurred on the test trees. The lack of PSHB attack may be due to their smaller size. Even though the beetles have been recorded attacking hosts with relatively small diameters, like castor bean (Eskalen et al. 2013), they may prefer larger hosts and only attack smaller hosts when no better alternatives exist or the dispersing populations are large enough to increase the probability that the beetles will coincidentally land on a smaller host. This was not a problem that occurred in the previous two studies as the trees were larger and already had existing PSHB infestations. As no natural attacks occurred on the trees, the trees were artificially infested using adult females collected from wood harvested from the Huntington Botanical Gardens and offspring grown in the lab derived from beetles that were collected from the same wood. The majority of beetles did not attack the tree they were placed on, with trees receiving less than an average of 3 hits per tree. Low overall attack rates may have been due to small tree size, which could provide poorer conditions such as insulation, and beetles would be more willing to attack larger, more mature trees. Alternatively, it is possible

that beetle success rate in the field was actually quite low and was normally compensated for with large numbers of invading beetles. Of the beetles that did attack the test trees there was no significant difference between the different irrigation levels with respect to attack rates. Trees that were water stressed were just as likely to get attacked as trees with lower levels of water stress. Furthermore, when beetles started to attack the trees they were just as likely to continue to form a gallery. Based on these findings it appears as if the irrigation status of the tree had no significant effect on its likelihood of being attacked.

Unlike other wood-boring insects the PSHB demonstrated no preference for less irrigated hosts. While some wood-boring insects preferentially attack stressed trees in order to bypass host defenses (Croise et al. 2001; Encina et al. 2012; Ferrell 1978; Paine et al. 1997) PSHB chooses to attack hosts regardless of their health. As long as the tree is living there is likely enough moisture to support fungal growth and as has been shown by the numerous attacks of PSHB on trees throughout southern California that the defenses of healthy trees are not enough to deter the beetle. As water stress, or lack thereof, did not appear to affect host preference there may be no selective pressure for PSHB to choose one tree over the other. These results, at first, seem to conflict with findings from Boland (2016) which indicated that beetle attacks favored trees closer to water. However, those results could be due to the water source being nutrient rich (Boland, 2016) and acting as a source of fertilizer for the trees. It has been shown that trees that received fertilizer were attacked more than trees that were just irrigated (Coyle et al, 2005), which may indicate

that beetles, *Dryoxylon onoharaensum*, *Euwallacea validus*, *Pseudopityophthorus minutissimus*, *Xyleborus atratus*, and *Xyleborus impressus*, were attracted to trees closer to the water due to the nutrient rich runoff that was fertilizing the trees.

It appears that irrigation level will not affect the probability that trees will be attacked. Visible damage in crown senescence occurred when trees were subjected to suboptimal conditions and appeared otherwise healthy when irrigated. It is important to note that irrigating trees won't prevent beetle damage and potentially the eventual death of the tree, but doing so will at least prevent earlier decline of tree health and potential mass exodus of PSHB leaving the dying host.

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## **Chapter 5**

### **Effects of the Fungal Symbionts of Polyphagous Shot Hole Borer**

## Introduction

Large body size in adult insects can confer fitness advantages; larger females can have higher fecundity or lay larger eggs (Alcock 1993; Kojima 2015; Thomas 1993). Larger eggs are advantageous as they result in greater initial larval size and higher larval survival (Fox and Czesak 2000; Kojima 2015). The size of wood-boring insects is significantly affected by the availability and quality of food in their larval environment (Andersen and Nilssen 1983; Haack and Slansky 1987; Hanks et al. 2005). Sufficient nutrition is monitored by target of rapamycin (TOR) pathways which regulate signals that promote transitioning into the next life stage based on reaching critical weight thresholds (Mirth and Riddiford 2007). As the larvae of wood-boring insects are unable to move to different hosts, the quality of their environment is set by their mother's oviposition site. Host quality can vary due to different levels of stress and age, which leads to variation in insect development (Andersen and Nilssen 1983).

