DISTRIBUTION AND POPULATION DYNAMICS OF THE ASIAN COCKROACH (*BLATTELLA ASAHINIA* MIZUKUBO) IN SOUTHERN ALABAMA AND GEORGIA

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Edward Todd Snoddy

A Thesis

Submitted to

the Graduate Faculty of

Auburn University

in Partial Fulfillment of the

Requirements for the

Degree of

Master of Science

Auburn, Alabama
May 10, 2007

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VITA

Edward Todd Snoddy was born in Auburn, Alabama on February 28, 1964 to Dr. Edward Lewis Snoddy and Lucy Mae Snoddy. He graduated Sheffield High School, Sheffield, Alabama in 1981. He attended Alexander Junior College from 1981 to 1983 at which time he transferred to Auburn University. He married Tracy Smith of Uchee, Alabama in 1984. He graduated Auburn University in 1987 with a Bachelor of Science degree in Integrated Pest Management, with a major in Entomology. In 1990 he earned a CCA (Certified Crop Adviser) certification. He is a graduate of the 1999 Class of Georgia Agri Business Leaders. In 1999 he went to work for Traylor Chemical Co. in Orlando, Florida and in 2004 became National Sales Manager of Agricultural Products. This allowed him to move to Auburn, Alabama where he entered graduate school to pursue a Masters of Science in Entomology. He has two children, Samantha Ann Snoddy who is the 3rd generation of the Snoddy family to attend Auburn University, and a son Edward Johnhenry Snoddy who is a senior at Auburn High School.

THESIS ABSTRACT

DISTRIBUTION AND POPULATION DYNAMICS OF THE ASIAN COCKROACH (*BLATTELLA ASAHINIA* MIZUKUBO) IN SOUTHERN ALABAMA AND GEORGIA

Edward Todd Snoddy

Master of Science, May 10, 2007
(B.S., Auburn University, 1987)

90 Typed Pages

Directed by Arthur G. Appel

In 1986, a new species of cockroach, the Asian cockroach, *Blattella asahinai* Mizukubo, was introduced into Florida. The Asian cockroach is very similar in appearance to the German cockroach, *B. germanica* (L.), but differs in behavior and habitat. Specimens of *B. asahinai* can only be distinguished from *B. germanica* by several morphological characters that are only present on adult males and visible under the microscope (Roth 1986). This species has become a peridomestic pest in Florida where homeowners spend large amounts of money for control. By 1999, the Asian cockroach had spread from three central counties in Florida to a total of 49 counties throughout the state (Kohler 1999, Donahoe 2005). In 2003, *B. asahinai* was discovered in large numbers in southeast Alabama (Hu et al., 2005).

We conducted a survey of southern Alabama and Georgia to determine to what extent *B. asahinai* had expanded its range northward from Florida. A total of 67 of the 159 counties in Georgia, and 33 counties of the 67 counties in Alabama were sampled. We found that *B. asahinai* is established in 7 counties in Georgia and 8 counties in Alabama. The area that *B. asahinai* has colonized in Alabama is double that of Georgia. The Asian cockroach has, not surprisingly, expanded its range utilizing human transportation. As it advances northward, cold temperatures only limit the range of *B. asahinai* during winter. We developed distribution maps to document the range of *B. asahinai.*

We also examined the population dynamics of *B. asahinai* in southern Alabama at a very large and established field population in Dothan, Alabama. For a 24 month period, we collected visual, arboreal, Berlese, and bucket samples on a bimonthly schedule during warm months until temperatures turned cold and population counts declined, we then sampled monthly. All stages of *B. asahinai* were counted and recorded and data were analyzed using ANOVA, ANCOVA, and multiple range tests with SAS Institute software. Results were then plotted using SigmaPlot. Data from 2005 and 2006 showed that visual and bucket sample populations began increasing in late May and reached their zenith in late August or early September. Berlese sample populations were low during the warm months but increased with the onset of cold weather and reached their zenith in late January. Berlese sample populations began to decline again with increasing warm temperatures. We estimate that three generations of *B. asahinai* develop during a typical year. Adult females and small and medium nymphs are the primary stages that over winter. Over wintering occurs in the soil or at the soil-mulch interface.

To better understand seasonal population dynamics and distribution patterns, we examined how *B. asahinai* responded to seasonal temperatures. We determined the CTMax and CTMin temperatures from the northern most established field population in Barbour County, Alabama. We took monthly samples of all available stages, and returned them to the laboratory for analysis. Over the 2 year period study, CTMax of field collected *B. asahinai* ranged from 25.3° ± 13.5°C for small nymphs on 29NOV2005 to 49.7° ± 0.70°C for large nymphs in 26OCT2005. CTMin values ranged between 0.8°±0.60°C for large nymphs on 19AUG2005 to 4.3°±0.44°C for adult females on 28JUL2006. We also determined how laboratory cultures responded to temperature changes by subjecting them to 10˚C or 35°C and measuring their CTMax and CTMin during acclimation (from 0-96 hours). Ranges for CTMin of laboratory acclimated *B. asahinai* at 10°C were 2.90 ± 0.18°C and 4.3 ± 0.30°C for females after 72 h and large nymphs at 0 h, respectively. For the 35°C acclimation experiment, CTMax of laboratory *B. asahinai* ranged between 45.55 ± 0.23°C and 50.65 ± 0.60°C for large nymphs at 0 h and large nymphs at 96 h, respectively. For the 35°C acclimation experiment, CTMin of laboratory *B. asahinai* ranged between 3.75 ± 0.62°C and 4.98 ± 0.27°C for large nymphs at 72 h and small nymphs at 48 h, respectively.

ACKNOWLEDGEMENTS

I would like to thank Dr. A.G. Appel for his support, ideas, and assistance in my research work, and for believing in me as a graduate student. I would like to thank my committee members, Dr. Micky Eubanks, and Dr. Ping Hu for their guidance, assistance with statistical analysis.

I would like to thank Dr. Wayne Clark, Dr. Gary Mullen, and Dr. Mike Williams for their excellent teaching abilities, which fostered my interest in entomology as an undergraduate and later was a determining factor to return as a graduate student. I would like to thank Dr. Steve "Bug" Brown, University of Georgia, for his assistance, support, and guidance through my career. I would like to thank Mr. Bill Traylor and Mr. Larry Adams of Traylor Chemical Co. for their support and allowing my time away from work to complete this research project.

Above all, I would like to greatly thank my wife Tracy, and children; Samantha Ann & Johnhenry, and my mother Lucy Mae, and John T. & Bettie Smith; whose love and encouragement, and unwavering support throughout my research allowed me to achieve this accomplishment.

I would like to dedicate this thesis to my late father Dr. Edward L. Snoddy, who opened my eyes at a young age to the wonderful world of insects and their importance, which ultimately inspired me to become an entomologist.

Style manual or journal used: Environmental Entomology

Computer software used: Microsoft Word®, SigmaPlot® 9.0, Excel®

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INTRODUCTION

History

Insects are thought to first have originated about 400 million years ago during the Late Silurian period (Grimaldi, 2005), which would make them some of the first terrestrial animals that appeared on the planet. There are about 925,000 known species of insects, but estimates run from 2.5 – 10 million species with an average estimate of about 5 million total species (Grimaldi, 2005). There are four orders; Coleoptera, Diptera, Hymenoptera, and Lepidoptera, that account for 80% of insect species (Grimaldi, 2005). Entomologists have generally avoided studying the early insects due to the difficulty in studying fossils. The space, time, and structures of fossils are difficult to master and understand. There are huge gaps in the geology history of many fossils groups, and the lack of detailed morphology of most insect fossils makes it difficult to positively identify characters and make precise assumptions concerning the relationship of fossil insects. When insects achieved flight, some 90-170 million years before vertebrates (Grimaldi, 2005), they began to achieve their greatest diversity. Flight allows dispersal, another means to avoid predation, and to become more efficient predators themselves. Wings allowed insects to develop more elaborate coloration, which in turn allowed them to advertise their toxins and venoms, and blend into the environment through mimicry and camouflage. Winged insects developed behaviors such as flying in loops which could have been an adaptation to avoid predation by bats, and other insects. Winged insects also integrated the use of wings into mating by using them in elaborate courtship behaviors, dispersal of pheromones, and simple flight to find a mate. Only insects have evolved to have complex eusocial societies such as those seen in termites, ants, and bees. Insects have been instrumental in the evolution plants. There are approximately 250,000 species of plants and 85% of them require pollination by insects (Grimaldi, 2005). Insects also evolved to feed on these plants and cause hundreds of million dollars in damage to many food and ornamental crops. Insects are not limited to interactions with plants; they have evolved to inhabit just about every niche on the planet with the exception of the benthic zone, and the marine environment; although there are a few insects that utilize the seashores and one genus of water striders, *Halobates* spp., which inhabits the open waters of the oceans (Grimaldi, 2005).

Insects have evolved over time to adapt and utilize their environment. Insects have evolved to feed on humans, and have played important roles in history. There are several diseases transmitted by insects that had significant roles in human history; epidemic typhus, Chaga's disease, sleeping sickness, malaria, yellow fevers, and plague. Insects and the diseases they vector have even played a role in human mutation and evolution (e.g., Sickle cell anemia) (Mullen and Durden, 2002). Insects play an important role in the ecology and life cycle of many diseases since they are the major vectors of many diseases (Mullen and Durden, 2002).

Cockroach origins date back 350-400 million years to the Devonian period of the Paleozoic era. There has been little change in their morphology since then. Paleoentomologists have grouped ancient cockroaches into an order named Dictyoptera. This order contained the ancient orders of Blattaria, Isoptera, Mantodea, and Paleozoic

Roachoids. The roachiods had large pronota covering the head, large flattened coxae for running, a dorso-ventrally flattened body, tegminous forewings, and a large external ovipositor. The only morphological difference between the modern cockroaches and the ancient roachoids is the presence of the long external ovipositor on the roachoids. Therefore, cockroaches have change very little over the last 300 million years. The Paleozoic roachoids first appeared in the early part of the late Carboniferous period (~ 320 million years ago) and lasted until the late Permian period (~ 255 million years ago) (Grimaldi, 2005). Schneider (1983, 1984) reviewed and revised the classification of the fossilized roachoids, and divided them into three families: Archimylarididae, Necymylacrididae, and Mylacrididae. As these families became extinct they gave rise to new families during the Mesozoic period such as Raphidiomimidae. This family was a predatory roachoid resembling a mantid, that had a long progonathus head, long palps, narrow pronotum that exposed the head, long slender wings, fore femur and fore tiba had two rows of spines and the fore legs appeared to be held forward (Grimaldi, 2005).

Cockroaches are thought to be closely related to termites. Some cockroach species have flagellated protozoa in their digestive systems similar to those of termites. Some species of the cockroach genus *Panesthia*, burrow into tunnels in the soil and loose their wings like termites, which would inhibit their movement in the burrows (Grimaldi, 2005). One genus of cockroach that is considered the “missing link” between the termites and cockroaches is *Cryptocercus*. *Cryptocercus* is so named because its cerci are recessed between the last abdominal segments (supraanal and subgenital plates). The young of *Cryptocercus* feed on their mother’s excretions that contain protists from her hindgut that digest cellulose (proctodeal trophallaxis). This behavior is similar to termites and how they pass along the protists to young. *Cryptocercus punctulatus* (Scudder) is the most common and well studied of this genus, but other species are found in several locations worldwide mostly in undisturbed temperate mountain forests. There are several species of *Cryptocercus* that have recently been discovered in the southeastern United States: *C. garciai* (Burnside, Smith and Kambhampati) from northwest Georgia, *C. darwini* (Burnside, Smith and Kambhampati) from Tennessee, Alabama, and North Carolina; and *C. wrighti* (Burnside, Smith and Kambhampati) from North Carolina and Tennessee. *Cryptocercus* spp. shares the same distinctive protists as two other families of termites (Termitidae and Rhinotermitidae) (Grimaldi, 2005).

Cockroaches are also related to Mantodea, in part, because both orders deposit their eggs within an ootheca. In the cockroaches, most oothecas are hard bean-like egg cases that have two rows of eggs and are deposited on the habitat substrate using a glue-like secretion. In the mantids, the ootheca is extruded onto the habitat substrate in a froth-like excretion that hardens to a foam consistency; mantid oothecae also contain two rows of eggs. Another morphological feature that ties all three orders together is the proventriculus. The proventriculus is a gizzard-like organ that aids in the digestion of food. The proventriculus is located between the foregut and mesentron in the digestive tract. The proventriculus has many folds in it called plicae. The plicae have sclerite tooth-like structures (acanthae) that help break up and digest the food. The morphology of the plicae and acanthae are very similar in the three orders.

Systematics and Taxonomy

The study of the diversity of organisms and the relationship between them is termed systematics and the related field of taxonomy classifies these organisms.

Cockroaches are characterized by their dorsoventrally flattened body where the head is largely concealed beneath the pronotum. Cockroaches have prominent cerci, chewing mouthparts, and prominent antennae. Cockroaches do not have enlarged hind femurs used for jumping as some of their closest relatives (Orthoptera) do, but are still very fast runners. Cockroaches, once considered members of the order Orthoptera (Helfer, 1987), have been in recent years placed in their own order of Blattodea (Triplehorn, 2005), although some taxonomists prefer Dictyoptera (Roth, 1986). Cockroaches differ from members of the order Orthoptera because they lack a prominent ovipositor. The order Dictyoptera contains five families; Cryptocercidae, Blattidae (e.g., oriental cockroach, American cockroach), Polyphagidae (e.g., sand cockroaches), Blattellidae (e.g., brownbanded cockroach, German cockroach), Blaberidae (e.g., giant cockroach), with 4000-5000 species worldwide. About 70 species occur in the United States, of which 24 species have been introduced. There are roughly 11 species that are commonly found in the southeastern United States; oriental cockroach, *Blatta orientalis* L., American cockroach, *Periplaneta americana* L., Australian cockroach, *Periplaneta australasiae* (Fab.), Brown cockroach, *Periplaneta brunnea* Burmeister, Smokybrown cockroach, *Periplaneta fuliginosa* (Serville), Florida woods cockroach, *Eurycotis floridana* (Walker), Brownbanded cockroach, *Supella longipalpa* (F.), German cockroach, *Blattella germanica* (L.), Asian cockroach, *Blattella asahinai* Mizukubo, Surinam cockroach, *Pycnoscelus surinamensis* (L.), Cuban cockroach, *Panchlora nivea* (L.).

Biology and Ecology

Cockroaches are generally tropical to subtropical insects, which live outdoors, except for the relatively few that have adapted to living domestically with humans.

Cockroaches are omnivores and because of their tendency to aggregate, cannibalism can occur. Cockroaches are typically nocturnal insects although; there are many brightly-colored diurnal species. Many female cockroaches produce a volatile blend of sex pheromones when they are ready to mate. These pheromones can attract males from some distance and cause the male to initiate courtship behavior. Many male cockroaches have a tergal gland located on their 7-8 abdominal segments, which is used to attract a female during mating. The male will unfold his wings and use them to fan the tergal gland excretion to attract the female. Once the female has approached the male and started feeding on the secretion of the tergal gland, the male will mate with the female. The female cockroach has an organ called a spermatheca, which serves as a storage vessel for the male's sperm. Once mated, the female has a life-long supply of sperm which will be used to produce multiple oothecae during her life span. Once the female has produced her eggs and fertilized them with the sperm from her spermatheca they are enclosed in an egg case or ootheca. The appearance of the ootheca varies among the cockroach species and has taxonomic importance. Depending on the species of the cockroach, oothecae are deposited in the habit of the cockroach or carried around by the female until the eggs hatch. Females can carry the oothecae exposed (e.g., *B. germanica* and *B. asahinai*) or they will carry the oothecae inside them until they hatch which is termed ovoviviparity (e.g., *Gromphadorhina portentosa* Schaum). Once the eggs hatch, the small cockroaches, which are called nymphs, are independent and generally receive no assistance from the adults. Cockroaches are paurometabolous insects and the juveniles (nymphs) closely resemble the adults morphologically. Developmental times depend on temperature, relative humidity, and food supply. Typically, oothecae can contain 6-40 eggs depending on the species. Like the German cockroach, the female Asian cockroach carries an ootheca until the time the eggs hatch. The eggs are sensitive to water loss and therefore the oothecae remain attached to the female so she can supply it ample moisture. There are normally 6-7 instars in cockroaches and the nymphs tend to aggregate. Developmental time (from egg to adult) varies by species and ranges from 52-103 days so there can be several generations per year. Nymphs generally inhabit the same environment as adults and may also interact with adults. Cockroaches that are domiciliary can go through many generations per year, whereas outdoor species cockroaches may be limited to 2-3 generations a year depending on environmental conditions. Cockroaches will inhabit locations, which are occupied by other cockroach species and compete with them for resources; but they may not necessarily interact with them.

