2011

Characterization of the subcortical interactions between larvae of the southern pine sawyer Monochamus titillator (F.) and the larvae of the southern pine beetle guild using molecular gut analyses

Erich N. Schoeller
Louisiana State University and Agricultural and Mechanical College, eschoeller@agcenter.lsu.edu

CHARACTERIZATION OF THE SUBCORTICAL INTERACTIONS BETWEEEN LARVAE OF THE SOUTHERN PINE SAWYER, *MONOCHAMUS TITILLATOR* (F.) AND THE LARVAE OF THE SOUTHERN PINE BEETLE GUILD USING MOLECULAR GUT ANALYSES

A Thesis

Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
Master of Science

in

The Department of Entomology

by
Erich N. Schoeller
B.S., University of Wisconsin-Whitewater, 2008
August, 2011

Epigraph

"Research is to see what everybody else has seen, and to think what nobody else has thought."- Albert von Szent-Györgyi

Acknowledgments

I would like to express my sincere appreciation to my major professor Dr. Jeremy Allison for his guidance, expertise, and endless patience. I would also like to express my thanks to my committee members Dr. Claudia Husseneder and Dr. Seth Johnson for sharing their time and knowledge in furthering my education. Many thanks to all of Dr. Husseneder's lab members for being accommodating to my needs and providing technical support in my work.

I would also like to thank Drs. Brian Sullivan and Jim Meeker, Wood Johnson, and Tim Haley (U.S. Forest Service Southern Research Station) and Carlton Cobb (Louisiana Department of Agriculture and Forestry) for their assistance with field collections. My thanks also goes out to the staff of the Bob R. Jones Idlewild Research Station and to the staff of the LSU Burden Research Plantation for providing me with additional trapping sites.

I am grateful for all the hard work and companionship that Jessica McKenney has provided in both the field and the lab. Finally, I would like to thank all our student workers and Hui-Ho Yang for their assistance.

Table of Contents

List of Tables

List of Figures

Abstract

The southern pine beetle guild (Coleoptera: Curculionidae: Scolytinae) is arguably the most destructive group of forest pests in the Southeastern United States. Laboratory assays suggest that larvae of wood borer associates from the genus *Monochamus* (Coleoptera: Cerambycidae) may be facultative intraguild predators of southern pine beetle guild. In this study the dynamics of the subcortical interactions between *M. titillator* (F.) and members of the southern pine beetle guild were examined using PCR-based molecular gut content analyses. Species-specific PCR primer sets were developed to work under multiplex PCR conditions to detect DNA of members of southern pine beetle guild in the gut contents of *M. titillator* larvae. The molecular half-life of the bark beetle *Ips grandicollis* (Eichhoff) was calculated as 6.89 hours post-consumption in the gut contents of *M. titillator* larvae under laboratory conditions. Comparison of the proportion of *M. titillator* larvae testing positive for each bark beetle species at 6.9 hours post-consumption showed that the proportion fed *Dendroctonus terebrans* (Olivier) differed significantly. A field study was performed to determine the detection frequencies of southern pine beetle guild DNA in the gut contents of *M. titillator* larvae under semi-natural conditions. A total of 271 *M. titillator* larvae were collected from experimental boles in the field. Twenty-six (9.6%) of the field-collected *M. titillator* larvae tested positive for DNA of members of the southern pine beetle guild. Of these larvae, 25 (96.2%), 1 (3.8%), 0 (0%), and 0 (0%) tested positive for *I. grandicollis*, *I. calligraphus* (Germar), *D. terebrans*, and *D. frontalis* (Zimmerman) DNA respectively. The species compositions of the southern pine beetle guild within the gut contents of the field-caught *M. titillator* larvae reflected those within the host, suggesting random predation. Results from this study support the hypothesis that *Monochamus* species may be facultative

intraguild predators of bark beetle larvae in the field. Additionally, this study demonstrates the capabilities of PCR in elucidating the predator-prey interactions of cryptic forest insects and provides a powerful tool to better understand mechanisms driving southern pine beetle guild population fluctuations.

1. Introduction and Literature Review

1.1 Biology and Attack Dynamics of the Southern Pine Beetle Guild

In the Southeastern United States five sympatric pine bark beetle species (Coleoptera: Curculionidae: Scolytinae) form what is known as the southern pine bark beetle guild. This guild includes the eastern six-spined engraver, *Ips calligraphus* (Germar); the eastern five-spined engraver, *I. grandicollis* (Eichhoff); the small southern pine engraver, *I. avulsus* (Eichhoff); the southern pine beetle, *Dendroctonus frontalis* (Zimmerman); and the black turpentine beetle, *D. terebrans* (Oliver) (Figure 1.1).

Both *Ips* and *Dendroctonus* beetles are attracted to recently felled, moribund, or weather damaged trees (Wood, S.L. 1982; Coulson and Witter, 1984). Not all trees selected by these beetles show signs of decline. Within the Scolytinae, species can be categorized as "primary pests" (e.g. *Dendroctonus* spp.), i.e. those that readily kill seemingly healthy trees and "secondary pests" (e.g. most *Ips* spp.), which normally only attack trees already in serious decline (Rudinsky, 1962). It has been estimated that *I. calligraphus* and *D. frontalis* have the potential to fly up to four miles while seeking new host material (Kinn, 1986) making the attack range of bark beetles significant. However, bark beetles tend to select the nearest suitable host tree rather than disperse over large distances (Gara and Coster, 1968; Coulson et al., 1978; Schowalter et al., 1981) possibly due to lowered risk of mortality (e.g. predation, fatigue, and environmental stressors). Members of the southern pine beetle guild are known to attack at least 16 *Pinus* spp. in the Southern United States (Conner and Wilkinson, 1983),

Figure 1.1 The southern pine beetle guild: *Dendroctonus frontalis* (Top Left), *Dendroctonus terebrans* (Top Right), *Ips grandicollis* (Right Center), *Ips calligraphus* (Left Center), and *Ips avulsus* (Bottom). Photographs by: Michael L. Ferro.

but their preferred hosts are loblolly pine (*P. taeda*) (L.) and shortleaf pine (*P. echinata*) (Miller) (Berisford and Franklin, 1971; Thatcher et al., 1980; Wagner et al., 1984).

The first individuals to arrive at a potential host tree [sometimes referred to as pioneer beetles (Borden, 1974)] locate suitable host material via either random landing tactics (Vité and Gara, 1962; Moeck et al., 1981) or primary attraction using host produced volatiles (Byers, 1995; Brattli et al., 1998). These pioneer beetles usually consist of a combination of reemerged parent adults and progeny adults originating from a single or multiple infested trees (Cooper and Stephen, 1978; Pope et al., 1980). Once a suitable host is located pioneer beetles begin excavating galleries through the outer bark and into the phloem tissue. This activity can be visualized externally by the presence of frass at the base of infested trees and by the presence of pitch tubes on the bole surface caused by sap exudation as a result of the trees natural defenses (Thatcher and Conner, 1985). The pioneer sex differs between the two genera. Males usually initiate attack in the three southern *Ips* species (Vité et al., 1972), while females initiate attack in the two southern *Dendroctonus* species (Coster and Vité, 1972; Godbee and Franklin, 1976). While boring into the outer bark and phloem the pioneer sex begins releasing aggregation pheromone components (see Section 1.3), which assist in attracting conspecifics. This behavior is thought to increase the number of attacking beetles and synchronize attack, ultimately facilitating establishment by overcoming the host's natural defenses (mass attack) (Wood, 1972; Coster et al., 1977; Payne, 1980). During endemic bark beetle population levels however, healthy host trees are likely to resist bark beetle colonization (Raffa and Berryman, 1983).

Mating occurs in the nuptial chambers constructed by the pioneer sex (Wagner et al., 1982). *I. calligraphus* and *I. grandicollis* practice harem polygyny (Kirkendall, 1983) and maintain an average harem size of three females (Cook et al., 1983; Haack et al., 1987;

Latty et al., 2009). Alternatively, *I. avulsus* exhibits a monogamous mating system (Cook et al., 1983), as do both *D. frontalis* and *D. terebrans* (Thatcher, 1960). Once mated, female *Ips* species initiate gallery formation following the grain of the wood (Haack et al., 1984; Raffa et al., 1993) and eggs are deposited at regular intervals along the margins of these galleries. *D. frontalis* females utilize a similar egg deposition strategy, but carve characteristic "serpentine" parental galleries that do not follow the grain of the wood. Unlike the other members of the southern pine beetle guild, *D. terebrans* exhibits a gregarious egg deposition strategy, where females may lay an average of 100 eggs in a single location (Mayfield and Foltz, 2005). This strategy is believed to have significant fitness advantages over traditional bark beetle larval feeding strategies, such as increased survivorship, shorter developmental periods, or higher quality resource utilization at the base of trees where larvae of this species usually feed (Grégoire, 1985; Six and Klepzig, 2004).

The developmental rates for the different species of the southern pine beetle guild are highly variable and temperature dependent. Summer conditions corresponding to approximately 25-35°C generally yield the optimal developmental rates for members of the southern pine beetle guild (Yearian and Wilkinson, 1967; Wagner et al., 1984, 1987, 1988). *I. calligraphus* and *I. grandicollis* exhibit similar developmental periods ranging from 25-27 days (Thatcher, 1960; Dixon, 1984) in the southern part of their range and as many as nine overlapping generations have been observed for *I. calligraphus* in Florida (Haack, 1985). The developmental period of *I. avulsus* is somewhat shorter at approximately 18-25 days with up to 10 overlapping generations in the southeastern part of its range (Baker, 1972). Each *Ips* species has three larval instars (Wilkinson, 1963) while the *Dendroctonus* species have four (Goldman and Franklin, 1977). The development of *D. frontalis* is the most temperature sensitive of the southern pine beetle guild (Wagner et al., 1984) and ranges from 26-110 days

with 6-8 overlapping generations in the southeastern part of its range (Thatcher and Pickard, 1967; Coulson, 1979; Ungerer et al., 1999). *D. terebrans* has the longest developmental time, ranging from 90-120 days with 2-4 overlapping generations per year (Smith and Lee, 1972).

1.2 Ecological and Economic Importance of the Southern Pine Beetle Guild

The southern pine beetle guild has been considered the most destructive group of forest pests in the Southeastern United States. Timber losses attributed to the southern pine beetle have been in excess of $237 million/year in parts of North America (Price et al., 1997). Between the years of 1999-2003 the southern pine beetle was attributed to in excess of $1.5 billion in timber losses over an area of 1.21 million acres in the Eastern U.S. (AL, KY, NC, SC, and TN) (Merten and Nowak, 2004). It has been widely reported that *D. frontalis* is a primary pest species and will readily attack healthy trees (Coulson, 1979; Wood, D.L. 1982) contributing to its status as a serious ecological and economic pest. The attack of vigorous trees usually does not occur at endemic population levels; rather it appears that epidemic levels are necessary for *D. frontalis* to kill healthy trees (Fargo et al., 1978; Paine et al. 1997). The ability of *D. frontalis* to overcome the host defenses of healthy trees is attributed not only to its complex chemical communication system, but also to its close relationship with phytopathogenic fungi (Raffa et al., 1993; Paine et al., 1997).

The three *Ips* species and *D. terebrans* are facultatively aggressive. Turpentine beetles (*D. terebrans*), rarely kill their hosts (Klepzig et al., 1991; Paine et al., 1997), but on occasion kill trees that have been mechanically injured or environmentally stressed (Kowal and Coyne, 1951; Merkel, 1981). Due to its relative scarcity in forests, *D. terebrans* is often overlooked by researchers and little is known of its roles in forest ecosystems. Since *D.*

terebrans normally infests stumps and the root systems of trees and rarely kills its hosts, it is of little economic concern except in high value trees. When tree mortality does occur, it is normally attributed to bluestain fungi vectored in the mycangia of *D. terebrans*. Like *D. terebrans* the three southern *Ips* species rarely attack healthy trees. However, when exceptional environmental conditions and/or plentiful host material allow *Ips* populations to reach epidemic levels they are capable of infesting and killing vigorous trees (Wood and Stark, 1968). The duration and scale of these outbreaks are usually much lower than those of *D. frontalis* (Paine et al., 1997). However, because of their much higher prevalence in space and time, the *Ips* species may play larger roles than *D. frontalis* in shaping forest ecosystems (Paine et al., 1981). The economic and ecological impacts of the southern pine beetle guild extend beyond the loss of raw materials. Loss of trees can affect wildlife diversity, disrupt the watershed in surrounding areas, provide new avenues for invasive species, and reduce the aesthetic value of affected stands (Leuschner, 1980).

1.3 Chemical Ecology of the Southern Pine Beetle Guild

Each member of the southern pine beetle guild produces pheromones during what is known as the concentration phase of attack (Wood, D.L. 1982). As mentioned previously, in combination with host volatiles these pheromone components attract conspecifics, which aid in overcoming host defenses and may assist in locating a fleeting resource (Vité and Francke, 1976). Some members of the southern pine beetle guild also release anti-aggregation pheromone components which have been shown to repel or "switch attack" of incoming conspecifics to prevent overcrowding when released in high concentrations (Rudinsky, 1973; Payne et al., 1978). Bark beetle semiochemicals can also function as kairomones or al-

lomones depending on the perceiving species. For example, the *D. frontalis* produced semiochemical frontalin has a kairomonal function for the parasitoid *Medetera bistriata* (Parent) (Diptera: Dolichopodidae) (Williamson, 1971), while R-(-)-ipsdienol produced by *I. pini* (Say) functions as an allomone for *I. paraconfusus* (Lanier) (Light and Birch, 1979). These semiochemicals also assist in mediating southern pine beetle guild interactions by dictating the temporal and spatial patterns of colonizing species (Birch and Svihra, 1979; Dixon and Payne, 1979; Birch et al., 1980; Svihra et al., 1980; Paine et al., 1981; Wagner et al., 1985). In addition to these functions, Wood (1970) suggests that these semiochemicals may play an important role in sexual isolation as reproductive isolating mechanisms. A brief review of the chemical ecology of the southern pine beetle guild is provided in the following subsections.

1.3.1 *Dendroctonus* Species

The chemical ecology of *D. frontalis* is arguably the best understood within the southern pine beetle guild and has been reviewed in detail by Smith et al., (1993). Once *D. frontalis* females land on a suitable host they begin releasing the aggregation pheromone frontalin. Frontalin is concentrated in the hindguts of *D. frontalis* females and released via defecation (Vité and Pitman, 1968). Vité and Pitman (1968) suggest that host defenses stimulate the release of frontalin and that cessation of resin flow and initiation of feeding ends production. Frontalin has been shown to attract large numbers of conspecifics of both sexes (Renwick and Vité, 1969). Payne et al., (1982) observed that *D. frontalis* was more attracted to (-)-frontalin than (+)-frontalin. In addition to frontalin females also produce the aggregation pheromone component *trans*-verbenol. *trans*-Verbenol is thought to function by orienting flying individuals to the host and to synergize the response to frontalin, particularly when host volatiles are absent (Renwick and Vité, 1969; Payne et al., 1978). *trans*-Verbenol is

autooxidized from α-pinene via feeding (Brattli et al., 1998) and the production halts once feeding has occurred for 12-16 hours (Vité and Pitman, 1968). Male *D. frontalis* may also play an additional role in conspecific aggregation via production of the pheromone component (+)-*endo*-brevicomin (Vité et al., 1985; Sullivan et al., 2007).

As the density of attacking beetles increases, male *D. frontalis* begin producing verbenone (females also produce verbenone, but in much lower quantities) and *endo*-brevicomin. *endo*-Brevicomin production is thought to be stimulated by the pairing of male and female *D. frontalis* and the (-)-isomer of *endo*-brevicomin has been shown to repel both sexes (Vité et al., 1985; Smith et al., 1990). These semiochemicals function as anti-aggregation pheromones which deter the arrival of both sexes when released in high concentrations (Payne et al., 1978; Borden et al., 1986). The concentration thresholds of verbenone and *endo*-brevicomin necessary to repel incoming beetles is unknown, however it is thought that their concentrations must exceed the level of attractive compounds by a particular amount [e.g. 15% higher concentrations of verbenone compared to *trans*-verbenol (Ryker and Yandell, 1983)] for anti-aggregation to occur. Alternatively, some bark beetle anti-aggregation pheromones such as *endo*-brevicomin may function by enhancing the attractiveness of more distant pheromone sources (Sullivan and Mori, 2009). No attraction by *D. frontalis* to semiochemicals produced by other members of the southern pine beetle guild has been demonstrated (Billings, 1985; Smith et al., 1990; however, Smith et al. (1990) speculate that *D. terebrans* aggregation pheromones may be attractive to *D. frontalis*.

The chemical ecology of *D. terebrans* appears similar to that of *D. frontalis*, however host volatiles appear to be necessary to mediate attraction to pheromones. For example, female *D. terebrans* produce the aggregation pheromones frontalin and *trans*-verbenol (Payne et al., 1987; Phillips et al., 1989). Frontalin has been shown to be weakly attractive to male

D. terebrans alone, but highly attractive when synergized by host volatiles (Payne et al., 1987; Phillips et al., 1989; Delorme and Payne, 1990). Similarly, *trans*-verbenol is only weakly attractive to *D. terebrans* alone, but highly attractive in combination with host-volatiles (Siegfried, 1984). Once male beetles arrive at the host tree they begin producing trace amounts of the pheromones *exo*- (Phillips et al., 1989) and *endo*-brevicomin (Payne et al., 1987). Although *endo*-brevicomin has been shown to be produced in low quantities by male *D. terebrans*, comparatively high quantities produced by *D. frontalis*, may be utilized by *D. terebrans* as a kairomone (Delorme and Payne, 1990). Once gallery formation is initiated both male and female *D. terebrans* produce verbenone. The behavioral function of verbenone in *D. terebrans* has yet to be demonstrated. The function of *trans*-verbenol, which is produced by both sexes is also unclear, however Dolorme and Payne (1990) suggest that both may have synergistic roles with host volatiles. While *D. terebrans* has been shown to respond to a variety of semiochemicals produced by *D. frontalis*, the *Ips* produced semiochemicals have been shown to only elicit weak responses by *D. terebrans* (Dolorme and Payne, 1990).

1.3.2 *Ips* Species

Male *Ips* spp. of the southern pine beetle guild arrive at a suitable host and begin production of aggregation pheromones: S-*cis*-verbenol, R-(-)-ipsdienol, and *trans*-verbenol (*I. calligraphus*) (Renwick and Vité, 1972; Vité et al., 1972); R-(-)-ipsdienol and lanierone (*I. avulsus*) (Vité et al., 1978; Teale et al., 1991; Miller et al., 2005); or S-(-)-ipsenol (*I. grandicollis*) (Vité and Renwick, 1971).

The antipodes of these aggregation pheromones have been shown to serve as anti-aggregation pheromones for the various *Ips* species. Vité et al. (1976a) observed that the presence of

the anti-aggregation pheromone component R-*cis*-verbenol did not affect the response of *I. calligraphus* when mixed with equal portions of S-*cis*-verbenol. However, when presented as 10 parts R-*cis*-verbenol to 1 part S-*cis*-verbenol a significant reduction in the response of *I. calligraphus* was observed (Vité et al., 1976a). The concentration of R-*cis*-verbenol necessary to elicit a negative response by *I. calligraphus* tested in this study may not be biologically plausible under natural conditions. The anti-aggregation pheromone component of *I. calligraphus* and *I. avulsus*, S-(+)-ipsdienol, has been shown to interrupt the response of *I. avulsus* to R-(-)-ipsdienol (Vité et al., 1978). This pattern was not observed by Strom et al., (2003) who found that racemic ipsdienol was more attractive to *I. avulsus* than the R-(-)-isomer alone. The antipode of S-(+)-ipsenol, R-(-)-ipsenol has been shown to reduce the response of *I. grandicollis* as well (Vité and Renwick, 1971; Vité et al., 1976b; Smith et al., 1990). Studies examining the enantiomeric ratios of the southern *Ips* species have observed a considerable effect of geographic location. For example, Kohnle et al. (1994) found that the enatiomeric ratio of ipsdienol produced by *I. avulsus* in East Texas was 90% S(+) and 10% R(-). Alternatively, a population of *I. avulsus* in Alabama was found to produce approximately 25% R-(-)-ipsdienol (Seybold et al., 1995).

Response of the southern *Ips* species to the semiochemicals produced by other members of the southern pine beetle guild appear to vary greatly among species. The presence of ipsenol has been shown to have a synergistic effect on the response of *I. avulsus* to ipsdienol (Hedden et al., 1976; Miller et al., 2005). Alternatively, the attractiveness of ipsenol to *I. calligraphus* is not well understood. Miller et al. (2005) found that racemic ipsenol had a synergistic effect on the response of *I. calligraphus* to racemic ipsdienol in Florida, but not in Georgia or Louisiana. Adding to the confusion are conflicting results from earlier studies showing attractiveness of material infested by *I. grandicollis* to *I. calligraphus* (Birch et al.,

1980) or no attraction (Svihra, 1982). Because both *I. avulsus* and *I. calligraphus* utilize R-(-)-ipsdienol as part of their aggregation pheromone, it is not surprising that *I. avulsus* and *I. calligraphus* are attracted to host material containing individuals of the other species (Birch et al., 1980; Svihra et al., 1980). No inhibition in response by *I. avulsus* to other sympatric *Ips* semiochemicals has been observed (Birgersson et al., 1995; Miller et al., 2005). Miller et al. (2005) found that the response of *I. grandicollis* to racemic ipsenol was not interrupted by the presence of racemic ipsdienol. No response to *Dendroctonus* produced semiochemicals was observed for *I. calligraphus* (Dixon and Payne, 1980; Smith et al., 1990) or *I. avulsus* (Birch et al., 1980; Svihra, 1982) in the field. Electroantennograms performed by Smith et al. (1988) showed that *I. calligraphus*, *I. avulsus*, and *I. grandicollis* responded to *endo*-brevicomin, frontalin, and verbenone. Unlike *I. avulsus* and *I. calligraphus*, *I. grandicollis* has been shown to respond to frontalin and *trans*-verbenol in the field (Dixon and Payne, 1980; Smith et al., 1990) and single cell recordings performed by Ascoli-Christensen et al. (1993) found that *I. grandicollis* responded to frontalin, *endo*-brevicomin, *trans*-verbenol, and verbenone (all produced by *D. frontalis*).

1.4 Traditional Associates of the Southern Pine Beetle Guild

Many natural enemies, parasitoids, and resource competitors (e.g. wood borers) utilize pheromone components of members of the southern pine beetle guild (see above) or host produced volatiles to locate suitable host material or potential prey (Overgaard, 1968; Vité and Williamson, 1970; Moser et al., 1971; Camors and Payne 1973; Dixon and Payne 1979; Billings and Cameron, 1984). These associates often overlap spatially and temporally with bark beetles in host material. The impact of natural enemies on population levels of the

southern pine beetle guild can be significant (Miller, 1986; Turchin et al., 1991; Turchin et al., 1999). Their effect is likely mediated by the presence of pheromones throughout much of the infestation stage, which natural enemies can utilize to locate their prey (Shepherd, 2004).

Many of the most influential traditional natural enemies of the southern pine beetle guild are coleopterous predators. These include but are not restricted to: *Temnochila virescens* (F.) (Coleoptera: Trogossitidae) (Mignot and Anderson, 1970; Billings and Cameron, 1984; Lawson and Morgan, 1993), *Thanasimus dubius* (F.) (Coleoptera: Cleridae) (Thatcher and Pickard, 1966; Frazier et al., 1981; Reeve, 1997), and histerids from the genera *Platysoma* (Leach) and *Plegaderus* (Erichson) (Goyer et al., 1980; Shepherd and Goyer, 2003). Adult *T. dubius* and *T. virescens* feed on adult scolytines on the surface of the bark and the larvae prey on the larvae in their galleries. Histerids from the genera *Platysoma* and *Plegaderus* traditionally feed on the eggs of the southern pine beetle guild.

Hymenopteran and dipteran parasitoids of scolytines are also numerous (Bushing, 1965). There are 6-10 known species of hymenopteran parasitoid associates of the southern pine beetle guild (Berisford, 1980; Stephen, 1995; Sullivan et al., 1997; Vanlaerhoven and Stephen, 2002) and >6 known dipteran parasitoids (Goyer et al., 1980). These parasitoids utilize host volatiles along with host pheromones to locate immature life stages of the southern pine beetle guild.

Resource competitors of the southern pine beetle guild include species of wood borers from the families Buprestidae and Cerambycidae. In Louisiana there are more than 25 species of buprestids and cerambycids that are associated with members of the southern pine beetle guild (E.N. Schoeller and J.D. Allison unpub. data). These beetles can consume

vast quantities of phloem material and potentially reduce the nutritional quality of host material for members of the southern pine beetle guild.

1.5 Biology of the Southeastern *Monochamus* Species

Two species of wood borers of the genus *Monochamus* (Megerle in Dejean) (Coleoptera: Cerambycidae) are of particular interest due to their close association with members of the southern pine beetle guild. These are the southern pine sawyer *M. titillator* (F.) and the Carolina sawyer *M. carolinensis* (Olivier). Peak flight periods of *M. titillator* and *M. carolinensis* in Louisiana last for approximately five months from late April until the middle of October (E.N. Schoeller and J.D. Allison unpub. data). Many *Monochamus* spp. (including the two southern *Monochamus* spp.) locate suitable hosts using a combination of host volatiles (Fatzinger, 1985; Phillips et al., 1988; Chénier and Philogène, 1989) and *Ips* and *Dendroctonus* pheromone components (Billings and Cameron, 1984; Billings, 1985; Fatzinger et al., 1987; Miller and Borden, 1990; Allison et al., 2001, 2003; Pajares, 2004; Miller et al., 2005; but see Fan et al., 2010). *M. titillator* has been shown to attack members of the pine family (Pinaceae) (Lindley) within its range including those of the genera *Pinus* (L.), *Abies* (Miller), and *Picea* (Link), while *M. carolinensis* appears to have a narrower host preference and only attacks trees from the genus *Pinus* (Webb, 1909; Lingafelter, 2007).

Monochamus species are attracted to fire-damaged, lightning-struck, wind-thrown, and insect-infested trees (Baker, 1972). Recently felled trees are usually not attractive until 5-7 days post-felling. Once suitable host trees are located females land on the bole where mating occurs. After mating, females begin carving ca. 3x8 mm elliptical oviposition niches with their mandibles through the outer bark (Pershing and Linit, 1986), where an average of 3-6 (*M. titillator*) or 1-3 (*M. carolinensis*) eggs are deposited (Alya and Hain, 1985; Dodds

A. **B.**

Figure 1.2 Adult *Monochamus titillator*. Image A: Male (32.2 mm body length). Image B: Female (27.2 mm body length). Photographs by: Jong-Seok Park

and Stephen, 2000) in a circular fashion (Webb, 1909; Dodds et al., 2002). The structure of these pits have been shown to vary depending on bark thickness with deep "cone-like" pits carved into thick bark and thin "slits" carved into thin bark (Walsh, 1983). The larvae hatch within 5-7 days and then feed on the phloem tissue for approximately three weeks. The number of instars of *M. titillator* is unknown; however Pershing and Linit (1988) found that *M. carolinensis* had 3-6 instars, with the 5^{th} and 6^{th} instars occurring rarely. Prior to pupation late instar larvae carve entrance holes into the sapwood and construct U-shaped pupal chambers that curve back to the surface (Webb, 1909; Pershing and Linit, 1986). The larvae continue feeding for a brief period after which they enter the chamber and pack the end with coarse debris. Typical generation times take 7-10 weeks for *M. carolinensis* and *M. titillator* (Linit, 1985; Pershing and Linit, 1986). Up to 85% of *M. titillator* and *M. carolinensis* have been shown to overwinter and emerge the subsequent spring if laid in the fall (Alya and Hain, 1985). *Monochamus titillator* has been observed to complete 1-2 generations per year in the south (Webb, 1909) and Pershing and Linit, (1986) observed two generations for *M. carolinensis* in Missouri.