In the case of ambrosia beetles, reliance on symbiotic fungi adds another layer of complexity to the larval environment. As the sole food source for these beetles, fungi supply nutrition that is difficult to obtain from wood, e.g. nitrogen and sterols (Bentz and Six 2006; Goldhammer et al. 1990). The growth of the symbiotic fungi will determine the level of nutrition available to ambrosia beetle larvae. Fungal growth and its availability to be used as a food source can be affected by temperature (Six and Bentz 2007). Temperature can also independently affect the size of developing ambrosia beetles. While

the growth rate of insects generally increases with temperature, the size of the adult has been shown to be affected in the opposite fashion, with larger adults emerging at cooler temperatures and smaller adults produced at higher temperatures (Atkinson 1994; Bauerfeind and Fischer 2013; Petersen 2000). Temperature and nutrition may both play potentially opposing roles in determining adult size, but there appears to be little interaction between the two (Bauerfeind and Fischer 2013; Petersen 2000). Even if fungal and host species may not always affect adult weight, they have been shown to have a significant effect on survival (Six and Paine 1998). These factors make choice of oviposition site and environment essential for reproductive success.

The invasive ambrosia beetle, *Euwallacea nr. fornicatus* (Coleoptera: Curculionidae; Scolytinae), commonly referred to as the Polyphagous Shot Hole Borer (PSHB), has become a pest in southern California since it was first found on black locust (*Robinia pseudoacacia*) in 2003 (Rabaglia et al. 2006). PSHB poses a threat to California forests due to its large host range that includes 49 reproductive hosts (Eskalen et al. <http://eskalenlab.ucr.edu/shotholeborerhosts.html>) and its transmission of a fungal symbiont that causes *Fusarium* dieback in infested trees (Freeman et al. 2013). In addition to *Fusarium euwallaceae*, the causal agent of *Fusarium* dieback, PSHB also forms a symbiotic relationship with *Graphium euwallaceae* and *Paracremonium pembeum* (Lynch et al. 2016). The three fungi are transported in the PSHB's mycangia (Lynch et al. 2016) to new hosts where it is cultivated by the beetle for food. In return for transport to new hosts, at least one of the fungi serves as the PSHB's source of nutrition.

It is unknown what exact role each fungi plays in its relationship with PSHB. In bark beetles it has been shown that having multiple fungal associations can be potentially advantageous rather than redundant. Multiple fungi may perform differently depending on temperature which ensures that at least one fungal associate will develop in different environments (Six and Bentz 2007). Other fungi are more prevalent at different times during larval development which means their importance to the beetle may change with time (Adams and Six 2007). It is possible that each of the three associated fungi of PSHB play a slightly different nutritional role, serving as the main source of food under specific conditions such as high or low temperatures.

The focus of this study was to determine the effects of the three fungal associates of PSHB on survival and fitness at three different temperatures in order to better define the role each of these fungal species plays in the PSHB life cycle.

## Materials and Methods

### Fungi

Fungal plates of *F. euwallaceae*, *G. euwallaceae*, and *P. pembeum* were obtained from the Eskalen laboratory at the University of California Riverside. Each fungus was collected and grown on potato dextrose agar plates by the Eskalen laboratory (Lynch et al. 2016; Eskalen pers. comm). Once sufficient fruiting bodies to sustain beetle

development were present the plates were transported to the Paine lab and kept at ambient temperature until use.

## PSHB

PSHB adults were obtained from infested *Quercus robur* wood collected from the Huntington Botanical Gardens in San Marino, CA. Infested wood consisted of multiple large branches cut down into billets between 1 to 2 feet in length using a handsaw. Infested wood was stored in clear plastic BugDorms (MegaView Science, Taiwan) at 27 °C. The use of bug dorms allowed for the infested wood to be completely enclosed while making it easy to collect emerging adults that aggregated along the top edges of the enclosure. The temperature was chosen based on the optimum developmental temperature for PSHB (Chapter 2). Adult female beetles were collected daily from the dorms and placed in tubes containing semi-artificial media based on the recipe developed by Biedermann et al. (2009). Males tend not to disperse away from their host wood and are easily distinguishable from females by their shorter, truncated abdomens. Female beetles typically mate with their siblings inside their natal galleries before emerging. Beetles were allowed to infest the tubes for approximately 2 weeks before the tubes were dissected in order to harvest larvae. Two weeks were given in order to allow enough time for the foundress to generate offspring which only happens after gallery formation and fungal growth inside has progressed sufficiently. Larvae were washed in White's solution (Barras 1973) in order to surface sterilize them and remove any fungal spores to prevent contamination of fungal test plates. Larvae used were harvested from colony tubes

between 2-3 weeks old and all larvae were approximately 1.5 mm in length in order to use larvae of about the same age. This larval size was chosen based on the age of the natal gallery and prior observations from dissections in Chapter 2 regarding development.