Economic Importance

Cockroaches can be an economic pest associated with the food industry and for homeowners. Cockroaches can mechanically vector or transmit certain pathogens such as *Salmonella spp***.** by physically contaminating food or food preparation surfaces. A cockroach infestation usually indicates poor sanitation practices. Infestations in homes do not necessarily imply poor housekeeping practices because cockroaches can build up populations outdoors, particularly large species such as the smokybrown cockroach, and move inside as they forage for food. Foodstuffs can be contaminated inside a house with cockroach feces, body parts, and pathogens. People can have allergies from cockroaches when populations are allowed to increase inside the home (Ebeling, 1978). Most of these allergies are asthmatic in nature and, in some cases, can be life threatening.

One particular species, *B. asahinai*, commonly known as the Asian cockroach was first described 1981 from Okinawa, Japan (Mizukubo, 1981), was introduced into Florida in 1986. The first account of *B. asahinai* was limited to 3 adjacent counties in Florida from Tampa to Lakeland (Brenner, 1988). By 1993, *B. asahinai* had spread to 30 counties in Florida. *Blattella asahinai* was also found in citrus groves in Florida as well, and several studies evaluated whether *B. asahinai* was a pest on citrus (Brenner, 1988). By 1999, *B. asahinai* was distributed in 48 counties in Florida from Dade (southern part of Florida peninsula) to Nassau (in northern Florida) (Koehler, 1999). Populations of *B. asahinai* have also been reported in the Florida panhandle in Santa Rosa County (Donahoe, 2005). As of 2007 every county in Florida is now thought to have an established population of *B. asahinai* (P.G. Koehler, personal communication, 2007).

In early 2003, *B. asahinai* was identified for the first time outside of Florida in Dothan, AL. (Hu et al., 2005). These cockroaches were found in homes, churches, and public parks. A review of the literature revealed that there have been no studies on the spread of *B. asahinai* outside the state of Florida. Some basic biological studies have been conducted in the laboratory, but few field studies have been attempted. The possibility of *B. asahinai* as a pest in citrus groves was examined by Persad and Hoy (2004), and an unpublished trap efficacy study was conducted on Kiawah Island, South Carolina (Sitthicharoenchai, 2002). Brenner (1988) examined behavior, and distribution of *B. asahinai* in a microhabitat, and found *B. asahinai* had preferences for leaf litter and shady areas. We know little of the habitat preferences, or population dynamics, and predator-prey interactions of *B. asahinai* in the southeastern United States.

One objective of this research was to determine the distribution of *B. asahinai* outside of the state of Florida. With the mass movement of people and products from Florida throughout the southeastern United States, *B. asahinai* has the opportunity to spread to Alabama, Georgia, and South Carolina and to become established. These southeastern states have relatively similar climates and habitats and could be ideal for establishment of populations of *B. asahinai.* Another objective of this research project was to evaluate the seasonal population dynamics of *B. asahinai* in the field. The last objective of this research was to evaluate the seasonal temperature sensitivity, measured as the CTMax and CTMin, of field populations and compare these values to those determined for laboratory cultures.

DISTRIBUTION OF *BLATTELLA ASAHINAI* MIZUKUBO IN SOUTHERN ALABAMA AND GEORGIA

Since the introduction of the Asian cockroach, *Blattella asahinai* Mizukubo, into central Florida in 1986 (Brenner, 1987), their range has increased dramatically. The Asian cockroach can become a pest to homeowners when it is attracted to lights inside the home (Roth, 1986). Originally *B. asahinai* was described from three counties but within five years had spread to 33 surrounding counties in Florida (Brenner, 1987). By 1999 *B. asahinai* had spread to 48 counties throughout Florida (Koehler 1999) and by 2005 had reached the furthest most Florida panhandle county of Santa Rosa (Donahoe 2005). This species has the potential to spread up the East coast and as far West as California and Washington limited only by cold temperatures (Koehler and Patterson, 1987). During the warm summer months *B. asahinai* has the potential to build up into large numbers and become a peridomestic pest problem for homeowners (Brenner, 1987). This species will fly at night to light sources and then find its way into the home where it becomes a nuisance for homeowners. If not properly identified the wrong pest control strategies may be employed yielding little or no control.

There has not been a formal distribution study of *B. asahinai* conducted outside of Florida except a dissertation that examined traps and populations of *B. asahinai* on

Kiawah Island, South Carolina (Sitthicharoenchai, 2002). The purpose of this study was to define the distribution of *B. asahinai* field populations in southern Alabama and Georgia. In August 2003, a county agent in Dothan, AL (Houston County) encountered what he described as a flying German cockroach, *B. germanica* (L.) and called Dr. A. G. Appel at Auburn University for information. It was later determined that the cockroach in question was *B. asahinai*. After several visits to Dothan, AL it was determined that a large field population was established in this area (Hu et al., 2005).

Since there are no previously published studies of the distribution of *B. asahinai* outside the state of Florida, this finding represented a new distribution of *B. asahinai*. Because *B. asahinai* was established in the wiregrass area of South Alabama, we needed to determine how far and to what extent *B. asahinai* had increased its range in AL and if it was also established in the bordering state of Georgia, and how its distribution might have expanded. We therefore sampled counties in the states of Alabama and Georgia to map the distribution of *B. asahinai*. From the distribution maps we suggest how *B. asahinai* has expanded its distribution so rapidly.

Materials and Methods

Survey Methods A visual survey for *B. asahinai* was conducted in the spring and summer of 2005 and 2006. The survey was conducted along main federal and state highways, stopping every 10 km, making periodic checks of habitats that would harbor populations of *B. asahinai*. This survey was conducted on sunny days to facilitate the observation of *B. asahinai*, and lasted until sunset. Since it is likely that *B. asahinai* populations spread by “hitching” rides on vehicles, we examined those places frequented by travelers such as motels, fueling stations, rest areas, and state parks. These locations had extensive landscaping including the use of leaf litter mulch (pine straw). Leaf litter and shady areas were the most likely areas to harbor populations of *B. asahinai* (Brenner, 1988). We sampled at least two locations in each county unless *B. asahinai* was found at the first site. If a county contained populations of *B. asahinai* then all counties contigous to that county were sampled for *B. asahinai*. In Alabama, we sampled by starting on US Highway 431/231 in Houston County at the Florida state line and traveling North. We also traveled along US Highway 231 northward when it split from US Highway 431. We also sampled all major cities in Alabama with populations of over 100,000 since they could be destinations for people traveling to and from Florida.

In Georgia, the survey of *B. asahinai* was conducted by starting on Interstate I-75, which enters the state from Florida, and transverses the state from North to South and continuing to the Tennessee line. We traveled along Interstate I-75 and made two stops per county to sample for the presence of *B. asahinai*. If the presence of *B. asahinai* was established, we sampled the surrounding counties until a county was reached that did not have *B. asahinai*. From I-75, each county adjacent to one with *B. asahinai* was sampled on both sides of the interstate. Interstate I-85 was surveyed from the Alabama state line across Georgia to the South Carolina state line. Every county along I-85 was sampled and no populations of *B. asahinai* detected. Interstate I-20 from Atlanta to the South Carolina state line was sampled and no populations of *B. asahinai* were detected. Interstate I-95 from the South Carolina state line south to the Florida state line was conducted and no populations of *B. asahinai* were detected.

Because species description for the genus *Blattella* are based on the morphology of adult males (Roth, 1986), only specimens of adult males were used to confirm the identification of *B. asahinai*. Field populations were positively identified by examination of the male dorsal abdominal tergal glands (Roth, 1986). The male tergal glands of *B. asahinai* (Fig 1A) differ considerable from *B. germanica* (Fig. 1B) in shape and presence or absence of posterior margins of the tergal gland. Once a field population was detected, male *B. asahinai* were collected and transported back to the laboratory for confirmation. The abdomens of the specimens were removed just behind the thorax. An excised abdomen was submerged in 10% KOH solution for 24-48 h. The cleared abdomen was removed from the 10% KOH solution and the 7th and 8th tergites were dissected from the rest of the abdomen by cutting lengthwise down the side of the abdomen through the lateral pleural membrane. The tergites were washed in distilled water for 15 min and dehydrated in a series of ethanol solutions. Tergites were bathed in 70% ETOH for 15 min then transferred to a 95% ETOH solution for 15 min, and finally to a 100% ETOH solution. The dehydrated tergal glands were transferred to a Xylene solution for 24-28 h. Tergal glands were mounted on a standard glass (VWR Scientific Inc.) microscope slide with Permount® (Fisher Scientific Co.). The slide was then placed on a GCA Corporation Precision microscope slide warmer and allowed to cure for 7 d at 32°C. The tergal glands were then examined under the microscope. The male tergal gland of *B. asahinai* has an open margin on the posterior edge of the glands in contrast to a closed, ridge like margin of *B. germanica* (Roth, 1986).

Results

We sampled a total of 32 counties in Alabama, and 62 counties in Georgia which account for 48% and 39% of the total counties in those states, respectively (Table 1). Out of these counties, we detected populations of *B. asahinai* in 7 counties in Alabama, and 8 counties in Georgia (Table 1). The counties in Alabama that contain populations of *B. asahinai* are: Baldwin, Barbour, Coffee, Dale, Geneva, Henry, and Houston. These counties are all located in the southeast corner of the state know as the wiregrass area. These counties are primarily classified as being in the coastal plain region, which is dominated by sandy-loam soils, pine trees, and herbaceous plants (Kush, 1998). No populations of *B. asahinai* were found North of Barbour County. The northern most established population of *B. asahinai* in Alabama is at Lake Point State Park, Barbour County (31°58’52.37” N, 85°06’06.32” W).

The counties in Georgia that contain populations of *B. asahinai* are: Cook, Crisp, Dooly, Houston, Lowndes, Tift, Turner, and Worth. These counties are all located on US Interstate I-75 which runs through the middle of the state from Florida to Tennessee. Every county on I-75 from the Florida line to Perry, GA. (32°26’16.12” N, 83°44’49.86” W) contained populations of *B. asahinai*. The northern most population of *B. asahinai* in Georgia is located at the Georgia State Fair Grounds, in Perry, Houston County (32°26’16.12” N, 83°44’49.86” W).

Discussion

In Alabama a total of 32 counties were sampled of which 7 had populations of *B. asahinai* (Table 1). The area of Alabama and Georgia that had populations of *B. asahinai* was 14,075 km^2 and 7492 km^2, respectively (Fig. 4). Alabama has more than twice the land area with *B. asahinai* as Georgia (Figure 5). In Georgia, a total of 62 counties were sampled 8 of which had populations of *B. asahinai*. These 8 counties are representing a land area of 7492 km^2. Figure 2 serves as a record of the distribution of *B. asahinai* in Alabama and Georgia during 2005-2006. The advancing northern distribution of *B.*

asahinai is not a broad, uniform advancement, instead, what appears are finger-like projections of advancing distribution into southern Alabama and Georgia (Fig.2).

By overlaying the major highways in Alabama, Georgia, and Florida on the distribution map of *B. asahinai*, it is evident that *B. asahinai* distribution is coincident with major interstates and highways (Fig. 2). It is likely that transportation vehicles facilitate the distribution of *B. asahinai*. Interstate I-75 that transverses most of Florida and all of Georgia (North to South) is a major transportation route for tourists and commerce. Since Florida is a major producer of fruits and vegetables, as well as many horticultural and ornamental crops, there is a large movement of commercial open flatbed trucks that are capable of transporting *B. asahinai* unknowing into other states throughout the year. Interstate I-10 that transverses Florida (East to West) in the Florida panhandle is a major transportation route that again would facilitate transportation of *B. asahinai* into other states. Interstate I-10 also travels through Baldwin County, AL where an established population of *B. asahinai* was detected. Interstate I-10 travels West from Florida through Alabama, Mississippi, and Louisiana, and through Houston, Texas. Mr. Jeffery Tucker submitted a *B. asahinai* specimen to Appel/ Snoddy for positive identification in 2006. The *B. asahinai* specimen was collected behind a car dealership on Interstate I-10 in Houston, Texas.

We propose that the large field population of *B. asahinai* in southeastern Alabama originated in Florida and entered Alabama via US Highway 431/231. This is a major transportation route for tourists that visit the crystal white sand beaches of the Gulf of Mexico. Tourists that come from Georgia, Tennessee or further North must use US Highway 431/231 to access the Gulf of Mexico beaches. Specimens could be unknowingly collected in nearby areas and transported back into Alabama. From Dothan, AL US 431/231 splits into different directions but both continue in a northerly direction facilitating further dissemination of *B. asahinai* into different areas of Alabama.

In Alabama, *B. asahinai* has not advanced its distribution as far North as it has in Georgia, this may be due in part to the winter temperatures (Fig. 3). The South Georgia costal plain climate is more humid and warmer in winter than the northern sections because it is influenced by both the Atlantic Ocean and the Gulf of Mexico (Fig. 2). The wiregrass area of Alabama, where field populations of *B. asahinai* are most prevalent, is influenced only by the Gulf of Mexico (Fig. 2). We propose that *B. asahinai* ranges further North in Georgia than in Alabama because of the more moderate winter climate. Other insects have similar distribution such as "Love bugs", *Plecia nearctica* (Hardy), and Eye Gnats, *Hippelates pusio* (Loew).

From 2005-2006 we observed no northerly shift in the range of *B. asahinai* past Houston County, Georgia; this is again, is probably due to seasonal temperatures climatic differences between North Georgia and South Georgia. The winter is colder in North Georgia with temperatures being as much as 4°C lower in the North than the South (AWIS, 2005). The temperature in North Alabama is cooler in the winter than in South Alabama area (Fig. 3). Based on our field surveys in 2005 and 2006, it appears that *B. asahinai* is restricted to the costal plains and gulf coasts regions of the continental United States.

The red imported fire ant, *Solenopsis invicta* Buren, is an introduced invasive species, brought into this country at the port of Mobile in the 1930s (Vinson, 1997). Since its introduction, *S. invicta,* has expanded its distribution throughout the southeastern

United States, and only is limited by climatic conditions (cold temperatures) (Vinson, 1997). The red imported fire ant is typically moved or transported in soil, but mated wing females have been detected in open beds of trucks (Vinson, 1997). Since its introduction into Florida in 1986, *B. asahinai* has expanded its distribution to four additional states in just 11 years, much like the *S. invicta*. It was originally thought that *S. invicta* would be limited in range by temperatures of -12.3°C, but more recently the estimate has been revised to include -17.8°C (Vinson, 1997). Under favorable environmental conditions *B. asahinai* could range along the Atlantic coast as far North as Maryland, and as far West as California and the Pacific coast of Washington (Brenner, 1988). With the recent detection of *B. asahinai* in AL, GA, and Houston, TX, it is plausible that the distribution of this species is facilitated by mechanical transportation. Based on the ability to utilize transportation, spread of approximately 1579 km in approximately 20 years, a relative direct transportation route (Interstate I-10), and similar habitats and climates, we could observe populations of *B. asahinai* in California by 2017. Similar to *S. invicta*, climatic conditions should be the only parameter governing the distribution of *B. asahinai* in the continental United States.