After emergence adult *M. titillator* and *M. carolinensis* begin an obligate period of maturation feeding that lasts 7-12 days (Walsh and Linit, 1985). Adults of *M. titillator* and *M. carolinensis* are long-lived, with typical adult life spans exceeding 70 days under laboratory conditions (Walsh and Linit, 1985; Zhang and Linit, 1998; Akbulut and Linit, 1999; E.N. Schoeller, per. obs.). Potential mortality factors of immature life stages include: host defenses, insect natural enemies, parasitoids, woodpeckers, cannibalism, poor host conditions, and resource depletion (Dodds and Stephen, 2000; Dodds et al., 2001; Akbulut et al., 2004). Predation by birds and small mammals, unfavorable weather conditions, and injuries from

mating competition (Hughes and Hughes, 1987; Ray et al., 2009) are likely mortality factors of adult *Monochamus*.

Monochamus species play important ecological roles in forest ecosystems as degraders of coarse woody debris. The activity of adult *Monochamus* feeding and larval boring however, can be of serious ecological and economic concern. Larval boring activity can potentially cause significant damage to trees due to the reduction of wood structural integrity. Additionally, the boring activity of *Monochamus* larvae can create points of entry for wood-rotting fungi (Rayner and Boddy, 1988) and pathogens (Linit, 1988). As a consequence, *Monochamus* have the potential to reduce timber yield and wood value (Wilson, 1962; Gardiner, 1975). For example, in British Columbia, Canada large woodborers (e.g. *M. scutellatus*) have the potential to cause up to $43.6 million in annual timber losses (Anonymous, 1997) and in China the pine wilt nematode *Bursaphelenchus xylophilus* (Steiner and Buhrer) Nickle vectored by *M. alternatus* was estimated to have killed more than 35 million trees between its discovery in 1982 and 2003 (Yang et al., 2003). Fears of *B. xylophilus* infestation have also caused economic damage in the form of embargos that restrict the import of coniferous wood originating from countries already infested with *B. xylophilus* (Bergdahl, 1988).

1.6 Subcortical Interactions Between *Monochamus* Species and the Southern Pine Beetle Guild

Reproduction is the most important aspect of an insect's life history and fecundity is affected by a number of factors such as adult size, egg production, adult longevity, immature survival, and larval and adult nutrition (Leather, 1995). Many studies have examined the fitness and fecundity of *Monochamus* species (Zhang and Linit, 1998; Akbulut and Linit, 1999;

Dodds and Stephen, 2000; Akbulut et al., 2004; Togashi et al., 2009). Few studies, however, have examined the consequences of the spatial and temporal overlap between cerambycids and bark beetles within the subcortical environment on cerambycid fitness (Schroeder and Weslein, 1994; Schroeder, 1997). No studies have examined the effects of the subcortical interactions between *M. titillator* and the southern pine beetle guild on *Monochamus* fitness and fecundity. There have, however, been studies on the effects of these subcortical interactions on members of the southern pine beetle guild (Coulson et al., 1976, 1980; Hennier, 1983; Miller, 1986; Flamm et al., 1989; Dodds et al., 2001).

In trees coinhabited by *M. titillator*, a marked reduction in the number of *D. frontalis* progeny was observed in areas foraged by *M. titillator* larvae (Coulson et al., 1976). Further work by Coulson et al., (1980) found that *D. frontalis* brood mortality was approximately 70% in areas foraged by *M. titillator* larvae and 14% across the entire tree. In addition they observed that the greatest *D. frontalis* brood mortality in the foraged areas occurred at the extremes of the infested bole heights. Hennier (1983) also examined the effects on *M. titillator* foraging on densities of *D. frontalis*, as well as *I. avulsus*, and *I. calligraphus*. She found that the highest mean percent mortalities in the areas foraged by *M. titillator* were 28.8%, 45.7%, and 60.6% for *D. frontalis*, *I. avulsus*, and *I. calligraphus* respectively. She found that *D. frontalis* mortality was highest within the areas foraged by *M. titillator* larvae at the lower portion of the infested bole height (2.0 m), which is in agreement with the findings of Coulson et al., (1980). She speculates that since *D. frontalis* arrives at the midbole first and later colonizes the periphery of its niche, that these individuals are at greater risk due to their prolonged presence in the tree, exposing them to the peak *M. titillator* foraging period. She also hypothesizes that the relatively higher mortality observed for *I. avulsus* and

I. calligraphus are due to their relative location in the tree (mid-upper bole), which coincides with higher *M. titillator* larval density.

Miller (1986) observed the effects of *M. titillator* foraging on *I. calligraphus* brood mortality using exclusion cages. He found that 51% of the average monthly mortality caused by insect associates of *I. calligraphus* was contributed by *M. titillator*. From May-July he found that the relative *I. calligraphus* mortality caused by other insect associates was higher than the relative mortality contributed by *M. titillator*. In the months of August-October he observed that the relative pattern of mortality shifted, with higher relative *I. calligraphus* mortality attributed to *M. titillator*. He hypothesized that this pattern was due to cooler months slowing *M. titillator*'s arrival to the tree and thus bark beetles can complete much of their development prior to peak *M. titillator* larval activity. *M. titillator* larvae have been shown to consume on average 40% of the phloem surface within the tree and up to 100% of the phloem surface at some bole heights (Coulson et al., 1980; Flamm et al., 1989). The *Ips* spp. and *D. terebrans* complete their development within the phloem. *D. frontalis* development is completed in the outer bark, where the larvae migrate during the 4^{th} instar (Goldman and Franklin, 1977). Miller (1986) hypothesized that the effects of foraging by *M. titillator* should be greater for *I. calligrahus* compared to *D. frontalis* due to this behavior. The rapid development of *Ips* spp. and the spatial shift in developing *D. frontalis* brood have been hypothesized to be behavioral adaptations by members of the southern pine beetle guild to reduce the impact of *M. titillator* larval foraging (Flamm et al., 1989), since the majority of *M. titillator* larval foraging occurs 25-30 days after initial bark beetle infestation (Hennier, 1983, Flamm et al., 1989).

Traditionally, the interactions between larval *Monochamus* and members of the southern pine beetle guild have been categorized as commensal (Flamm et al., 1989) or competitive

(Coulson, 1980). High mortality rates observed in the studies described above have been attributed to the asymmetrical competitive advantage of the much larger and more mobile *Monochamus* larvae (Flamm et al., 1989). Additional evidence provided by more recent studies however, suggests that *M. titillator* and *M. carolinensis* may be facultative intraguild predators of bark beetles.

Dodds et al., (2001) studied the behavior of *M. carolinensis* larvae towards *I. calligraphus* larvae under laboratory conditions. Prior to this study all bark beetle mortality observed had been attributed to *M. titillator* larval foraging (i.e. competition). Dodds et al., (2001) found that the majority of *I. calligraphus* larvae placed within phloem disks containing foraging *M. carolinensis* larvae were attacked and consumed by *M. carolinensis* larvae. Of the *I. calligraphus* larvae encountered by *M. carolinensis* larvae, they observed that 74.1% were attacked and 48.1% of those attacked were at least partially ingested. Based on these observations they proposed the hypothesis that *M. carolinensis* larvae are facultative intraguild predators.

1.7 Research Goals

The primary goal of this thesis was to better understand the dynamics of the subcortical interactions between members of the southern pine beetle guild and *Monochamus* wood borers. Specifically, it empirically tested the hypothesis that *M. titillator* larvae are intraguild predators of the southern pine beetle guild. To accomplish this DNA-based molecular tools were developed to screen the gut contents of *M. titillator* larvae for DNA of each member of the southern pine beetle guild under laboratory conditions. Once developed these tools were used to screen the gut contents of field-collected *M. titillator* larvae. Results from these studies support the growing body of evidence that suggests *M. titillator* as well as *M. caro-*

linensis are facultative intraguild predators of the southern pine beetle guild. Additionally, results from this study provide a better understanding of the potential roles *Monochamus* larvae play in southern pine beetle guild population dynamics.

2. Molecular Tool Development and Determination of the Molecular Half-life of Bark Beetle DNA in the Gut Contents of *Monochamus titillator* Larvae

2.1 Introduction

2.1.1 The Study of Predator-Prey Interactions in Cryptic Systems

The study of trophic interactions provides an invaluable source of information on many ecosystem processes including predator-prey interactions, species composition, ecosystem stability, and ecosystem resilience (Juen and Traugott, 2006). Predator-prey dynamics remain some of the most difficult ecosystem processes to study; however, several techniques exist for studying them under natural conditions (reviewed by Sunderland, 1988). In many vertebrate systems the target predator and prey taxa are easily observed facilitating the collection of critical data such as predation rates, predator and prey densities, population structures, and behaviors. Alternatively, most invertebrate taxa are typically small and/or live in cryptic environments (e.g. leaf litter, soil, within plants, aquatic environments) making gathering of the aforementioned data exceedingly difficult. Attempts to alter conditions to facilitate direct observation may disturb the natural system making interpretation of predator-prey interaction results difficult (Symondson, 2002). Since both immature *Monochamus* and members of the southern pine beetle guild live in a cryptic environment (the subcortical layer of trees) investigations of the potential predator-prey dynamics between these taxa present a significant challenge.

There have been many indirect methods developed to facilitate the study of the predator-prey dynamics of cryptic invertebrates. The most practical indirect methods involve gut content analyses of predators. These analyses are invaluable when attempting to determine trophic structures and/or determine the frequencies of predatory and scavenging events (Foltan et al., 2005). In addition, these techniques may be useful in determining if intraguild predation dampens the magnitude of prey population fluctuations (Harwood et al., 2007).

The techniques utilized for performing gut analyses over the years have advanced rapidly since their first implementation. The most basic techniques utilized fecal and microscope dissections to examine the gut contents of predators for identifiable remains of prey (Sunderland, 1975; Sunderland et al., 1987; Breene et al., 1990). These techniques however have some disadvantages. For example, many invertebrate predators are fluid feeders (e.g. Diptera and Hemiptera) and leave no identifiable prey remains making morphological identification of prey nearly impossible (Sunderland, 1988). Secondly, the time requirement associated with meticulously mounting fecal and gut contents onto microscope slides and identifying the contents to a specific taxon is not often viable.

2.1.2 Molecular Techniques

The advancement of molecular technology has provided new methods for analyzing predator gut contents for prey remains. Molecular techniques offer new levels of specificity and accuracy compared to dissection and observation techniques when analyzing predator-prey interactions. These approaches include enzyme-linked serological assays (ELISA) utilizing monoclonal (Greenstone and Morgan, 1989; Hagler and Naranjo 1994; Symondson and Liddell, 1996; Agustí et al., 1999a; Symondson et al., 1999; Schenk and Bacher, 2004; Calder et al., 2005) and polyclonal (Dennison and Hodkinson, 1983; Chiverton 1987; Sunderland

et al., 1987) antibodies, enzyme electrophoresis (Lister et al., 1987; Solomon et al., 1996; Camara et al., 2003; Traugott, 2003), and DNA-based techniques.

Recently, the primary DNA-based technique utilized by researchers performing molecular gut analyses has been polymerase chain reaction (PCR). The use of many PCR-based methods is represented in the molecular gut analysis literature. These methods include: the use of single or multiple PCR reactions containing one primer set to test for a single or groups of prey species (singleplex-PCR) (Zaidi et al., 1999; Chen et al., 2000; Hoogendoorn and Heimpel, 2001; Agustí et al., 2003a, 2003b; Foltan et al., 2005; Admassu et al., 2006); the use of single or multiple PCR reactions containing multiple primer sets allowing rapid screening for multiple prey species simultaneously (multiplex-PCR) (Harper et al., 2005; Juen and Traugott, 2006; Traugott et al., 2006; King et al., 2010); the use of TaqMan real-time PCR (RT-PCR) assays, which utilize fluorogenic probes to quantify prey DNA during the amplification process (Zhang et al., 2007a); and the use of sequence characterized amplified region markers (SCARs) derived from bands obtained from the random amplification of polymorphic DNA (RAPD-PCR) (Agustí et al., 1999b, 2000; de León et al., 2006; Zhang et al., 2007b).

2.1.3 Benefits of Polymerase Chain Reaction

PCR offers many advantages over the other molecular techniques described above, with particular reference to ELISA (Symondson, 2002; King et al., 2008). Prior to the rapid shift towards the utilization of PCR-based techniques, ELISA was the state of the art technique utilized by researchers performing molecular gut analyses. The development and the advantages/disadvantages of ELISA have been reviewed by Greenstone (1996). The primary benefits of PCR over ELISA are the significantly reduced cost and time necessary to develop

PCR-based probes (see Chen et al., 2000). This is often achieved by the fact that many of the components necessary to perform PCR analyses are readily available in kits and that once PCR primers have been designed they can be easily uploaded to the internet and are available to other researchers studying similar taxa (Admassu et al., 2006). Additionally, many target genes along with information on their conserved and specific regions have been characterized for a wide array of invertebrate taxa facilitating the development of primers (Greenstone and Shufran, 2003).

In addition to the lower financial and labor costs of PCR over ELISA, PCR primers can be designed to amplify a single species or groups of species (Admassu et al., 2006), making PCR invaluable for studying trophic links of predators that consume multiple food sources. However, great care must be taken when designing species-specific or group-specific primers by ensuring target specificity via cross-testing on an array of non-target species to prevent false positives (Symondson, 2002; Admassu et al., 2006). One disadvantage of PCR-based gut content analyses compared to ELISA is that PCR is not able to distinguish prey to developmental stage (Greenstone and Morgan, 1989; Hagler et al. 1994; Greenstone 1995). Both ELISA and PCR-based techniques suffer from the fact that they are unable to distinguish between prey that has been scavenged or actively predated (Calder et al., 2005; Foltan et al., 2005; Juen and Traugott, 2005) or detect the occurance of secondary predation (Harwood et al. 2001; Sheppard et al. 2005; Hosseini et al., 2008). However Foltan et al. (2005) suggest if a researcher's goal is simply to identify the prey taxa being consumed, that not being able to differentiate between prey that have been scavenged or actively predated is not a serious issue. Because of the factors mentioned above, PCR has rapidly replaced ELISA as the dominant molecular approach utilized in performing molecular gut analyses.

2.1.4 Research Goals

In order to overcome the difficulties associated with studying the subcortical interactions between immature *M. titillator* and the five members of the southern pine beetle guild (i.e. their cryptic subcortical lifestyles) PCR was used to analyze the gut contents of *M. titillator* larvae. The goals of this study were to:

1) Develop the species-specific primers necessary to screen the gut contents of *M. titillator* larvae for DNA of each member of the southern pine beetle guild.

2) Design efficient and robust multiplex-PCR reactions to incorporate the designed species-specific primers.

3) Determine the molecular half-life of bark beetle DNA in the digestive systems of laboratory fed *M. titillator* larvae, which would provide a frame of reference when interpreting predation data obtained from field-caught *M. titillator* larvae (see Chapter 3) and provide an additional source of ecologically useful data.

2.2 Materials and Methods

2.2.1 Insects and Rearing

In order to guarantee an accessible and continuous supply of bark beetles, field-collected beetles were used to establish colonies for each *Ips* species. Adult *Ips* engraver beetles were collected using 12-unit Lindgren funnel traps [(Pherotech Inc. (now Contech Enterprises Inc.) Delta, B.C., Canada)] at the Bob R. Jones-Idlewild Research Station (Clinton, LA) and Burden Research Plantation (Baton Rouge, LA) from 5/11/09-8/25/09 and 3/17/10-4/15/10, respectively. Six traps were hung at each site and baited with combinations of either racemic ipsenol and ipsdienol (both 50:50 blends of the plus and minus enantiomers) to attract *I. avulsus* and *I. grandicollis* or racemic ipsdienol and *cis*-verbenol (13:87 blend

of the plus and minus enantiomers) to attract *I. calligraphus*. Traps were emptied weekly from dry cups and live individuals were placed into 4 oz plastic cups lined with damp filter paper. Adult *Ips* were identified and separated by species using differences in the elytral declivital morphologies (Wood, S.L. 1982) and were stored at 10°C until needed (no more than 3 days).

Every few weeks a single ca. 20 year old *P. taeda* 25-33 cm dbh was felled at the Bob R. Jones-Idlewild Research Station. Bolts (15-18 cm in diameter, 35 cm in length) were brought back to the laboratory for bark beetle rearing material. The ends of the bolts were dipped in hot paraffin wax to prevent desiccation and infested within 2 weeks of harvest. Bark beetle infested bolts were housed individually in 5 gallon opaque, plastic buckets (Lowe's Co. Mooresville, NC) with a single glass mason jar attached to facilitate collection of emerging adults (Figure 2.1). The glass mason jars were lined with damp paper towel to give the beetles a surface they could walk on to prevent self-injury and reduce stress. The inside of the rearing containers were lined with aluminum screening to allow beetles to climb to collection jars. Two ca. 2 cm holes were cut near the tops of each bucket to allow natural airflow to occur and reduce excessive moisture buildup in the containers (metal screening was placed on both sides to prevent escape). Bark beetles are positively phototrophic so rearing containers were placed with the collecting jar facing the laboratory windows. Each log was infested with ca. 3 unsexed individuals per 1 dm^2 of bark surface area and maintained at approximately 26-30°C, 70-80% RH, and ambient (approximately 13:11 L:D) conditions. Once adult emergence began, jars were emptied daily for 2 weeks and then the bolt was dissected by carefully peeling away the bark to remove any remaining adults.

As adult *Ips* spp. population numbers in the laboratory increased (>500 individuals of each species) logs were dissected and a minimum of 250 3^{rd} instar larvae of each species

removed 15 days after initial infestation. Collected larvae were placed into 10 ml plastic vials. In order to remove external sources of contamination from the bodies of larvae, the 10 ml vials were half filled with distilled water and vortexed at 1800 rpm for 20 seconds. The water was removed using a strainer and the larvae placed onto kimwhipes and allowed to dry for 5 minutes before being placed collectively into 1.5 ml microcentrifuge tubes and stored at -80°C until needed for experimental trials.

Adult and larval *D. terebrans* were hand collected on 9/15/09 from ∼2 week old stumps at the Bob R. Jones-Idlewild Research Station (Clinton, LA) and on 9/22/09 at the Kisatchie National Forest - Catahoula Ranger District (Pineville, LA) where recent logging activity had taken place. Additional larvae were obtained on 11/2/09 from an ongoing mass attack on live *P. taeda* and *P. palustris* (Miller) from Butler, AL. Larvae taken from the field were placed into 4 oz plastic cups and placed over ice to be transported back to the laboratory where they were stored at -80°C. Adult *D. terebrans* were reared in containers identical to those described above, with the addition of sand burying the lower 10 cm portion of the bolts (Godbee and Franklin, 1978). Bolts used to rear *D. terebrans* were 35 cm long with 25-30 cm diameters. Individuals were sexed using stridulatory behavior and three pairs of beetles were allowed to infest each bolt. Once a suitable laboratory population had been established late instars were removed every 90 days and cleaned and stored as described above until more than 250 larvae were obtained.

Adult *D. frontalis* were collected from infested material taken from the Homochitto National Forest in Mississippi and used to establish a laboratory population. The ends of 6 freshly cut *P. taeda* bolts (35 cm long with 10-15 cm diameters) were dipped in hot paraffin wax to prevent desiccation. Six holes were drilled lengthwise every 90° across the bolt's surface using a 3.1 mm diameter drill bit down to the bark/phloem interface. This was

A. **B.**

Figure 2.1 Insect laboratory rearing containers. Image A: Five-gallon bucket used to rear bark beetles. Image B: 46x46x61 cm screened cage used to rear *Monochamus* species.

necessary because most of the adults were unwilling to initiate galleries without the presence of holes. The bolts were then stood on end in 46x46x61 cm screened rearing cages and forty unsexed individuals added to each cage. Bolts were maintained at 25°C ± 2°C, 55% ± 2% RH, and ambient light conditions. Twenty-five days after infestation four of the bolts were dissected and 4^{th} instars removed from the outer bark until at least 100 larvae were obtained. Larvae were cleaned and stored as described above until needed.

Adult *M. titillator* used to establish laboratory populations were collected from the same traps used to collect the *Ips* spp. described above. Additional beetles were hand collected from host material decked in the field twice a week until no more beetles were observed (approximately 3 weeks). The host material was then taken back to the lab and placed into 75.7 liter metal emergence chambers in order to collect the emerging *Monochamus* at a later date (Figure 2.2).

Adult *Monochamus* collected from the field were identified to species using a combination of apical elytral spine characters (Lingafelter, 2007) and size differences (Pershing and Linit, 1985). Beetles were sexed using differences in antennal length and by the presence of female pubescence at the apex of the abdomen (Linsley and Chemsak, 1984). The elytral morphology of *M. titillator* and *M. carolinensis* is sometimes variable between the two species, with the apical spine morphology of *M. titillator* often appearing similar to that of *M. carolinensis*, but not vice versa. This made it difficult to confidently use this character to separate individuals to species. Although genital morphology differs between the two species (Pershing and Linit, 1985), this character could only be used to check proper identification of males after they had died.

To account for these morphological inconsistencies individual mating behavior was used to confirm identifications of individuals with uncertain elytral characters. In brief, male and

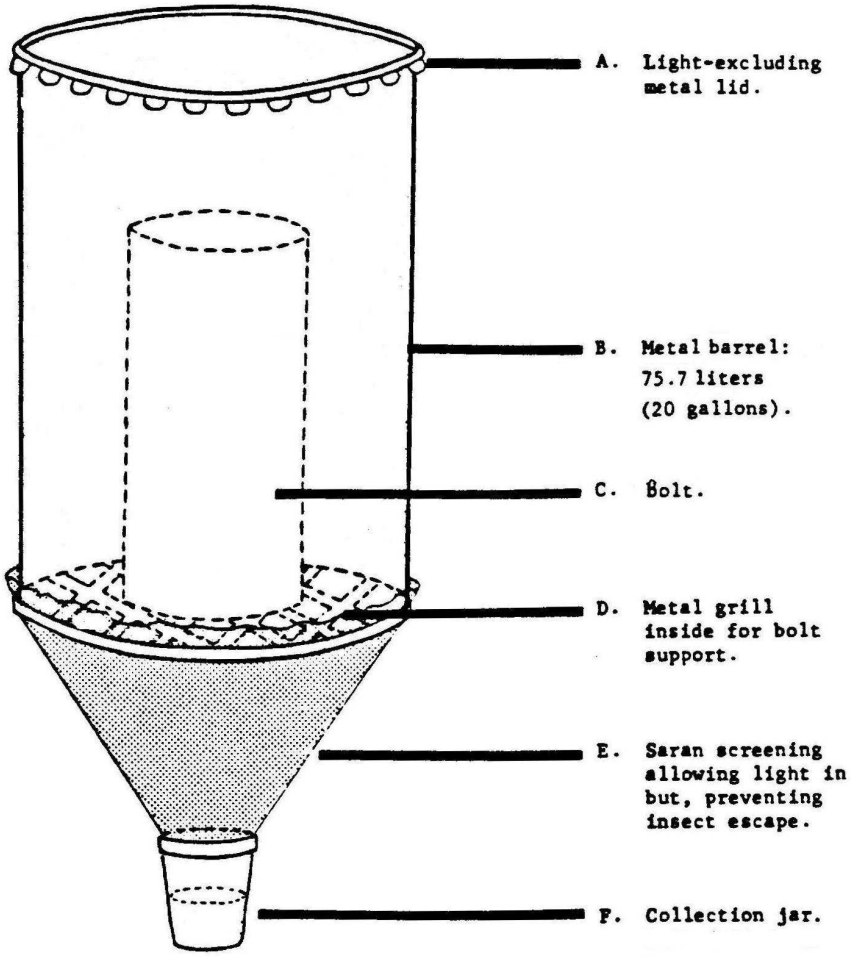


Figure 2.2 Metal emergence chamber used to collect adult *Monochamus* spp. from field-infested host material [(Modified from Riley (1983)].

female beetles of known identity were placed into 150 mm plastic petri dish mating arenas. Individuals of questionable identity were then placed into these arenas with an individual of known identity and the opposite sex and mating behavior observed for 5 minutes. If no mating occurred within the 5 minute timeframe they were placed with a member of the opposite sex of the second species and mating behavior observed an additional 5 minutes. A copulatory event was considered mounting of the female by the male and insertion of its adeagus into the female. If the individual refused to mate with either of the two species it was excluded from the breeding population. Only a small population of *M. carolinensis* was maintained in the laboratory for species identification purposes. To confirm that interspecific copulation does not occur, 5 individuals from each sex were placed with the opposite sex of both species sequentially and observed for 5 minutes for interspecific copulation. Interspecific copulation was never observed.

Host material used for rearing the laboratory population of *M. titillator* was taken from the mid and upper bole of *P. taeda* felled for bark beetle rearing purposes. The lower 2 meters of the bole was not used since this section contains thick bark that may inhibit oviposition by *Monochamus* spp. (Linit et al., 1983; Walsh and Linit, 1985). Bolts used varied in size with 43-47 cm lengths and 17-20 cm diameters. The ends of each bolt were dipped in hot paraffin wax to prevent desiccation and stored a minimum of three days, to make them more attractive before being placed with the adult *M. titillator*. Bolts were placed upright into 46x46x61 cm mesh wire rearing cages (Figure 2.1). Each cage contained fresh sprigs of *P. taeda* placed into a 0.47 l jar of water packed with paper toweling to prevent beetles from drowning. Water was replaced every other day and foliage as needed. Each cage contained a minimum of 10 individuals but no more than 40 at a time to prevent stress due to overcrowding.

Beetles were allowed to oviposit until ca. 20 oviposition sites (Linit, 1985) were observed (72-96 hours). Bolts were then taken out of the screened cages and labeled by stapling paper tags to one end, which contained the species ID and bolt number. The labeled bolts were placed on the lab floor until no more chewing sounds and frass buildup was observed (35-50 days). Once larval activity appeared to have ceased the bolts were placed into 46x46x61 cm emergence cages and emerging adults collected daily. Sixty days after the first beetle emerged bolts were discarded.