Sterilized offspring were placed on a plate containing one of the three fungal symbionts. Plates contained five larvae and were stored in one of three temperatures. Temperatures tested were 16, 27, and 32 °C based on temperatures that were near the beetle's minimum, optimum and maximum developmental ranges, respectively (Chapter 2). No light cycle was used as the larvae inside the galleries would not naturally be exposed to light. A total of 520 offspring were tested, with at least 20 offspring per fungus and temperature. Each plate was checked three times per week to monitor the growth of the PSHB. Plates were sealed with parafilm in order to reduce the amount of moisture being lost from the agar and if condensation formed it was removed with a Kimwipe (Kimberly-Clark Professional, Roswell GA) to prevent larvae from getting trapped in droplets. All life stages were moved to new fungal plates when the quality of the fungal plates started decline, determined by thinning of agar and shriveling of fungi, in order to ensure that beetle mortality was not caused by factors such as gradual desiccation. Upon reaching adulthood, female PSHB were placed inside separate vials containing 95% ethanol. Beetles were subsequently dried in a Thelco Model 17 Laboratory Oven (Precision Scientific Company, Chicago) at 65°C for 96 hours and weighed on a Vibra HT-224 R Analytical Balance (Intelligent Weighing Technology, CA). Survival rate was calculated as the number of larvae reaching adulthood divided by the total number of



larvae tested for that treatment. Due to a malfunction with the 16 °C temperature cabinet and the length of time required to complete development at that temperature there were not enough adult beetle specimens to collect sufficient dry weight data to perform a reliable analysis on the effects of fungi on fitness at that temperature, but enough overall survival to analyze the effect of fungi on survival at 16 °C. Survival was defined as the larvae reaching adulthood and the fitness effects examined were development time and dry weight.

### Statistical Methods

To determine if there was a significant difference in mean survival rate between fungal treatments a logistic regression was conducted at  $\alpha = 0.05$  (PROC Logistic, SAS Institute Inc 2010) with pairwise comparisons using least squares means with Bonferroni adjustment to determine if PSHB on any of the three fungal symbionts had greater rates of survival. No larvae raised on *P. pembeum* survived to adulthood. Consequently, survival data from *P. pembeum* was not compared with the other two fungal symbionts as it would skew the analysis. Development time and adult dry weight were analyzed using an ANOVA conducted at  $\alpha = 0.05$  (PROC GLM, SAS Institute Inc 2010) to determine if fungal symbiont significantly affected potential fitness. Tukey's pairwise comparison tests were used to distinguish differences between fungal symbionts and to determine which fungal symbionts conferred the greater fitness advantages.

## Results

### PSHB survival

Because no larvae survived on a diet of *P. pembeum* this treatment is excluded from further analysis. Only temperature had a significant effect on survival rate (df=2, Wald  $\chi^2=6.6637$ , p=0.0357). When comparing *F. euwallacea* and *G. euwallacea* there was no significant effect on survival (df=1, Wald  $\chi^2=1.387$ , p=0.2389). Fungi and temperature did not have a significant interaction (df=2, Wald  $\chi^2=2.8911$ , p=0.2356). PSHB showed significant differences in the rate of survival on *F. euwallacea* at 27 °C compared to *G. euwallacea* (z=2.43, p=0.0149), but not at any other temperature (Fig. 5.1). Both were different from *P. pembeum* which did not have any offspring surviving to maturity (Fig. 5.1). Mean survival on both *F. euwallacea* and *G. euwallacea* was low with only a fraction of the larvae progressing all the way to adulthood.