Table 1. Comparisons of Alabama and Georgia.

	Alabama	Georgia
Total Land Area	131,426 km^2	153,873 km^2
Total Land Area with *B. asahinai*	14,075 km^2	7,492 km^2
Total Number of Counties	67	159
Number of Counties Sampled	32	62
Number of Counties with *B. asahinai*	7	8

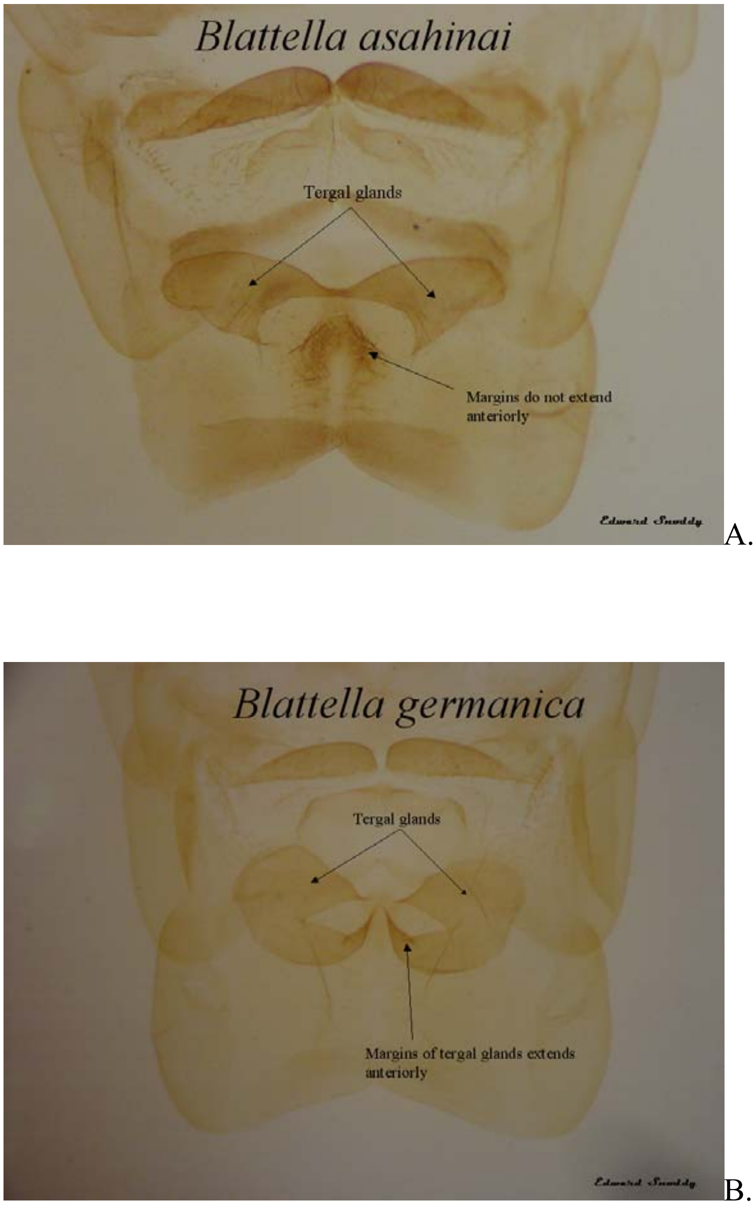

Figure 1A and 1B. Microscope view (10X magnification) of dissected male abdominal tergal glands of A., *B. asahinai* and B., *B. germanica*.

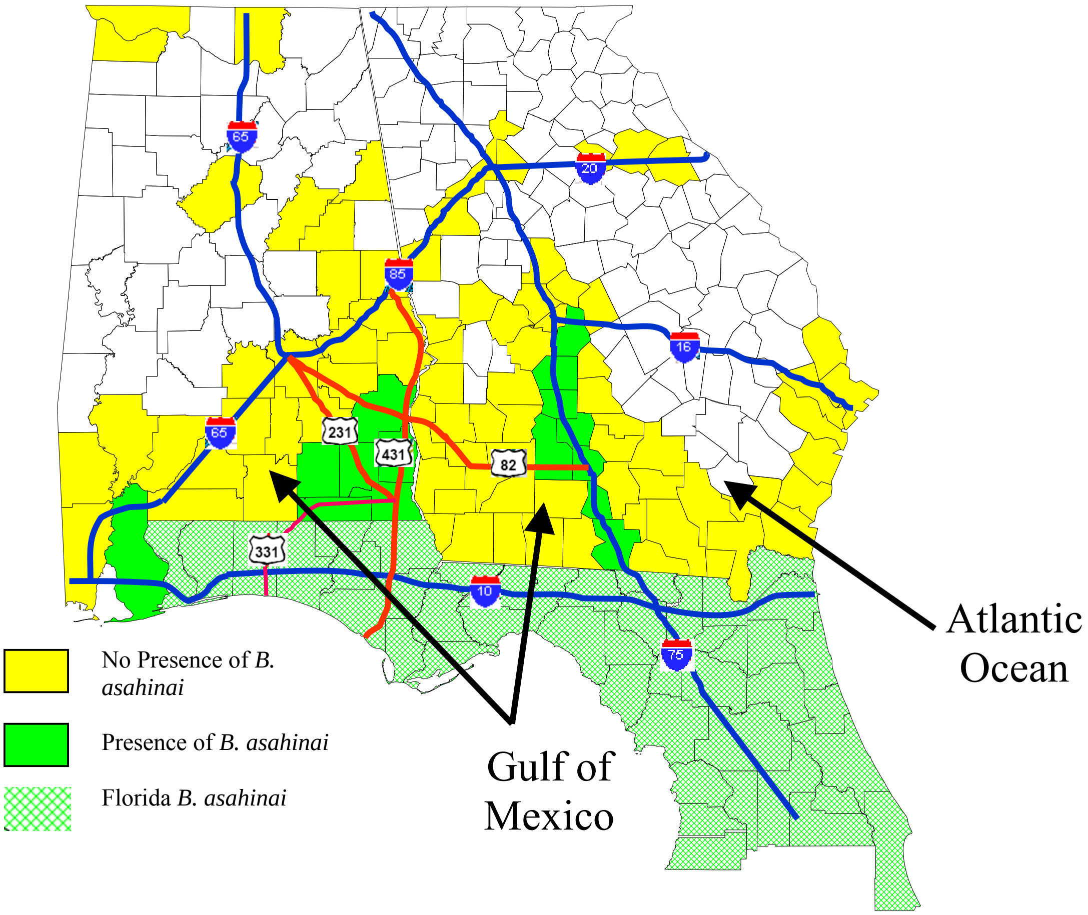

Figure 2. Distribution of the Asian cockroach, *B. asahinai* in Alabama, Georgia, and northern Florida.

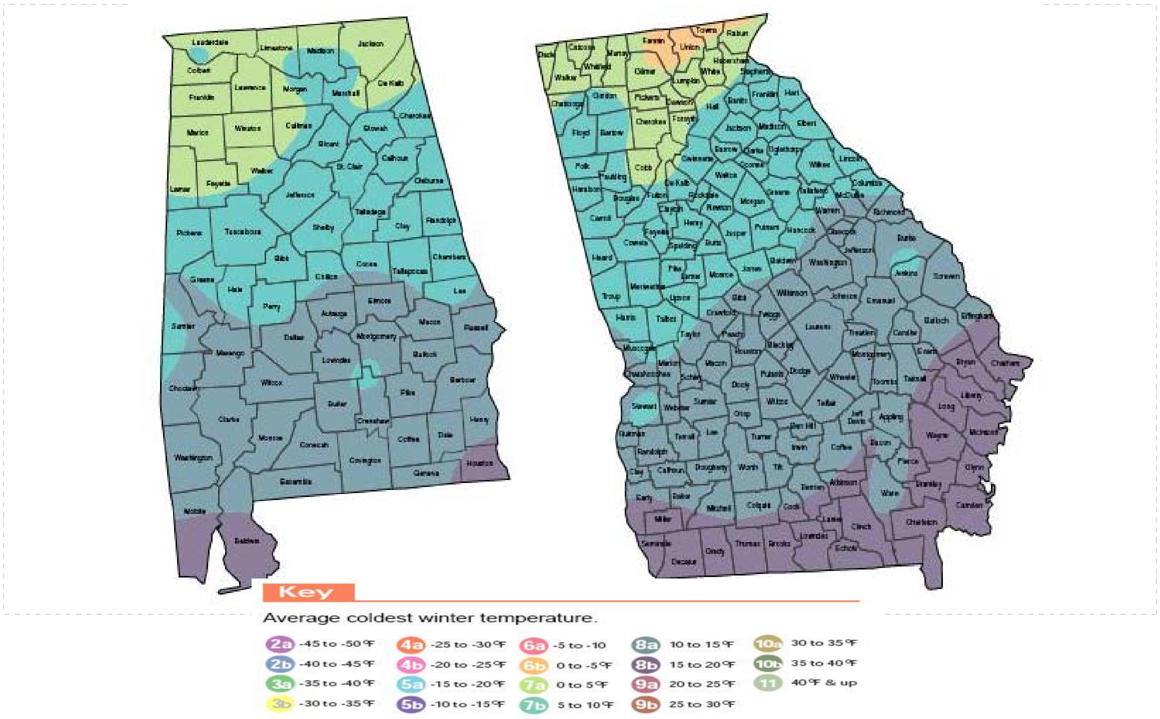

Figure 3. Winter temperatures and plant hardiness zones of Alabama and Georgia.

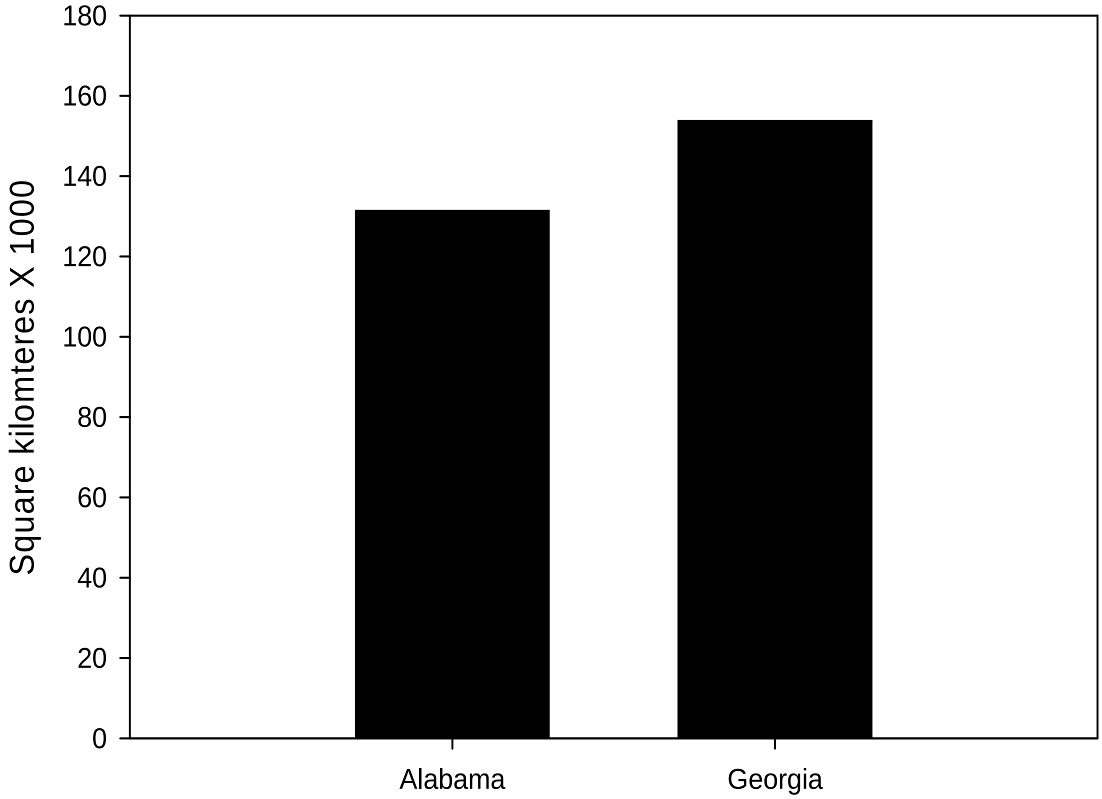

Figure 4. Total land area of Alabama and Georgia.

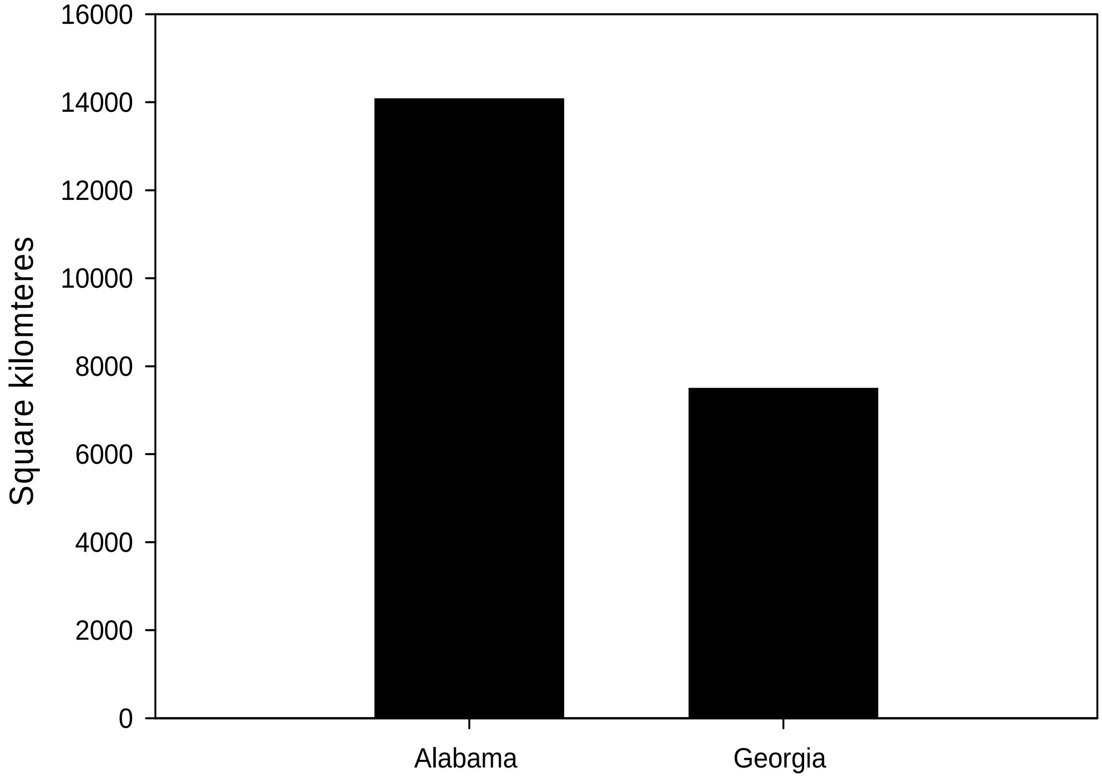

Figure 5. Total land area by state that contains *B. asahinai*.