2.2.2 Sample Preparation

DNA was extracted from whole adults of each bark beetle species or just the digestive systems of the *M. titillator* larvae using the DNeasy Blood and Tissue Kit (Qiagen Inc. Valencia, CA) according to the manufacturer's protocol for animal tissues. Only the digestive tracts of *M. titillator* were used in order to decrease the amount of extraneous, nontarget predator DNA present in the sample. In order to remove the digestive systems of the *M. titillator* larvae they were placed onto sterile dissection trays, stretched and pinned with their ventral surface facing up, and then anteriorally-posteriorally dissected using a pair of microdissection scissors. A portion of the digestive system was removed by severing the esophagus directly behind the head capsule and by severing the hindgut 1-2 millimeters anterior to the anal opening. DNA sample purities were assessed at the 260/280 nm ratio and their concentrations calculated using absorbance by a Nanodrop ND-1000 spectrophotometer (Nanodrop Technologies). DNA solutions were stored at -20°C until needed.

During a feeding trial pilot study, PCR inhibitors present in the *M. titillator* DNA samples were found to cause false negative results for bark beetle consumption. The inhibitors present were likely caused by compounds present in the phloem tissue consumed by *M. tit-*

illator larvae. Determining the identity of these inhibitors in fecal material is a lengthy and complex process (Monteiro, 1997). The most common inhibitors of PCR reactions in fecal and plant materials are humic acids and complex polysaccharides, but other less abundant inhibitors can also be present (reviewed by Wilson, 1997). Under normal circumstances the effects of these inhibitors on the performance of the *Taq* polymerase can be reduced to acceptable levels by reducing the concentration of the inhibitors in the DNA sample via dilution. Although dilution is an easy and cost-effective solution for preventing PCR inhibition, sample dilution in molecular gut analysis can cause increased false negative results. Prey DNA is often present in such small quantities within the predators that diluting DNA samples may result in prey DNA reaching undetectable concentration levels (King et al., 2008).

In order to remove excess humic acids and polysaccharides that may have been present in the predator DNA samples, a post-extraction purification step was performed using a combined polyvinylpolypyrrolidone (PVPP) (Acros Organics) and Sepharose®4B (Sigma-Aldrich) spin column technique developed by Arbeli and Fuentes (2007). Pierce 0.8 ml spin columns (Thermo Scientific Waltham, MA) were loaded with 25 mg of PVPP followed by the addition of 400 μl of the Sepharose 4B solution. Excess liquids were first removed from the columns by inserting them into 1.5 ml microcentrifuge tubes (Eppendorf AG, Hamburg, Germany) for centrifugation at 1100 g for 2 minutes. Columns were then washed with 450 μl of TE solution (10 mM Tris-HCl, 1 mM sodium EDTA, pH 8.0) three times by centrifugation at 1100 g for 2 minutes. DNA samples were purified by placing 200 μl of the DNA solution into the prepared PVPP/Sepharose 4B columns placed into a new 1.5 ml collection tube and migrated by centrifugation at 1500 g for 4 minutes. The purified DNA samples were stored at -20°C until needed.

2.2.3 Sequencing and Primer Design

Partial bark beetle cytochrome oxidase subunit I (COI) gene sequences were obtained from GenBank (Accession numbers: EF115512, AF113331, AF113330, AF113335, AF113336, AF113352, AF113351, AF113350, AF113349, AY570903, AF067986, AF375315, and AF068003). Haplotype sequences were included when available to aid in the design of species specific primers. Sequence alignments were performed using CLUSTALW in the MEGA 4.0 program (Tamura et al., 2007). The aligned sequences were manually checked for regions of high variability among the bark beetle species sequences and multiple sites were chosen for potential primer development.

Potential bark beetle primers were checked for secondary structure formation, and their annealing temperatures determined using the web programs FastPCR (Kalendar, 2009) and NetPrimer (PREMIER Biosoft International, Palo Alto, CA). Primers were designed to amplify only a single member of the southern pine beetle guild using variation in the COI gene sequences and to prevent non-specific amplification of *M. titillator* DNA. Each potential species-specific bark beetle primer set was designed to create a PCR product of variable base pair length to allow easy discrimination between species on agarose gels.

It was necessary to develop species-specific primers to correctly identify field-collected *M. titillator* larvae (see Chapter 3), since there are no known differences in larval morphology between *M. titillator* and *M. carolinensis*. Universal primers were first developed to amplify a portion of the COI gene for *M. titillator* and *M. carolinensis*, because COI gene sequences were already available for several other *Monochamus* species. Additionally, the use of the COI gene provided a greater chance of creating species specific primers by allowing direct comparison of the gene sequences used for both predator and prey. *Monochamus* primers were designed using partial COI gene sequences from *M. alternatus* (Hope), *M. sutor* (L.),

M. galloprovincialis (Olivier), *M. guerryi* (Pic), *M. sartor* (F.), *M. saltuarius* (Gebler), and *M. urussovi* (Fischer) (Genbank Acession numbers: AB083740, DQ861321, EU556542, AB439140, AY260838, AY260842, and AY260844 respectively). The partial *Monochamus* COI sequences were aligned as described above and a universal *Monochamus* primer pair MCF1 (5'-GCT CAT AGT GGT TCA TCA GTT G-3') and MCR1 (5'-TGT TCG GCA GGA GGT AAA TG-3') was designed to amplify conserved regions within the *Monochamus* COI gene.

Partial COI gene sequences were obtained for *M. titillator* and *M. carolinensis* using three individuals from each species. These individuals were taken from the laboratory population and killed by freezing them at -20°C for 10 minutes. The prothoracic legs from each individual were removed using sterile dissection scissors and placed into 1.5 ml microcentrifuge tubes. The legs were homogenized using liquid nitrogen and a sterile pestle. DNA was extracted and quantified as described above (see Section 2.2.2) before being stored at -20°C for sequencing purposes.

An initial PCR reaction was performed to amplify the *M. titillator* and *M. carolinensis* COI gene sequences using an Amplitronyx 6 gradient thermocycler (Nyx Technik Inc. San Diego, CA). The reaction was run using the following cycling conditions: an initial denaturation period at 94°C for 2 minutes followed by 35 cycles of a denaturation period at 94°C for 1 minute, an annealing period at 58°C for 2 minutes, and an extension period at 72°C for 1 minute. There was a final extension period of 72°C for 7 minutes. The PCR products were subjected to gel electrophoresis for 120 minutes using a 1% agarose gel and visualized using UV light to check for successful amplification. The PCR products were purified using the QIAquick PCR Purification Kit (Qiagen Inc. Valencia, CA) according to the manufacturer's protocol. Sequencing of the purified PCR products was performed at the Louisiana

State University gene lab using a BigDye Terminator version 3.1 sequencing kit (Applied Biosystems) in an ABI PRISM 3130 Genetic Analyzer. Samples were sequenced across both strands using the designed universal *Monochamus* primer pair. Sequence data was analyzed using Sequence Scanner software v1.0 (Applied Biosystems).

The *Monochamus* COI sequences obtained were aligned as described above and manually checked to determine differences in the COI gene sequences between *M. titillator* and *M. carolinensis*. Once promising regions were identified, the bark beetle COI gene sequences aligned with the *Monochamus* COI sequence in order to assist in eliminating regions with little variability between the bark beetle species and *M. titillator* COI gene sequences. The *M. titillator* primer pair was designed to work in a separate reaction to reduce primer-primer conflicts with the bark beetle primer sets.

2.2.4 PCR Analysis

All PCR optimization reactions were performed using a PTC-200 DNA Engine gradient thermocycler (MJ Research, South San Francisco, California) and optimized reactions were run using an Amplitronyx 6 gradient thermocycler. Different combinations of primer pairs from the list of potential primers (see Section 2.2.2) were first tested and amplification success or failure as well as amplification robustness determined under singleplex PCR conditions for each species of interest. The highest quality primer pair for each bark beetle species was then combined into multiplex reactions to reduce the total number of reactions necessary to screen *M. titillator* larvae for the presence of bark beetle DNA.

Initial PCR reaction conditions were optimized with the aid of a PCR Optimizer Kit (Invitrogen, Inc., Carlsbad, California). During PCR optimization all DNA template solutions were diluted to 25 ng/μl using nuclease-free H_2O. Each was tested in the buffers

provided by the PCR optimization Kit (A-J) at 52°C according to the manufacturer's protocol. Optimal PCR annealing conditions were determined by running each PCR reaction on a temperature gradient (50-60°C). The final conditions of the PCR reactions were adjusted using 5 Prime (5 Prime GmbH, Hamburg, Germany) hot start technology according to the manufacturer's protocol. After optimization the final PCR reaction solutions (50 μl total volume) contained 5 μl of dNTPs (0.4 mM final concentration), 1 μl of each primer (0.04 μM final concentration), 0.2 μl of HotMaster *Taq* DNA polymerase (1.0 unit), 5 μl of 10X HotMaster *Taq* Buffer (containing 25 mM Mg^{2+}), 2 μl of each DNA template, and brought up to volume using nuclease-free H_2O. PCR products were run on ethidium bromide-stained 2.5% w/v agarose gels (bark beetle multiplex reactions) or EtBr-stained 1.5% w/v agarose gels (*Monochamus* singleplex) at 120 V for 45 minutes. Gels were visualized by UV transillumination.

2.2.5 Primer Specificity

The specificity of each bark beetle primer pair was tested on each target bark beetle species as well as the other non-target bark beetle species and *M. titillator*. The specificity of the *M. titillator* primer pair was tested against each bark beetle species as well as *M. carolinensis*. Each bark beetle primer pair was tested on at least five individuals of each non-target species using the optimized multiplex reactions. The *M. titillator* primer pair was tested in its optimized singleplex reaction on at least five individuals for each bark beetle species and fifteen individuals of *M. carolinensis*. Each set of reactions contained a positive control consisting of target species DNA and a negative control of nuclease-free H_2O to check for reagent contamination.

2.2.6 Primer Sensitivity

Trials were performed in order to quantify the sensitivity of the primer pair of each species of bark beetle in the presence of *M. titillator* DNA under singleplex and multiplex PCR conditions. This was done by serially diluting the bark beetle DNA in a standard solution of *M. titillator* DNA. In an attempt to approximate a biologically relevant mixing ratio of predator to prey DNA an initial mixing ratio of 5:1 (125 ng:25 ng) *M. titillator* DNA to bark beetle DNA was used. This ratio reflected the magnitudinal difference between the mean DNA concentration from the five bark beetle species with weights equivalent to a 3^{rd} instar *I. grandicollis* and the digestive tract of a late instar *M. titillator*. Since multiple bark beetle species may be consumed by a single *M. titillator* larvae, bark beetle DNA solutions were mixed at 1:1:1 (25:25:25 ng/μl) concentration ratio (multiplex A) or 1:1 (25:25 ng/μl) (multiplex B) concentration ratio before being mixed with the *M. titillator* DNA standard solution during the multiplex sensitivity analyses. The sensitivity of each bark beetle primer pair was tested at DNA concentrations ranging fom 500-0.0005 pg/μl in both the singleplex and multiplex PCR sensitivity analyses. The final concentration of *M. titillator* DNA in all the PCR sensitivity reactions was standardized to 2.5 ng/μl.

The mean weight of 3^{rd} instar *I. grandicollis* used to compare the DNA concentrations of each bark beetle species as mentioned above was determined using *I. grandicollis* larvae from the population maintained in the LSU forest entomology laboratory. One bolt containing *I. grandicollis* was dissected 15 days post-infestation by carefully peeling the bark away and removing larvae that were forming pupal chambers using sterile techniques. Larvae (N=20) were weighed to the nearest 0.0001 g. The DNA concentrations for *I. grandicollis* and the remaining four bark beetle species (N=10 per species) with weights equivalent to the mean weight found for 3^{rd} instar *I. grandicollis* were then determined as described above (see

Section 2.2.2). The DNA solutions were measured in groups of ten according to species and vortexed immediately prior to measurement. The ND-1000 spectrophotometer was blanked between each group of DNA solutions. The mean of the pooled DNA concentrations from all five bark beetle species was used to calculate the biologically relevant DNA mixing ratio mentioned above.

The mean DNA concentration of the digestive system of a late instar *M. titillator* was obtained by removing 10 late instar *M. titillator* from laboratory infested material 21 days post-infestation. The digestive system from each larva was removed and the DNA extracted and quantified as described above. In order to obtain the biologically relevant DNA mixing ratio the mean of the pooled bark beetle DNA concentrations was compared to the mean DNA concentration of the *M. titillator* digestive systems.

2.2.7 Bark Beetle and *M. titillator* Haplotype Screening

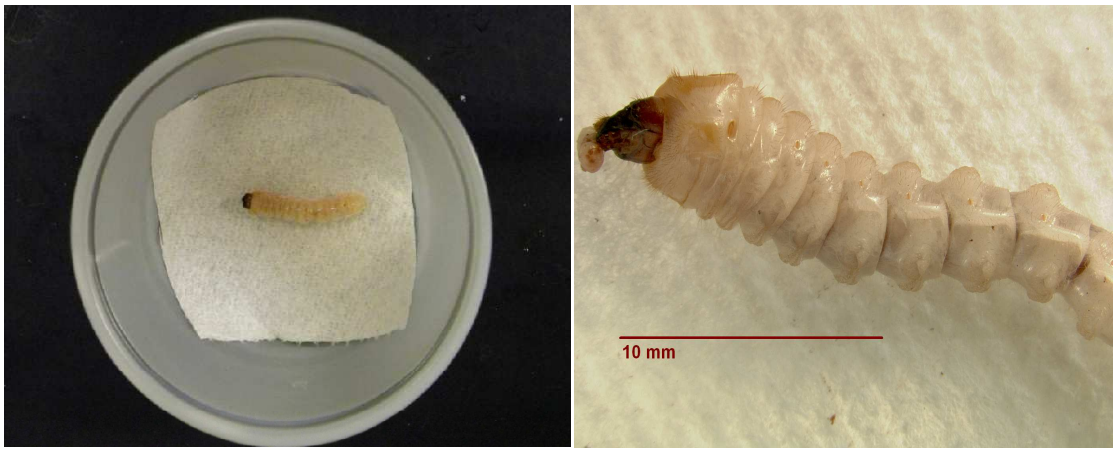
A haplotype experiment was performed in order to determine the effect of sequence variation that might exist among and within bark beetle and *M. titillator* populations. A total of fifteen adults were used for each bark beetle species. Individuals originated from two different populations except for *D. frontalis*, which originated from one population due to its local rarity. *I. calligraphus*, *I. avulsus*, and *I. grandicollis* samples were obtained from populations located at the LSU Burden Research Plantation (Baton Rouge, LA) and Bob R. Jones Research Plantation (Clinton, LA). *D. terebrans* samples were obtained from populations at the Bob R. Jones Research Plantation and Butler, AL. *D. frontalis* samples were obtained from a population located at the Oconee National Forest (GA). PCR reactions were performed as described above (see Section 2.2.4) and visualized on agarose gel. *M. titillator* were collected from the LSU Burden Research Plantation, Bob R. Jones Research

Plantation, and Kisatchie National Forest-Catahoula Ranger District (Pollock, LA) and tested as described above.

2.2.8 Molecular Half-Lives of Bark Beetle DNA in *M. titillator* Digestive Systems

Pairs, (2-3) of adult *M. titillator* were placed into eight 46x46x61 cm screened oviposition cages. Each cage was supplied with one *P. taeda* bolt every 5 days to produce four cohorts of infested bolts. Twenty-five days after bolts were exposed to *M. titillator* oviposition, larvae were collected from bolts by carefully peeling the bark away. Larvae were haphazardly assigned to labeled 4 oz plastic cups with damp filter paper and placed into an environmental chamber maintained at 30°C, 75% RH, and 12:12 L:D conditions. Larvae were starved for 48 hours prior to the feeding trials. This was done to ensure that their digestive systems didn't contain any plant material, which may have contained PCR inhibitors, as well as increase their motivation to consume the bark beetle remains offered to them (see below). The feeding arena and feeding technique are depicted in Figure (2.3).

After 48 hours of starvation each *M. titillator* larva was removed from the environmental chamber. Larvae where then placed into a new sterile identically labeled 4 oz plastic cup with their ventral side facing up to begin feeding assays. After being placed into the new plastic cup, larvae were allowed to rest for 10 minutes at room temperature in order to reduce their stress levels from handling, which negatively impacted their willingness to consume prey. Meals were offered to the *M. titillator* larvae in sterile forceps by placing the bark beetle remains immediately in front of their mandibles. On occasion it was necessary to agitate the *M. titillator* larvae by probing them gently with a dissection pick to get them to open their mandibles and begin consumption. If the larva refused to consume the bark beetle remains within 5 minutes they were discarded and replaced.



A. **B.**

Figure 2.3 Laboratory feeding assays. Image A: A 4 oz plastic cup lined with paper towel used as a feeding arena with *M. titillator* larva inside. Image B: A larva of *M. titillator* feeding on a larva of *I. grandicollis*.

Prior to initiation of the feeding assays, meal sizes for all bark beetle species were standardized to the mean weight of a 3^{rd} instar *I. grandicollis* ($\pm$ 1 SD). This was done using one of three methods depending on the size of the 3^{rd} instar of each bark beetle species. Meals consisting of *I. calligraphus* or *D. terebrans* were prepared by removing tissue from each larva using a scalpel and sterile techniques. Meals consisting of *D. frontalis* and *I. grandicollis* were prepared using individual larvae with weights equivalent to that of the 3^{rd} instar *I. grandicollis*. Meals consisting of *I. avulsus* larvae were not prepared due to issues discussed below (see Section 2.3.1). After the meal sizes were standardized they were stored at -80°C until feeding assays began. Meals were removed from the freezer and thawed at 30°C for 10 minutes in an environmental chamber prior to all feeding assays.

To construct a detection half-life model for bark beetle DNA in the digestive systems of *M. titillator* larvae, *M. titillator* larvae (N=120; 15 per treatment) were fed 3^{rd} instar *I.*

grandicollis. After being assigned haphazardly to the 4 oz cups, the *M. titillator* larvae were randomly grouped by seven or eight individuals (approximately 1/2 of the total treatment sample size), since it was necessary to use two of the four *M. titillator* larval cohorts to reach the necessary 120 larvae sample size. Each group of seven or eight *M. titillator* larvae was randomly assigned to one of the eight treatment groups of variable time post-feeding larval mortality. Once the *M. titillator* larvae had finished consuming their entire meal (visualized by no chewing motions for 1 minute) they were returned to the environmental chamber and maintained at 30°C, 75% RH, and 12:12 L:D conditions. A pilot study using a limited sample size suggested that the half-life of *I. grandicollis* in the gut contents was between 6-7 hours post-consumption, so *M. titillator* larvae were fed and killed at 0,3,4,5,6,7,8, or 10 hours post-consumption. Larvae were killed by placing them into 10 ml plastic vials containing 70% ethanol pre-chilled to -20°C to prevent regurgitation of their meals (Weber and Lundgren, 2009). Larvae were processed for DNA extraction within 24 hours post-mortem. Larvae were prepared for DNA extraction via dissection (see Section 2.2.2) and their digestive tracts extracted for subsequent PCR analysis.

The molecular half-life of *I. grandicollis* DNA in the guts of *M. titillator* larvae was determined using the proportion of *M. titillator* larvae testing positive for *I. grandicollis* DNA at each post-ingestion mortality interval. The median detection time (Y=50%) was calculated using Probit analysis (Chen et al., 2000) performed in Minitab v.15 (Minitab, 2008).

To test whether or not there were differences in the detection half-lives between the bark beetle species, 25 *M. titillator* larvae (N=100 total) were fed either *I. grandicollis*, *D. frontalis*, *D. terebrans*, or *I. calligraphus* larvae and then killed at the time corresponding to the detection half-life of *I. grandicollis* determined above. Due to the lack of positively

identified *I. avulsus* larvae (PCR failure), this species was not included in the analysis (see Section 2.3.1). To test the null hypothesis that the proportion of *M. titillator* larvae testing positive did not differ between treatments χ^2-analysis was performed followed by a Monte Carlo simulation (5000 iterations) to confirm significance. If the null hypothesis was rejected the Marascuilo procedure was implemented to determine which treatments differed significantly. These tests were performed in XLSTAT (Addinsoft, 2011).

2.3 Results

2.3.1 Primer Design and PCR Optimization

The designed bark beetle and *M. titllator* primer sets along with their characteristics are displayed in Table 2.1. The designed bark beetle species-specific primers yielded PCR products ranging in size from 122 (*D. frontalis*) to 427 base pairs in length (*D. terebrans*). PCR products sizes did not exceed 500 bp in length following the recommendations of Chen et al. (2000) (see Section 2.3.4). All species-specific primer pairs yielded PCR products of the expected sizes. Unfortunately, during the molecular half-life experiments it was discovered that the species-specific *I. avulsus* primer set only amplified DNA from adult *I. avulsus*. As a result, this species was not included in the half-life comparison experiments. The reason behind this phenomenon will be subject to further investigations.

The *M. titillator* and *M. carolinensis* COI gene sequencing results are depicted in Figure 2.4. Out of the 969 base pairs amplified from the COI gene sequences there was only 32 (3.3%) base pair differences between the two species. The low base pair variation between the two *Monochamus* species made it difficult to develop a species-specific primer pair for *M. titillator*. The most variable of all the potential primer pairs chosen for testing only incorporated a portion of the gene template with a five base pair difference. Not surprisingly

Table 2.1. Designed species-specific PCR primers

Name	Primer Sequence (5'→3')	Annealing Temp. (°C)	Target Species	Product Size (Bp)
IGF1	CCACTATTTACAGGACTTACAC	50.5	*I. grandicollis*	145
IGR1	CATCAGGGTAATCTGAATAACG	50.8	-	-
ICF1	GCTTACTTGGTTTCGTAGTAT	51.2	*I. calligraphus*	331
ICR1	GCAATAATAGCAAAGACTGC	49.6	-	-
DTF1	GAGCCTATTTCACATCTGC	50.8	*D. terebrans*	427
DTR1	GGATAATCAGAGTAACGACG	49.9	-	-
IAF1	GCTCACTTCCATTATGTCCTT	52.4	*I. avulsus*	168
IAR1	GAAAATGTTGAGGGAAGAAG	49.1	-	-
DFF1	GCTTACTTCACATCAGCCAC	53.8	*D. frontalis*	122
DFR1	CCAATAGCTCATAAAGAGGAGG	52.5	-	-
MTF1	ATCCAGCAGGAGGAGGAGAT	57.3	*M. titillator*	277
MTR1	CTTTAATTCCTGTTGGAACGG	51.7	-	-

this was the only primer pair that yielded a product for *M. titillator*, but not *M. carolinensis*. The *M. titillator* species-specific primer set selected yielded a PCR product 277 base pairs in length.

A total of two multiplex reactions were designed to screen *M. titillator* larvae for DNA of the five members of the southern pine beetle guild. This was the minimum number of reactions feasible without causing excessive primer-primer conflicts, which reduced the overall performance of the reactions. The first multiplex reaction (denoted multiplex "A" hereafter) contained the primer pairs ICF1/ICR1, IGF1/IGR1, and DTF1/DTR1 and was designed to screen for *I. calligraphus*, *I. grandicollis*, and *D. terebrans* DNA respectively. The second reaction (denoted multiplex "B" hereafter) contained the primer pairs DFF1/DFR1 and IAF1/IAR1 and was designed to screen for *D. frontalis* and *I. avulsus* DNA respectively. The *M. titillator* specific primer set MTF1/MTR1 was placed into a single reaction, since its addition to either multiplex reaction reduced the overall quality of these reactions to an unacceptable level. The variation in PCR product sizes within each multiplex PCR reaction

```
  1  TTCTTCTATT TTAGGTGCAG TAAATTTTAT TACAACCGTA ATTAATATAC GACCTTCAGG
     .......... C......... .......... .......... .......... .....AA...
 61  AATAAATTTA GATCGTTTAC CTTTATTTGT TTGAGCAGTA AAAATTACTG CAATTCTTCT
     .......... .......... .......... .......... .....C.... ..........
121  TCTTCTTTCT TTACCAGTTC TAGCCGGAGC AATTACTATA CTTTTAACAG ATCGAAATTT
     .......... .........T ....T..... T......... ........T. ..........
181  AAATACATCA TTCTTTGATC CAGCAGGAGG AGGAGATCCA ATTTTATATC AACATTTATT
     .......... .......... .......G.. ...T...... .......... ..........
241  TTGATTTTTT GGTCACCCAG AAGTTTATAT TCTTATTCTT CCAGGATTTG GAATAATTTC
     .......... .......... .......... .........C .......... ..........
301  TCATATTATT AGACAAGAAA GAGGTAAAAA AGAAACTTTT GGAACTTTAG GAATAATTTA
     .......... ..T....... .......... .......... .......... ..........
361  TGCTATAATA GCAATTGGTT TATTAGGATT TGTAGTTTGA GCTCATCATA TATTTACTGT
     .......... .......... .......T.. .......... .....C.... ..........
421  AGGAATAGAT GTTGATACCC GAGCATATTT TACTTCAGCA ACTATAATTA TTGCCGTTCC
     .......... .......... .......... .......... .......... ....T..A..
481  AACAGGAATT AAAGTTTTTA GATGATTAGC AACTTTTCAT GGAACACAAC TTTTATATAG
     ......T... .......... .......... ......C... ..T....... ..........
541  ACCTGTATCT TTATGAGCAT TAGGTTTTGT ATTTTTATTT ACAGTCGGAG GATTAACAGG
     .......... .......... .......... .......... .....T.... ..........
601  AGTAGTTTTA GCTAATTCTT CTTTAGATAT TATTCTTCAT GATACATATT ATGTTGTTGC
     .......... .......... .......C.. .......... .....C.... ..........
661  TCATTTTCAT TATGTTCTTT CAATAGGAGC AGTATTTGCT ATTATAGCTG GATTAGTTCA
     .......... .......... .T..G..... .......... .......... ..........
721  ATGATTCCCT TTATTTACAG GTTTAACTCT TCACAGAAAA TTTTTAAAAA TTCAATTCTT
     .......... .......... .......... ...T...... .......... .......T..
781  TATTATATTT ATTGGAGTTA ATATAACATT TTTTCCACAA CATTTTTTAG GATTAAGAGG
     .G.A...... .......... .......... ......T... .......... ..C.......
841  AATACCACGA CGATATTCTG ATTATCCTGA TGCTTTTACT CTATGAAATA TTGTTTCATC
     .......... .......... .......... .......... .......... ..........
901  TATTGGATCT TTAATTTCCT TAGTAAGAGT AACATTATTA TTATACATTT TTTGAGAAGC
     .......... ........T. .......... .......... .....T.... .C........
961  ATTTGCTGTT CATCGA???? ????????
     .........? ......AAAA CACTAGCT
```

Figure 2.4 Partial cytochrome oxidase subunit I gene sequences for *Monochamus titillator* (Top) and *M. carolinensis* (Bottom). The target sequences of the *M. titillator* species-specific primer pair is highlighted in yellow. Question marks signify unknown nucleotides

allowed for easy determination of the presence or absence of DNA from each bark beetle species in the gut contents of *M. titillator* larvae.