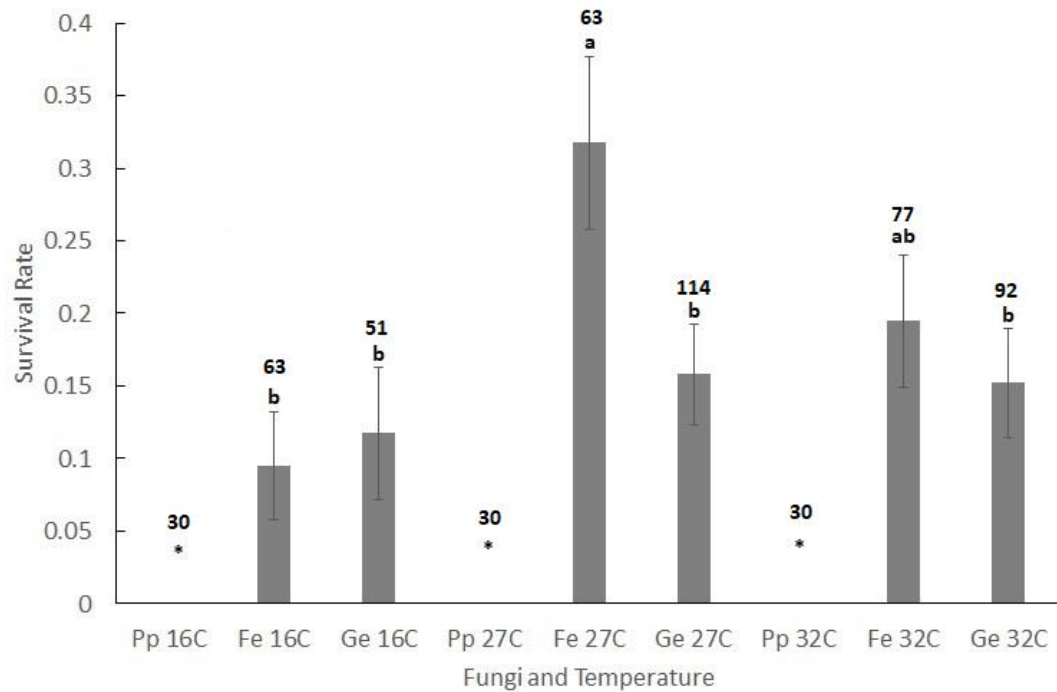


Figure 5.1. Survival rate for PSHB larvae to adult stage raised on one of three fungi at 16, 27, or 32 °C. Pp is *Paracremonium pembeum*, Fe is *Fusarium euwallaceae*, and Ge is *Graphium euwallaceae*. Numbers indicate the sample size for each treatment. Same letters indicate no significant difference between treatments. \* as no larvae survived to adulthood *P. pembeum* was not compared to the other treatments.

#### PSHB development

Development time was significantly affected by fungi (df=1, F=25.08,  $p<0.0001$ ) (Fig. 5.2). There was a significant interaction between fungi and temperature (df=1, F=4.96,  $p=0.0302$ ). Larvae on *F. euwallaceae* at 27°C took significantly longer to develop than those raised on *G. euwallaceae* at either temperatures (Fig. 5.2) Larvae raised on *G. euwallaceae* had shorter development time at 27 °C than those raised on *F. euwallaceae* at either temperatures (Fig. 5.2). Mean development time at 27°C was approximately 7 days shorter on *G. euwallaceae*, compared to *F. euwallaceae*. Both fungi did not

significantly differ in larval development rates within species regardless of temperature and between species at 32°C (Fig. 5.2). Adult dry mass was significantly affected by fungi (df=1, F=164.68, p<0.0001). However, temperature did not significantly affect adult dry mass (df=1, F=2.2, p=0.1439) (Figure 5.3) and there was no interaction between fungi and temperature (df=1, F=2.9, p=0.0945). Larvae raised on *F. euwallaceae* were significantly larger than those raised on *G. euwallaceae* at both temperatures (Fig. 5.3).