POPULATION DYNAMICS OF *BLATTELLA ASAHINAI* MIZUKUBO IN SOUTHERN ALABAMA

Since the introduction of the Asian cockroach, *Blattella asahinai* Mizukubo, into Florida in 1986, there has been little field work other than some basic ecology and behavior (Brenner et al., 1988). Basic biology studies were conducted in the laboratory to determine the mean development time (Atkinson et al., 1999). In 1999, Atkinson et al. found that mean development and adult longevity of *B. asahinai* at 25°C with 50% RH were 67.8 and 103.5 d for females and 65.7 and 48.5 d for males. Environmental factors and anthropogenic factors such as low temperature or low humidity may skew developmental time of *B. asahinai* in the field. Brenner et al. (1988) used numerous traps place at ground level around a home, and examined distribution patterns and habitat preferences. *Blattella asahinai* showed a preference for shady areas with abundance of leaf litter (Brenner et al.,, 1988). Populations of *B. asahinai* can reach between 30,000-205,000 per acre in Florida (Richman, 2005). In 1990, Brenner reported that adults were not present in field populations during January and December, and again in June and July. This suggests there are two broad generations per year in central Florida. Since *B. asahinai* has expanded its range into southern Alabama and Georgia (Chapter 1) there have been no published studies on population dynamics in these states. Since temperature and anthropogenic factors may influence cockroach population, and the climate of

Alabama and Georgia is different than that of central Florida, and the dynamics of field populations of *B. asahinai* may be different.

Since the most closely related species to *B. asahinai* is *B. germanica* (L.), and *B. germanica* has been studied extensively, similar parameters may apply to *B. asahinai*. There are differences in the dynamics of foraging among stages of *B. germanica* (Cloarec and Rivault, 1991). Younger, immature stages of *B. germanica* will not forage as far as adults, this may also be true for *B. asahinai*, especially since *B. asahinai* has the ability to fly and *B. germanica* (L.) cannot.

The objective of this study was to determine the population dynamics of *B. asahinai* in the field. We compared the seasonal abundance of populations and developmental stages (means per m^2) and the distribution of different stages of *B. asahinai* in peridomestic habitats.

Materials and Methods

Three sites were selected based on habitat and location in Dothan, Houston County, AL that had established populations of *B. asahinai*. All sites were public parks owned by the City of Dothan. Site I was selected because it contained predominately pine straw mulch and no litter. Site II was selected because it contained predominately turf habitat and a moderate amount of litter. Site III was selected because it was covered with predominately hardwood leaf litter and heavily littered with residential trash. The sites were sampled for *B. asahinai* every 14 d starting on 26MAY2005 and were sampled continuously until September 2006 when populations plummeted due to the onset of cool weather. Sample intervals were reduced to every 30 d during winter months when *B. asahinai* was relatively inactive. Three random locations were sampled at each site consisting of population counts of all stages of *B. asahinai*. In each of the random samples, three areas were sampled: vegetation, ground surface, and leaf litter. Ambient temperature and relative humidity, as well as time of day, and weather conditions were recorded at each site. A digital hydrothermograph with a probe (Model 3700, Cole-Palmer, NJ) was used to record the temperature and RH.

A sweep net was used to sample vegetation for *B. asahinai*; five sweeps of the vegetation for each sample. Next, a standard white 5-gallon (~19 L) plastic paint bucket (Lowe's Home Improvement, Opelika, AL) was used to sample the ground surface and leaf litter. We removed the bottom of the bucket and sharpened the sides to produce a sampling device. For sampling, the bucket was placed on the ground and driven into the leaf litter. This procedure allowed *B. asahinai* to fly from the enclosed leaf litter to the sides of the bucket and to run upwards on the inside walls. The cockroaches and other arthropods could then be counted. Nymphs of *B. asahinai* would also run up the side of the bucket and could be easily counted. Driving the bucket into the leaf litter assured an accurate count of *B. asahinai* in that confined space with a minimum of escapees. Temperature and relative humidity readings were taken under the leaf litter inside the bucket. The probe was inserted and temperature and RH readings recorded at the mulch-soil interface. Next, the leaf litter enclosed by the bucket was placed in plastic bags (Hefty Ziploc® freezer bags), labeled, and returned to the laboratory in a cooler. The bucket was left in place and a 1 min 30 sec visual count was taken for cockroaches in a 1-meter radius circle. Leaf litter samples were placed in a Berlese funnel for complete collection of the cockroaches in that sample. A screen mesh was placed over the top of the funnel to contain the cockroaches. Leaf litter samples were exposed to a 60-watt light bulb for 48 h and the specimens were collected into 70% ethanol. Specimens of *B. asahinai* were categorized according to sex of the adults, and size of the nymphs. Nymphs from 1-3 mm were categorized as small, 4-7 mm as medium, and > 7 mm were considered large nymphs.

Statistical Analysis

All data were converted to cockroaches/m^2 for comparisons using an Excel spreadsheet. Population means (± SE) were correlated with temperature and RH using SAS software (SAS Institute, 2007). Results of data analysis were plotted using SigmaPlot (ver. 9.0) software.

Results

For the 2 yr of this study (2005-2006), all sampling methods and sampling sites, a total of 1173 *B. asahinai* were collected. Overall, the collections consisted of 24% adult females, 16% adult males, 10% small nymphs, 28% medium nymphs, and 22% large nymphs. In 2005, there were a total of 725 *B. asahinai* collected which consisted of 20% adult females, 14% adult males, 9% small nymphs, 33% medium nymphs, and 24% large nymphs (Fig. 6). In 2006, only 448 total *B. asahinai* were collected which consisted of 32% adult females, 20% adult males, 10% small nymphs, 19% medium nymphs, and 19% large nymphs (Fig. 6).

In 2005, visual count means for adult female *B. asahinai* ranged from 0 m^2 on 07JUL2005 to 10.22 ± 5.11 per m^2 on 15SEP2005 (Table 6), whereas bucket means ranged from 0 m^2 to 2.77 ± 1.46 per m^2 for 07JUL2005 to 09JUN2005, respectively (Table 4), and Berlese means ranged from 0 per m^2 on 26MAY2005 to 0.55± 0.55 per m^2 on 29SEP2005 (Table 2). Visual count means for adult males ranged from 0 per m^2 to

7.08 ± 2.96 per m^2 for 07JUL2005 to15SEP2005, respectively (Table 6), whereas bucket means ranged from 0 per m^2 on 25OCT2005 to 2.78 ± 1.68 per m^2 on 02SEP2005 (Table 4), and Berlese means ranged from 0 per m^2 to 1.11 ± 0.73 per m^2 for 26MAY2005 to 27DEC2005, respectively (Table 2). Visual count means for small nymphs ranged from 0 per m^2 on 27DEC2005 to 7.47 ± 3.49 per m^2 on 02SEP2005 (Table 6), whereas bucket means ranged from 0 per m^2 to 3.89 ± 2.32 per m^2 for 27DEC2005 to 24JUN2005, respectively (Table 4), and Berlese means ranged from 0 per m^2 on 26MAY2005 to 1.67 ± 1.17 per m^2 on 07JUL2005 (Table 2). Visual count means for medium nymphs of ranged from 0 per m^2 to 10.62 ± 3.79 per m^2 for 27DEC2005 to 02SEP2005, respectively (Table 6), whereas bucket means ranged from 0 per m^2 on 27DEC2005 to 8.33 ± 1.86 per m^2 on 02JUL2005 (Table 4), and Berlese means ranged from 0 per m^2 to 1.67 ± 1.17 per m^2 for 09JUN2005 to 24JUN2005, respectively (Table 2). Visual count means for large nymphs of ranged from 0 per m^2 on 26MAY2005 to 12.98 ± 3.40 per m^2 on 02SEP2005 (Table 6), whereas bucket means ranged from 0 per m^2 to 7.22 ± 2.37 per m^2 for 26MAY2005 to 02SEP2005, respectively (Table 4), and Berlese means ranged from 0 per m^2 on 26MAY2005 to 1.11 ± 1.11 per m^2 on 20JUL2005 (Table 2).

In 2006, visual count means for adult female *B. asahinai* ranged from 0 per m^2 to 9.83 ± 3.87 per m^2 for 27JAN2006 to 18AUG2006, respectively (Table 7), whereas bucket means ranged from 0 per m^2 on 27JAN2006 to 1.67 ± 1.17 per m^2 on 19JUL2006 (Table 5), and Berlese means ranged from 0 per m^2 to 2.78 ± 2.77 per m^2 for 09JUN2006 to 24MAY2006, respectively (Table 3). Visual count means for adult males ranged from 0 per m^2 on 27JAN2006 to 6.68 ± 1.04 per m^2 on 03AUG2006 (Table 7), whereas bucket means ranged from 0 per m^2 on 27JAN2006 to 2.22 ± 1.46 per m^2 on 19JUL2006 (Table

5), and Berlese means ranged from 0 per m^2 to 1.11 ± 1.11 per m^2 for 09JUN2006 to 24MAY2006, respectively (Table 3). Visual count means for small nymphs ranged from 0 per m^2 on 27JAN2006 to 3.93 ± 1.04 per m^2 on 18AUG2006 (Table 7), whereas bucket means ranged from 0 per m^2 on 27JAN2006 to 0.55 ± 0.55 per m^2 on 19JUL2006 (Table 5), and Berlese means ranged from 0 per m^2 to 4.44 ± 2.27 per m^2 for 09JUN2006 to 27JAN2006, respectively (Table 3). Visual count means for medium nymphs of ranged from 0 per m^2 on 27JAN2006 to 6.29 ± 1.71 per m^2 on 03AUG2006 (Table 7), whereas bucket means ranged from 0 per m^2 on 27JAN2006 to 3.89 ± 1.82 per m^2 on 19JUL2006 (Table 5), and Berlese means ranged from 0 per m^2 to 2.78 ± 1.21 per m^2 for 09JUN2006 to 27JAN2006, respectively (Table 3). Visual count means for large nymphs ranged from 0 per m^2 on 27JAN2006 to 7.47 ± 3.93 per m^2 03AUG2006 (Table 7), whereas bucket means ranged from 0 per m^2 on 27JAN2006 to 1.67 ± 1.66 per m^2 on 07JUL2006 (Table 5), and Berlese means ranged from 0 per m^2 on 09JUN2006 to 2.78 ± 1.68 per m^2 on 27JAN2006 (Table 3).

Blattella asahinai had a positive correlation with temperature ($P < 0.0001$) for total stages. However, when examined as independent stages small nymphs were the only stage that didn’t have a significant correlation ($P=0.09$) with temperature. During the 2005-2006 sampling periods, average daily temperatures ranged from 8.9°C to 28.5°C (AWIS, 2005 and 2006).

Discussion

During 2005 and 2006, mean visual populations of all stages of *B. asahinai* reached their zenith in late August-early September (Fig. 7). All visual counts were at ground level; *B. asahinai* was never observed or collected in sweep net samples. When disturbed *B. asahinai* would fly to escape, and when an individual encountered vegetation it would land, but quickly return to ground level. In 2005 mean visual populations for all stages of *B. asahinai* started increasing in early May with overlapping generations observed before populations declined sharply with the onset of cool weather in September (Fig. 7). In 2006, mean visual counts of all stages of *B. asahinai* began to increase in early May but dropped sharply in early June (Figure 7). It is possible that the sudden decline in counts was in part due to a prolong absence of rainfall (± 8 weeks) (AWIS, 2006). Data show a concurrent increase in Berlese counts at this time in June. By late June 2006, when normal precipitation resumed, mean visual counts of all stages of *B. asahinai* resumed their growth rate and reached their zenith in late August, and then decline sharply with the onset of cool weather (Figure 7). During 2005-2006 there was a significant positive correlation between total means of *B. asahinai* and temperature ($P < 0.0001$). There was also a significant positive correlation between total means of *B. asahinai* and RH ($P < 0.0001$).

In early May 2005, *B. asahinai* populations were predominately adults (50% females and 25% males), with some nymphs (25%), and higher populations of nymphs occurring in the next two generations (Figure 7). Again in 2006, we observed a similar adult to nymph ratio during the same time period (Figure 7). Interestingly, during the drought conditions of June 2006, when we observed a sharp decline in visual counts of all stages of *B. asahinai*, there was a sharp increase in Berlese counts during the same time period (Figure 9). The increase in Berlese sample counts demonstrates that all stages of *B. asahinai* moved from the ground level surface habitat and leaf litter into a cooler and moister habitat. This movement demonstrates that *B. asahinai* has the ability to escape adverse environmental conditions (e.g., temperature and low RH) buy burrowing down into cooler and moister leaf litter and into the soil. In the 2005-2006 bucket samples, we observed distributions and population sizes similar to those of the 2005-2006 visual means (Figure 8). In contrast to the visual and bucket means we observed higher population numbers of the different stages of *B. asahinai* during the colder months of 2005-2006 in the Berlese samples (Figure 9). This demonstrates again that *B. asahinai* has the ability to burrow down into the soil when faced with cold temperatures during the winter months.

We observed that laboratory and field populations of different stages of *B. asahinai* have the ability to acclimatize and acclimate to their environment (Chapter 3). Temperature sensitivity varies with environmental temperatures; higher environmental temperatures result in higher CTMax and CTMin values. Lower environmental temperatures result in lower CTMax and CTMin values. Acclimation enables *B. asahinai* to better tolerate increasing or decreasing ambient temperatures (Chapter 3).

The Berlese samples were highly skewed towards the nymphal stages (Fig.9). Berlese samples suggest that when *B. asahinai* is faced with adverse environmental conditions such as low temperature and low humidity, they will burrow into the substrate to escape those conditions. By recording the different stages of *B. asahinai* using three different sampling methods, we were able to show the stage distributions as they occur over time in the field. During January through June the population of *B. asahinai* is skewed towards adult females and nymphs (small, medium, and large). Populations build from early May through late August when they decline sharply; females and nymphs (small, medium, and large) over-winter in the substrate. In January, populations are ~10%

adults and ~90% nymphs, but his changes into ~100% nymphs from February through May, when the large nymphs go through eclosion into adults. During January, *B. asahinai* can only be detected in Berlese samples since they over wintering in the leaf litter and soil. As temperatures warm in the spring, Berlese sample counts start to decline and visual counts start to increase. There is an overlap between the Berlese samples and visual counts during the transition into warmer weather in the spring, as adults are much more mobile than nymphs, and adults are picked up more frequently in the visual and bucket samples at that time.

Field observations during 2005 and 2006 show that *B. asahinai* has 3 generations per year in Alabama, which differs from the observations of Brenner et al. (1988). We did observe, like Brenner et al. (1988), that visual and bucket counts were absent of adults during certain times of the year. But concurrent Berlese samples detected some adult populations. Brenner et al. (1988) examined a microhabitat whereas our observations were recorded from three different macrohabitats. The ability of *B. asahinai* to acclimatize and burrow down to avoid adverse conditions would allow *B. asahinai* to expand and colonize new habitats that are similar. With three general generations per year, *B. asahinai* has the ability to rapidly expand and colonize new ranges in the coastal plains and gulf coast regions

Table 2: Berlese sample means (± SE) for different stages of *B. asahinai* in Dothan, Alabama 2005.

Date	n	♀	♂	Small	Medium	Large
26MAY	9	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.55 ± 0.55	0.0 ± 0.00
09JUN	9	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00
24JUN	9	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	1.67 ± 1.17	0.0 ± 0.00
07JUL	9	0.55 ± 0.55	0.0 ± 0.00	1.67 ± 1.17	1.67 ± 1.17	0.0 ± 0.00
20JUL	9	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	1.11 ± 1.11
04AUG	9	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00
18AUG	9	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.55 ± 0.55	0.55 ± 0.55
02SEP	9	0.0 ± 0.00	0.0 ± 0.00	0.55 ± 0.55	0.0 ± 0.00	0.55 ± 0.55
15SEP	9	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00
29SEP	9	0.55 ± 0.55	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00
13OCT	9	0.55 ± 0.55	0.55 ± 0.55	0.0 ± 0.00	0.55 ± 0.55	0.55 ± 0.55
25OCT	9	0.55 ± 0.55	0.55 ± 0.55	1.11 ± 0.73	0.0 ± 0.00	0.55 ± 0.55
29NOV	9	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00
27DEC	9	0.0 ± 0.00	1.11 ± 0.73	1.11 ± 0.73	0.55 ± 0.55	0.0 ± 0.00

Table 3: Berlese sample means (± SE) for different stages of *B. asahinai* in Dothan, Alabama 2006.