The optimal PCR buffer utilized during the early stages of PCR development was found to be buffer "C" (12.5 mM $MgCl_2$, pH=8.5). This buffer was later replaced by the 5-prime HotMaster 10X *Taq* Buffer, which releases Mg^{2+} ions as required by the reaction. This was shown to increase PCR robustness over buffer "C". The optimal annealing temperature for multiplex reactions A and B was calculated as 58°C. Temperatures >60°C caused these reactions to yield no products. The optimal annealing temperature for the *M. titillator* singleplex reaction was calculated as 60°C. However, temperatures ≤60°C caused non-specific amplification of *M. carolinensis* DNA, so an annealing temperature of 62°C was selected to avoid this issue.

Using the optimal temperatures the final optimized PCR cycling parameters were as follows: an initial denaturation period of 94°C for 2 minutes followed by 35 cycles of a denaturation period at 94°C for 1 minute, an extension period at 58°C (multiplex reactions A and B) or 62°C (*Monochamus* singleplex reaction) for 2 minutes, an elongation period at 72°C for 1 minute, and a final extension period at 72°C for 7 minutes.

2.3.2 Primer Specificity and Haplotype Tests

The designed species-specific primer sets in their optimized PCR reactions exhibited no amplification of non-target DNA in any of the primer specificity tests performed (Figure 2.5). As mentioned previously (see Section 2.3.1) the primer set MTF1/MTR1 did amplify *M. carolinensis* DNA but this was prevented by increasing the annealing temperature of the reaction. During the haplotype experiments 100% amplification success was observed for all bark beetle species and *M. titillator* confirming the functionality of the designed species-

specific primer pairs for amplification of the partial COI gene sequence from southern pine beetle guild populations in Louisiana and some neighboring regions.

2.3.3 Primer Sensitivity Tests

The mean weight of a 3^{rd} instar *I. grandicollis* used for determining the ratio of predator:prey DNA in the sensitivity analyses was calculated 4.00x10^{-3} g (SD= 3.25x10^{-4}). The DNA concentrations from all five bark beetles species with weights equivalent to 4.00x10^{-3} g as well as the DNA concentrations from late instar *M. titillator* digestive tracts are presented in Table 2.3.3. The mean of the pooled DNA concentrations of all five bark beetles species was calculated as 82.04 ng/μl (SD=34.06). The mean DNA concentration calculated from the digestive system from a late instar *M. titillator* was 406.29 ng/μl (SD=215.25). The mean DNA concentration of the digestive system from a late instar *M. titillator* is approximately 4.95 times higher than the mean DNA concentrations from the pooled bark beetles with weights equivalent to that of a 3^{rd} instar *I. grandicollis*. This number was rounded to 5 for convenience.

The bark beetle primer pairs ICF1/ICR1, IGF1/IGR1, IAF1/IAR1, DTF1/DTR1, and DFF1/DFR1 were tested for their capability of amplifying target DNA under singleplex and multiplex PCR conditions in predator:prey DNA mixing ratios ranging 5:1 to 5,000,000:1. Gel images depicting the results from the multiplex bark beetle primer sensitivity analyses are presented in Figure 2.6. A summary of the results from the singleplex and multiplex sensitivity analyses are presented in Table 2.3.3. Under singleplex PCR conditions the lower detection limits were 0.05 pg/μl of DNA for primer sets ICF1/ICR1, IGF1/IGR1, DTF1/DTR1, and DFF1/DFR1 and 50 pg/μl of DNA for primer set IAF1/IAR1. Under multiplex PCR conditions the lower detection limits were 0.05 pg/μl for primer pair

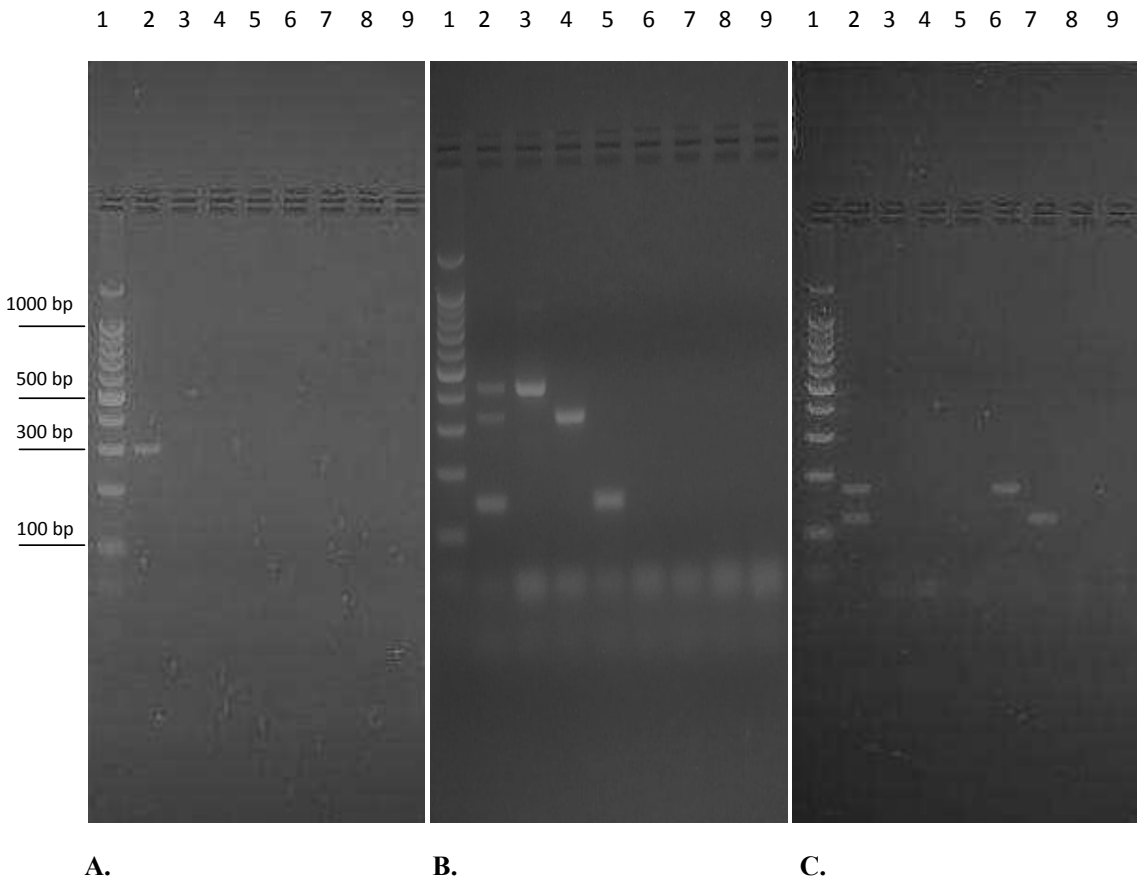


Figure 2.5 Primer specificity analyses. Image A: *M. titillator* singleplex. Image B: Multiplex "A". Image C: Multiplex "B". Gel Lanes: (1) DNA ladder, (2) Positive control (All target templates), (3) *D. terebrans* DNA, (4) *I. calligraphus* DNA, (5) *I. grandicollis* DNA, (6) *I. avulsus* DNA, (7) *D. frontalis* DNA, (8) *M. carolinensis* DNA (Image A) or *M. titillator* DNA (Images B and C), (9) Negative control (Nuclease-free H_2O).

Table 2.2 DNA concentration (ng/μl) analyses of members of the southern pine beetle guild with equivalent weights ($4.00\text{x}10^{-3}$ g) and the digestive tract of late-instar *M. titillator* used to calculate a biologically relevent predator:prey DNA mixing ratio during primer sensitivity analyses.

	Species					
Sample	***I. grandicollis***	***I. calligraphus***	***I. avulsus***	***D. terebrans***	***D. frontalis***	***M. titillator***
1	74.3	113.9	109.7	38.5	60.2	314.2
2	81.2	79.9	116.2	117.3	66.8	909.4
3	113.3	37.2	111.7	55.1	88.3	445.6
4	101.1	116.3	132.9	80.2	69.1	403.4
5	69.8	105.4	127.4	86.7	61.8	426.3
6	50.6	84.5	120.4	66.4	57.6	269.5
7	57.2	78.1	119.9	45.3	91.6	546.2
8	67.5	43.0	114.0	39.3	51.8	267.5
9	24.8	159.2	123.8	22.6	63.1	97.5
10	88.2	108.8	144.9	9.2	55.9	383.3
Mean	72.8	92.6	122.1	56.1	66.6	406.3
SE	8.05	11.47	3.40	10.22	4.21	68.07

Table 2.3 Comparison of primer sensitivities under singleplex and multiplex PCR conditions.

Reaction	Sensitivity Levels	Magnitudinal Difference
Multiplex A	All species: 5.0 pg/µl-0.5 pg/µl	[redacted]
Multiplex B	*I. avulsus*: 500.0 pg/µl-50 pg/µl *D. frontalis*: 0.05 pg/µl-0.005 pg/µl	[redacted]
***I. grandicollis* Singleplex**	0.05 pg/µl-0.005 pg/µl	100X
***I. calligraphus* Singleplex**	0.05 pg/µl-0.005 pg/µl	100X
***I. avulsus* Singleplex**	50.0 pg/µl-5.0 pg/µl	10X
***D. terebrans* Singleplex**	0.05 pg/µl-0.005 pg/µl	100X
***D. frontalis* Singleplex**	0.05 pg/µl-0.005 pg/µl	0X

DFF1/DFR1, 5 pg/μl for primer pairs ICF1/ICR1, IGF1/IGR1, DTF1/DTR1, and 500 pg/μl for primer pair IAF1/IAR1. An increase in primer sensitivities under singleplex PCR conditions was observed, ranging from as low as 0X (*D. frontalis*) and 10X (*I. avulsus*) to as high as 100X (*I. grandicollis*, *I. calligraphus*, and *D. terebrans*) over primer sensitivites observed under multiplex PCR conditions.

2.3.4 Molecular Half-lives of Bark Beetle DNA

The molecular half-life of *I. grandicollis* DNA in the guts of *M. titillator* larvae calculated using Probit analysis was found to be 6.89 h post-consumption (95% fiducial limits 6.21 and 7.70) (Figure 2.7). The Probit model fit the data well based on Pearson's goodness-of-fit

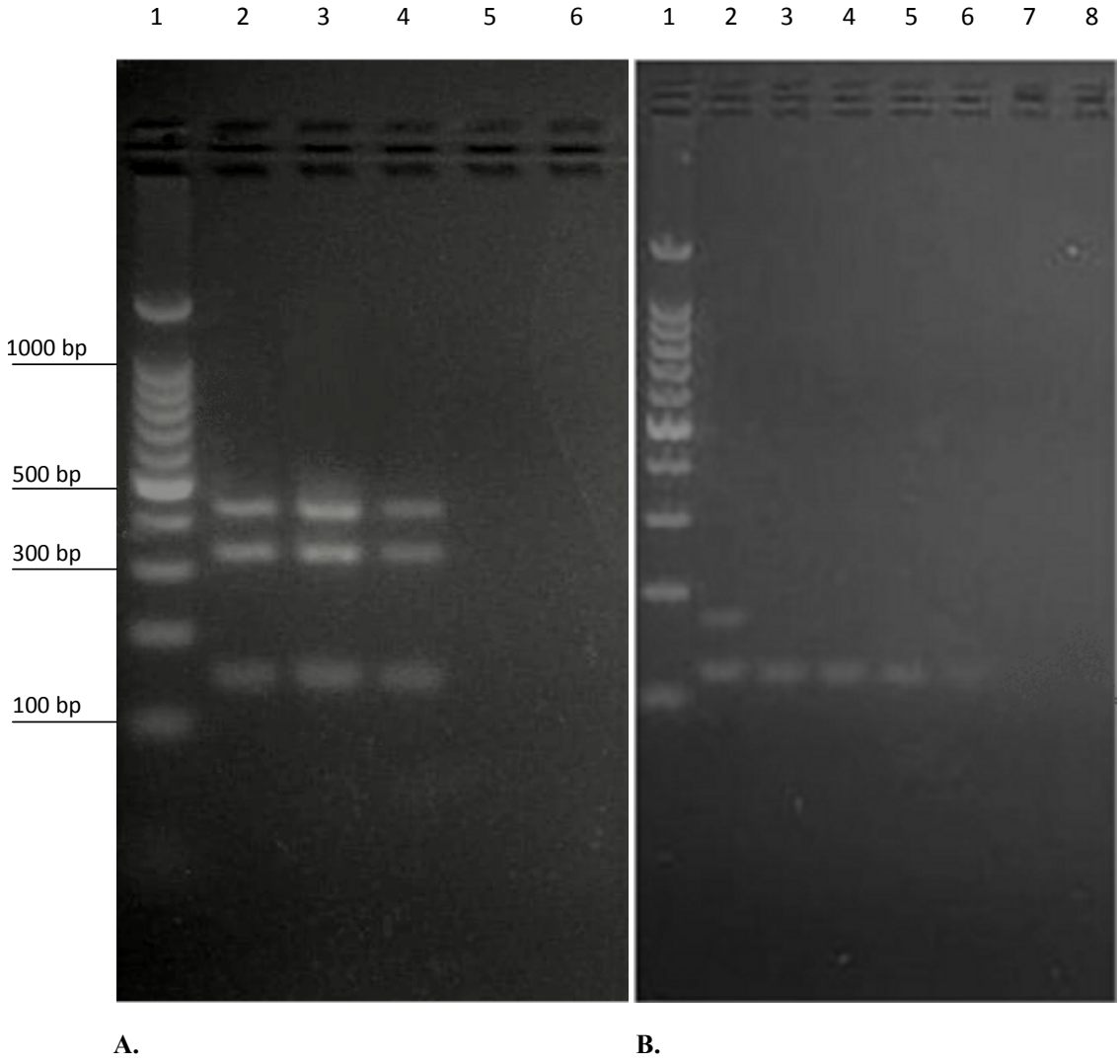


Figure 2.6 Gel images from the multiplex sensitivity analyses. Image A. Bands: (Top *D. terebrans* Middle *I. calligraphus* Bottom *I. grandicollis*) Multiplex "A". Image B. Bands: (Top *I. avulsus* Bottom *D. frontalis*) Multiplex "B". Gel Lanes: (1) DNA Ladder. Bark Beetle DNA concentrations of (2) 500, (3) 50, (4) 5, (5) 0.5, (6) 0.05, (7) 0.005, and (8) 0.0005 pg/μl. Bark beetle DNA templates were mixed in a constant concentration of 2.5 ng/μl of *M. titillator* DNA stock solution.

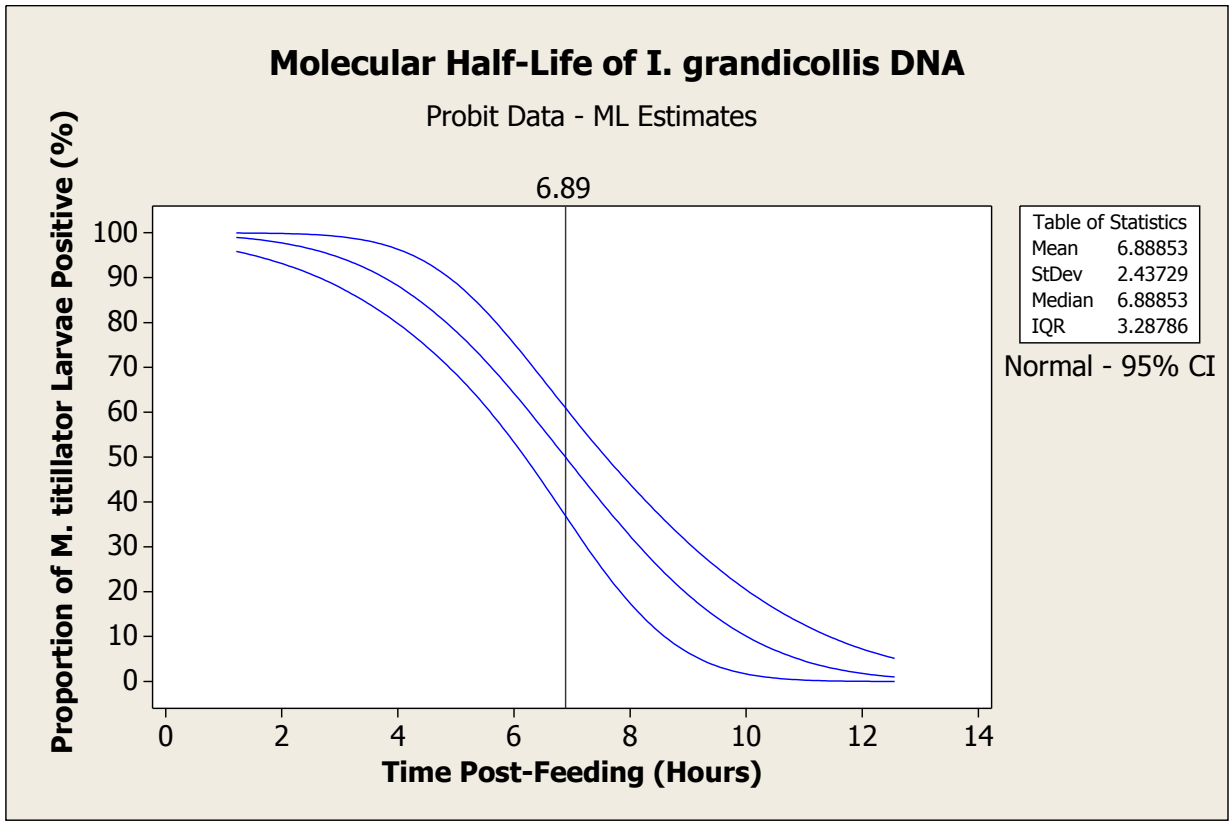


Figure 2.7 The molecular half-life of *I. grandicollis* DNA in the gut contents of *M. titillator* larvae (N=120) calculated using Probit analysis (95% fiducial limits shown).

test (Chi-square value=1.455, P=0.962). Amplification of *I. grandicollis* DNA in the gut contents of *M. titillator* was detectible up to 10 hours post-consumption. The proportion of *M. titillator* larvae testing positive for *I. grandicollis* DNA ranged as high as 100% (0 and 3 hours post-consumption) to as low as 13.3% (10 hours post-consumption). Larvae generally responded well to hand feeding with 55.2% of larvae (N=221) completely consuming the offered meals. This number may have been higher, but larvae preparing to molt or those that had recently molted would not feed.

The proportion of the 25 *M. titillator* larvae testing positive for four of the bark beetle species killed at 6.9 hours post-consumption was 0.64, 0.68, 0.16, and 0.64 for *I. grandicollis*, *I. calligraphus*, *D. terebrans* and *D. frontalis*, respectively. The Chi-square analysis for multiple proportions found a significant difference between prey species (Chi-square value=18.426, DF=3, P<0.001, α=0.05). The Monte Carlo simulation confirmed significance (P<0.001). Since the null hypothesis of proportional equality was rejected the Marascuilo procedure was implemented (Table 2.4). The pairwise comparison of these proportions showed that the proportion of *M. titillator* larvae testing positive for *I. grandicollis*, *I. calligraphus* and *D. frontalis* at 6.9 hours did not differ significantly. However, the proportion of *M. titillator* larvae testing positive for *D. terebrans* did differ significantly from the proportion of *M. titillator* testing positive for the other three bark beetle species.

2.4 Discussion

2.4.1 Primer Design and PCR Optimization

Species-specific primer sets were successfully developed for adults of all five members of the southern pine beetle guild as well as a primer set capable of separating *M. titillator* larvae from *M. carolinensis* larvae. However, for an unknown reason the *I. avulsus* species-

Table 2.4. The Marascuilo procedure

Contrast	Difference	Critical Value	Significant?
|p(*I. grandicollis*) - p(*I. calligraphus*)|	0.04	0.374	No
|p(*I. grandicollis*) - p(*D. terebrans*)|	0.48	0.338	Yes
|p(*I. grandicollis*) - p(*D. frontalis*)|	0.00	0.380	No
|p(*I. calligraphus*) - p(*D. terebrans*)|	0.52	0.332	Yes
|p(*I. calligraphus*) - p(*D. frontalis*)|	0.04	0.374	No
|p(*D. terebrans*) - p(*D. frontalis*)|	0.48	0.338	Yes

specific primer set was capable of amplifying DNA of adults and not larvae. The COI gene is present in both adults and the immatures. At this time it is not known why the primers work on adults and not larvae. Most likely there is something inherent with the larvae that prevented the primers from binding to the DNA. Given the similarities in the biologies of all five members of the southern pine beetle guild, it is surprising larvae from the other bark beetle species did not have this same issue. Unfortunately, the inability to amplify the DNA of *I. avulsus* larvae prevented it from being included in the molecular half-life comparison.

Due to the fact that the five prey species used in this study were represented by two closely related genera, the partial COI gene sequences of the southern pine beetle guild provided few regions of high variation to design species-specific primers. Combining these primer sets into working multiplex reactions presented further difficulties. The time and cost benefits of multiplex PCR over traditional singleplex PCR outweighed the additional effort required to develop species-specific primers for the southern pine beetle guild. This study represents an extreme scenario in the utilization of molecular gut analyses to study closely related prey taxa and further demonstrates the capabilities of PCR. Until recently few studies have utilized multiplex PCR in molecular gut content analysis studies.

The primer sensitivities in this study are high with the exception of the *I. avulsus* species-

specific primer set, when compared to other molecular gut content analysis studies (Zhu and Williams, 2002; Agustí et al., 2003b; Traugott et al., 2006). For example, Zhu and Williams (2002) observed a lower sensitivity limit of approximately 2.4 pg for primers designed to amplify DNA of the wasp *Anaphes iole* (Girault) (Hymenoptera: Mymaridae). Previous studies have also observed an increase in primer sensitivies under singleplex PCR conditions compared to multiplex PCR conditions. Traugott et al. (2006) found that the lower sensitivity limit of a primer pair designed to amplify the DNA of the parasitoid *Diadegma semiclausum* Hellen (Hymenoptera: Ichneumonidae) under singleplex PCR conditions was 0.59 pg. This was four-fold higher than the primer sensitivity observed under multiplex PCR conditions (2.34 pg of DNA). The low sensitivity limit observed for *I. avulsus* is not of concern as the observed sensitivity limits for the species-specific primer set of this species corresponds to approximately 244-2441 larval equivalencies (i.e. the DNA content corresponding to an extracted larva) based on the mean DNA concentration of an *I. avulsus* larvae with a weight equivalent to $4.00\text{x}10^{-3}$ g (mean weight of 3^{rd} instar *I. grandicollis*).

2.4.2 Molecular Half-lives of Bark Beetle DNA

Greenstone and Hunt (1993) suggest that in order for molecular-based predation studies to provide ecologically useful data, assays must be able to provide per-capita estimates of the number of prey consumed by predators per unit of time. This is a challenging metric to obtain. It is difficult to relate detection of bark beetle DNA in the gut contents of *M. titillator* larvae to predation rates as this detection could represent a large meal (i.e. a meal larger than that utilized in molecular half-life assays in my study) eaten longer than 6.9 hours ago or a small meal eaten recently (Harwood and Obrycki, 2005). PCR analysis however,

does not allow for the quantification of prey consumed or their masses (King et al., 2008) making discrimination among these alternatives impossible.

The molecular half-life provides a means of quantifying predation rates by describing the time where half of the predation events should be detectable for any given predator and prey. This model will serve as an important metric for interpreting positive and negative detection events of DNA in the gut contents of field-collected *M. titillator* larvae. The short molecular half-lives detected for DNA of members of the southern pine beetle guild in the gut contents of *M. titillator* larvae are comparable to those of other studies dealing with active predators. Examples of observed detection limits and half-lives for active predators have ranged as low as less than 1 hour post-consumption for dragonflies (Morales et al., 2003), <5 hours for carabids (Zaidi et al., 1999), and < 7 hours for coccinellids (Chen et al., 2000; Hoogendorn and Heimpel, 2001; Weber and Lundgren, 2009) to greater than 30 hours observed for anthocorids (Agustí et al., 2003b), > 25 hours for carabids (Harper et al., 2005; Juen and Traugott, 2005), and > 24 hours for phlaeothripids (Jaramillo et al., 2010).

A significant difference in the proportion of *M. titillator* testing positive for *D. terebrans* was observed at 6.9 hours post-consumption. Although the four bark beetle species had similar sensitivities under multiplex PCR conditions, their respective PCR product sizes varied. The relatively large PCR product formed by the *D. terebrans* species-specific primer set is likely the cause of the lower half-life observed for this species due to larger PCR products generally exhibiting lower prey detection frequencies (see Section 2.3.3). Differences between the expected proportion (50%) of *M. titillator* testing positive for *I. grandicollis* DNA calculated from first half-life experiment and the observed proportion testing positive for *I. grandicollis* DNA during the half-life comparison experiment (64%) are likely due to lower sample sizes utilized in the half-life comparison studies. Another possible reason

for the difference in proportions that was observed was the temporal lag between the two studies. There was however, only a one week lag between when the molecular half-life of *I. grandicollis* DNA study and the half-life comparison study was performed, making this an unlikely scenario.

2.4.3 Factors Affecting Prey Detection Rates in the Guts of Predators

Factors that affect the detection length and probability of prey remains in the guts of predators can be broadly classified in to three main causal groups: biological factors, environmental (physical) factors, and methodological factors (Hosseini et al., 2008). Some of the most critical aspects of these factors are discussed below.

Numerous environmental factors have been demonstrated to influence prey detection lengths and probabilities. For example, studies examining the effects of temperature on prey detection rates and durations have found that in general, higher temperatures lead to a decrease in prey detection lengths and probabilities (Hagler and Cohen, 1990; Hagler and Naranjo, 1994; Hoogendorn and Heimpel, 2001; Hosseini et al., 2008). This is likely due to an increase in the predator's digestion rates resulting in a decrease in the molecular half-life of prey DNA. Taxon-specific digestion rates also appear to influence prey detection periods, with metabolically "active" predators (e.g. predacious beetles) (Harper et al., 2005; Sheppard et al., 2005) exhibiting generally shorter prey detection periods compared to metabolically "slow" predators such as spiders that have considerably lower resting digestion rates than most invertebrates (Greenstone and Bennett, 1980; Greenstone, 1983; Ragsdale et al., 1981; Harwood et al., 2001). This pattern however can be highly variable based on a combination of the experimental variables described above and below (e.g. prey taxa, probe sensitivity, temperature, and target gene). The effects of meal size on prey detection

rates and duration is not completely clear with some studies observing an effect of meal size (Hagler and Naranjo, 1997), while others observed no effect (Zaidi et al., 1999; Hoogendorn and Heimpel, 2001; Juen and Traugott, 2005; Staudacher et al., 2011). Other predator characteristics such as sex, size, gender, and developmental stage appear to have little impact on the probability or duration of prey detection within their gut contents (Harwood et al., 2001; Hoogendorn and Heimpel, 2001; Sheppard et al., 2005; Hosseini et al., 2008; but see Symondson et al., 1999).