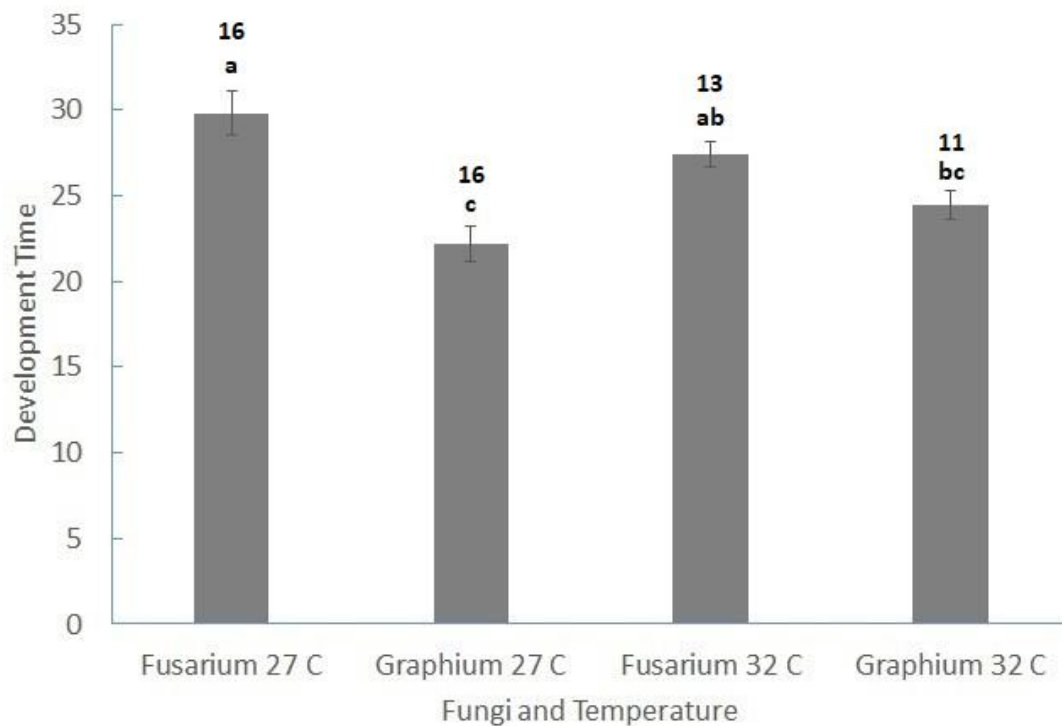


Figure 5.2. Mean developmental time in days for *F. euwallaceae* and *G. euwallaceae* at 27 and 32 °C. Numbers above bars indicate sample size and same letters indicate no significant difference between treatments.

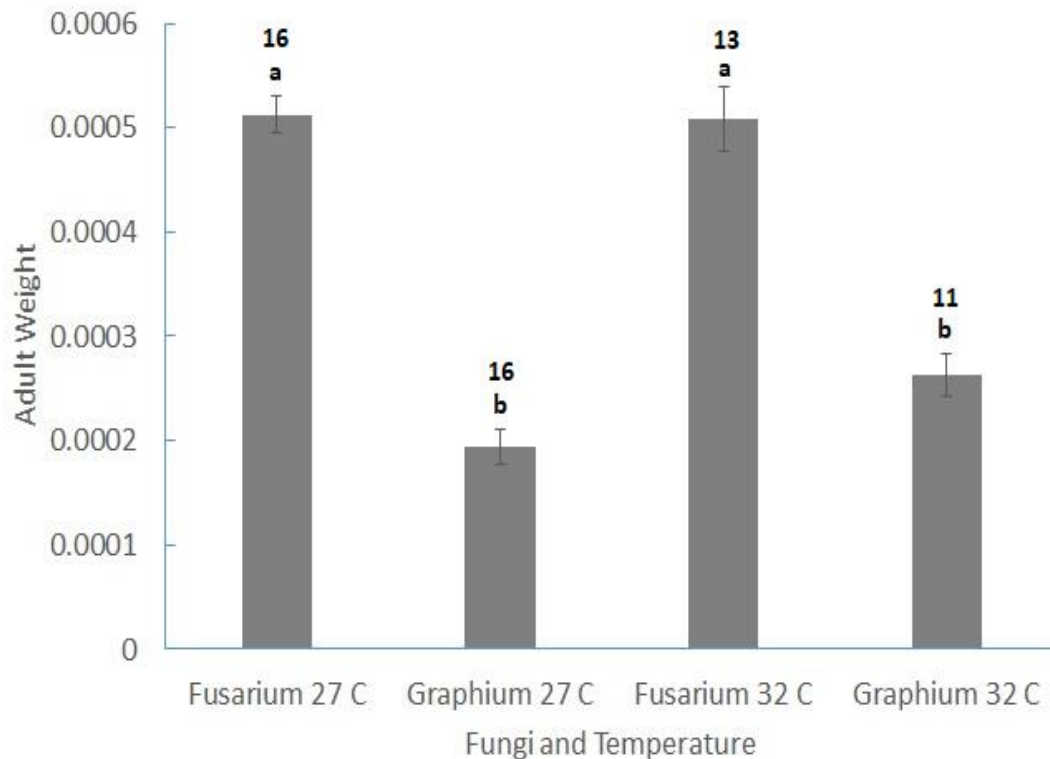


Figure 5.3. Mean dry weight, in grams, of adult PSHB developing on *F. euwallaceae* and *G. euwallaceae* at either 27 or 32°C. Numbers above bars indicate sample size and same letters indicate no significant difference between treatments.