Date	n	♀	♂	Small	Medium	Large
27JAN	9	0.55 ± 0.55	0.55 ± 0.55	4.44 ± 2.27	2.22 ± 1.21	2.78 ± 1.68
01MAR	9	0.0 ± 0.00	0.0 ± 0.00	1.67 ± 1.17	2.78 ± 1.21	2.22 ± 1.21
24MAR	9	0.0 ± 0.00	0.0 ± 0.00	3.89 ± 2.00	1.67 ± 1.17	0.0 ± 0.00
14APR	9	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	1.11 ± 0.73	2.22 ± 1.21
01MAY	9	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.55 ± 0.55	0.0 ± 0.00
12MAY	9	1.11 ± 0.73	0.55 ± 0.55	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00
24MAY	9	2.78 ± 2.77	1.11 ± 1.11	0.55 ± 0.55	0.0 ± 0.00	2.22 ± 1.46
12JUN	9	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00
23JUN	9	0.55 ± 0.55	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.55 ± 0.55
07JUL	9	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.55 ± 0.55	1.11 ± 1.11
19JUL	9	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.55 ± 0.55	0.0 ± 0.00
03AUG	9	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00
18AUG	9	0.55 ± 0.55	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00

Table 4: Bucket sample means (± SE) for different stages of *B. asahinai* in Dothan, Alabama 2005

Date	n	♀	♂	Small	Medium	Large
26MAY	9	2.22 ± 0.87	1.67 ± 0.83	0.0 ± 0.00	1.11 ± 0.73	0.0 ± 0.00
09JUN	9	2.77 ± 1.46	0.55 ± 0.55	0.0 ± 0.00	1.11 ± 0.73	0.55 ± 0.55
24JUN	9	0.55 ± 0.55	0.55 ± 0.55	3.89 ± 2.32	1.11 ± 0.73	0.55 ± 0.55
07JUL	9	0.0 ± 0.00	0.55 ± 0.55	3.33 ± 2.20	11.67 ± 3.99	3.89 ± 2.32
20JUL	9	1.11 ± 1.11	1.11 ± 0.73	1.11 ± 1.11	8.33 ± 1.86	5.00 ± 2.50
04AUG	9	0.55 ± 0.55	1.67 ± 0.83	1.11 ± 1.11	3.89 ± 2.00	5.00 ± 2.88
18AUG	9	1.11 ± 0.73	2.78 ± 1.46	0.55 ± 0.55	2.78 ± 1.46	2.78 ± 1.88
02SEP	9	1.11 ± 0.73	2.78 ± 1.68	0.0 ± 0.00	7.22 ± 2.64	7.22 ± 2.37
15SEP	9	2.22 ± 1.68	0.55 ± 0.55	0.0 ± 0.00	3.33 ± 2.20	2.78 ± 1.68
29SEP	9	1.67 ± 1.17	0.0 ± 0.00	0.55 ± 0.55	2.22 ± 1.68	0.55 ± 0.55
13OCT	9	0.55 ± 0.55	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00
25OCT	9	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00
29NOV	9	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00
27DEC	9	0.55 ± 0.55	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00

Table 5: Bucket sample means (± SE) for different stages of *B. asahinai* in Dothan, Alabama 2006

Date	n	♀	♂	Small	Medium	Large
27JAN	9	0.55 ± 0.55	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00
01MAR	9	0.0 ± 0.00	0.0 ± 0.00	0.55 ± 0.55	0.55 ± 0.55	0.0 ± 0.00
24MAR	9	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00
14APR	9	0.55 ± 0.55	0.55 ± 0.55	0.55 ± 0.55	1.11 ± 0.73	0.0 ± 0.00
01MAY	9	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.55 ± 0.55
12MAY	9	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00
24MAY	9	1.67 ± 1.17	1.67 ± 1.17	0.0 ± 0.00	0.55 ± 0.55	1.11 ± 1.11
12JUN	9	0.0 ± 0.00	0.55 ± 0.55	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00
23JUN	9	1.11 ± 0.73	0.0 ± 0.00	0.0 ± 0.00	1.11 ± 0.73	1.11 ± 0.73
07JUL	9	0.55 ± 0.55	0.55 ± 0.55	0.55 ± 0.55	2.78 ± 1.68	1.67 ± 1.66
19JUL	9	1.67 ± 1.17	2.22 ± 1.46	0.55 ± 0.55	3.89 ± 1.82	0.0 ± 0.00
03AUG	9	0.55 ± 0.55	2.22 ± 0.87	0.0 ± 0.00	3.89 ± 1.61	1.67 ± 0.83
18AUG	9	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00

Table 6: Visual means (± SE) for different stages of *B. asahinai* in Dothan, Alabama 2005.

Date	n	♀	♂	Small	Medium	Large
26MAY	9	0.79 ± 0.39	0.39 ± 0.39	0.0 ± 0.00	0.39 ± 0.39	0.0 ± 0.00
09JUN	9	1.57 ± 0.78	1.57 ± 1.04	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00
24JUN	9	1.18 ± 1.18	0.78 ± 0.78	1.57 ± 1.14	1.96 ± 1.96	0.0 ± 0.00
07JUL	9	0.0 ± 0.00	0.0 ± 0.00	2.36 ± 1.18	10.22 ± 3.21	1.18 ± 0.68
20JUL	9	0.78 ± 0.78	0.78 ± 0.78	1.96 ± 1.04	9.83 ± 2.57	3.93 ± 2.08
04AUG	9	3.93 ± 0.39	1.18 ± 0.68	1.96 ± 0.39	6.68 ± 2.83	5.90 ± 2.96
18AUG	9	4.32 ± 2.18	4.72 ± 2.36	0.0 ± 0.00	5.90 ± 1.80	4.72 ± 1.18
02SEP	9	8.26 ± 3.12	6.29 ± 2.39	7.47 ± 3.49	10.62 ± 3.79	12.98 ± 3.4
15SEP	9	10.22 ± 5.11	7.08 ± 2.96	1.57 ± 0.78	8.65 ± 2.75	7.08 ± 2.72
29SEP	9	8.65 ± 3.87	4.32 ± 1.71	0.78 ± 0.78	2.36 ± 0.39	3.93 ± 0.39
13OCT	9	3.93 ± 2.75	3.54 ± 2.96	1.18 ± 0.68	2.75 ± 0.39	1.96 ± 0.78
25OCT	9	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.39 ± 0.39	0.39 ± 0.39
29NOV	9	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00
27DEC	9	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00

Table 7: Visual means (± SE) for different stages of *B. asahinai* in Dothan, Alabama 2006.

Date	n	♀	♂	Small	Medium	Large
27JAN	9	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00
01MAR	9	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00
24MAR	9	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00
14APR	9	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00
01MAY	9	1.18 ± 0.68	0.39 ± 0.39	0.0 ± 0.00	0.39 ± 0.39	0.0 ± 0.00
12MAY	9	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.78 ± 0.39
24MAY	9	6.68 ± 3.42	2.75 ± 0.78	0.0 ± 0.00	0.78 ± 0.39	1.18 ± 0.68
12JUN	9	1.57 ± 1.57	3.14 ± 1.14	1.57 ± 0.78	0.78 ± 0.39	0.0 ± 0.00
23JUN	9	0.39 ± 0.39	0.39 ± 0.39	0.0 ± 0.00	0.0 ± 0.00	0.39 ± 0.39
07JUL	9	8.65 ± 3.99	2.75 ± 1.71	0.0 ± 0.00	2.75 ± 1.41	1.18 ± 1.18
19JUL	9	8.26 ± 4.46	5.11 ± 2.57	0.39 ± 0.39	1.57 ± 1.57	2.75 ± 2.18
03AUG	9	8.65 ± 1.41	6.68 ± 1.04	2.36 ± 0.68	6.29 ± 1.71	7.47 ± 3.93
18AUG	9	9.83 ± 3.87	5.5 ± 2.18	3.93 ± 1.04	3.54 ± 0.00	5.11 ± 1.41

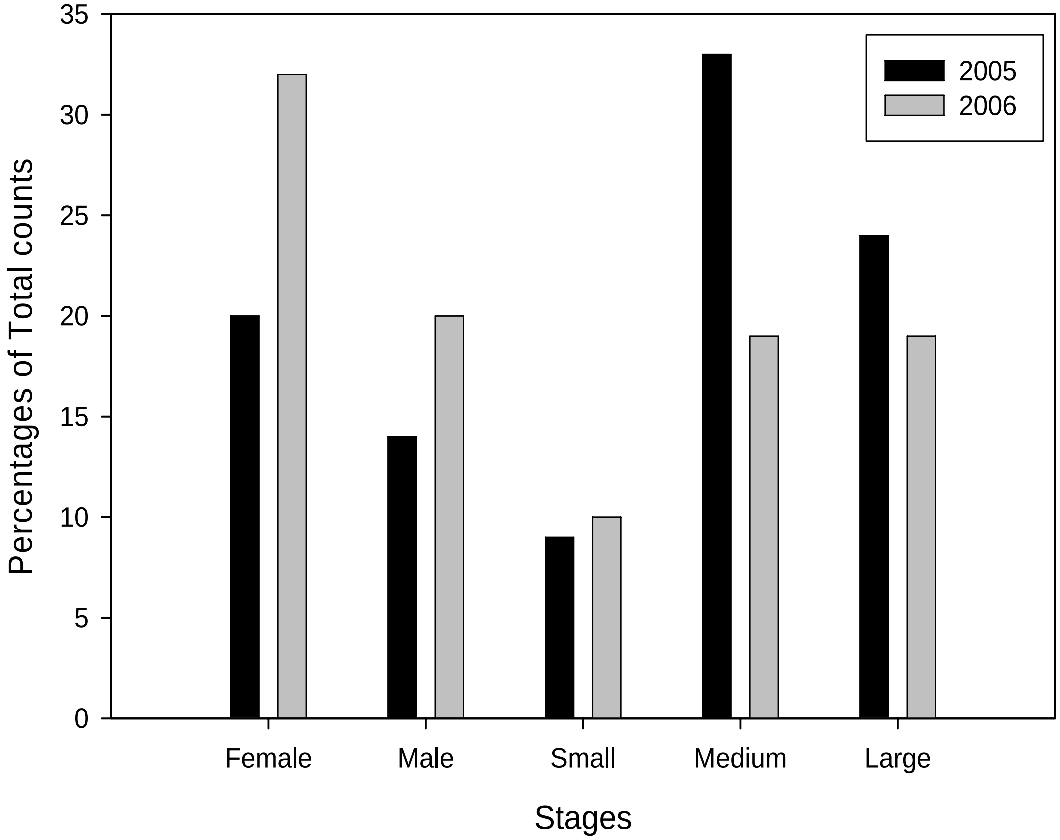

Figure 6. 2005-2006 Percentage by stage of counts of *B. asahinai* in Dothan, Alabama.

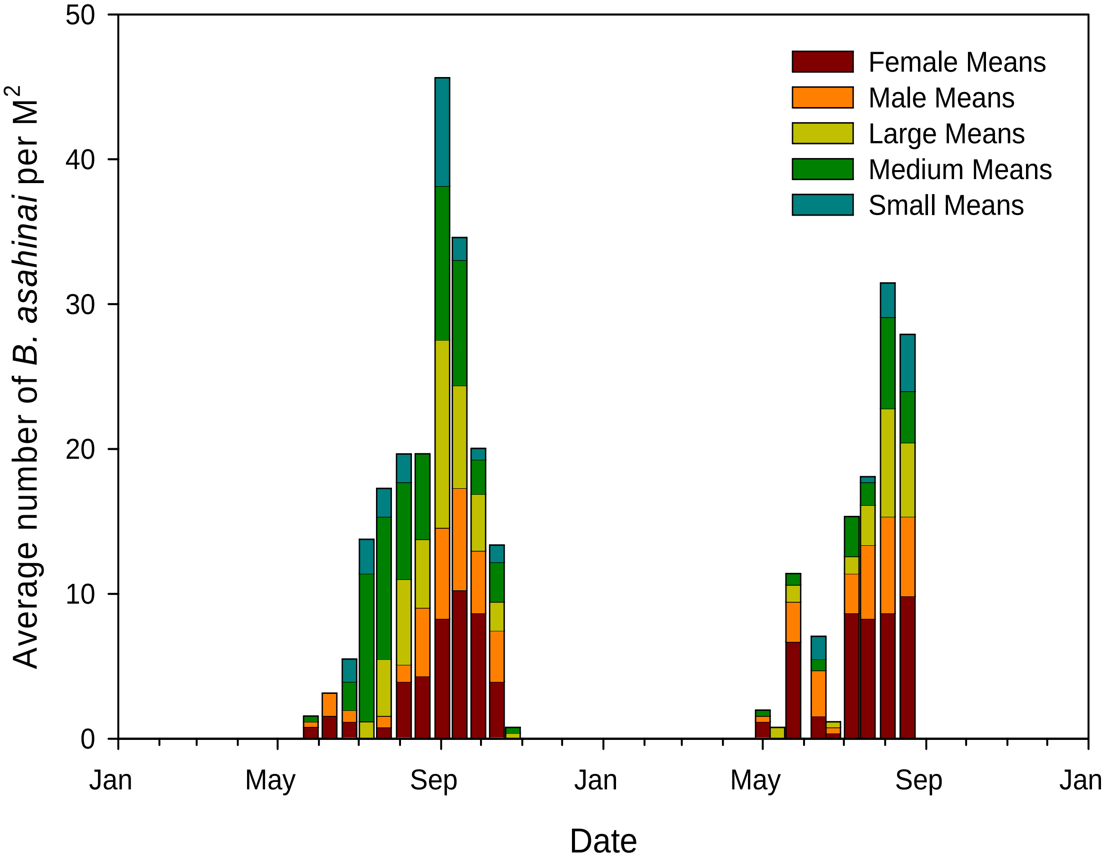

Figure 7. 2005-2006 Visual population means of all stages of *B. asahinai* in Dothan, Alabama.

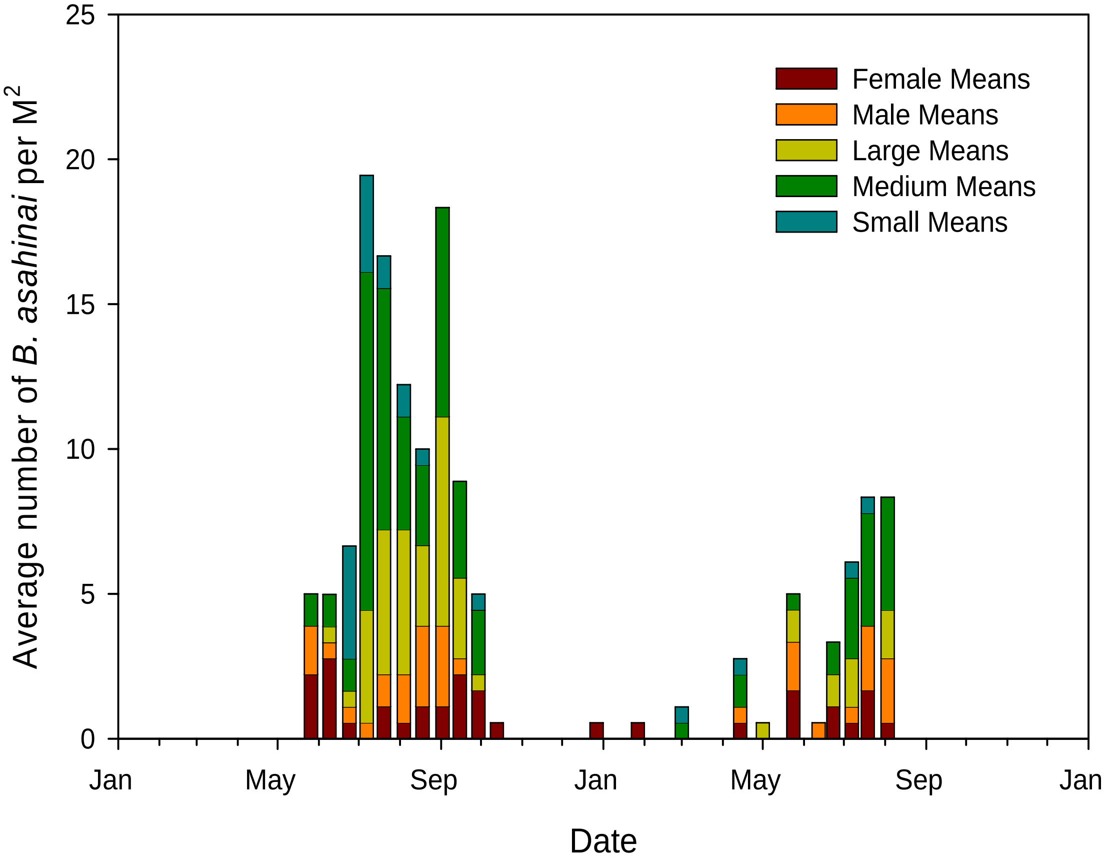

Figure 8. 2005-2006 Bucket Sample means for all stages of *B. asahinai* in Dothan, Alabama.