Methodological factors such as the choice of target gene appear to play a major role in the observed detection length and probability of prey remains. Both nuclear (Zaidi et al., 1999; Hoogendoorn and Heimpel, 2001) and mitochondrial (Chen et al., 2000; Agustí et al., 2003b) multi-copy genes have been shown to work extremely well in molecular gut analyses. Mitochondrial genes are particularly useful due to their presence in hundreds or thousands of copies per cell (Hoy, 1994). Mitochondrial genes are also useful when attempting to develop species- and group-specific primers due to their relatively high mutation rates compared to nuclear genes (Simon et al., 1994). Another methodological factor influencing the detection rates of prey is the length of the amplified PCR products. Previous studies have shown that prey detection half-lives or rates are inversely related to product length (Agustí et al., 1999b, 2000, 2003b; Zaidi et al., 1999; Chen et al., 2000; Hoogendoorn and Heimpel, 2001; Foltan et al., 2005; Juen and Traugott, 2005). Primers designed to amplify PCR product sizes <500 base pairs are generally suitable for gut content analyses, since DNA in the guts of predators is usually degraded making smaller target sequences more likely to persist. Finally, antigen and primer quality may play a role in the observed prey detection rates and duration (Juen and Traugott, 2005; Admassu et al., 2006; de León et al., 2006).

2.5 Summary

Species-specific PCR primer sets were successfully developed for adults and larvae of all five members of the southern pine beetle guild, with exception to *I. avulsus* larvae. Species-specific PCR primers were also designed that were capable of seperating *M. titillator* larvae from *M. carolinensis* larvae. The five bark beetle primer sets were successfully combined into two multiplex PCR reactions. The size differences in the PCR products amplified from these primer sets allowed easy determination of the presence or absence of DNA from each bark beetle species. The designed primers were highly sensitive to target DNA under multiplex PCR conditions.

The molecular half-life of *I. grandicollis* DNA in the gut contents of *M. titillator* larvae was calculated as 6.89 hours post-consumption. A comparison of the proportion of *M. titillator* larvae fed larvae of four of the five bark beetles species killed at 6.9 hours post-consumption showed that the proportion fed *D. terebrans* differed significantly. This was most likely due the large PCR product formed for this species. Results from the molecular half-life studies will provide a frame of reference when attempting to interpret results on the observed predation frequencies of field-collected *M. titillator* larvae for members of the southern pine beetle guild presented in the following chapter.

3. Predatory Dynamics Between *Monochamus titillator* Larvae and the Southern Pine Beetle Guild in the Field

3.1 Introduction

Southern pine beetle guild populations undergo dramatic fluctuations between endemic and epidemic levels. In the case of *D. frontalis* these fluctuations appear to have a periodicity (Turchin, 1990). The underlying mechanisms involved in these fluctuations have recieved considerable attention. Factors driving the reduction in southern pine beetle guild populations are poorly understood compared to those leading to an increase in population levels. Regulation via bottom-up effects driven by host resistance appears to be the dominant force preventing bark beetle populations from reaching epidemic levels. Host resistance can be affected by stand, site, and climactic conditions (Lorio and Hodges, 1968; Lombadero et al., 2000), mechanical stress (Ruel et al., 1998), and bark beetle-induced stress (Lorio et al. 1995). When favorable conditions allow, host resistance can be overcome by bark beetle mass attacks. This eventually leads to the production of more individuals who can in turn, facilitate the breach of the host's defenses and ultimately lead to an explosion in population levels.

There has been an increase in awareness of the roles insect associates play in southern pine beetle guild population dynamics. The negative impacts of natural enemies on populations of members of the southern pine beetle guild have been widely demonstrated (Linit and Stephen, 1983; Miller, 1984). Interpretation of results from some studies observing a negative impact of insect associates on southern pine beetle guild populations however, must be

viewed critically due to the fact that they simultaneously excluded competitors and natural enemies (Miller, 1986; Riley and Goyer, 1986). It is believed that predation by natural enemies may affect bark beetle populations in a delayed density-dependent manner (Turchin et al., 1999). Alternatively, competition with phloem-inhabiting insects may generate direct negative feedback (Reeve and Turchin, 2002). Further studies quantifying the impact of competitors and natural enemies on southern pine beetle guild population dynamics are needed.

The field of molecular-based predation studies has rapidly expanded in the last decade, providing many examples of its successful application to a wide array of invertebrate taxa. A large proportion of early molecular-based predation studies were calibratory laboratory studies, which examined factors affecting prey DNA detection rates in the guts of predators. These factors include: temperature (Hagler and Naranjo, 1997; Hosseini et al., 2008), meal size (Hagler and Naranjo, 1997; Agustí et al., 1999b; Juen and Traugott, 2005; Weber and Lundgren, 2009), predator species (Hagler and Naranjo, 1997; Hosseini et al., 2008), predator gender and size (Hosseini et al., 2008), time since feeding (Hagler and Naranjo, 1997; Hosseini et al., 2008; Weber and Lundgren, 2009), sample processing and visualization techniques (Juen and Traugott, 2006; Sint et al., 2011), predator digestion morphology (Hosseini et al., 2008), sample preservation (Weber and Lundgren, 2009), and effect of chaser meal (Weber and Lundgren, 2009). These studies identified factors that may influence the performance of the molecular tools developed. The ultimate goal of these tools is their application to the study of trophic interactions in the field.

The first study to use DNA-based molecular gut analyses of field-collected samples to study predator-prey interactions of cryptic species under natural conditions examined predation rates of Collembola species by spiders in arable fields (Agustí et al., 2003a). Since

then, DNA-based molecular gut analyses have been used to study many systems including aquatic (Saitoh et al., 2003; Deagle et al., 2005; Suzuki et al., 2006), soil (Read et al., 2006; Staudacher et al., 2011), grassland (Foltan et al., 2005; Juen and Traugott, 2007), and agroecosystems (Wallace, 2004; Traugott et al., 2006; Harwood et al., 2007, 2009; King et al., 2010; Eitzinger and Traugott, 2011). Until now, no other studies are known to have utlilized DNA-based molecular gut content analyses to elucidate food-webs in forest ecosystems, with exception to a study by Muilenburg et al. (2008) who observed predation of eggs of the cerambycid *Enaphalodes rufulus* (Haldeman) by multiple ant species. Field studies are important when attempting to characterize predator-prey dynamics, because it is difficult to reproduce environmental conditions and predator/prey compositions in the laboratory that approximate those in the field (Symondson, 2002). This is particularly true of the subcortical environment of pines. Study of predator-prey interactions in this environment would require reproduction of the intricate communication system driving adult bark beetle and *M. titillator* arrival to the host, and their within-host larval densities and distributions. Because of this, DNA-based molecular gut content analyses of the gut contents of field-collected *M. titillator* are important for elucidating the subcortical interactions between *M. titillator* larvae and the southern pine beetle guild.

The primary goal of this study was to use the tools devloped in Chapter 2 to survey the gut contents of field-collected *M. titillator* larvae to test the capability of the molecular tools developed in the laboratory. This was done by obtaining semi-quantitative measurements of predation events by *M. titillator* larvae on members of the southern pine beetle guild under semi-natural conditions. This study tested the hypothesis that the frequency of predation events by *M. titillator* larvae on members of the southern pine beetle guild is high enough to allow their detection using the molecular tools developed. In addition, this study

attempted to further characterize the subcortical interactions between larval *M. titillator* and the southern pine beetle guild by comparing the species composition of members of the southern pine beetle guild within the host to that observed within the gut contents of the *M. titillator* larvae to see if they exhibit prey choice.

3.2 Materials and Methods

3.2.1 Field Predation Pilot Study

A pilot study was performed to determine the relationship between bole surface location (top, bottom, sides) and height, on the distribution of woodborer oviposition niches and bark beetle and woodborer larvae. This was done in order to optimize sampling efforts (i.e., samples were taken from areas characterized by high densities of both *M. titillator* and bark beetle larvae).

On 5/17/10 a single healthy ∼20 year old *P. taeda* (dbh=1.2 m, height=21 m) was felled. The lowest 1.75 meters of the bole was cut and discarded since the thick bark in this region has been shown to inhibit *Monochamus* oviposition (Linit et al. 1983; Walsh and Linit 1985). The remaining portion of the bole was cut into 1 m long sections until 9 sections were obtained. The remaining upper portion of bole and crown of the tree was discarded. The 1 m sections were elevated 30.5 cm off the ground by placing log sawhorses underneath each section. The bole sections were elevated to help protect the bole sections against fire ant infestation, flooding, and to provide attacking insects easy access to the entire bole surface (Riley, 1983). Additionally, reduced *Monochamus* oviposition density has been shown on portions of bolts touching other bolts when decked or in contact with the ground (Raske, 1975). Thus, elevating the logs off the ground potentially allowed for a more natural oviposition distribution on the experimental bolts. The bole sections were aligned end to

end as tightly as possible to help prevent desiccation according to their original position on the tree. The bole was placed parallel to the edge of a *P. taeda* stand in an open field.

Ten days post felling, the number of oviposition niches were counted across the surface of each bole section. This was done by dividing the surface area into quarters (see Peddle, 2000; Allison and Borden, 2001). Each quarter section was designated as either the top, bottom, left side (facing pine stand), or right side (facing open field). The quarter divisions were visualized by hammering a nail every 90° at both ends of each bolt section (starting at 45°) and running cotton string from one end to the other. The number of oviposition niches were then counted and recounted for each quarter section of each bolt section until a consensus count was reached. The number of oviposition niches for each bole quarter area were pooled across the height of the bole and the percentage of oviposition niches occupying each quarter area determined. Additionally, the total number of oviposition niches was pooled across the four quarters within sections and the percentage of oviposition niches occupying each bole section compared.

In addition to determining the optimal bole heights and surface areas on the boles to sample, the optimal time post-felling to sample was determined by monitoring bark beetle and wood borer development in the bole sections. Every five days for 30 days post-felling small samples of bark (< 1 dm^2) were carefully removed from the top, sides, and bottom quarters of the bole sections using a hatchet. Bark beetle and wood borer larval and gallery development were observed and recorded. Specifically, the time post-felling when within host bark beetle populations were high and *Monochamus* foraging had begun to overlap significantly with bark beetle galleries was determined. In order to prevent excessive destruction of the bolts due to the bark sampling, even and odd numbered bolts were checked on alter-

nating sampling dates. Thirty days post-felling all bolt sections were stripped of their bark and removed from the area.

3.2.2 Field Predation Survey

On 6/25/10 four healthy ∼20 year old *P. taeda* (mean dbh=0.321 m, SD=0.026) were felled and the initial 1.75 meters of the bole discarded. The remaining portion of the felled bole was cut up into 1 m long sections until 9 sections were obtained. The first four sections from each tree were discarded and the remaining five sections (N=20) moved to the experimental area described above (see Section 2.2.8). Each bole section was numbered according to its original position on the tree and marked to facilitate alignment of the upper surfaces of consecutive bolts. The five sections from each tree were grouped and tightly aligned end to end to prevent excessive desiccation according to height. The bole sections were elevated 30.5 cm off the ground by placing log sawhorses underneath each section. The bole section groups from each tree were at least 20 m away from the other tree bole sections situated parallel approximately 1.5 m out along the edge of the tree line.

One of the original goals of this study was to determine the effect of bark beetle density on their observed detection frequencies within the gut contents of *M. titillator* larvae. However, due to the small sample size available this was not possible. Since the *M. titillator* recovered from this attempt were included in the predation detection frequency analysis, the sampling methodolgy is described below.

Twenty-five days post felling, two 1 dm^2 bark disks were extracted per side and bottom area of each bole section using a 114 mm diameter hole saw. To collect the samples each bole section was carefully lifted off of its supports and placed onto the ground with the side originally facing the open field facing upwards. A single bark disk sample was taken 0.33

m from each end of the bole section 90° from the mark used to designate the upper surface of the bole section (Figure 3.1). The bole section was then rolled 90° counterclockwise to expose the bottom surface and 180° (an additional 90°) to sample the other side. This sampling method was repeated for each bole section until the sides and bottom areas of each bole section had been sampled (N=120). Cerambycidae larvae were collected from the areas exposed by the hole saws and placed individually into labeled collection vials containing 95% ethanol and placed over ice to halt digestive enzymes until they could be transported back to the laboratory for identification and subsequent DNA extraction.

It is possible that other larvae from the subfamily (Lamiinae) were collected during the bark disk sampling portion of the experiment due to the lack of *Monochamus*-specific morphological characters at the time of dissection. This was not of concern however, due to the very low numbers of the other adult Lamiinae [except for *Acanthocinus obsoletus* (Olivier), N=227] observed in multiple-funnel traps at the same location and time of year the bolts were sampled (E.N. Schoeller and J.D. Allison unpub. data). To ensure that the primers developed to amplify *M. titillator* DNA did not amplify *A. obsoletus* DNA, we tested the primers on five individuals of *A. obsoletus*. Since no unspecific amplification occurred it was concluded that any non-*Monochamus* in the samples, that may give false positives, would be statistically insignificant. Due to the low number of Cerambycidae larvae recovered from this sampling method it was possible to screen all larvae using the species-specific *M. titillator* primer set.

Three days after the bark disk samples were collected (6/28/10); supplementary *M. titillator* larvae were obtained by sampling a 0.5 m long portion from each of the 20 bole sections. This was done by cutting 0.25 m long sections from both ends of each bole section. These 0.25 m sections were labeled according to their tree ID and section numbers, and transported

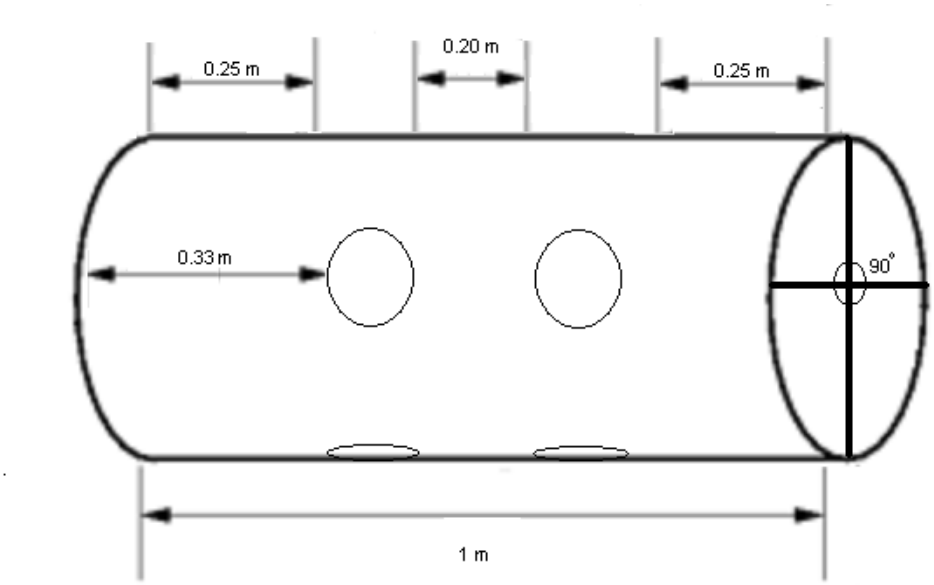


Figure 3.1 Diagram of the 1 meter bole sections. Measurements indicate: Distance from ends of each section that the 1 dm^2 bark disk samples were removed (0.33 m), length of each section where supplementary Cerambycidae larvae were removed (0.25 m x2), and the portion of each section taken back to laboratory to collect emerging saphrophagous insects to determine southern pine beetle guild species composition (0.2 m).

back to the laboratory to be hand dissected by carefully peeling off the bark and removing Cerambycidae larvae. The Cerambycidae larvae were identified as either *Monochamus* or non-*Monochamus* using characters provided by Craighead (1923) and Böving and Craighead (1931). These larvae were added to the larvae obtained from the density sampling efforts to be used for the predation detection frequency analyses.

Some *M. titillator* larvae were too small (<10 mm) and brittle due to ethanol dehydration to dissect using normal dissection methods. In these cases the DNA was extracted from the entire larva, with the exception of the head capsule. Head capsules were excluded from DNA extractions, since their inclusion may have lead to false positives for bark beetle consumption by *M. titillator* larvae that may have bitten but not consumed bark beetle larvae. The use of whole larvae as a source of template DNA was of concern due to the possibility of prey DNA being present on the cuticle of the *M. titillator* larvae. It was hypothesized that any potential bark beetle DNA attached to the cuticle of the *M. titillator* larvae would be suspended in the 95% ethanol storage solution and that further rinsing with 70% ethanol would be sufficient to remove any remaining attached DNA.

To test the potential effectiveness of this cleaning method an experiment was designed which exposed larvae to two prey DNA contamination methods. Prior to the experiment 25 *M. titillator* larvae were removed from the laboratory population 15 days post infestation and killed by freezing them at -20°C for 10 minutes. Larvae were thawed for 10 minutes at room temperature prior to use. The larvae were randomly assigned to one of 5 treatment groups (N=5 larvae per treatment). The first two treatments consisted of *M. titillator* larvae dipped into a solution of *I. grandicollis* DNA and either cleaned as described above or not cleaned prior to DNA extraction. The third and fourth treatments consisted of *M. titillator* larvae rubbed against active *I. grandicollis* larval galleries and either cleaned as described

above or not cleaned prior to DNA extractions. The final treatment consisted of a control group of *M. titillator* larvae not exposed to *I. grandicollis* DNA.

In the solution dipping treatments the *M. titillator* larvae were grasped with a pair of sterile forceps and dipped into 1.5 ml microcentrifuge tube containing 1 ml of 25 ng/μl *I. grandicollis* DNA solution. The larvae were submerged up to a few millimeters below the head capsules and held in the solution for 15 seconds. In the rubbing treatments the *M. titillator* larvae were grasped with a pair of sterile forceps and rubbed against a piece of *P. taeda* phloem with galleries of actively feeding *I. grandicollis* larvae for 15 seconds. The larvae from both types of DNA application procedures, which were assigned to the rinse treatments, were rinsed in 70% ethanol for 10 seconds prior to DNA extraction. Larvae from all five treatments were dissected and their DNA extracted for subsequent PCR analysis. Only 3/5 *M. titillator* larvae from the dipped and unwashed treatment tested positive for *M. titillator* DNA, and no larvae tested positive from the rubbed and unwashed treatments. Because no *I. grandicollis* DNA was detected on washed larvae, it was concluded that this washing method was sufficient to remove any DNA contamination that may be present on the cuticle surface. This assumption appeared valid after observing the detection rates of bark beetle DNA in the field (see Section 3.3.3).

After disk and supplementary field *M. titllator* larvae were washed with 70% ethanol they were dissected and their gut contents extracted for subsequent PCR analysis (except for *M. titillator* larvae under 10 mm in length, which were processed whole). The field caught larvae were first screened to species in order to determine which individuals were *M. titillator* using the singleplex PCR reaction containing the *M. titillator* species-specific primer pair MTF1/MTR1. Samples that were confirmed as *M. titillator* were then sampled for bark beetle DNA using the optimized multiplex reactions PCR reactions containing the

species-specific bark beetle primer sets and the presence or absence of prey DNA in their gut contents recorded.

3.2.3 Bark Beetle Species Compositions

An experiment was performed to see if differences in species compositions existed for the southern pine beetle guild in host material and in the gut contents of the field collected *M. titillator* larvae. This was tested by comparing the species composition of members of the southern pine beetle group represented in the experimental bolts to the species composition detected in the gut contents of the field-collected *M. titillator* larvae. In brief, a 20 cm portion of each bole section was removed from between the 1 dm^2 areas sampled (Figure 3.1) and taken back to the laboratory and placed into 5 gallon bucket rearing containers. Emerging adult bark beetles were counted and identified to species. The ratios of the southern pine beetle guild species observed in the 20 cm bole sections were assumed to reflect the southern pine beetle species composition in the semi-adjacent 0.25 m areas removed from the ends of each bole section. The number of emerged southern pine beetle guild members was pooled across all 20 bole sections. These species ratios were then compared to the observed ratios of bark beetle species in the guts of the field-collected *M. titillator* larvae. *Ips avulsus* was not included in the final comparison due to the inability of the primer set to detect larvae of this species (see Section 2.3.1). Differences in the southern pine beetle guild species composition within the host and within the gut contents of the field-collected *M. titillator* larvae were compared using a contingency table and singificance calculated using Fisher's exact test in Minitab v.15.

Table 3.1 Distribution of wood borer oviposition niches across various heights and surface areas of the bole used in the field sampling pilot study.

Section	Area					Total	Percent
	Top	Bottom	Left	Right			
1	0	13	11	10		**34**	**2.6**
2	1	21	19	11		**52**	**4.0**
3	0	43	35	12		**90**	**6.9**
4	0	41	38	31		**110**	**8.5**
5	0	42	32	47		**121**	**9.3**
6	0	74	82	53		**209**	**16.1**
7	2	90	50	54		**196**	**15.1**
8	4	80	89	52		**225**	**17.3**
9	5	77	112	67		**261**	**20.1**
Total	**12**	**481**	**468**	**337**			
Percent	**0.9**	**37.1**	**36.1**	**25.9**			

3.3 Results

3.3.1 Field Sampling Pilot Study

A total of 1298 wood borer oviposition niches were counted across the 9 bolt sections of the pilot study tree. Bolt sections 5-9 contained approximately 78% of the total number of oviposition niches and the sides and bottom quarters contained >99% of the oviposition niches on the bole (Table 3.3.1). Thus, subsequent sampling efforts were restricted to these corresponding bole heights and surfaces.

Observations made during the development and species composition portion of the field

pilot study agree with those of Dodds and Stephen (2000) for the within tree development of *M. titillator* in the field. Based on these observations trees were sampled 25 days post-felling. This sample time was convenient as the majority of field-collected *M. titillator* larvae were of similar age to those used in the laboratory assays. Beyond 25 days post-felling bark beetle gallieries were almost completely destroyed by wood borer larval foraging. The upper surface of the pilot study tree was the only area that had visible bark beetle activity past this time, where woodborer densities were lowest.

3.3.2 Larval Cerambycidae Identification

A total of 764 cerambycid larvae were collected from 0.5 m bole section portions taken from the 4 experimental trees (Table 3.2). Of these, 362 (47.38%) were identified as *Monochamus* using morphology. Using the *M. titillator*-specific PCR reaction, 219 (60.49%) of the 362 larvae identified as *Monochamus* were further identified as *M. titillator*. An additional 52 *M. titillator* larvae taken from the areas exposed by the hole saw were included, yielding a total of 271 *M. titillator* to be used for molecular gut content analyses. The proportion of *Monochamus* larvae identified as *M. titillator* (62.88%) was significantly less (Z=-4.81, P<0.001) than the proportion of adult *Monochamus* identified as *M. titillator* (78.71%) (N=310) trapped over the same period and location as the field predation survey (E.N. Schoeller and J.D. Allison unpub. data). The highest proportion of cerambycid larvae that were identified as *M. titillator* larvae were collected within bole sections 2 and 3 having mean diameters of 0.27 m (SD=0.016) and 0.24 m (SD=0.022) respectively. These sections correspond to bole heights ranging from 2.75-4.75 meters. The proportion of pooled cerambycid larvae that were identified as *M. titillator* ranged from as little as 17.61% in section 5 (bole heights 5.75-6.75 m) to as high as 37.01% in section 2 (bole heights 2.75-3.75 m)(Figure 3.2).

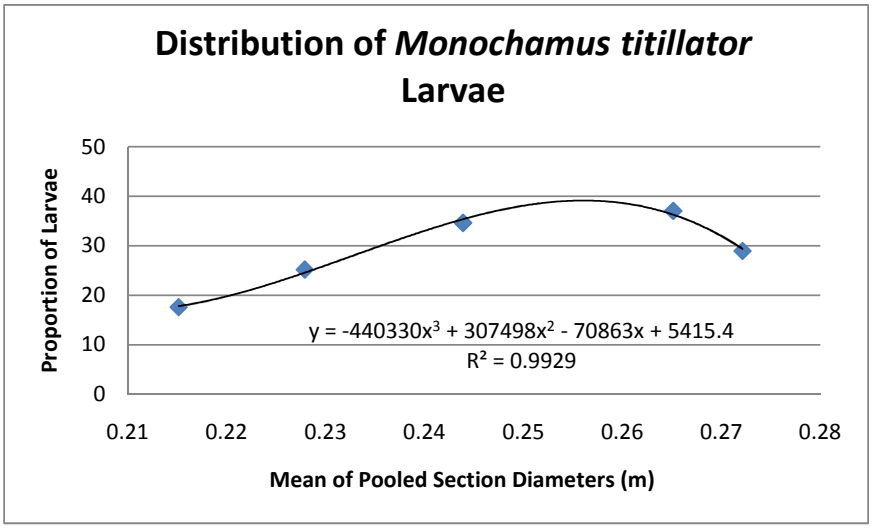


Figure 3.2 The proportion of pooled Cerambycidae larvae identified as *M. titillator* larvae compared against the mean diameters of the pooled bole sections.

3.3.3 Detection of Southern Pine Beetle Guild DNA in the Gut Contents of *M. titillator* Larvae in the Field

Using the developed bark beetle multiplex PCR reactions a total of 26 (9.6%) *M. titillator* larvae tested positive for DNA of members of the southern pine beetle guild in their gut contents. Of these larvae 25 (96.2%) tested positive for *I. grandicollis* DNA, 1 (3.8%) for *I. calligraphus* DNA, and 0 (0%) for *D. terebrans* and *D. frontalis* DNA.

3.3.4 Southern Pine Beetle Guild Species Compositions

A total of 200 adults of the southern pine beetle guild emerged from the 0.2 m bolt sections in the laboratory. Of these individuals, 15 (7.5%) were *I. avulsus*, 177 (88.5%) *I. grandicollis*, and 8 (4%) were *I. calligraphus* (Table 3.3.4). No adult *D. terebrans* or *D. frontalis* adults emerged from these bolts. After elimination of *I. avulsus* from the data, the proportion of *I. grandicollis* and *I. calligraphus* in the host material increased to 95.7% and

Table 3.2. Summary of the field-collected Cerambycidae larvae removed from the 0.5 m portion of each bole section.

Tree-Section	Total Larvae (Cerambycidae)	Number of Larvae (*M. titillator*)	Percent Larvae (*M. titillator*)
Tree(1)-Section(1)	41	17	41.5
T1-S2	44	22	50.0
T1-S3	44	12	27.3
T1-S4	25	11	44.0
T1-S5	48	13	27.1
T2-S1	35	9	25.7
T2-S2	49	13	26.5
T2-S3	24	19	79.2
T2-S4	55	15	27.3
T2-S5	44	8	18.2
T3-S1	60	13	21.7
T3-S2	28	13	46.4
T3-S3	45	10	22.2
T3-S4	38	3	7.9
T3-S5	45	5	11.1
T4-S1	16	5	31.3
T4-S2	33	9	27.3
T4-S3	43	13	30.2
T4-S4	25	7	28.0
T4-S5	22	2	9.1
Total	764	219	-

4.3% respectively. Using these proportions, no differences between the species compositions of the southern pine beetle guild within the experimental host material and those in the gut contents of the field-collected *M. titillator* larvae were observed (P=0.99).