## Discussion

Larvae of PSHB were able to fully complete development on *F. euwallaceae* and *G. euwallaceae*. However, no larvae survived to adulthood on *P. pembeum* (Fig. 5.1), suggesting either lack of nutritional quality or lack of larval feeding on this species.

PSHB larvae grown on *F. euwallaceae* and *G. euwallaceae* appeared to have similar survival rates with the exception of larvae grown on *F. euwallaceae* at 27°C which had greater rates of survival than the rest of the treatments (Fig. 5.1). As a food source, both

provided adequate nutrition independently and are not required to supplement each other. It is uncertain what role *P. pembeum* played in the PSHB life cycle and may function as a supplemental resource or an opportunistic associate of PSHB. *P. pembeum* may have an indirect effect on PSHB by affecting its other two fungal symbionts. Adams et al. (2008) demonstrated that certain yeasts associated with the bark beetle *Dendroctonus ponderosae* facilitated the growth of one of the beetle's fungal symbionts, *Ophistioma montium*, while inhibiting the growth of another fungal symbiont, *Grosmania clavigera*. In this respect, *P. pembeum* may be enhancing the growth of *F. euwallaceae* and/or *G. euwallaceae*, which is why its association with PSHB is maintained despite its lack of nutritional value. *P. pembeum* may also be an opportunistic associate that provides no benefit to PSHB. It is not unheard of to have a fungal associate that is in a commensal or antagonistic relationship with a wood-boring beetle. In the *Dendroctonus frontalis* system, the blue stain fungus *Ceratocystis minor* has been found associated with the beetle in attacked trees, but provides no nutritional benefit and may have a negative effect on beetle fitness (Goldammer et al. 1990; Six and Wingfield 2011).

Overall larval survival was low even amongst the two fungal symbionts that supported beetle development (Fig. 5.1). There is a level of mortality to be expected from surface sterilization (Barras 1972), but it would not account for the low overall levels of survival. As the levels of mortality experienced by ambrosia beetles inside their galleries are relatively undocumented and difficult to record, it is uncertain how different the levels of mortality experienced in this laboratory study differ from the field. The soft-bodied

larvae decompose quickly and it is impossible to determine how many larvae were originally in a natural gallery after the larvae have died. Greater levels of exposure may have affected larval survival, as the larvae were not sealed within a narrow gallery which confines the larvae into closer proximity with each other and creates a different microclimate. There could potentially be an effect of adult care on survival, but this is difficult to test as adults introduced into the plates with the larvae burrowed into the agar and left the larvae unattended.

PSHB raised on *G. euwallaceae* developed significantly faster than those raised on *F. euwallaceae* (Fig. 5.2), but PSHB raised on *F. euwallaceae* were significantly larger than those raised on *G. euwallaceae* at all temperatures tested (Fig. 5.3). This trend follows the tradeoff of faster growth with smaller size and slower growth with larger size at 27°C (Atkinson 1994; Bauerfeind and Fischer, 2013; Petersen 2000). The longer an insect spends developing the more time it will have to grow, which creates a positive correlation between developmental time and body size (Nijhout et al. 2010). Though the trend between sizes of beetles and the development time generally follows the expected correlation between species, it appears to deviate at higher temperatures. PSHB raised on *G. euwallaceae* were dramatically lighter than those raised on *F. euwallaceae*, even at 32 °C where developmental times were more similar. This could indicate that, while *G. euwallaceae* promoted faster growth, *F. euwallaceae* was potentially more nutritious as beetles developing in a similar amount of time were significantly different in mass. The difference in development time could be due to difference in the ability of the larvae to

process the different fungi, with *F. euwallaceae* taking longer to eat or digest which may causes the beetle to take longer to gain the nutrition necessary to reach the next life stage.