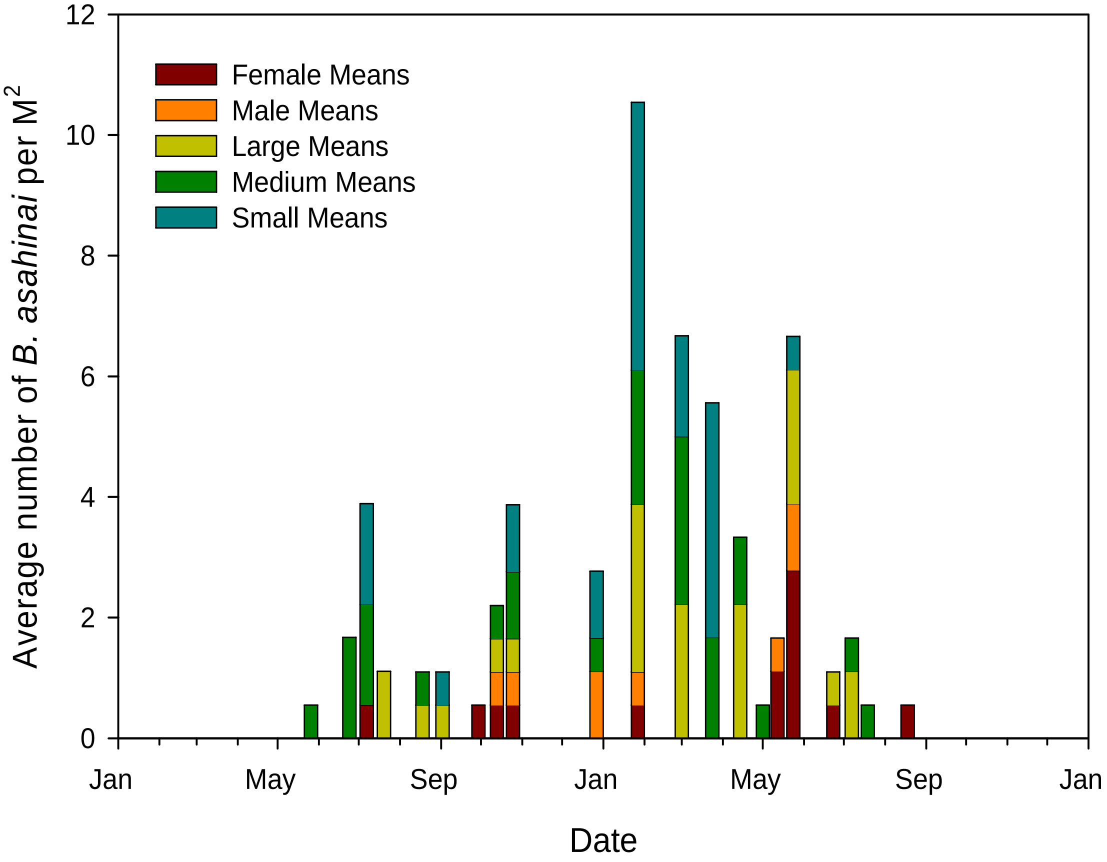

Figure 9. 2005-2006 Berlese sample means for all stages of *B. asahinai* in Dothan, Alabama.

SEASONAL TEMPERATURE SENSITIVITY OF FIELD POPULATIONS OF *BLATTELLA ASAHINAI* MIZUKUBO

Since the introduction of the Asian cockroach, *Blattella asahinai* Mizukubo, into the state of Florida in 1986, its range has expanded northward (Koehler, 1999). Originally, *B. asahinai* was introduced into three central Florida counties, but has since spread to 49 counties in Florida from Dade County in the South to Nassau County in the North (Koehler, 1999), and across the panhandle West to Santa Rosa County (Donahoe, 2005). In a recent examination of the distribution of *B. asahinai* in southern Alabama and Georgia, populations have become established as far North as Houston County, Georgia, and Barbour County, Alabama. Additional populations of *B. asahinai* have also been reported from as far East as Kiawah Island, South Carolina (Sitthicharoenchai, 2001), and as far West as Houston, Texas (Tucker 2006, J. Austin, personal communication 2006).

There have been no studies conducted on over wintering of *B. asahinai* in the United States or in its native range of Taiwan and Japan (Roth, 1986), or studies that suggest the temperature ranges in which it can survive. Several studies have shown a positive correlation between the temperature sensitivity and the habitat temperatures of cockroaches (Tsuji and Mizumo 1973, Appel et al. 1983) and many other animals (Slabber, 2007). Critical thermal maxima (CTMax) and critical thermal minima (CTMin) are the upper most, and lower most temperatures, respectively, that an organism can escape from which ultimately would lead to its death (Lutterschmidt, 1999).

The range of CTMax and CTMin can vary among closely related taxa of insects. For example the native species of Collembola in Antarctica have a lower CTMin and lower CTMax than an introduced species of Collembola (Slabber, 2007). During the current climatic warming of Antarctica, the introduced species of Collembola with its higher CTMax has been able to survive warmer summers and displace the native species (Slabber, 2007). The range of temperature sensitivity of introduced species can provide a competitive advantage and allow expansion of range and displacement of native species, or exploit unused niches in the new habitat.

Observations of field populations over a 2 year period indicate that only certain stages and sexes of *B. asahinai* were observed at certain times of the year. In addition, we have observed that some individuals will burrow into the substrate, possibly to avoid adverse environmental conditions such as temperature and humidity. There may be therefore differences in the temperature sensitivity among different stages and sexes of *B. asahinai*. CTMax per se is probably not relevant to the survival of an organism, as it seldom reaches the CTMax in the environment (Lutterschmidt and Hutchison, 1997). CTMin is better realistic indicator of how well *B. asahinai* might survive in the field because the CTMin temperature is more likely to be encountered than the CTMax temperature. Tolerance to low temperatures may be the most important factor in governing geographic distribution of a species (Lutterschmidt and Hutchison, 1997) because temperatures affect behavior, reproduction, and physiology of ectothermic species (Slabber, 2007).

This study reports the seasonal temperature sensitivity of *B. asahinai* as measured by the CTMax and CTMin. The range of CTMax and CTMin in field collected populations and laboratory culture *B. asahinai* was compared. We examined possible correlations between environmental temperatures and CTMax and CTMin since changes in temperature sensitivity of other closely related organisms, such as termites, correlate with seasonal changes in temperature (Hu and Appel, 2004).

Materials and Methods

Weather Data. Weather data were collected from AWIS Weather Services on the website http://www.awis.com. The weather data were collected from sites within same county as the field populations. Weather data consisted of the high and low temperatures for each day as well average daily and monthly temperatures.

Field Populations. Field specimens of *B. asahinai* were collected monthly during 2005 and 2006 from Lake Point State Park, Barbour County, Alabama and returned to the laboratory within 12 hours for CTMax and CTMin determinations. Since field populations of *B. asahinai* at this location were minimal from January through May, most specimens were collected from June through December. In 2005 and 2006, populations of *B. asahinai* declined in late August and early September; gravid females and large and medium nymphs remained in large numbers until January when practically no specimens of *B. asahinai* could be collected. To quickly collect field populations of *B. asahinai*, a Black & Decker® Dust Buster 14.4V™ (Model CHV1400 Type I) was modified for insect collection. The Dust Buster™ was connected to a collection reservoir and specimens were vacuumed up through a clear 13 mm (diameter) hose. The collection reservoir was made by taking a Ziploc® 956 ml storage container with screw top and drilling two 13 mm diameter holes in the lid. Then two 13mm hose barbs were inserted into the top and secured with retaining nuts. A piece of window screen mesh 1 mm by 1 mm was positioned on the inside of the hose barb connected to the vacuum to prevent insects and trash from being sucked into the vacuum. A clear polyethylene 13 mm diameter hose 127 cm long connected the storage reservoir to the vacuum. Another collecting hose 127 cm long was attached to the second hose barb on the collection reservoir. The vacuum was then turned on which created a vacuum that would suck up specimens and deposit them into the storage reservoir unharmed. When a storage reservoir was full it was simply unscrewed and an empty reservoir reconnected. All specimens were placed into a wide mouth 3.8 liter glass jar which contained corrugated cardboard harborage, a water source, and several pieces of dry dog food. Cockroaches were taken to the laboratory and tested for CTMax and CTMin temperatures within 12 hours of collection.

Laboratory Cultures. Laboratory cultures of *B. asahinai* were also used for CTMax and CTMin determinations. Laboratory cultures were derived from specimens collected in 2004 from Dothan, Alabama and have been reared continuously for a 24 month period at 27° ± 3°C and 70 ± 10% RH and a photoperiod of 12:12 (L:D). They were reared in 3.7 liter glass jars with cardboard harborages and free access to water and Purina Dog Chow®.

Laboratory cultures of *B. asahinai* were maintained in temperature controlled incubators (Revco® BOD30) for up to 96 h to determine how quickly they acclimatized to the new temperatures. Laboratory cultures were placed in temperature controlled incubators at 10° ± 1°C or 35° ± 1°C and CTMax and CTMin values were determined at 24, 48, 72, and 96 h of exposure to the new temperature.

Determination of CTMax and CTMin. Critical temperatures were determined by randomly selecting specimens from field collections or acclimatized colonies, individually weighing each specimen to the nearest 0.01 mg with a digital balance (Mettler scale, DeltaRange® AX205), and then placing them in a thermal chamber. Specimens were placed in Solo® (plastic 29.1 ml, No. P100 with tops PL1) cups that had the bottom and tops cut out and replaced with 1 mm^2 window screen wire mesh. One female, male, large nymph, medium nymph, and small nymph was used for each replicate; there were 6 replicates for each critical thermal temperature, stage, and time period. The thermal chamber was a custom-designed microprocessor controlled device that raised or lowered the temperature at a constant rate (Hu and Appel, 2004). The specimens were placed in the thermal chamber and the temperature raised (CTMax) or lowered (CTMin) at 1°C/min until each specimen reached its critical thermal temperature. Critical thermal temperatures were determined by visual observation, when a specimen collapsed onto its back or side and could not right itself or move when probed, for a 3 sec; it was deemed that the specimen had reached its critical thermal temperature.

Statistical Analysis. We used a randomized complete block design that consisted of 3 blocks with 6 specimens of each stage in each block. With field collected specimens the number of specimens varied depending upon time of year and abundance of *B. asahinai* in the field. We used SAS software (SAS Institute, 2007) ANOVA table to analysis the data and plotted results on SigmaPlot (ver. 9.0) software.

Results.

Field Populations. Average 2005 and 2006 monthly temperatures in Eufaula, Alabama ranged from 2°C for NOV to 32°C for AUG (AWIS Weather Services, 2005). Monthly temperatures for the 2005 collection period had averages from 7.7°C to 25.7°C (Fig 10). Average 2005 monthly temperatures declined steadily from 29NOV2005 until 27DEC2005. Monthly temperatures for the 2006 collection period had averages from 10.6°C OCT 2006 to 15.44°C AUG 2006.

Over the 2 year period study, CTMax of field collected B. asahinai ranged from 25.3° ± 13.5°C for small nymphs collected on 29NOV2005 to 49.7° ± 0.70°C for large nymphs collected on 26OCT2005 (Table 8). CTMin values ranged between 0.8°±0.60°C for large nymphs collected on 29AUG2005 to 4.3°±0.44°C for adult females collected on 28JUL2006 (Table 9). Over the entire study, small nymphs had the greatest variation in CTMax (23.5°C) whereas medium nymphs had the least variation (0.67°C) (Table 8). However, small nymphs had the least seasonal variation in CTMin values (1.08°C) while adult females had the greatest variation (3.28°C) (Table 9).

There were no significant differences in CTMin among collection dates for female *B. asahinai*, however, there were significant differences CTMax values (P=0.0008). There were significant differences in CTMax of the different dates of 2005 field collected small and large nymphs of *B. asahinai* (P=0 .0008) (Fig 11). The 29AUG2005 and 29NOV2005 collections differed significantly from the 29SEP2005 and 26OCT2005 collections (P= 0.0147). There were no significant differences among CTMin or CTMax collection dates for male, large nymphs, and medium nymphs. There were also no differences in CTMax among collection dates for small nymphs, however there were significant differences in CTMin among collection dates ($P<0.001$).

There was a CTMin temperature effect on date of 2005 field collected small nymphs that was significant (P-value 0.0008). There was a significant effect of mass of small nymphs on CTMin ($P= 0.0048$). There was a significant ($P= 0.0006$) effect of the stage by date interaction on CTMin values. There was a CTMin temperature effect on mass by stage interaction of field collected small nymphs that was significant ($P= 0.001$). There was a CTMin temperature effect on mass by date interaction of field collected small nymphs that was significant ($P< 0.0001$).

Laboratory Cultures. CTMax of laboratory *B. asahinai* for the 10°C acclimation experiment ranged between 46.5 ± 0.55°C for small nymphs after 96 h and 49.53 ± 0.30°C for large nymphs after 24 h (Table 10). Ranges for CTMin of laboratory acclimated *B. asahinai* at 10°C were 2.90 ± 0.18°C and 4.3 ± 0.30°C for females after 72 h and large nymphs at 0 h, respectively (Table 11). For the 35°C acclimation experiment, CTMax of laboratory *B. asahinai* ranged between 45.55 ± 0.23°C and 50.65 ± 0.60°C for large nymphs at 0 h and large nymphs at 96 h, respectively (Table 10). For the 35°C acclimation experiment, CTMin of laboratory *B. asahinai* ranged between 3.75 ± 0.62°C and 4.98 ± 0.27°C for large nymphs at 72 h and small nymphs at 48 h, respectively (Table 11).

There were significant differences in CTMax among *B. asahinai* stages acclimated to 10°C ($F= 4.93$, $P= 0.0011$). There were also significant differences in body masses among stages ($F= 4.22$, $P= 0.0426$). There was a significant stage by time interaction effect for CTMax ($F= 1.84$, $P= 0.0364$) as well as mass by stage by time interaction (F= 1.96, P= 0.0229). For small nymphs, there was a significant difference, though only ~2°C, in their CTMax between 0-96 h acclimation period (Fig. 12). Overall acclimation periods, the CTMax of small nymphs was significantly different than other stages (F=7.53, P= 0.0012). There was no significant difference between adults CTMax. There was a significant difference in the stage by time interaction effect for nymphs (F= 2.05, P= 0.05).

Across all the stages of *B. asahinai* in the 10°C acclimation experiment, there were significant differences in CTMin among the different stages (F= 3.16, P= 0.0172). All stages had greater CTMin at 0 h than at 96 h demonstrating acclimation (Table 11). There was no significant difference in CTMin between females and males or among nymphs. There was no significant difference in CTMax among any of the stages in the acclimation experiment. There was an increase in the CTMax of all stages of *B. asahinai* CTMax over time (Fig. 13). There was a significant difference between the mean temperatures and hours of acclimation for small nymphs (F= 5.81, P= 0.0019), medium nymphs (F= 2.75, P= 0.05), and large nymphs (F= 3.62, P= 0.0185). There was a significant difference in CTMin among the stages and acclimation periods (F= 4.31, P= 0.0029). There was a significant difference in CTMin for females and males of *B. asahinai* between the time acclimation periods (F= 5.72, P= 0.001).