3.4 Discussion

3.4.1 Field Sampling Pilot Study

Previous studies have found that *Monochamus* species tend to oviposit preferentially on the shaded sides and bottom of raised horizontal boles (Peddle, 2000; Allison and Borden, 2001) probably due to reduced desiccation and temperature fluctuations that affect larval survival (Rose, 1957). The results of the field collection pilot study support these results. It was not possible to identify the sources of the oviposition niches, however the general pattern observed in the pilot study was most likely driven by *Monochamus* species since they were the dominant cerambycid taxa observed during the time of sampling.

In standing trees, the oviposition pit densities of *M. titillator* exhibit a bimodal distribution (peaking at 25% and 75% of the sequentially infested bole height) (Hennier, 1983) surrounding the region where *D. frontalis* typically infest first (Fargo et al., 1978, Coulson et al., 1979). In other *Monochamus* species, high oviposition pit densities have been observed in the middle and upper portions of the bole (Yoshikawa, 1987; Nakamura et al., 1995). Within these areas, the oviposition niches appear evenly distributed (Hennier, 1983; Shibata, 1984), potentially due to avoidance behavior of consepecifics (Shibata, 1984; Peddle et al., 2002). It is possible that the oviposition behavior of *M. titillator* is chemically-mediated by bark beetles. For example, the cerambycid *A. aedilis* (Linné) has been observed to oviposit preferentially on logs and in or near entrance holes infested with the bark beetle *Tomicus piniperda* (L.) (Schroeder, 1997). Schroeder (1997) hypothesizes that oviposition in or near

Table 3.3 Number of members of the southern pine beetle guild emerging from the 0.2 m bole sections taken back to the laboratory. This data was used to calculate the within-host southern pine beetle guild species composition.

	Species				
Section	***I. avulsus***	***I. grandicollis***	***I. calligraphus***	***D. terebrans***	***D. frontalis***
T1-S1	0	0	0	0	0
T1-S2	0	1	0	0	0
T1-S3	0	4	0	0	0
T1-S4	4	40	0	0	0
T1-S5	4	36	0	0	0
T2-S1	0	2	0	0	0
T2-S2	2	13	5	0	0
T2-S3	0	0	0	0	0
T2-S4	0	15	0	0	0
T2-S5	0	1	0	0	0
T3-S1	1	2	0	0	0
T3-S2	1	16	0	0	0
T3-S3	0	21	0	0	0
T3-S4	0	5	2	0	0
T3-S5	0	1	0	0	0
T4-S1	1	1	1	0	0
T4-S2	0	10	0	0	0
T4-S3	0	2	0	0	0
T4-S4	1	7	0	0	0
T4-S5	1	0	0	0	0
Total	**15**	**177**	**8**	**0**	**0**
Total (%)	**7.5**	**88.5**	**4.0**	**0.0**	**0.0**

T. piniperda entrance holes could be due to the physical presence of a hole or due to increased concentrations of host volatiles released from these areas. The oviposition behavior of *M. titillator* however, is more likely mediated by conspecific- (Anbutsu and Togashi, 2001; Li and Zhang, 2006), and/or host-produced chemicals in order to select areas of high resource quality and/or avoid areas with increased risk of intra- and inter-specific competition. In the case of *M. titillator*, Hennier (1983) hypothesizes that the observed distribution of *M. titillator* oviposition niches on the bole is likely an adaptation in order to avoid early arriving *D. frontalis* which may reduce the nutritional quality of phloem material available to the *M. titillator* larvae.

The proportions of *M. titillator* larvae observed across the various bole heights in this field study suggest that, future sampling efforts could be even further restricted to the portion of the bole with diameters ranging 0.24-0.27 meters. Phloem thickness in the portion of the bole with diameters <0.24 meters may be too thin to support the large *M. titillator* larvae and smaller species such as *A. obsoletus* may outcompete *M. titillator* in these areas. Additionally, the thin bark associated with these areas may increase risk of predation or parasitism (Hennier, 1983). Alternatively, areas of the bole with diameters >0.27 meters tend to have thicker bark, which may deter *M. titillator* oviposition due to increased energy costs associated with carving the deep oviposition pits necessary to reach the phloem interface.

3.4.2 Detection of Southern Pine Beetle Guild DNA in the Gut Contents of *M. titillator* Larvae in the Field

The short molecular half-lives (see Section 2.3.4) found for bark beetle DNA in the gut contents of *M. titillator* larvae provided only a short timeframe for observing potential predatory interactions in the field. Additionally, the molecular half-lives of bark beetle DNA in the gut contents of *M. titillator* were calculated from starved individuals. Starvation

often leads to reduced metabolic rates [and potentially digestion rates (Greenstone and Hunt, 1993)] in some invertebrates (Anderson, 1970; Greenstone and Bennet, 1980; Lövei et al., 1985). This fact could have potentially caused some disparity between the observed half-lives under laboratory conditions and the actual half-lives in the field, since field-sampled *M. titillator* larvae were most likely not suffering from starvation. Another factor that may have contributed the low number of *M. titillator* larvae testing positive for prey remains was the temperature utilized for the laboratory molecular half-life tests. Larvae utilized for calculating the molecular half-life of *I. grandicollis* DNA and for comparing the proportions of *M. titillator* larvae testing positive for each of the four bark beetle species at 6.9 hours post-consumption were maintained at 30°C under laboratory conditions. Ambient temperatures observed in the field during the dates of collection were 33°C and 34°C on the first and second collection dates respectively. The temperature of the phloem tissue was not measured in this study, however the temperature of the phloem is normally higher than the ambient temperature due to radiant thermal energy being absorbed by the tree (Powell, 1967; Logan and Powell, 2001). As mentioned previously, higher temperatures have been shown to reduce the half-lives of prey DNA in the gut contents of predators (see Section 2.4.3). Hosseini et al. (2008) found that a 5°C increase in temperature (25-30°C) caused a 30-48% reduction in prey detection frequences in the gut contents of the coccinellid *Hippodamia variegata* (Goeze) at five different time intervals post-consumption. Hagler and Naranjo (1997) observed similar results, with observed half-lives declining (38.3, 26.2, 26.5, 4.5, and 1.8 hours) for prey DNA in the gut contents of the anthocorid *Orius insidiosus* (Say) (held at 15, 20, 25, 30, and 35°C respectively). Additionally, an increase in temperature has been shown to increase predation rates in some invertebrate taxa (Néve, 1994; Ayre, 2001). Thus, *a priori*, it is difficult to predict what, if any, effect the temperature differences may have had.

This study demonstrates that DNA-based molecular gut content analyses are capable of detecting DNA of members of the southern pine beetle guild in the gut contents of field-collected *M. titillator* larvae. The fact that no *M. titillator* larvae tested positive for *I. avulsus* DNA may mean that although possible, consumption of adult bark beetles within the phloem is an infrequent event. The interactions between adult bark beetles that come into contact with foraging *M. titillator* larvae is something that needs to be examined in order to understand how *M. titillator* larvae may impact this bark beetle lifestage. Given the extreme local rarity of *D. frontalis*, it is not surprising that it was not detected within the gut contents of the field-collected *M. titillator* larvae. Additionally, sections of the bole used in this study were from outside the normal within-host distribution of *D. terebrans* (i.e the root system and base of infested trees) making detection of this species also unlikely. Because of its normal distribution within the host, *D. terebrans* is likely to interact infrequently with foraging *M. titillator* larvae. Additionally, since no adult *D. terebrans* or *D. frontalis* emerged from host material brought back to the laboratory, the presence of these species in the bole heights sampled was unlikely.

3.4.3 Southern Pine Beetle Guild Species Composition Comparison

The within-tree species composition of southern pine beetle guild members emerging from the 20 cm bole sections are somewhat similar to those observed by Berisford (1974), who observed an *Ips* species composition of approximately 90% *I. grandicollis*, 6% *I.calligraphus*, and 4% *I. avulsus* in *P. taeda* from Georgia. Differences in *Ips* spp. composition observed between these studies could be attributed to: 1) differences in the study area microhabitats, 2) tree size, 3) climactic differences, and 4) differences in *Ips* phenologies due to geographic area. In bole sections with the greatest relative proportion of *M. titillator* larvae (2.75-4.75

m) the greatest number (relative to the other bole section heights) of emerging *I. calligraphus* adults was observed. It is interesting to note that the only *M. titillator* larva testing positive for *I. calligraphus* DNA was collected from one of three bole sections with emerging adult *I. calligraphus* observed in the laboratory. Unfortunately, this study was not able to directly test for an effect of bark beetle density on the observed frequency of bark beetle DNA in the gut contents of *M. titillator* lavae. This was due to the limited number of *M. titillator* larvae collected and low number testing positive for prey remains (i.e. only 1 of the 52 *M. titillator* larvae) removed from the areas exposed by the hole saw.

Results from the southern pine beetle guild species composition tests were not surprising. It is unlikely that *M. titillator* larvae exhibit prey choice as they are primarily phytophagous on phloem tissue and most likely consume bark beetle larvae indiscriminately as they forage. *M. titillator* larvae are highly agressive and will attack anything in close proximity including conspecifics [(which they often attempt to cannibalize (Dodds et al., 2001)] and other subcortical insects. Some studies that have utilized molecular gut content analyses to compare differences in prey compositions is the field to those observed in the gut contents of their predators of interest have observed prey choice (Agustí et al., 2003a), while others have not (King et al., 2010). Prey choice is a metric that incorperates many factors such as predator:prey encounter rates, and the quality of predator attack and prey defense strategies (Harwood et al., 2004). Prey choice is an important metric to elucidate in the study of trophic interactions as it allows the quantification of the relative importance of a particular prey species in regulating the dynamics of predator populations and vice versa. The potential for prey choice in this system is likely limited due to temporal and spatial isolating mechanisms that dictate the arrival of southern pine beetle guild members and *M. titillator* to the host and their within-host distributions (Dixon and Payne, 1979; Birch et al., 1980;

Paine et al., 1981). Within a particular area, *M. titillator* larval-bark beetle interactions are likely restricted to a single dominant member of the southern pine beetle guild or to a mixture of two species providing *M. titillator* larvae a limited opportunity to descriminate between potential prey.

3.4.4 Southern Pine Beetle Guild Population Dynamics

The ability to detect DNA in the gut contents of field-collected *M. titillator* larvae is significant as it provides further insight into the potential mechanisms driving the reduction in bark beetle numbers in areas foraged by *M. titillator* larvae observed in previous studies (Coulson et al., 1976, 1980; Hennier, 1983; Flamm et al., 1989). These results also demonstrate that facultative intraguild predation previously observed by *Monochamus* larvae in the laboratory (Dodds et al., 2001) also occurs under natural conditions.

Observations on the seasonal abundance of members of the southern pine beetle guild in Louisiana found that greater numbers of *D. frontalis* emerged in the early spring and late fall than in the summer, which may have been a result of the greater numbers of observed interspecific interactions during the summer with wood borers and other bark beetle species (Moore and Thatcher, 1973). Other studies have observed an increase in *Ips* and *Monochamus* numbers coinciding with the onset of *D. frontalis* population collapse (Clarke and Billings, 2003). These studies support the hypothesis that competitive interactions with larvae of *Monochamus* species may be a significant driving force in southern pine beetle guild population collapse. Additionally, results from this study and those of Dodds et al. (2001) suggest that these interactions could be classified as predatory rather than strictly competition for phloem resources.

3.5 Summary

This study demonstrated the molecular tools developed and used successfully in Chapter 2 are capable of amplifying DNA of members of the southern pine beetle guild in the gut contents of field-collected *M. titillator* larvae. A total of 271 *M. titillator* larvae were screened for DNA of members of the southern pine beetle guild. Twenty-six larvae tested positive for bark beetle DNA in their gut contents. The observed proportion of bark beetle species within the gut contents of the field-collected *M. titillator* larvae were 96.2% *I. grandicollis* and 3.8% *I. calligraphus*, and 0% *D. terebrans* and *D. frontalis*. This reflected the observed southern pine beetle species composition within the experimental host material, suggesting *M. titillator* were not exhibiting prey choice. Results from this study provide emperical evidence that larvae of *M. titillator* are consuming members of the southern pine beetle guild in the field, and demonstrate the capability of PCR in studying food-webs of cryptic species in forest ecosystems.

4. Summary and Conclusions

4.1 Summary of Results

Results from this study provide additional evidence to support the hypothesis that *M. titillator*, and likely *M. carolinensis* as well, are facultative intraguild predators. They also provide a better understanding of the dynamics of the subcortical interactions between immatures of the southern pine beetle guild and *M. titillator*. Species-specific PCR primers were successfully developed to amplify DNA from adults of all five members of the southern pine beetle guild. Unfortunately, these primers did not amplify larval *I. avulsus* DNA, but did amplify larval DNA from the other four species. Species-specific primers were also sucessfully developed to facilitate seperation of field-collected *M. titillator* and *M. carolinensis* larvae. These bark beetle-specific primer sets were successfully combined into two multiplex PCR reactions, which significantly reduced the cost and time necessary to screen the gut contents of field-collected *M. titillator* larvae for DNA of the southern pine beetle guild.

The sensitivities of the developed bark beetle species-specific primers were tested under both singleplex and multiplex PCR conditions. An increase in primer sensitivities was observed under singleplex PCR conditions compared to multiplex (except for the primer set designed to amplify *D. frontalis*, which was not observed to differ). This was not of concern however, since the primer sentivities under multiplex PCR conditions corresponded to >200 larval equivalencies for all bark beetle species, which were sufficient for the purpose of this study. Using the developed bark beetle multiplex PCR reactions the half-life for *I. grandicollis* DNA in the gut contents of *M. titillator* larvae was calculated under laboratory

conditions, to be 6.89 hours post-consumption. Although short, this half-life was reasonable due to the active lifestyle of *Monochamus* larvae. The proportion of *M. titillator* larvae with detectable bark beetle DNA at 6.9 hours post-consumption were 0.64, 0.68, 0.16, and 0.64 for larvae fed *I. grandicollis*, *I. calligraphus*, *D. terebrans* and *D. frontalis* respectively. The proportion of *M. titillator* larvae testing positive for *D. terebrans* DNA differed significantly from the proportion of larvae fed the other three bark beetle species. The results of this half-life study provide a basis for interpreting results on the detection frequencies of DNA of the southern pine beetle guild in the gut contents of field-collected *M. titillator* larvae.

A total of 915 cerambycid larvae were collected from the four experimental boles in the field. Of these larvae, 271 were identified as *M. titillator* using the *M. titillator*-specific primer set. Twenty-six of the 271 *M. titillator* larvae screened positive for DNA of members of the southern pine beetle guild. The low number of larvae testing positive for bark beetle DNA was likely dictated by short molecular half-lives observed for bark beetle DNA in the gut contents of *M. titillator*. Of the 26 *M. titillator* larvae that tested positive 96.2%, 3.8%, 0%, and 0%, tested positive for *I. grandicollis*, *I. calligraphus*, *D.terebrans*, and *D. frontalis* DNA respectively. It was not suprising that consumption of neither *D. terebrans* (due to utilization of the bole outside its normal distribution within the host) or *D. frontalis* (due to its local rarity) was observed.

The within host species composition of the southern pine beetle guild in the experimental boles sections was 95.7% *I. grandicollis*, 4.3% *I. calligraphus* and 0% *D. terebrans* and *D. frontalis* when *I. avulsus* was excluded from the data. The species composition of members of the southern pine beetle guild in the gut contents of field-collected *M. titillator* larvae reflects the within-host species composition of members of the southern pine beetle guild.

This suggests that *M. titillator* are feeding indiscriminately on bark beetle species as they forage.

4.2 Significance and Future Research

This study is a first step towards developing a better understanding of the mechanisms driving southern pine beetle guild population dynamics. Previous studies have observed reduced southern pine beetle guild brood production due to predation by natural enemies, competition with other insect associates, and unfavorable climatic factors. The information provided by this study may enable researchers to further refine current population growth models for the southern pine beetle guild to include mortality factors driven by the interactions with *Monochamus* wood borers. Further, this research may promote increased interest into other *Monochamus*-bark beetle systems in the U.S. and around the world. In North America nearly every major bark beetle pest is associated with a *Monochamus* species. The occurance of facultative predatory interactions in these systems would be of great interest due to the implications this could have on the population dynamics of these pest species.

The potential use of *M. titillator* as a biocontrol agent is intriguing. It may be possible to develop novel IPM tactics such as silvicultural techniques, that promote healthy *Monochamus* populations. This could reduce the observed time-lag between bark beetle and *Monochamus* colonization. A reduction in the colonization period of *Monochamus* species may dampen the severity and frequency of bark beetle outbreaks. This could come about in two ways; 1) prolonged disturbance of the natural within-host distributions of the southern pine beetle guild, thus leading to an increase in bark beetle brood mortality caused by unforvorable interactions with other associates or 2) a reduction in bark beetle brood survivorship due to prolonged exposure to predatory interactions with *M. titillator* larvae. Hennier (1983) and

Flamm et al. (1989) suggest that for a significant reduction in bark beetle brood production to occur due to *M. titillator* larval foraging, that 1) *M. titillator* adults must either oviposit prior to bark beetle arrival; 2) *M. titillator* infestation duration must be shortened; or 3) *M. titillator* larvae must develop at a faster rate than members of the southern pine beetle guild.

Most biological control programs utilize specialist predators or aggressive generalist predators to control pest populations of interest. These polyphagous predators are usually primarily zoophagous and secondarily phytophagous, which is not the case for *Monochamus* species. Facultative feeding on plant material in the case of primarily zoophagous predators arise primarily when prey densities are low. Later as prey densities increase these predators can switch their diets and regulate prey populations early in the season (Chiverton, 1987; Butler and O'Niel, 2007; Harwood et al., 2007, 2009). The use of generalist predators in biological control programs is best suited as preventative measures (Albajes and Alomar, 1999), such as maintaining endemic bark beetle population levels. This concept probably fits the potential biological control profile for *Monochamus* species. The risks involved by utilizing polyphagous predators in biological control programs can be high so we must understand the risks involved in diet shifts and their circumstances (Alomar, 2002). Additionally, before any biological control strategies can be taken into consideration the obstacles proposed by Hennier (1983) and Flamm et al. (1989) must be overcome. Anecdotal evidence exists for *Monochamus* species colonizing trees prior to bark beetles during the height of infestations. This suggests that it may be possible to augment the initial infestation rate of *Monochamus* species to help regulate bark beetle populations.

Potential hypotheses for the facultative predatory behaviors of *Monochamus* larvae are discussed by Dodds et al., (2001). These include icreased survivorship due to: 1) increased

access to nitrogen, which is a limited resource in phloem tissue (Ayres et al., 2000) compared to insects (Fagan et al., 2002); 2) reduced resource competition via direct elimination of competitors (e.g. bark beetle larvae); 3) shortened developmental times as a result of superior nutrition; and 4) utilization of supplemental nutrition when the phloem is of poor quality.

The ecological risks associated with using *M. titillator* as a control agent seem minimal as this ecological system has likely been in place for tens of thousands of years. Some potential risks include increased timber value reduction due to increased *Monochamus* activity and the reduction in the impacts from other bark beetle natural enemies (see Miller, 1986). Future studies examining interactions between *Monochamus* density and the ability of other natural enemies to regulate bark beetle populations are needed to determine optimal densities of *Monochamus* larvae to achieve maximum combined bark beetle mortality. Additionally, *Monochamus* larvae are cannibalistic (Dodds et al., 2001) and studies examining the effects of larval density on cannibalism rates, which may reduce bark beetle mortality rates, are needed. Currently, studies are underway to examine the phenological synchrony between *Monochamus* spp. and members of the southern pine beetle guild. This may lead to identification of times associated with low bark beetle-*Monochamus* population interactions, which may be suitable for biological control.

Bibliography

Addinsoft, 2011. XLSTAT software version 13.2 (online). Available from http://www.xlstat.com.

Admassu, B., A. Juen, and M. Traugott. 2006. Earthworm primers for DNA-based gut content analysis and their cross-reactivity in a multi-species system. Soil Biol. Biochem. 38:1308-1315.

Agustí, N., J. Aramburu, and R. Gabarra. (1999a) Immunological detection of *Helicoverpa armigera* (Lepidoptera: Noctuidae) ingested by heteropteran predators: time-related decay and effect of meal size on detection period. Ann. Entomol. Soc. Am. 92:56-62.

Agustí, N., M.C. de Vicente, and R. Gabarra. (1999b) Development of sequence amplified characterized region (SCAR) markers of *Helicoverpa armigera*: a new polymerase chain reaction-based technique for predator gut analysis. Mol. Ecol. 8:1467-1474.

Agustí, N., C. de Vicente, and R. Gabarra. 2000. Developing SCAR markers to study predation on *Trialeurodes vaporariorum*. Insect Mol. Biol. 9:263268.

Agustí, N., S.P. Shayler, J.D. Harwood, I.P. Vaughan, K.D. Sunderland, and W.O.C. Symondson. 2003a. Collembola as alternative prey sustaining spiders in arable ecosystems: prey detection within predators using molecular markers. Mol. Ecol. 12:3467-3475.

Agustí, N., T.R. Unruh, and S.C. Welter. 2003b. Detecting *Cacopsylla pyricola* (Hemiptera: Psyllidae) in predator guts using COI mitochondrial markers. Bull. Entomol. Res. 93:179-185.

Akbulut, S., and M.J. Linit. 1999. Flight performance of *Monochamus carolinensis* (Coleoptera: Cerambycidae) with respect to nematode phoresis and beetle characteristics. Environ. Entomol. 28:1014-1020.

Akbulut, S., W.T. Stamps, and M.J. Linit. 2004. Population dynamics of *Monochamus carolinensis* (Col., Cerambycidae) under laboratory conditions. J. Appl. Entomol. 128:17-21.

Albajes, R., and O. Alomar. 1999. Current and potential use of polyphagous predators. *In*: R. Albajes, M. Lodovica-Gullino, J.C. Van Lenteren, and Y. Elad, (eds.), Integrated pest and disease management in greenhouse crops. Dordrech: Kluwer. pp. 265-275.

Allison, J.D., and J.H. Borden. 2001. Observations on the behavior of *Monochamus scutellatus* (Say) (Coleoptera: Cerambycidae) in Northern British Columbia. J. Entomol. Soc. B.C. 98:195-200.

Allison, J.D., J.H. Borden, R.L. McIntosh, P. de Groot, and R. Gries. 2001. Kairomonal response by four *Monochamus* species (Coleoptera: Cerambycidae) to bark beetle pheromones. J. Chem. Ecol. 27:633-646.

Allison, J.D., W.D. Morewood, J.H. Borden, K.E. Hein, and I.M. Wilson. 2003. Differential bioactivity of *Ips* and *Dendroctonus* pheromone components for *Monochamus clamator* and *M. scutellatus* (Coleoptera: Cerambycidae). Environ. Entomol. 32:23-30.

Alomar, O. 2002. Facultative predation as a biological control. *In*: D. Pimentel, (ed.), Encylopedia of pest management. New York: Marcel Dekker, Inc. pp. 1-3.

Alya, A.B., and F.P. Hain. 1985. Life histories of *Monochamus carolinensis* and *M. titillator* (Coleoptera: Cerambycidae) in the piedmont of North Carolina. J. Entomol. Sci. 20:390-397.

Anbutsu, H., and K. Togashi. 2001. Oviposition deterrent by female reproductive gland secretion in Japanese pine sawyer, *Monochamus alternatus*. J. Chem. Ecol. 27:1151-1162.

Anderson, J.F. 1970. Metabolic rates of spiders. Comp. Biochem. Physiol. 33: 51-72.

Anonymous, 1997. Phero Tech Inc. 7572 Progress Way, RR5, Delta, British Columbia. V4G 1E9, Canada. Damage Assessment of Woodborers in the Interior of British Columbia. unpubl. report.

Arbeli, Z., and C.L. Fuentes. 2007. Improved purification and PCR amplification of DNA from environmental samples. FEMS Microbiol. Lett. 272:269-275.

Ascoli-Christensen, A., S.M. Salom, and T.L. Payne. 1993. Olfactory receptor cell responses of *Ips grandicollis* (Eichhoff) (Coleoptera: Scolytidae) to intra- and interspecific behavioral chemicals. J. Chem. Ecol. 19:699-712.

Ayre, K. 2001. Effects of predator size and temperature on the predation of *Deroceras reticulatum* (Müller) (Mollusca) by carabid beetles. J. Appl. Entomol. 125:389-395.

Ayres, M.P., R.T. Wilkens, J.J. Ruel, M.J. Lombardero, and E. Vallery. 2000. Nitrogen budgets of phloem-feeding bark beetles with and without symbiotic fungi (Coleoptera: Scolytidae). Ecology 81:2198-2210.

Baker, W.L. 1972. Eastern Forest Insects. U.S. Department of Agriculture, Forest Service Misc. Publ. No. 1175. 642 pp.

Bergdahl, D.R. 1988. Impact of the pine wood nematode in North America: present and future. J. Nematol. 20:260-265.

Billings, R.F. 1985. Southern pine bark beetles and associated insects. Effects of rapidly-released host volatiles on response to aggregation pheromones. Z. Angew. Entomol. 99:483-491.

Billings, R.F., and R.S. Cameron. 1984. Kairomonal responses of Coleoptera, *Monochamus titillator* (Cerambycidae), *Thanasimus dubius* (Cleridae), and *Temnochila virescens* (Trogositidae), to behavioral chemicals of southern pine beetles (Coleoptera: Scolytidae). Environ. Entomol. 13:1542-1548.

Birch, M.C., and P. Svihra. 1979. Exploring olfactory interactions between species of Scolytidae. *In*: W.E. Waters, (ed.), Current topics in forest entomology: selected papers from the XV^{th} international congress of entomology. [Washington, D.C., Aug. 1976.] U.S. Dep. Agric. For. Serv., Gen. Tech. Rep. WO-8. pp. 135-138.

Birch, M.C., P. Svihra, T.D. Paine, and J.C. Miller. 1980. Influence of chemically mediated behavior on host tree colonization by 4 cohabiting species of bark beetles. J. Chem. Ecol. 6:395-414.

Birgersson, G., M.J. Dalusky, and C.W. Berisford. 1995. Interspecific attraction and inhibition among four species of *Ips* bark beetles in Southeastern U.S.A. *In*: F.P. Hain, S.M. Salom, W.F. Ravlin, T.L. Payne, and K.F. Raffa, (eds.), Behavior, population dynamics, and control of forest insects. Joint IUFRO Working Party Conference, 6 Feb. 1994, Maui HI. Ohio State University Press, Columbus, OH. pp. 12-18.

Berisford, C.W. 1974. Parasite abundance in *Ips* spp. infestations as influenced by the southern pine beetle. Environ. Entomol. 3:695-696.

Berisford, C.W. 1980. Natural enemies and associated organisms. *In*: R.C. Thatcher, J.L. Searcy, J.E. Coster, and G.D. Hertel, (eds.), The southern pine beetle. USDA Expanded Southern Pine Beetle Research and Applications Program. Forest Service, SEA Technical Bulletin no. 1631. pp. 31-52.

Berisford, C.W., and R.T. Franklin. 1971. Attack patterns of *Ips avulsus* and *I. grandicollis* (Coleoptera: Scolytidae) on four species of southern pines. Ann. Entomol. Soc. Am. 64:894-897.