The choice between *F. euwallaceae* and *G. euwallaceae* would be an interesting trade-off between speed and size as both fungi are grown inside the gallery. Depending on the characteristics of the fungal symbionts the choice may already be made for the larvae. Similar to *Dendroctonus ponderosae*, another fungus feeding wood-boring beetle, the temporal and temperature variation of fungi within the galleries may dictate the prevalent food source (Adams and Six 2007) and depending on conditions one fungi may be present in greater abundance over the other. These factors can be seen in the existence of an interaction between temperature and fungal type on developmental rate.

These results indicate that only *F. euwallaceae* and *G. euwallaceae* provided enough nutritional benefit to allow larvae of PSHB to reach adulthood. *P. pembeum* may provide some benefit to PSHB, but if it does it is indirectly. Between these two fungal species there was a tradeoff between developmental rate and adult weight. Near optimum temperature beetles will develop faster on *G. euwallaceae*, but *F. euwallaceae* will result in larger adults at optimum and higher temperatures. PSHB feeding on *F. euwallaceae* will be larger and potentially more fit than those just feeding on *G. euwallaceae*, but the effects of eating both may provide synergistic benefits. The basis for the difference between development on the two fungi cannot be fully understood until a more detailed study is conducted on the characteristics of the fungi themselves. Future work is needed



to determine what promotes faster growth when feeding on *G. euwallaceae* and why it results in such a large drop in body mass. Furthermore, there may be interactions between the three fungal symbionts that promote their co-occurrence, such as facilitating each other's growth, inhibiting competitors, or providing more balanced nutrition. Additionally, work to determine if the fitness trends seen here are repeated at lower temperatures.

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## **Chapter 6**

## **Conclusion**

Polyphagous Shot Hole Borer (PSHB) (Coleoptera: Curculionidae: Scolytinae), *Euwallacea* sp. near *forficatus*, poses a significant threat to its invasive range. Due to its symbiotic relationship with the pathogenic fungi, *Fusarium euwallaceae*, PSHB is responsible for spreading Fusarium dieback to trees (Freeman et al. 2013) and causing severe damage in southern California and Israel (Mendel et al. 2012). With a broad and increasing reproductive host range that has more than doubled since the initial formation of a host list in 2013 and includes multiple California native species (Eskalen et al. 2013; Eskalen et al. <http://eskalenlab.ucr.edu/shotholeborerhosts.html>), PSHB is a serious invasive threat to both urban and wild forests. As relatively little is known about this ambrosia beetle the goal of these studies was to gather information on its life history in order to aid the development of efficient management strategies.

Based on studies at a number of different temperatures I have found the range of temperatures at which PSHB is able to complete development. The range of suitable temperatures for beetle development falls between 15.00 - 33.08 °C with the optimal temperature at 27.58°C. It is important to note that while this is the ranges for development, they are not necessarily lethal temperatures and beetles can persist for extended periods at 15°C. They also do not necessarily reflect air temperatures in the natural environment because the larvae are developing within an insulated environment within the wood of the host. PSHB require 398.41 degree days to complete development from egg to adult. Degree days are a unit that can be calculated as the lower temperature threshold subtracted from the average daily temperature with the possibility of multiple

degree days accumulating in a single day. At or near optimal temperatures PSHB need only approximately 3 weeks to complete their development to adults. Understanding PSHB's development rates will be helpful in planning monitoring efforts and predicting periods of increased beetle activity. By knowing degree days and progression of life stages, predictions of emergence can be made. The data gained was also integral to creating climate models in order to estimate PSHB's potential range of invasion.

As the beetle is sheltered inside its host trees and grows its own food, climate is one of the few factors that may limit its distribution. The temperature data gained from Chapter 2 was used in the formation of climate matching maps created from the software CLIMEX and MaxEnt (Sutherst and Maywald 1985; Phillips et al 2006). These programs used temperature and location data to generate maps that show areas that could potentially allow for beetle development. CLIMEX and MaxEnt models successfully recovered the native range of the beetle. Based on the climate models it was predicted that PSHB could spread through California's Central Valley into northern California and that California can support two to four generations of PSHB per year. On a broader scale, the southern portion of North America extending all the way into the middle of South America were highly suitable for PSHB development based on climate. Mexico is potentially at high risk due to its favorable climate, which can support multiple generations of PSHB per year, and it supports a substantial avocado industry.