There was a significant difference in CTMin for females and males between the stage by time interaction effect (F= 4.54, P= 0.0042). There was also a significant difference for females and males of *B. asahinai* between the mass by time interaction effect in the CTMin 35°C acclimation experiment (F= 4.96, P= 0.0025). There was a significant difference for females and males of *B. asahinai* between the mass*stage*time effect in the CTMin 35°C acclimation experiment (F-value 4.68, P-value 0.0035). There was no significant difference between any stages of the nymphs of *B. asahinai* in the CTMin 35°C acclimation experiment.

Discussion

Environmental temperatures play an important role in determining the ability of an organism surviving in a given habitat. If an organism is able to survive extreme temperature swings such as is experienced during winter or summer, this increases their likelihood to colonize that habitat. When organisms experience different stressors including subleathal exposure to high or low temperatures they produce certain proteins called "heat shock proteins (HSP)" (Lutterschmidt and Hutchison, 1997). HSP allows an organism to protect itself during periods of temperature stress and allows recovery on a cellular level. HSP are found in many organisms from bacteria to mammals (Lutterschmidt and Hutchison, 1997).

It has been demonstrated that organisms have the ability to increase or decrease their CTMin and CTMax in response to environmental temperatures (Slabber et al., 2007). It has been show that certain insects, such as termites, have the ability to acclimate to their environmental temperature (Hu and Appel, 2004). Since cockroaches are closely related to termites, they to, should have the propensity to acclimatize to their environment. Several studies have shown a positive correlation between the temperature sensitivity and the habitat temperatures of cockroaches (Tsuji and Mizumo 1973, Appel et al. 1983) and many other animals (Slabber et al., 2007).

As previously stated in this paper *B. asahinai* will burrow into the substrate to escape adverse conditions like temperature or low humidity. We hypothesize that *B.*

asahinai can acclimate to surrounding temperatures to a certain point at which time they burrow down into the substrate to escape extreme temperatures. This may account for field observations in which only certain stages (nymphs) of *B. asahinai* are present and able to be collected. The ability to withstand higher CTMaxa and lower CTMina would allow these individuals in a population extra time to seek refuge or borrow into the substrate leaving less tolerate individuals to perish.

During the 2005-2006 seasons we collected field populations of *B. asahinai* to determine their CTMax and CTMin values in the field. It was observed that during certain times of the year, especially during colder months, that nymphs seemed to be the predominate stage in the field population. It would appear that *B. asahinai* is over wintering in the nymphal stage, which is supported by the Berlese samples taking during the same time period (Snoddy and Appel, 2007). CTMax and CTMin varied with ambient temperature in 2005-2006 field collected populations of *B. asahinai* (Fig. 10, 11, 17, 18). Large and small nymphs showed the biggest decrease in CTMax with the onset of cooler temperatures, which demonstrates acclimatization (Fig. 11). Large nymphs showed the biggest decline in CTMin with onset of cooler temperatures, which again demonstrates the ability to acclimatize (Fig. 10). Likewise we observed an increase in both CTMax and CTMin for nymphs with the onset of warmer temperatures in June and July 2006 (Fig. 17 and 18). Since the adults had the highest CTMax and CTMin this suggests that they cannot acclimatize as well as the nymphs.

To investigate possible differences in critical temperatures among stages we examined rates of acclimation of all stages of *B. asahinai*. We determined CTMax and CTMin after acclimation at a low temperature of 10°C and a high temperature of 35°C.

When acclimated at 10°C, the CTMin of all stages of *B. asahinai* declined with exposure time (Fig 19), but when acclimated to low temperatures the CTMax of all stages, with the exception of small nymphs, remained unchanged. Only small nymphs of laboratory populations showed a decline in CTMax when acclimated to low temperatures (Fig. 20). These are the same results we saw in the field populations of *B. asahinai* during the 2005-2006 seasons (Fig. 17).

When acclimated to high temperatures (35°C), the CTMax of all stages of *B. asahinai* increased with acclimation time (Fig. 13). The CTMin increased over the first 72 hours during the acclimation to higher temperatures (Fig. 21). This 35°C acclimation experiment demonstrated that all stages of *B. asahinai* acclimated to higher temperatures, or that *B. asahinai* increased its CTMax and CTMin temperature. We observed an increase in CTMax in all stages with the onset of warmer temperatures in the 2005-2006 field populations.

We demonstrated that certain stages (nymphs) of *B. asahinai* have the ability to acclimate more rapidly than others. This may explain why there are only nymphs of *B. asahinai* present at particular times of the year. Certain individuals in *B. asahinai* can withstand greater temperature change allowing them the extra time needed to seek refuge or borrow down into the substrate to escape possible death.

Table 8: 2005-2006 Mean CTMax (°C) for field collected *B. asahinai* in Eufaula, Alabama.

Year	Date	♀	♂	Large	Medium	Small
2005	29AUG	46.2 ± 0.35 n = 2	48.3 ± 0.55 n = 2	47.6 ± 1.15 n = 2	48.5 ± 0.45 n = 2	48.8 ± 0.20 n = 2
	29SEP	48.5 ± 0.33 n = 4	48.0 ± 0.30 n = 5	48.6 ± 0.28 n = 3	48.4 ± 0.35 n = 3	48.4 ± 0.26 n = 3
	26OCT	48.0 ± 0.35 n = 6	48.7 ± 0.0 n = 1	49.7 ± 0.10 n = 2	49.1 ± 0.70 n = 2	48.2 ± 0.72 n = 3
	29NOV	46.2 ± 0.35 n = 2	48.3 ± 0.55 n = 6	32.2 ± 15.4 n = 3	48.5 ± 0.45 n = 2	25.3 ± 13.5 n = 4
2006	12JUN	48.3 ± 0.35 n = 3	48.7 ± 1.45 n = 2	48.7 ± 1.08 n = 3	48.7 ± 1.45 n = 2	a
	28JUL	48.9 ± 1.25 n = 2	49.8 ± 0.00 n = 1	48.9 ± 0.47 n = 10	a	a
	29AUG	47.9 ± 0.12 n = 3	48.6 ± 0.08 n = 3	48.4 ± 0.20 n = 3	48.9 ± 0.47 n = 3	48.4 ± 1.25 n = 2
	12OCT	49.3 ± 0.15 n = 4	a	48.6 ± 0.65 n = 4	49.0 ± 0.14 n = 6	47.3 ± 0.17 n = 3

a=no collection of this stage of *B. asahinai*

Table 9: 2005-2006 Mean CTMin (°C) for field collected *B. asahinai* in Eufaula, Alabama.

Year	Date	♀	♂	Large	Medium	Small
2005	29AUG	2.0 ± 0.90 n = 2	2.5 ± 0.25 n = 2	0.8 ± 0.60 n = 2	1.6 ± 0.00 n = 1	1.8 ± 0.25 n = 2
	29SEP	2.9 ± 0.43 n = 5	2.8 ± 0.20 n = 5	2.7 ± 0.72 n = 3	2.1 ± 0.15 n = 3	2.9 ± 0.54 n = 3
	26OCT	1.0 ± 0.18 n = 7	1.5 ± 0.15 n = 2	1.3 ± 0.00 n = 1	1.5 ± 0.15 n = 2	1.4 ± 0.05 n = 3
	29NOV	2.0 ± 0.25 n = 2	2.5 ± 0.25 n = 3	a	1.6 ± 0.00 n = 1	a
2006	12JUN	2.0 ± 0.36 n = 3	3.7 ± 0.00 n = 1	3.0 ± 0.30 n = 7	2.5 ± 0.00 n = 1	2.4 ± 1.25 n = 2
	28JUL	4.3 ± 0.44 n = 2	4.2 ± 0.00 n = 1	3.8 ± 0.14 n = 10	3.8 ± 0.35 n = 2	a
	29AUG	4.0 ± 0.44 n = 2	2.5 ± 0.18 n = 3	2.9 ± 0.28 n = 3	3.8 ± 0.46 n = 3	2.9 ± 0.00 n = 1
	12OCT	2.8 ± 0.26 n = 5	3.1 ± 0.00 n = 1	3.0 ± 0.00 n = 4	3.7 ± 0.11 n = 3	3.2 ± 0.10 n = 2

a=no collection of this stage of *B. asahinai*

Table 10: CTMax (°C) of *B. asahinai* laboratory cultures from acclimation experiment.

Acclimation Temperature	h	n	♀	♂	Small	Medium	Large
10°C	0	6	48.10 ± 0.18	48.85 ± 0.37	48.56 ± 0.27	48.16 ± 0.41	48.6 3 ± 0.37
	24	6	48.36 ± 0.37	48.53 ± 0.44	47.85 ± 0.35	49.00 ± 0.39	49.53 ± 0.34
	48	6	48.18 ± 0.21	47.88 ± 0.14	48.10 ± 0.39	49.01 ± 0.47	48.06 ± 0.79
	72	6	47.40 ± 0.40	48.10 ± 0.27	47.63 ± 0.30	48.58 ± 0.46	49.00 ± 0.26
	96	6	48.03 ± 0.19	48.60 ± 0.33	46.50 ± 0.55	49.18 ± 0.37	49.08 ± 0.48
35°C	0	6	47.00 ± 0.22	48.63 ± 0.47	46.73 ± 0.51	47.48 ± 0.50	45.55 ± 0.23
	24	6	47.70 ± 0.31	48.00 ± 0.54	47.51 ± 0.94	49.00 ± 0.68	48.03 ± 0.82
	48	6	48.30 ± 0.50	49.45 ± 0.27	49.95 ± 0.30	49.70 ± 0.57	50.01 ± 0.31
	72	6	48.23 ± 0.42	49.18 ± 0.28	48.30 ± 0.64	49.38 ± 0.34	49.96 ± 0.36
	96	6	48.81 ± 0.90	49.84 ± 1.09	50.00 ± 0.40	49.35 ± 0.45	50.65 ± 0.60

Table 11: CTMin (°C) of *B. asahinai* laboratory cultures from acclimation experiment.

Acclimation Temperature	h	n	♀	♂	Small	Medium	Large
10°C	0	6	4.03 ± 0.21	4.05 ± 0.10	4.10 ± 0.31	4.23 ± 0.21	4.33 ± 0.30
	24	6	3.55 ± 0.29	3.43 ± 0.38	3.85 ± 0.40	3.36 ± 0.41	3.48 ± 0.44
	48	6	3.01 ± 0.31	3.18 ± 0.33	3.63 ± 0.40	3.18 ± 0.20	3.00 ± 0.30
	72	6	2.90 ± 0.18	2.91 ± 0.23	3.40 ± 0.24	3.18 ± 0.35	3.26 ± 0.32
	96	6	3.48 ± 0.33	3.46 ± 0.31	4.00 ± 0.20	3.61 ± 0.20	3.38 ± 0.18
35°C	0	6	3.90 ± 0.29	4.10 ± 0.18	4.46 ± 0.36	4.30 ± 0.26	4.16 ± 0.15
	24	6	4.38 ± 0.31	4.60 ± 0.26	4.71 ± 0.18	4.61 ± 0.23	4.43 ± 0.26
	48	6	4.85 ± 0.52	4.46 ± 0.42	4.98 ± 0.27	4.96 ± 0.58	4.51 ± 0.46
	72	6	3.76 ± 0.80	3.80 ± 0.58	4.55 ± 1.11	4.23 ± 1.03	3.75 ± 0.62
	96	6	4.60 ± 0.52	4.34 ± 0.80	4.55 ± 0.59	4.45 ± 0.57	4.23 ± 0.70

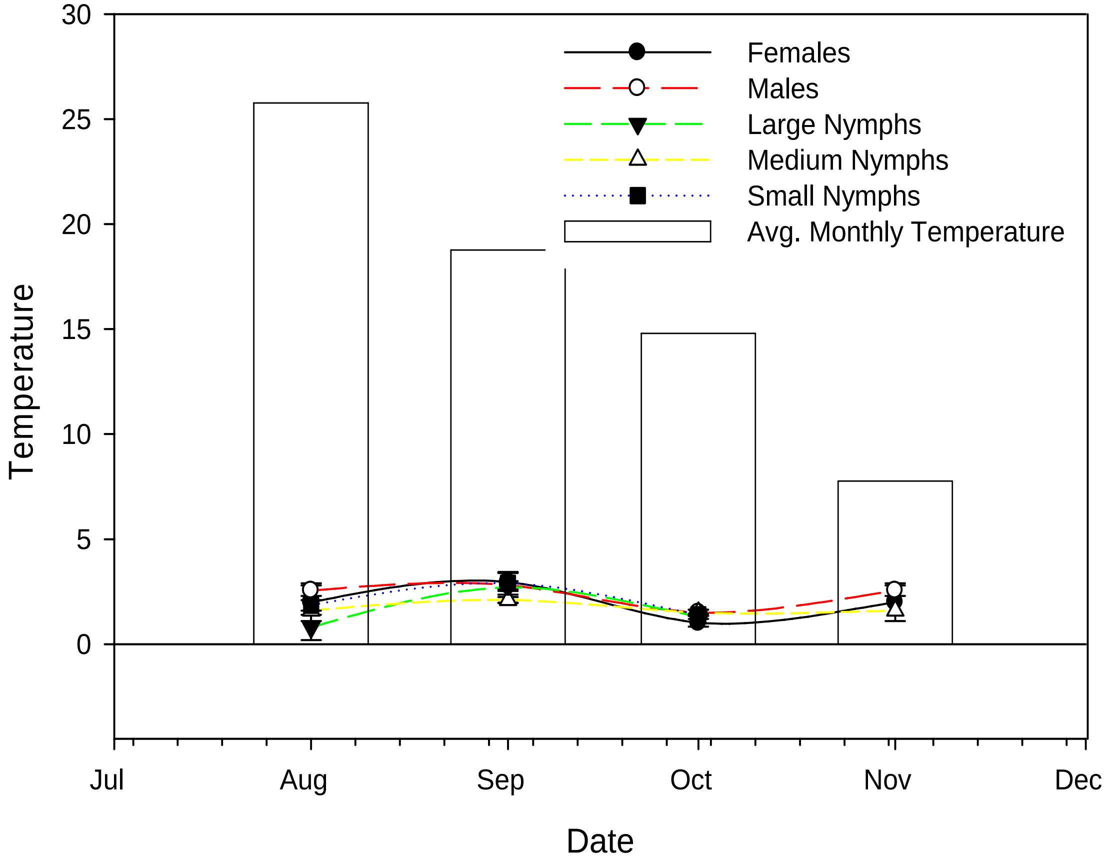

Figure 10. 2005 Field populations of *B. asahinai* CTMin temperature means for all stages.