Borden, J.H. 1974. Aggregation pheromones in the Scolytidae. *In*: M.C. Birch, (ed.), Pheromones. North-Holland Publ. Co., Amsterdam. pp. 135-160.

Borden, J.H., D.W.A. Hunt, D.R. Miller, and K.N. Slessor. 1986. Orientation in forest Coleoptera: an uncertain outcome of responses by individual beetles to variable stimuli. *In*: T. L. Payne, M. C. Birch, and C.E.J. Kennedy, (eds.), Mechanisms in insect olfaction. Clarendon Press, Oxford, United Kingdom. pp. 97-109.

Böving, A.G., and F.C. Craighead. 1931. An illustrated synopsis of the principal larval forms of the order Coleoptera. Entomol. Am. (N.S). 11:1-351.

Brattli, J.G., J. Anderson, and A.C. Nilssen. 1998. Primary attraction and host tree selection in deciduous and conifer living Coleoptera: Scolytidae, Curculionidae, Cerambycidae and Lymexylidae. J. Appl. Entomol. 122:345-352.

Breene, R.G., M.H. Sweet, and J.K. Olson. 1990. Analysis of the gut contents of naiads of *Enallagma civile* (Odonata: Coenagrionidae) from a Texas pond. J. Am. Mosquito Contr. 6:547-548.

Bushing, R.W. 1965. A synoptic list of the parasites of Scolytidae (Coleoptera) in North America north of Mexico. Can. Entomol. 97:449-492.

Butler, C.D., and R.J. O'Neil. 2007. Life history characteristics of *Orius insidiosus* (Say) fed diets of soybean aphid, *Aphis glycines* (Matsumura) and soybean thrips, *Neohydatothrips variabilis* (Beach). Biol. Control. 40:339-346.

Byers, J.A. 1995. Host-tree chemistry affecting colonization in bark beetles. *In*: R.T. Carde and W.J. Bell, (eds.), Chemical ecology of insects 2. Chapman and Hall, New York. pp. 154-213.

Calder, C.R., J.D. Harwood, and W.O.C. Symondson. 2005. Detection of scavenged material in the guts of predators using monoclonal antibodies: a significant source of error in measurement of predation. Bull. Entomol. Res. 95:57-62.

Camara, M., C. Borgemeister, R.H. Markham, and H-M. Poehling. 2003. Electrophoretic analysis of the prey spectrum of *Teretrius nigrescens* (Lewis) (Col., Histeridae), a predator of *Prostephanus truncatus* (Horn) (Col., Bostrichidae), in Mexico, Honduras, and Benin. J. Appl. Entomol. 127:360-368.

Camors Jr., F.B., and T.L. Payne. 1973. Sequence of arrival of entomophagous insects to trees infested with the southern pine beetle. Environ. Entomol. 2: 267-270.

Chen, Y., K.L. Giles, M.E. Payton, and M.H. Greenstone. 2000. Identifying key cereal aphid predators by molecular gut analysis. Mol. Ecol. 9:1887-1898.

Chénier. J.V.R., and B.J.R. Philogène. 1989. Field responses of certain forest Coleoptera to conifer monoterpenes and ethanol. J. Chem. Ecol. 15:1729-1745.

Chiverton, P.A. 1987. Predation of *Rhopalosiphum padi* (Homoptera, Aphididae) by polyphagous predatory arthropods during the aphids' pre-peak period in spring barley. Ann. Appl. Biol. 111:257-269.

Clarke, S.R., and R.F. Billings. 2003. Analysis of the southern pine beetle suppression program on the national forests in Texas in the 1990s. South. J. Appl. For. 27:122-129.

Connor, M.D., and R.C. Wilkinson. 1983. *Ips* bark beetles in the south. USDA Forest Service, Washington, D.C. Forest Insect and Disease Leaflet 129. 8 p.

Cook, S.P., T.L. Wagner, R.O. Flamm, J.C. Dickens, and R.N. Coulson. 1983. Examination of sex ratios and mating habits of *I. avulsus* and *I. calligraphus* (Coleoptera: Scolytidae). Ann. Entomol. Soc. Am. 76:56-60.

Cooper, M.E., and F.M. Stephen. 1978. Parental adult reemergence in southern pine beetle populations. Environ. Entomol. 7:574-577.

Coster, J.E., and J.P. Vité. 1972. Effects of feeding and mating on pheromone release in the southern pine beetle. Ann. Entomol. Soc. Am. 65:263-266.

Coster, J.E., T.L. Payne, E.R. Hart, and L.J. Edson. 1977. Aggression of the southern pine beetle in response to attractive host trees. Environ. Entomol. 6:627-631.

Coulson, R.N. 1979. Population dynamics of bark beetles. Annu. Rev. Entomol. 24:417-447.

Coulson, R.N., A.M. Mawasi, J.L. Foltz, and F.P. Hain. 1976. Interspecific competition between *Monochamus titillator* and *Dendroctonus frontalis*. Environ. Entomol. 5:235-247.

Coulson, R.N., W.S. Fargo, P.E. Pulley, J.L. Foltz, D.N. Pope, J.V. Richerson and T.L. Payne. 1978. Evaluation of the reemergence process of parent adult *Dendroctonus frontalis* (Coleoptera: Scolytidae). Can. Ent. 110:475-486.

Coulson, R.N., D.N. Pope, J.A. Gagne, W.S. Fargo, P.E. Pulley, L.J. Edson, and T.L. Wagner. 1980. Impact of foraging by *Monochamus titillator* (Col: Cerambycidae) on within-tree populations of *Dendroctonus frontalis* (Col: Scolytidae). Entomophaga. 25:155-170.

Coulson, R.N., and J.A. Witter. 1984. Forest entomology: ecology and management. John Wiley and Sons Inc. pp. 315-318.

Craighead, F.C. 1923. North American Cerambycidae larvae. A classification and biology of the North American Cerambycidae larvae. Canada Dept. Agr. Ent. Bull. 23:1-238. 44 pls.

Deagle, B.E., S.N. Jarman, D. Pemberton, and N.J. Gales. 2005. Genetic screening for prey in the gut contents from a giant squid (*Architeuthis* sp.). J. Hered. 96:417-423.

Delorme, J.L., and T.L. Payne. 1990. Antennal olfactory responses of black turpentine beetle, *Dendroctonus terebrans* (Olivier), to bark beetle pheromones and host terpenes. J. Chem. Ecol. 16:1321-1329.

Dennison, D.F., and I.D. Hodkinson. 1983. Structure of the predatory beetle community in a woodland soil ecosystem. I. Prey selection. Pedobiologia. 25:109-115.

Dixon, W.N. 1984. *Ips* engraver beetles. FDACS, Division of Forestry. Forest and Shade Tree Pests Leaflet No. 2. 2 pp.

Dixon, W.N. and T.L. Payne. 1979. Sequence of arrival and spatial distribution of entomophagous and associated insects on southern pine beetle infested trees. Texas Agric. Exp. Stn. Bull. MP-1432. 27 p.

Dixon, W.N. and T.L. Payne. 1980. Attraction of entomophagous and associate insects of the southern pine beetle to beetle- and host tree-produced volatiles. J. Ga. Entomol. Soc. 15:378-389.

Dodds, K.J. and F.M. Stephen. 2000. Partial age-specific life tables for *Monochamus titillator* in *Dendroctonus frontalis* infested loblolly pines. Entomol. Exp. Appl. 97:331-338.

Dodds, K.J., C. Graber, and F.M. Stephen. 2001. Facultative intraguild predation by larval Cerambycidae (Coleoptera) on bark beetle larvae (Coleoptera: Scolytidae). Environ. Entomol. 30:17-22.

Dodds, K.J., C. Graber, and F.M. Stephen. 2002. Oviposition biology of *Acanthocinus nodosus* (Coleoptera: Cerambycidae) in *Pinus taeda*. Fla. Entomol. 85:452-457.

Eickwort, J.M., A.E. Mayfield, and J.L. Foltz. 2006. *Ips* engraver beetles (Coleoptera: Curculionidae: Scolytinae). Florida Dept. Agric. Div. Plant Ind. Entomol. Circ. No. 417. 4 p.

Eitzinger, B., and M. Traugott. 2011. Which prey sustains cold-adapted invertebrate generalist predators in arable land? Examining prey choices by molecular gut analysis. J. Appl. Ecol. 48:591-599.

Fagan, W.F., E.H. Siemann, R.F. Denno, C. Mitter, A. Huberty, H.A. Woods, and J.J. Elser. 2002. Nitrogen in insects: implications for trophic complexity and species diversification. Am. Nat. 160:784-802.

Fan, J.T., D.R. Miller, L.W. Zhang, and J.H. Sun. 2010. Effects of bark beetle pheromones on the attraction of *Monochamus alternatus* to pine volatiles. Insect Sci. 17:553-556.

Fargo, W.S., R.N. Coulson, P.E. Pulley, D.N. Pope, and C.L. Kelley. 1978. Spatial and temporal patterns of within-tree colonization by *Dendroctonus frontalis*. Can. Entomol. 110:1213-1232.

Fatzinger, C.W. 1985. Attraction of the black turpentine beetle (Coleoptera: Scolytidae) and other forest Coleoptera to turpentine-baited traps. Environ. Entomol. 14:768-775.

Fatzinger, C.W., B.D. Siegfried, R.C. Wilkinson and J.L. Nation. 1987. *trans*-Verbenol, turpentine, and ethanol as trap baits for the black turpentine beetle, *Dendroctonus terebrans*, and other forest Coleoptera in North Florida. J. Entomol. Sci. 22:201-209.

Flamm, R.O., R.N. Coulson, P. Beckley, P.E. Pulley, and T.L. Wagner. 1989. Maintenance of a phloem-inhabiting guild. Environ. Entomol. 18:381-387.

Foltan, P., S. Sheppard, M. Konvicka, and W.O.C. Symondson. 2005. The significance of facultative scavenging in generalist predator nutrition: detecting decayed prey in the guts of predators using PCR. Mol. Ecol. 14:4147-4158.

Frazier, J.L., T.E. Nebeker, R.F. Mizell, and W.H. Calvert. 1981. Predatory behavior of the clerid beetle *Thanasimus dubius* (Coleoptera: Cleridae) on the southern pine beetle (Coleoptera: Scolytidae). Can. Entomol. 113:35-43.

Gara, R.I. and J.A. Coster. 1968. Studies on the attack behavior of the southern pine beetle. III. Sequence of tree infestation within stands. Contrib. Boyce Thompson Instit. 24:77-86.

Gardiner, L.M. 1975. Insect attack and value loss in wind-damaged spruce and jack pine stands in Northern Ontario. Can. J. For. Res. 5:87-398.

Godbee, J.F. Jr., and R.T. Franklin. 1976. Attraction, attack patterns and seasonal activity of the black turpentine beetle. Ann. Entomol. Soc. Am. 69:653-655.

Godbee, J.F. Jr., and R.T. Franklin. 1978. Sexing and rearing the black turpentine beetle (Coleoptera: Scolytidae). Can. Entomol. 110:1087-1089.

Goldman, S.E., and R.T. Franklin. 1977. Development and feeding habits of southern pine beetle larvae. Ann. Entomol. Soc. Am. 70:54-56.

Goyer, R.A., G.J. Lenhard, T.E. Nebeker, and L.D. Jarrard. 1980. How to identify common insect associates of the southern pine beetle. USDA Agric., Comb. For. Pest Res. Develop. Prog. Handbook No. 563. 33 pp.

Greenstone, M.H. 1983. Site-specificity and site tenacity in a wolf spider: a serological dietary analysis. Oecologia. 56:79-83.

Greenstone, M.H. 1995. Bollworm or Budworm? Squashblot immunoassay distinguishes eggs of *Helicoverpa zea* and *Heliothis virescens*. J. Econ. Entomol. 88:213-218.

Greenstone, M.H. 1996. Serological analysis of arthropod predation: past, present and future. *In*: W.O.C. Symondson, and J.E. Liddell, (eds.), The ecology of agricultural pests-biochemical approaches. Chapman and Hall, London. pp. 265-300.

Greenstone, M.H. and A.F. Bennett. 1980. Foraging strategy and metabolic rate in spiders. Ecology. 61:1255-1259.

Greenstone, M.H., and C.E. Morgan. 1989. Predation on *Heliothis zea*: an instar-specific ELISA for stomach analysis. Ann. Entomol. Soc. Am. 82:45-49.

Greenstone, M.H., and J.H. Hunt. 1993. Determination of prey antigen half-life in *Polistes metricus* using a monoclonal antibody-based immunodot assay. Entomol. Exp. Appl. 68:1-7.

Greenstone, M.H., and K.A. Shufran. 2003. Spider predation: species-specific identification of gut contents by polymerase chain reaction. J. Arachnol. 31:131-134.

Grégoire, J.C. 1985. Host colonization strategies in *Dendroctonus*: larval gregariousness vs. mass attack by adults? *In*: L.S. Safranyik, (ed.), The role of the host in the population dynamics of forest insects. Proceedings of the IUFRO Working Parties S2.07-05 and S2.07-06 Conference, Banff, Canada, September 1983. Canadian Forestry Service and USDA Forest Service, Victoria, Canada. pp. 147-154.

Haack, R.A., R.C. Wilkinson, J.L. Foltz, and J.A. Corneil. 1984. Gallery construction and oviposition by *Ips calligraphus* (Coleoptera: Scolytidae) in relation to slash pine phloem thickness and temperature. Can. Entomol. 116:625-632.

Haack, R.A. 1985. Voltanism and diurnal emergence-flight pattern of *Ips calligraphus* (Coleoptera: Scolytidae) in Florida. Fla. Entomol. 68:658-667.

Haack, R.A., R.C. Wilkinson, J.L. Foltz, and J.A. Corneil. 1987. Spatial attack pattern, reproduction, and brood development of *Ips calligraphus* (Coleoptera: Scolytidae) in relation to slash pine thickness: a field study. Environ. Entomol. 16:28-436.

Hagler, J.R., and A.C. Cohen. 1990. Effects of time and temperature on digestion of purified antigen by *Geocoris punctipes* (Hemiptera: Lygaeidae) reared on artificial diet. Ann. Entomol. Soc. Am. 83:1177-1180.

Hagler, J.R., S.E. Naranjo, D. Bradley-Dunlop, F.J. Enriquez, and T.J. Hennberry. 1994. A monoclonal antibody to pink bollworm (Lepidoptera: Gelechiidae) egg antigen: a tool for predator gut analysis. Ann. Entomol. Soc. Am. 87:85-90.

Hagler, J.R., and S.E. Naranjo. 1997. Measuring the sensitivity of an indirect predator gut content ELISA: detectability of prey remains in relation to predator species, temperature, time and meal size. Biol. Control. 9:112-119.

Harper, G.L., R.A. King, C.S. Dodd, J.D. Harwood, D.M. Glen, M.W. Bruford, and W.O.C. Symondson. 2005. Rapid screening of invertebrate predators for multiple prey DNA targets. Mol. Ecol. 14:819-827.

Harwood, J.D., S.W. Phillips, K.D. Sunderland, and W.O.C. Symondson. 2001. Secondary predation: quantification of food chain errors in an aphid-spider-carabid system using monoclonal antibodies. Mol. Ecol. 10:2049-2057.

Harwood, J.D., K.D. Sunderland, and W.O.C. Symondson. 2004. Prey selection by linyphiid spiders: molecular tracking of the effects of alternative prey on rates of aphid consumption in the field. Mol. Ecol. 13:3549-3560.

Harwood, J.D., and J.J. Obrycki. 2005. Quantifying aphid predation rates of generalist predators in the field. Eur. J. Entomol. 102:335-350.

Harwood, J.D., N. Desneux, H.J.S. Yoo, *et al.* 2007. Tracking the role of alternative prey in soybean aphid predation by *Orius insidiosus*: a molecular approach. Mol. Ecol. 16:4390-4400.

Harwood, J.D., H.J.S. Yoo, M.H. Greenstone, D.L. Rowley, and R.J. O'Neil. 2009. Differential impact of adults and nymphs of a generalist predator on an exotic invasive pest demonstrated by molecular gut-content analysis. Biol. Invasions. 11:895-903.

Hedden, R., J.P. Vité, and K. Mori. 1976. Synergistic effect of a pheromone and a kairomone on host selection and colonization by *Ips avulsus*. Nature. 261:696-697.

Hennier, P.B. 1983. *Monochamus titillator* (F.) (Coleoptera: Cerambycidae) colonization and influence on populations of *Dendroctonus frontalis* Zimmmerman, *Ips avulsus* Eichhoff and *Ips calligraphus* Germar (Coleoptera: Scolytidae). Master's Thesis. Texas A&M Univ., College Station. 126 pp.

Hoogendoorn, M., and G.E. Heimpel. 2001. PCR-based gut content analysis of insect predators: using ribosomal ITS-I fragments from prey to estimate predation frequency. Mol. Ecol. 10:2059-2068.

Hosseini, R., O. Schmidt, and M.A. Keller. 2008. Factors affecting detectability of prey DNA in the gut contents of invertebrate predators: a polymerase chain reaction-based method. Entomol. Exp. Appl. 126:194-202.

Hoy, M.A. 1994. Insect molecular genetics: an introduction to principles and applications. Academic Press, San Diego, California. 560 pp.

Hughes, A.L., and M.K. Hughes. 1987. Asymmetric contests among sawyer beetles (Cerambycidae: *Monochamus notatus* and *Monochamus scutellatus*). Can. J. Zool. 65:823-827.

Jaramillo, J., E.G. Chapman, F.E. Vega, and J.D. Harwood. 2010. Molecular diagnosis of a previously unreported predatorprey association in coffee: *Karnyothrips flavipes* Jones (Thysanoptera: Phlaeothripidae) predation on the coffee berry borer. Naturwissenschaften. 97:291-298.

Juen, A., and M. Traugott. 2005. Detecting predation and scavenging by DNA gut-content analysis: a case study using a soil insect predator-prey system. Oecologia 142:344-352.

Juen, A., and M. Traugott. 2006. Amplification facilitators and multiplex PCR: tools to overcome PCR-inhibition in DNA-gut content analysis of soil-living invertebrates. Soil Biol. Biochem. 38:1872-1879.

Juen, A. and M. Traugott. 2007. Revealing species-specific trophic links in below-ground invertebrate communities: the predator guild of scarab larvae identified by diagnostic PCR. Mol. Ecol. 16:1545-1557.

Kalendar, R., D. Lee, and A.H. Schulman. 2009. FastPCR software for PCR primer and probe design and repeat search. Available from http://www.biocenter.helsinki.fi/bi/programs/fastpcr.htm

King, R.A., D.S. Read, M. Traugott, and W.O.C. Symondson. 2008. Molecular analysis of predation: a review of best practice for DNA-based approaches. Mol. Ecol. 17:947-963.

King, R.A., I.P. Vaughan, J.R. Bell, D.A. Bohan, and W.O.C. Symondson. 2010. Prey choice by carabid beetles feeding on an earthworm community analysed using species- and lineage-specific PCR primers. Mol. Ecol. 19:1721-1732.

Kinn, D.N. 1986. Studies on the flight capabilities of *Dendroctonus frontalis* and *Ips calligraphus*: preliminary findings using tethered beetles. USDA Forest Service Research Note SO-324. 3 p.

Kirkendall, L.R. 1983. The evolution of mating systems in bark and ambrosia beetles (Coleoptera: Scolytidae and Platypodidae). Zool. J. Linnean. Soc. 77:293-352.

Klepzig, K.D., K.F. Raffa, and E.B. Smalley. 1991. Association of an insect-fungal complex with red pine decline in Wisconsin. Forest Sci. 37:1119-1139.

Kohnle, U., J.P. Vité, H. Meyer, and W. Francke. 1994. Response of four American engraver bark beetles *Ips* spp. (Col., Scolytidae), to synthetic racemates of chiral pheromones. J. Appl. Entomol. 117:451-456.

Kowal, R.J., and J.F. Coyne. 1951. The black turpentine beetle can kill trees. Am. Turpentine Farmers Assoc. J. 13:14-15.

Latty, T.M., M.J.L. Magrath, and M.R.E. Symonds. 2009. Harem size and oviposition behaviour in a polygynous bark beetle. Ecol. Entomol. 34:562-568.

Lawson, S.A., and F.D. Morgan. 1993. Prey specificity of adult *Temnochila virescens* F. (Col., Trogositidae), a predator of *Ips grandicollis* Eichh. (Col., Scolytidae). J. Appl. Entomol. 115:139-144.

Leather, S.R. 1995. Factor affecting fecundity, fertility, oviposition, and larviposition in insects. *In*: S.R. Leather and J. Hardie, (eds.), Insect reproduction. CRC, Boca Raton, Florida. pp. 143-174.

de León J.H., V. Fournier, J.R. Hagler, and K.M. Daane. 2006. Development of molecular diagnostic markers for sharpshooters *Homalodisca coagulata* and *Homalodisca liturata* for use in predator gut-content examinations. Entomol. Exp. Appl. 119:109-119.

Leuschner, W.A. 1980. Impacts of the southern pine beetle. *In*: R.C. Thatcher, J.L. Searcy, J.E. Coster, and G.D. Hertel, (eds.), The southern pine beetle. USDA Forest Service Technical Bulletin 1631. pp. 137-151.

Li, S.Q., and Z.N. Zhang. 2006. Influence of larval frass extracts on the oviposition behaviour of *Monochamus alternatus* (Col., Cerambycidae). J. Appl. Entomol. 130:178-182.

Light, D.M., and M.C. Birch. 1979. Inhibition of the attractive pheromone response in *Ips paraconfusus* by (R)-(-)-ipsdienol. Naturwissenschaften. 66:159-160.

Lingafelter, S.W. 2007. Illustrated key to the longhorned woodboring beetles of the Eastern United States. Special Publication No. 3. The Coleopterists Society Miscellaneous Publication. 206 pp.

Linit, M.J. 1985. Continuous laboratory culture of *Monochamus carolinensis* (Coleoptera: Cerambycidae) with notes on larval development. Ann. Entomol. Soc. Am. 78:212-213.

Linit, M.J. 1988. Nematode-vector relationships in the pine wilt disease system. J. Nematol. 20:227-235.

Linit, M.J., and F.M. Stephen. 1983. Parasite and predator components of within-tree southern pine beetle (Coleoptera: Scolytidae) mortality. Can. Entomol. 115:679-688.

Linit, M.J., E. Kondo, and M.T. Smith. 1983. Insects associated with the pinewood nematode, *Bursaphelenchus xylophilus* (Nematoda: Aphelechoididae), in Missouri. Environ. Entomol. 12:467-470.

Linsley, E.G. and J.K. Chemsak. 1984. The Cerambycidae of North America, Part VII, No.1: taxonomy and classification of the subfamily Lamiinae, tribes Parmenini through Acanthoderini. Univ. Calif. Publ. Entomol., Vol. 102. 258 pp.

Lister. A., M.B. Usher, and W. Block. 1987, Description and quantification of field attack rates by predatory mites: an example using an electrophoresis method with a species of Antarctic mite. Oecologia. 72:185-191.

Logan, J.A., and J.A. Powell. 2001. Ghost forests, global warming, and the mountain pine beetle (Coleoptera: Scolytidae). Am. Ent. 47:160173.

Lombardero, M.J., M.P. Ayres, P.L. Lorio, Jr. and J.J. Ruel. 2000. Environmental effects on constitutive and inducible resin defenses of *Pinus taeda*. Ecol. Lett. 3:329-339.

Lorio Jr., P.L. and J.D. Hodges. 1968. Microsite effects on oleoresin exudation pressure of large loblolly pines. Ecology 49:1207-1210.

Lorio Jr., P.L., F.M. Stephen, and T.D. Paine. 1995. Environment and ontogeny modify loblolly pine response to induced acute water deficits and bark beetle attack. Forest Ecol. Manag. 73:97-110.

Lövei, G.L., E. Monostori, and I. Ando. 1985. Digestion rate in relation to starvation in the larva of a carabid predator, *Poecilus cupreus*. Entomol. Exp. Appl. 37:123-127.

Mayfield III, A.E. and J.L. Foltz. 2005. The black turpentine beetle, *Dendroctonus terebrans* (Olivier) (Coleoptera: Curculionidae: Scolytinae). Division of Plant Industry, FDACS. Entomology Circ. 405:4.

Merkel, E.P. 1981. Control of the black turpentine beetle. Georgia Forest Research Paper 15. Georgia Forestry Commission. 4 pp.

Merten, P. and D. Nowak. 2004. Overview of consequences of the SPB-killed pines in the Southern Appalachians and Cumberland Plateau. After the southern pine beetle. SAMAB conference, 24 Jan. 2004, Murphy, NC.

Mignot, E.C., and R.F. Anderson. 1970. Bionomics of the bark beetle predator, *Temnochila virescens* Mann. (Coleoptera: Ostomidae). Entomol. News. 81:85-89.

Miller, D.R., and J.H. Borden. 1990. β-Phellandrene: kairomone for pine engraver, *Ips pini* (Say) (Coleoptera: Scolytidae). J. Chem. Ecol. 16:2519-2531.

Miller, D.R., C. Asaro, and C.W. Berisford. 2005. Attraction of southern pine engravers and associated bark beetles (Coleoptera: Scolytidae) to ipsenol, ipsdienol, and lanierone in Southeastern United States. J. Econ. Entomol. 98:2058-2066.

Miller, M.C. 1984. Mortality contribution of insect natural enemies to successive generations of *Ips calligraphus* (Germar) (Coleoptera, Scolytidae) in loblolly pine. Z. Angew. Entomol. 93:495-500.

Miller, M.C. 1986. Survival of within-tree *Ips calligraphus* (Col.: Scolytidae): effect of insect associates. Entomophaga. 31:39-48.

Moeck, H.A., D.L. Wood, and J.K.Q. Lindahl. 1981. Host selection behavior of bark beetles (Coleoptera: Scolytidae) attacking *Pinus ponderosa*, with special emphasis on the western pine beetle, *Dendroctonus brevicomis*. J. Chem. Ecol. 7:49-83.

Monteiro, L., D. Bonnemaison, A. Vekris, K.G. Petry, J. Bonnet, R. Vidal, J. Cabrita, and F. Mégraud. 1997. Complex polysaccharides as PCR inhibitors in feces: *Helicobacter pylori* model. J. Clin. Microbiol. 35:995-998.

Moore, G.E., and R.C. Thatcher. 1973. Epidemic and endemic populations of the southern pine beetle. RP-SE-111. U.S. Department of Agriculture, Forest Service, Southern Research Station, Atlanta, GA. 11 pp.

Morales, M.E., D.M. Wesson, I.W. Sutherland, D.E. Impoinvil, C.M. Mbogo, J.I. Githure, and J.C. Beier. 2003. Determination of *Anopholes gamibiae* larval DNA in the gut of insectivorous dragonfly (Libellulidae) nymphs by polymerase chain reaction. J. Am. Mosq. Control Assoc. 19:163-165.

Moser, J.C., R.C. Thatcher, and L.S. Pickard. 1971. Relative abundance of southern pine beetle associates in Texas. Ann. Entomol. Soc. Am. 64:72-77.