Climate is only one of the important factors for determining whether PSHB could spread. The distribution maps generated by CLIMEX were refined further using data on 14 reproductive hosts and native California plant communities. By incorporating these data, the validity of the previously generated climate maps was checked as lack of hosts could be a limiting factor in the spread of PSHB. Based on the continuous presence of at least one reproductive host throughout the predicted climatic range of PSHB I conclude that host availability is unlikely to limit the spread of PSHB and that the climate maps are a conservative predictor of potential invasive range. Maps are conservative not only because urban forests are unaccounted for and contain numerous hosts, but also because temperature extremes can be partially mitigated by the insulating effects of the tree host. As PSHB is predicted to be able to successfully develop much further north from its current invaded area, it is imperative that as much as possible is done to slow or halt its spread. Unfortunately, there are no current methods to curatively control this beetle in existing infestations. The best options are to monitor susceptible areas along the borders of its range, prevent the spread of infested wood, and removal of infested trees by chipping (Eatough-Jones and Paine 2015).

Besides predicting the invasive range of PSHB, I also sought to gather information on host preference to aid in choosing management practices and monitoring choices. I looked at the effect of water stress on two different sets of trees to determine if the trees exhibit any differences in beetle attacks. Many wood-boring insects prefer water-stressed trees (Mattson and Haack 1987; Waring and Cobb 1992), but it was uncertain if reliance



on moist environments for fungal growth would also play a role in host selection. I found that PSHB exhibits no preference between trees with or without water stress and that trees are selected randomly, regardless of watering status. Based on these data we cannot recommend any particular management strategies to reduce the chance of PSHB on trees. While no recommendations can be made with regard to PSHB preference, it appears that keeping trees sufficiently irrigated reduces the level of crown senescence and may extend the lifespan of the infected tree.

PSHB is associated with three different fungal symbionts, *Fusarium euwallaceae*, *Paracremonium pembeum* and *Graphium euwallaceae* (Lynch et al. 2016). I observed the effects each fungus has on survival and development when presented as the sole food source for immature PSHB to determine if each fungus plays an equally important role. Of the three fungal symbionts, only *F. euwallaceae* and *G. euwallaceae* provided enough nutrition for PSHB larvae to survive to adulthood. It is possible that *P. pembeum* plays an indirect role in PSHB development that promotes its continual association. *F. euwallaceae* had noticeably higher survival at 27°C compared to all other treatments. With regards to fitness, PSHB exhibited a trade-off when feeding on the different fungi at 27°C. Larvae feeding on *F. euwallaceae* took longer to reach adulthood but were larger than those raised on *G. euwallaceae*. However, at 32°C developmental rate was similar, but *F. euwallaceae* still produced larger PSHB adults. Based on these findings it would appear that *F. euwallaceae* has higher nutritional value than *G. euwallaceae*, but it is unclear as to why they take longer to develop at 27°C. Further investigation into the characteristics of the

fungus symbionts is necessary to determine why the developmental time and adult size trade-off occurs near optimal temperature and to find any synergistic effects that may be occurring when the fungi are grown together.

## Summary

PSHB has been shown to develop in a range of temperatures that will allow it to spread throughout the southern portion of the United States of America and southward all the way to South America. Its range includes all but the most northern parts of California. Throughout California there are suitable hosts to facilitate its gradual spread and allow it to establish anywhere that the climate promotes development. The proposed range is likely to be conservative due to limited information and environmental effects that will buffer the beetle from extreme temperatures. As the spread of PSHB throughout California is a real threat it is important that we acquire information useful to developing methods of control or, at the very least, reduce risk of attack. Unlike many wood-boring beetles that preferentially attack water-stressed trees, PSHB does not appear to have a preference for trees with any particular level of water stress. This means that irrigation practices are unlikely to increase or decrease risk of attack by PSHB. With regards to the food source of PSHB, only two of the three fungus symbionts are directly used for nutrition. Of these two it seems that *F. euwallacae* provides the most nutritional benefit, but *G. euwallacae* allows beetles to emerge up to a week earlier. If these fungi are targeted in order to control the PSHB if both cannot be eliminated these benefits would

need to be taken into account with regards to which one should be prioritized for removal. More information needs to be obtained regarding the fungal symbionts themselves to understand their interactions with the each other and the beetle in order to fully elucidate how they are contributing to PSHB's development.

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