Muilenburg, V.L., F.L. Goggin, S.L. Hebert, L. Jia, and F.M. Stephen. 2008. Ant predation on red oak borer confirmed by field observation and molecular gut-content analysis. Agric. For. Entomol. 10:205-213.

Nakamura, H., N. Tsutsui, and H. Okamoto. 1995. Oviposition habit of the Japanese pine sawyer, *Monochamus alternatus* Hope (Coleoptera: Cerambycidae) II. Effects of bark thickness of making of oviposition scars. Jpn. J. Entomol. 63:739-745.

Néve, G. 1994. Influence of temperature and humidity on activity of three *Carabus* species. *In*: K. Desender, M. Dufrene, M. Loreau, M.L. Luff, and J.-P. Maelfait, (eds.), Carabid beetles: ecology and evolution. Kluwer Academic Publishers, Dordrecht. pp. 189-192.

Overgaard, N.A. 1968. Insect associates with the southern pine beetle in Texas, Louisiana, and Mississippi. J. Econ. Entomol. 61:1197-1201.

Paine, T.D., M.C. Birch, and P. Svihra. 1981. Niche breadth and resource partitioning by four sympatric species of bark beetles (Coleoptera: Scolytidae). Oecologia. 48:1-6.

Paine, T.D., K.F. Raffa, and T.C. Harrington. 1997. Interactions among scolytid bark beetles, their associated fungi, and live host conifers. Ann. Rev. Entomol. 42:179-206.

Pajares, J.A., F. Ibeas, J. J. Diez, and D. Gallego. 2004. Attractive responses by *Monochamus galloprovincialis* (Col., Cerambycidae) to host and bark beetle semiochemicals. J. Appl. Entomol. 128:633-638.

Payne, T.L., J.E. Coster, J.V. Richerson, L.J. Edson, and E.R. Hart. 1978. Field response of the southern pine beetle to behavioral chemicals. Environ. Entomol. 7:578-582.

Payne, T.L. 1980. Life history and habits. *In*: R.C. Thatcher, J.L. Searcy, J.E. Coster, and G.D. Hertel, (eds.), The southern pine beetle. USDA Forest Service Technical Bulletin 1631. pp. 7-28.

Payne, T.L., J.V. Richerson, J.C. Dickens, J.R. West, K. Mori, C.W. Berisford, R.L. Hedden, J.P. Vité, and M.S. Blum. 1982. Southern pine beetle: olfactory receptor and behavior discrimination of enantiomers of the attractant pheromone frontalin. J. Chem. Ecol. 8:873-881.

Payne, T.L., R.F. Billings, J.D. Delorme, N.A. Andryszak, J. Bartels, W. Francke, and J.P. Vité. 1987. Kairomonal-pheromonal system in the black turpentine beetle, *Dendroctonus terebrans* (Ol.). J. Appl. Entomol. 103:15-22.

Peddle, S.M. 2000. Host selection, oviposition behavior, and inter- and intra-specific competition in the white-spotted sawyer beetle, *Monochamus scutellatus* (Say) (Coleoptera: Cerambycidae). Master's Thesis. Univ. of Toronto, Toronto. 95 pp.

Peddle, S., P. de Groot, S. Smith. 2002. Oviposition behavior and response of *Monochamus scutellatus* (Coleoptera: Cerambycidae) to conspecific eggs and larvae. Agric. For. Entomol. 3:217-222.

Pershing, J.C. and M.J. Linit. 1985. A structural difference in the male genitalia of *Monochamus carolinensis* (Oliver) and *M. titillator* (Fabricius) (Coleoptera: Cerambycidae). J. Kans. Entomol. Soc. 58:543-546.

Pershing, J.C., and M.J. Linit. 1986. Biology of *Monochamus carolinensis* (Coleoptera: Cerambycidae) on Scotch pine in Missouri. J. Kansas Entomol. Soc. 59:706-711.

Pershing, J.C., and M.J. Linit. 1988. Variation in number of instars of *Monochamus carolinensis* (Coleoptera: Cerambycidae). J. Kans. Entomol. Soc. 61:370-378.

Phillips, T.W., A.J. Wilkening, T.H. Atkinson, J.L. Nation, R.C. Wilkinson, and J.L. Foltz. 1988. Synergism of turpentine and ethanol as attractants for certain pine-infesting beetles (Coleoptera). Environ. Entomol. 17:456-462.

Phillips, T.W., J.L. Nation, R.C. Wilkinson, and J.L. Foltz. 1989. Secondary attraction and field activity of beetle-produced volatiles in *Dendroctonus terebrans*. J. Chem. Ecol. 15:1513-1533.

Pope, D.N., R.N. Coulson, W.S. Fargo, J.A. G, and C.W. Kelley. 1980. The allocation process an between-tree survival probabilities in *Dendroctonus frontalis* infestations. Res. Popul. Ecol. 22:197-210.

Powell, J.M. 1967. A study of habitat temperatures of the bark beetle, *Dendroctonus ponderosae* Hopkins, in lodgepole pine. Agric. Meterol. 4:189-201.

Price, T.S., H.C. Dogget, J.M. Pye, and B. Smith. 1997. A history of southern pine beetle outbreaks in the Southestern United States. Georgia Forestry Commision, Macon, Georgia. 72 pp.

Raffa, K.F., and A.A. Berryman. 1983. The role of host plant resistance in the colonization behavior and ecology of bark beetles (Coleoptera: Scolytidae) Ecol. Monogr. 53:27-49.

Raffa, K.F., T.W. Phillips, and S.M. Salom. 1993. Strategies and mechanisms of host colonization by bark beetles. *In*: T.D. Schowalter and G. Filip, (eds.), Beetle pathogen interactions in conifer forests. Academic Press, London, UK. pp. 103-128.

Ragsdale, D.W., A.D. Larson, and L.D. Newsome. 1981. Quantitative assessment of the predators of *Nezara viridula* eggs and nymphs within a soybean agroecosystem using an ELISA. Environ. Entomol. 10:402-405.

Raske, A.G. 1975. Distribution of *Monochamus* entrance holes in lodgepole pine. Environment Canada Forestry Service Bi-Monthly Research Notes. 31:33.

Ray, A.M., M.D. Ginzel, and L.M. Hanks. 2009. Male *Megacyllene robiniae* (Coleoptera: Cerambycidae) use multiple tactics when aggressively competing for mates. Env. Entomol. 38:425-432.

Rayner, A.D.M., and L. Boddy. 1988. Fungal decomposition of wood: its biology and ecology. Wiley, New York. 587 pp.

Read, D.S., S.K. Sheppard, M.W. Bruford, D.M. Glen, and W.O.C. Symondson. 2006. Molecular detection of predation by soil micro-arthropods on nematodes. Mol. Ecol. 15:1963-1972.

Reeve, J.D. 1997. Predation and bark beetle dynamics. Oecologia. 112:48-54.

Reeve, J.D., and P. Turchin. 2002. Evidence for predator-prey cycles in a bark beetle. *In*: A.A. Berryman, (ed.), Population cycles: evidence for trophic interactions Oxford University Press, New York. pp. 92-108.

Renwick, J.A.A., and J.P. Vité. 1969. Bark beetle attractants: mechanism of colonization by *Dendroctonus frontalis*. Nature. 224:1222-1223.

Renwick, J.A.A. and J.P. Vité. 1972. Pheromones and host volatiles that govern aggregation of the six-spined engraver beetle, *Ips calligraphus*. J. Insect Physiol. 18:1215-1219.

Riley, M.A. 1983. Insect enemies of *Ips calligraphus* (Germar) on felled loblolly (*Pinus taeda* L.) and slash (*P. elliottii* Engelmann) pines in Louisiana. Master's Thesis. Louisiana State University, Baton Rouge. 94 pp.

Riley, M.A., and R.A. Goyer. 1986. Impact of benecial insects on *Ips* spp. (Coleoptera: Scolytidae) bark beetles in felled loblolly and slash pines in Louisiana. Environ. Entomol. 15:1220-1224.

Rose, A.H. 1957. Some notes on the biology of *Monochamus scutellatus* (Say) (Coleoptera: Cerambycidae). Can. Entomol. 89:547-553.

Rudinsky, J.A. 1962. Ecology of the Scolytidae. Ann. Rev. Entomol. 7:327-348.

Rudinsky, J.A. 1973. Multiple functions of the southern pine beetle pheromone verbenone. Environ. Entomol. 2:511-514.

Ruel, J.J., M.P. Ayres, and P.L. Lorio Jr. 1998. Loblolly pine responds to mechanical wounding with increased resin flow. Can. J. For. Res. 28:596602.

Ryker, L.C., and K.L. Yandell. 1983. Effect of verbenone on aggregation of *Dendroctonus ponderosae* (Coleoptera: Scolytidae) to synthetic attractant. Z. Angew. Entomol. 96:452-459.

Saitoh, K., M. Takagaki, and Y. Yamashita. 2003. Detection of Japanese flounder-specific DNA from gut contents of potential predators in the field. Fish. Sci. 69:473-477.

Schenk, D., and S. Bacher. 2004. Detection of shield beetle remains in predators using a monoclonal antibody. J. Appl. Entomol. 128:273-278.

Schroeder, L.M. 1997. Oviposition behavior and reproductive success of the cerambycid *Acanthocinus aedilis* in the presence and absence of the bark beetle *Tomicus piniperda*. Entomol. Exp. Appl. 82:9-17.

Schroeder, L.M., and J. Weslein. 1994. Interactions between the phloem-feeding species *Tomicus piniperda* (Col.: Scolytidae) and *Acanthocinus aedilis* (Col.: Cerambycidae) and the predator *Thanasimus formicarius* (Col.: Cleridae) with special reference to brood production. Entomophaga. 39:149-157.

Seybold, S.J., T. Ohtsuka, D.L. Wood, and I. Kubo. 1995. Enantiomeric composition of ipsdienol: a chemotaxonomic character for North American populations of *Ips* spp. in the pini subgeneric group (Coleoptera: Scolytidae). J. Chem. Ecol. 21:995-1016.

Shepherd, W.P., and R.A. Goyer. 2003. Seasonal abundance, arrival, and emergence patterns of predaceous hister beetles (Coleoptera: Histeridae) associated with *Ips* engraver beetles (Coleoptera: Scolytidae) in Louisiana. Entomol. Sci. 38:612-620.

Shepherd, W.P. 2004. Biology and host finding of predaceous hister beetles (Coleoptera: Histeridae) associated with *Ips* spp. (Coleoptera: Scolytidae) in loblolly pine (*Pinus taeda* L.). Ph.D. Dissertation. Louisiana State University, Baton Rouge. 108 pp.

Sheppard, S.K., J. Bell, K.D. Sunderland, J. Fenlon, D. Skervin, and W.O.C. Symondson. 2005. Detection of secondary predation by PCR analyses of the gut contents of invertebrate generalist predators. Mol. Ecol. 14:4461-4468.

Showalter, T.D., D.N. Pope, R.N. Coulson, and W.S. Fargo. 1981. Patterns of southern pine beetle (*Dendroctonus frontalis* Zimm.) infestation enlargement. For. Sci. 27:837-849.

Shibata, E. 1984. Spatial distribution pattern of the Japanese pine sawyer, *Monochamus alternatus* Hope (Coleoptera: Cerambycidae), on dead pine trees. Appl. Entomol. Zool. 19:361-366.

Siegfried, B.D. 1984. Attraction of the black turpentine beetle, *Dendroctonus terebrans*, to host and insect-produced volatiles. Master's Thesis, Univ. of Florida, Gainesville. 57 pp.

Simon, C., F. Frati, A. Beckenbach, B. Crespi, H. Liu, and P. Flook. 1994. Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. Ann. Entomol. Soc. Am. 87:651-701.

Sint, D., L. Raso, R. Kaufmann, and M. Traugott. 2011. Optimizing methods for PCR-based analysis of predation. Mol. Ecol. Resour. 11.

Six, D.L., and K.D. Klepzig. 2004. *Dendroctonus* bark beetles as model systems for studies on symbiosis. Symbiosis. 37:207-232.

Smith, M.T., G.R. Busch, T.L. Payne, and J.C. Dickens. 1988. Antennal olfactory responsiveness of three sympatric *Ips* species [*Ips avulsus* (Eichhoff), *Ips calligraphus* (Germar), *Ips grandicollis* (Eichhoff)], to intra- and interspecific behavioral chemicals. J. Chem. Ecol. 14:1289-1304.

Smith M.T., T.L. Payne, and M.C. Birth. 1990. Olfactory-based behavioral interactions among five species in the southern pine bark beetle group. J. Chem. Ecol. 16:3317-3332.

Smith, M.T., S.M. Salom, and T.L. Payne. 1993. The southern bark beetle guild: an historical review of the research on the semiochemical-based communication system of the five principle species. VA. Agric. Exp. Stn., Bull. 93-4. Blacksburg, VA. 106 pp.

Smith, R.H., and R.E. Lee III. (1972). Black turpentine beetle. USDA Forest Service. Forest Pest Leaflet 12. 8 pp.

Solomon, M.G., J.D. Fitzgerald, and R.A. Murray. 1996. Electrophoretic approaches to predator-prey interactions. *In*: W.O.C. Symondson and J.E. Liddell, (eds.), The ecology of agricultural pests-biochemical approaches. Chapman and Hall, London. pp. 457-468.

Staudacher, K., C. Wallinger, N. Schallhart, and M. Traugott. 2011. Detecting ingested plant DNA in soil-living insect larvae. Soil Biol. Biochem. 43:346-350.

Stephen, F.M. 1995. Potential for suppressing southern pine beetle populations by enhancing effectiveness of their hymenopteran parasitoids. *In*: F.P. Hain, S.M. Salom, W.F. Ravlin, T.L. Payne and K.F. Raffa, (eds.), Proceedings: behavior, population dynamics and control of forest insects. Ohio State University, Ohio Agricultural Research and Development Center: Wooster, Ohio. pp. 226-240.

Strom, B.L., S.R. Clarke, and L.M. Roton. 2003. Attraction of *Ips avulsus* (Eichhoff) to varying enantiomeric composition of ipsdienol in commercially available lures. J. Entomol. Sci. 38:137-139.

Sullivan, B.T., C.W. Berisford, and M.J. Dalusky. 1997. Field response of southern pine beetle parasitoids to some natural attractants. J. Chem. Ecol. 23:837-856.

Sullivan, B.T., W.P. Shepherd, D.S. Pureswaran, and K. Mori. 2007. Evidence that (+)-*endo*-brevicomin is a male produced aggregation pheromone component of the southern pine beetle, *Dendroctonus frontalis*. J. Chem. Ecol. 33:1510-1527.

Sullivan, B.T., and K. Mori. 2009. Spatial displacement of release point can enhance activity of an attractant pheromone synergist of a bark beetle. J. Chem. Ecol. 35:1222-1233.

Sunderland, K.D. 1975. The diet of some predatory arthropods in cereal crops. J. Appl. Ecol. 12:507-515.

Sunderland, K.D. 1988. Quantitative methods of detecting invertebrate predation occurring in the field. Ann. Appl. Biol. 112:201-224.

Sunderland, K.D., N.E. Crook, D.L. Stacey, and B.J. Fuller. 1987. A study of feeding by polyphagous predators on cereal aphids using ELISA and gut dissection. J. Appl. Ecol. 24:907-933.

Suzuki, N., K. Murakami, H. Takeyama, and S. Chow. 2006. Molecular attempt to identify prey organisms of lobster phyllosoma larvae. Fish. Sci. 72:342-349.

Svihra, P. 1982. Influence of opposite sex on attraction produced by pioneer sex of four bark beetle species cohabiting pine in the Southern United States. J. Chem. Ecol. 8:373-378.

Svihra, P., T.D. Paine, and M.C. Birch. 1980. Interspecific olfactory communications in southern pine beetles. Naturwissenschaften. 67:518-519.

Symondson, W.O.C. 2002. Molecular identification of prey in predator diets. Mol. Ecol. 11:627-641.

Symondson, W.O.C., and J.E. Liddell. 1996. A species-specific monoclonal antibody system for detecting the remains of field slugs, *Deroceras reticulatum* (Müller) (Mollusca: Pulmonata), in carabid beetles (Coleoptera: Carabidae). Biocontrol Sci. Techn. 6:91-99.

Symondson, W.O.C., M.L. Erickson, and J.E. Liddell. 1999. Development of a monoclonal antibody for the detection and quantification of predation on slugs within the *Arion hortensis* agg. (Mollusca: Pulmonata). Biol. Control. 16:274-282.

Tamura, K., J. Dudley, M. Nei, and S. Kumar. 2007. MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. Mol. Biol. Evol. 24:1596-1599.

Teale, S.A., and G.N. Lanier. 1991. Seasonal variability in response of *Ips pini* (Coleoptera: Scolytidae) to ipsdienol in New York. J. Chem. Ecol. 17:1145-1158.

Thatcher, R.C. 1960. Bark beetles affecting southern pines: a review of current knowledge. USDA Forest Service Southern Forest Exp. Station, Occ. Pap. 180. 25 pp.

Thatcher, R.C. and L.S. Pickard. 1966. The clerid beetle, *Thanasimus dubius*, as a predator of the southern pine beetle. J. Econ. Entomol. 59:955-957.

Thatcher, R.C. and L.S. Pickard. 1967. Seasonal development of the southern pine beetle in East Texas. J. Econ. Entomol. 60:656-658.

Thatcher, R.C., J.L. Searcy, J.E. Coster, and G.D. Hertel. 1980. The southern pine beetle, USDA, expanded southern pine beetle research and application program. Forest Service, Science, and Education Administration, Pineville, LA, Tech. Bull. 1631. 265 pp.

Thatcher, R.C., and M.D. Conner. 1985. Identification and biology of southern pine bark beetles. USDA Forest Service, Washington D.C. Handbook No. 634. 14 pp.

Togashi, K., J.E. Appleby, H. Oloumi-Sadeghi, and R.B. Malek. 2009. Age-specific survival rate and fecundity of adult *Monochamus carolinensis* (Coleoptera: Cerambycidae) under field conditions. Appl. Entomol. Zool. 44:249-256.

Traugott, M. 2003. The prey spectrum of larval and adult *Cantharis* species in arable land: an electrophoretic approach. Pedobiologia. 47:161-169.

Traugott, M., P. Zangerl, A. Juen, N. Schallhart, and L. Pfiffner. 2006. Detecting key parasitoids of lepidopteran pests by multiplex PCR. Biol. Control. 39:39-46.

Turchin, P., P.L. Lorio Jr, A.D. Taylor, and R.F. Billings. 1991. Why do populations of southern pine beetles (Coleoptera: Scolytidae) fluctuate? Environ. Entomol. 20:401-409.

Turchin, P., A.D. Taylor, and J.D. Reeve. 1999. Dynamical role of predators in population cycles of a forest insect: an experimental test. Science. 285:1068-1071.

Ungerer, M.J., M.P. Ayres, and M.J. Lombardero. 1999. Climate and the northern distribution limits of *Dendroctonus frontalis* Zimmermann (Coleoptera: Scolytidae). J. Biogeogr. 26:1133-1145.

Vanlaerhoven, S.L., and F.M. Stephen. 2002. Height distribution of parasitoids of the southern pine beetle complex. Environ. Entomol. 31:982-987.

Vité, J.P., and R.I. Gara. 1962. Volatile attractants from ponderosa pine attacked by bark beetles (Coleoptera: Scolytidae). Contrib. Boyce Thompson Inst. 21:251-254.

Vité, J.P., and G.B. Pitman. 1968. Bark beetle aggregation: effects of feeding on the release of pheromones in *Dendroctonus* and *Ips*. Nature. 218:169-170.

Vité, J.P., and D.L. Williamson. 1970. *Thanasimus dubius*: prey perception. J Insect. Physiol. 106:233-239 .

Vité, J.P., and J.A.A. Renwick. 1971. Population aggregating pheromone in the bark beetle *Ips grandicollis*. J. Insect. Physiol. 17:1699-1704.

Vité, J.P., A. Bakke, and J.A.A. Renwick. 1972. Pheromones in *Ips* (Coleoptera: Scolytidae): occurrence and production. Can. Entomol. 104:1967-1975.

Vité, J.P., and W. Francke. 1976. The aggregation pheromones of bark beetles: progress and problems. Naturwissenschaften. 63:550-555.

Vité, J.P., R. Hedden, and K. Mori. 1976a. *Ips grandicollis*: field response to the optically pure pheromone. Naturwissenschaften. 63:43-44.

Vité, J.P., D. Klimetzek, G. Loskant, R. Hedden, and K. Mori. 1976b. Chirality of insect pheromones: response interruption by inactive antipode. Naturwissenschaften. 63:582-583.

Vité, J.P., G. Ohloff, and R.F. Billings. 1978. Pheromonal chirality and integrity of aggregation response in southern species of the bark beetle *Ips* sp. Nature. 272:817-818.

Vité, J.P., R.F. Billings, C.W. Ware, and K. Mori. 1985. Southern pine beetle: Enhancement or inhibition of aggregation response mediated by enantiomers of *endo*-brevicomin. Naturwissenschaften. 72:99-100.

Wagner, T.L., W.S. Fargo, L.L Keeley, R.N. Coulson, and J.D. Cover. 1982. Effects of sequential attack on gallery construction, oviposition and re-emergence by *Dendroctonus frontalis* (Coleoptera: Scolytidae). Can. Entomol. 114:491-502.

Wagner, T.L., J.A. Gagne, P.J.H. Sharpe, and R.N. Coulson. 1984. A biophysical model of southern pine beetle *Dendroctonus frontalis* (Coleoptera: Scolytidae) development. Ecol. Model. 21:125-147.

Wagner, T.L., R.O. Flamm, and R.N. Coulson. 1985. Strategies for cohabitation among the southern pine beetle species: comparisons of life-process biologies. *In*: S.J. Branham, and R.C. Thatcher, (eds.), Integrated pest management research symposium: the proceedings. USDA-FS, S. For. Exp. Stn. New Orleans, Louisiana. Gen. Tech. Rep. SO-56. pp. 87-101.

Wagner, T.L., R.O. Flamm, H. Wu, W.S. Fargo, and R.N. Coulson. 1987. Temperature-dependent model of life cycle development of *Ips calligraphus* (Coleoptera: Scolytidae). Environ. Entomol. 16:497-502.

Wagner. T.L., P.B. Hennier, R.O. Flamm, and R.N. Coulson. 1988. Development and mortality of *Ips avulsus* (Coleoptera: Scolytidae) at constant temperatures. Environ. Entomol. 17:181-191.

Wallace, S.K. 2004. Molecular gut analysis of carabids (Coleoptera: Carabidae) using aphid primers. Master's thesis, Montana State University, Bozeman. 69 pp.

Walsh, K.D. 1983. Oviposition and host preference of *Monochamus carolinensis* (Coleoptera: Cerambycidae). Master's Thesis. University of Missouri, Columbia. 74 pp.

Walsh, K.D. and M.J. Linit. 1985. Oviposition biology of the pine sawyer, *Monochamus carolinensis* (Coleoptera: Cerambycidae). Ann. Entomol. Soc. Am. 78:81-85.

Webb, J.L. 1909. Some insects injurious to forests. The southern pine sawyer. USDA, Bull. 58, part IV. Gov't. Publ. Office, Washington, D.C. 56 pp.

Weber, D.C., and J.G. Lundgren. 2009. Quantification of predation using qPCR: effect of prey quantity, elapsed time, chaser diet, and sample preservation. J. Insect Sci. 9:41 12 pp.

Wilkinson, R.C. 1963. Larval instars and head capsule morphology in three southeastern *Ips* bark beetles. Fla. Entomol. 46:19-22.

Williamson D.L. 1971: Olfactory discernment of prey by *Medetera bistriata* (Diptera, Dolichopodidae). Ann. Entomol. Soc. Am. 64:586-589.

Wilson, I.G. 1997. Inhibition and facilitation of nucleic acid amplification. Appl. Environ. Microbiol. 63:3741-3751.

Wilson, L.F. 1962. Insect damage to field-piled pulpwood in northern Minnesota. J. Econ. Entomol. 55:510-516.

Wood, D.L. 1970. Pheromones of bark beetles. *In*: D.L. Wood, R.M. Silverstein, and M. Nakajima, (eds.), Control of insect behavior by natural products. Acedemic Press, New York. pp. 301-316.

Wood, D.L. 1972. Selection and colonization of ponderosa pine by bark beetles. Symp. Royal Entomol. Soc. London. 6:10-17.

Wood, D.L. 1982. The role of pheromones, kairomones, and allomones in the host selection and colonization behavior of bark beetles. Annu. Rev. Entomol. 27:411-446.

Wood, D.L., and R.W. Stark. 1968. The life history of *Ips calligraphus* with notes on its biology in California. Can. Entomol. 100:145-151.

Wood, S.L. 1982. The bark and ambrosia beetles of North and Central America (Coleoptera: Scolytidae), a taxonomic monograph. Great Basin Nat. Mem. 6. 1359 pp.

Yang, B.J., H.Y. Pan, J. Tang, Y.Y. Wang, L.F. Wang, and Q. Wang. 2003. *Bursaphelenchus xylophilus*. Chinese Forestry Press, Beijing. pp. 6-143.

Yearian, W.C. and R.C. Wilkinson. 1967. Development of three *Ips* bark beetles on a phloem-based rearing medium. Fla. Entomol. 50:43-45.

Yoshikawa, K. 1987. A study of the subcortical insect community in pine trees. II. Vertical distribution. Appl. Entomol. Zool. 22:195-206.

Zaidi, R.H., Z. Jaal, N.J. Hawkes, J. Hemingway, and W.O.C. Symondson. 1999. Can the detection of prey DNA amongst the gut contents of invertebrate predators provide a new technique for quantifying predation in the field? Mol. Ecol. 8:2081-2087.

Zhang G.F., Z.C. Lü, and F.H. Wan. 2007a. Detection of *Bemisia tabaci* remains in predator guts using a sequence-characterised amplified region marker. Entomol. Exp. Appl. 123:81-90.

Zhang, G.F., Z.C. Lü, F.H. Wan, and G. Lövei. 2007b. Real-time PCR quantification of *Bemisia tabaci* (Homoptera: Aleyrodidae) B-biotype remains in predator guts. Mol. Ecol. 7:947-954.

Zhang, X. and M.J. Linit. 1998 Comparison of oviposition and longevity of *Monochamus alternatus* and *Monochamus carolinensis* (Coleoptera: Cerambycidae) under laboratory conditions. Environ. Entomol. 27:885-891.

Zhu, Y.C., and L. Williams III. 2002. Detecting the egg parasitoid *Anaphes iole* (Hymenoptera: Mymaridae) in tarnished plant bug (Heteroptera: Miridae) eggs by using a molecular approach. Ann. Entomol. Soc. Am. 95:359-365.

Vita

Erich Nicholas Schoeller is the son of Nicholas and Julie Schoeller. He was born on October 8^{th} 1984 in Janesville, Wisconsin. Erich graduated from Milton High School in 2003. Erich obtained his Bachelor of Science degree in biology with a minor in chemistry from the University of Wisconsin at Whitewater in 2008. In 2011 Erich obtained his Master of Science in entomology at Louisiana State University and A&M College under the direction of Dr. Jeremy D. Allison.