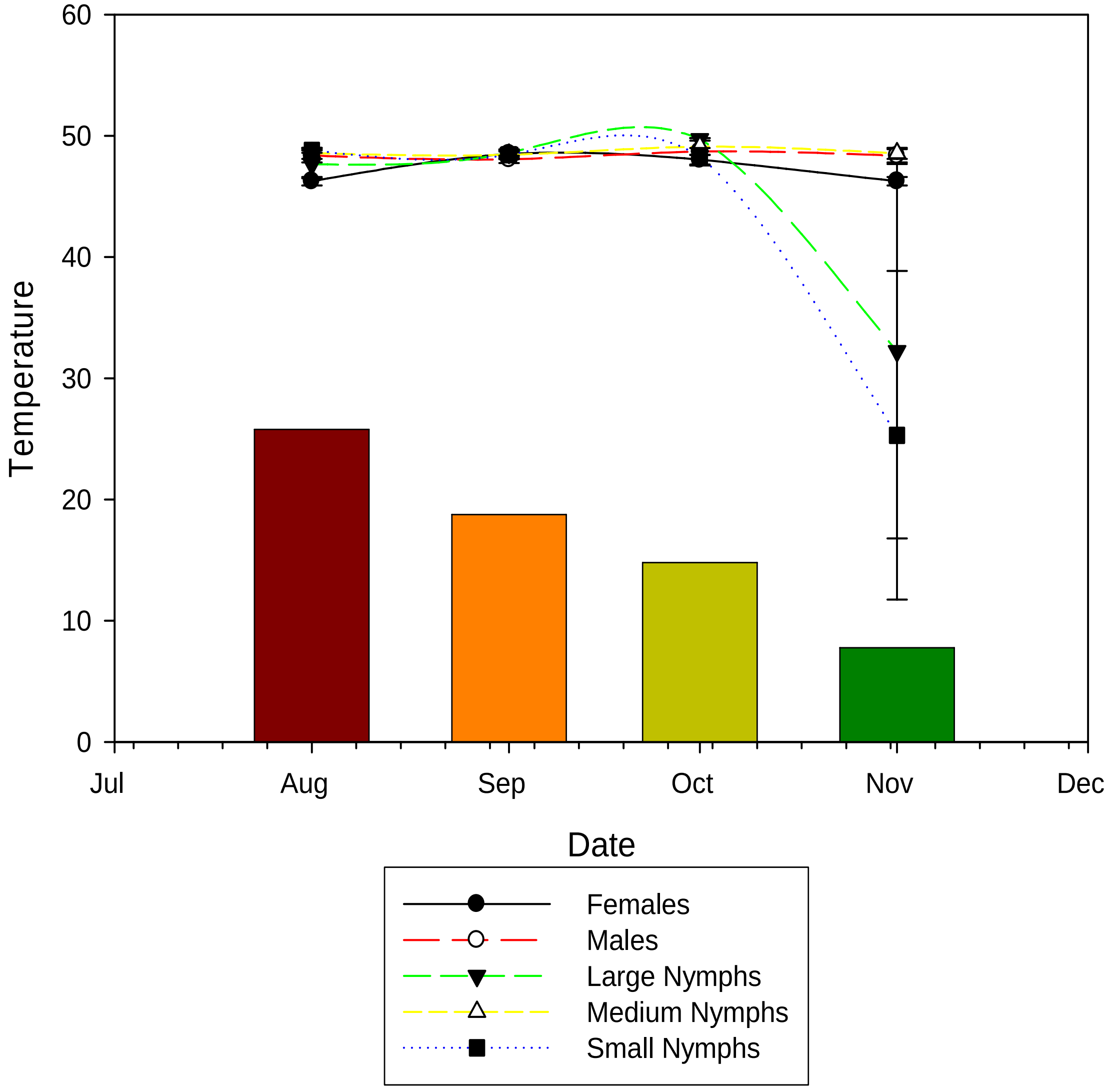

Figure 11. 2005 Field populations of *B. asahinai* CTMax temperature means for all stages.

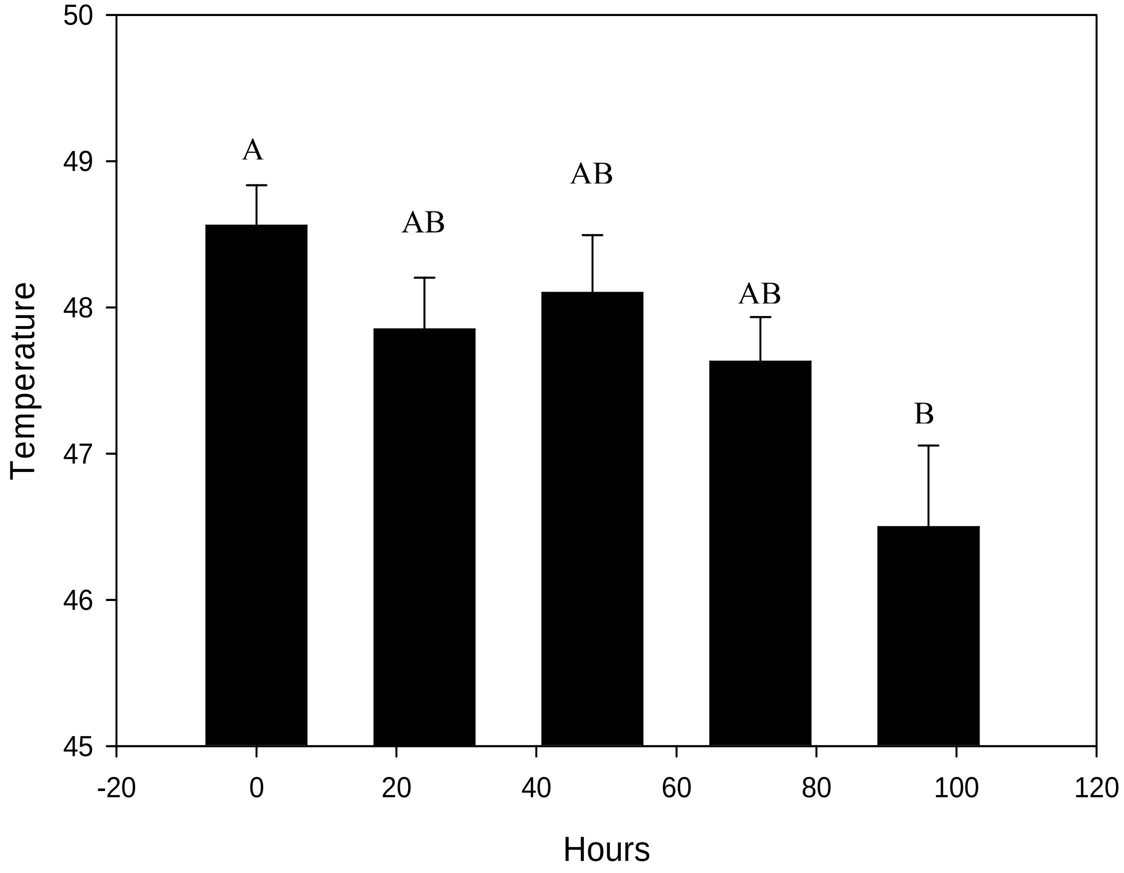

Figure 12. 10°C CTMax temperatures of *B. asahinai* small nymphs.

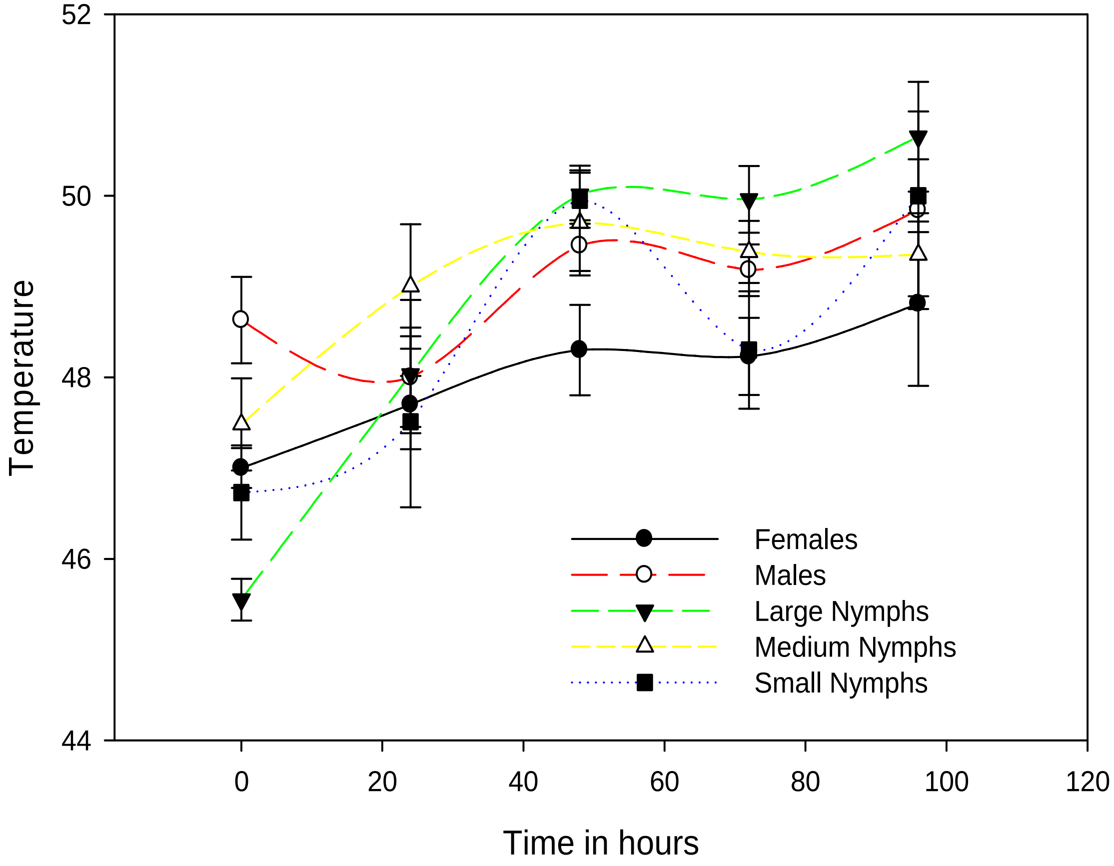

Figure 13. *B. asahinai* 35°C acclimation experiment CTMax mean temperatures for all stages.

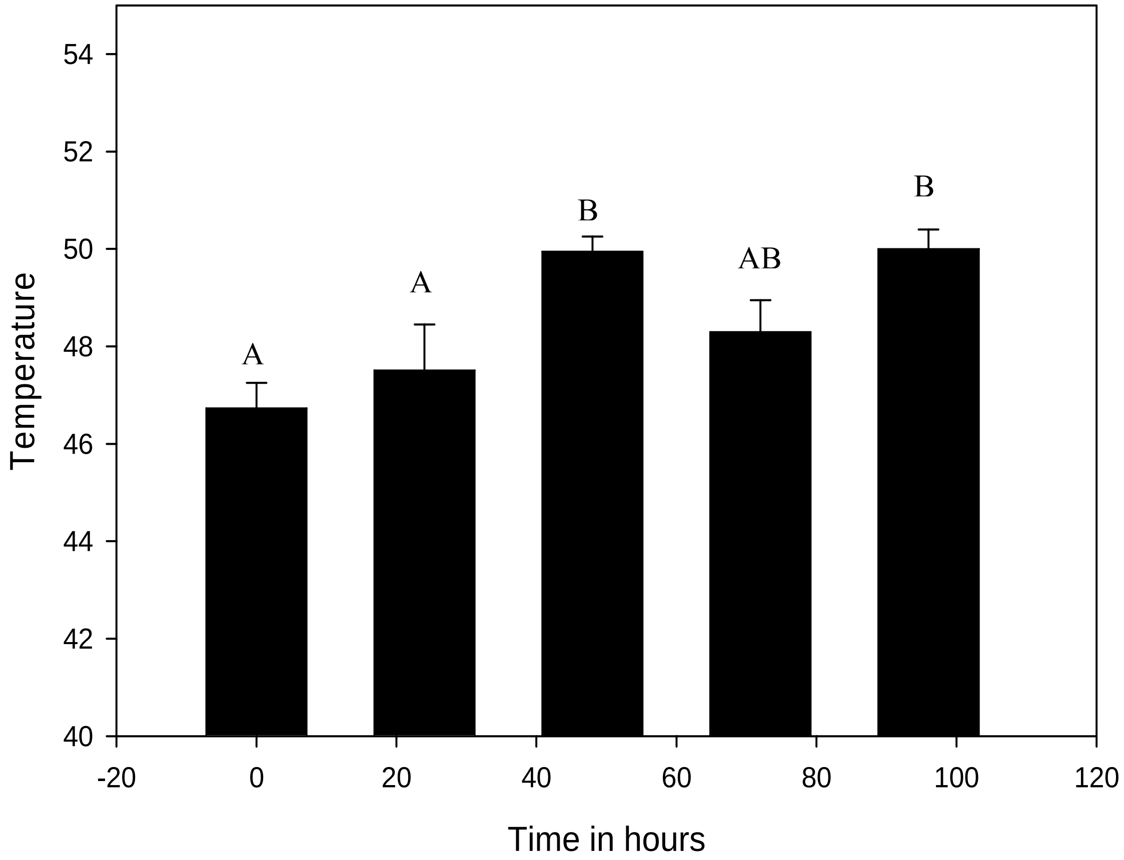

Figure 14. *B. asahinai* 35°C acclimation experiment CTMax mean temperatures for small nymphs.

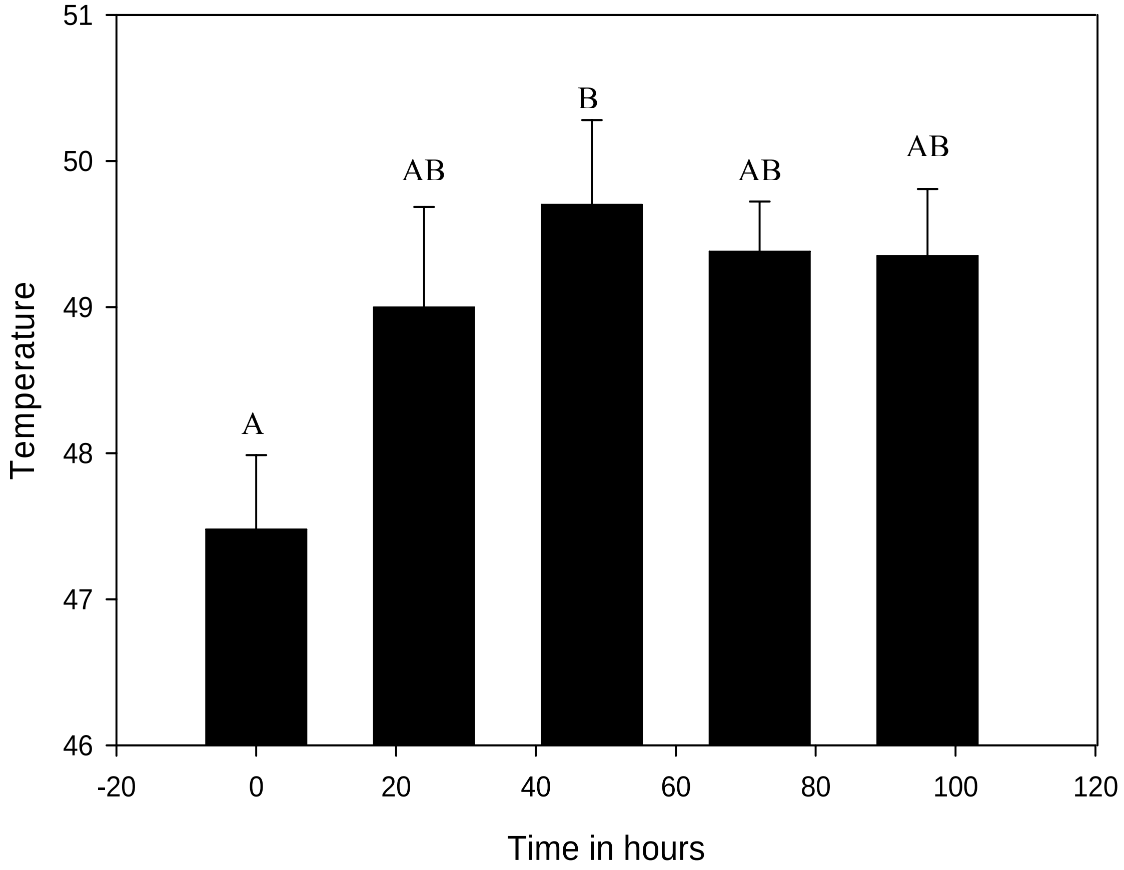

Figure 15. *B. asahinai* 35°C acclimation experiment CTMax mean temperatures for medium nymphs.

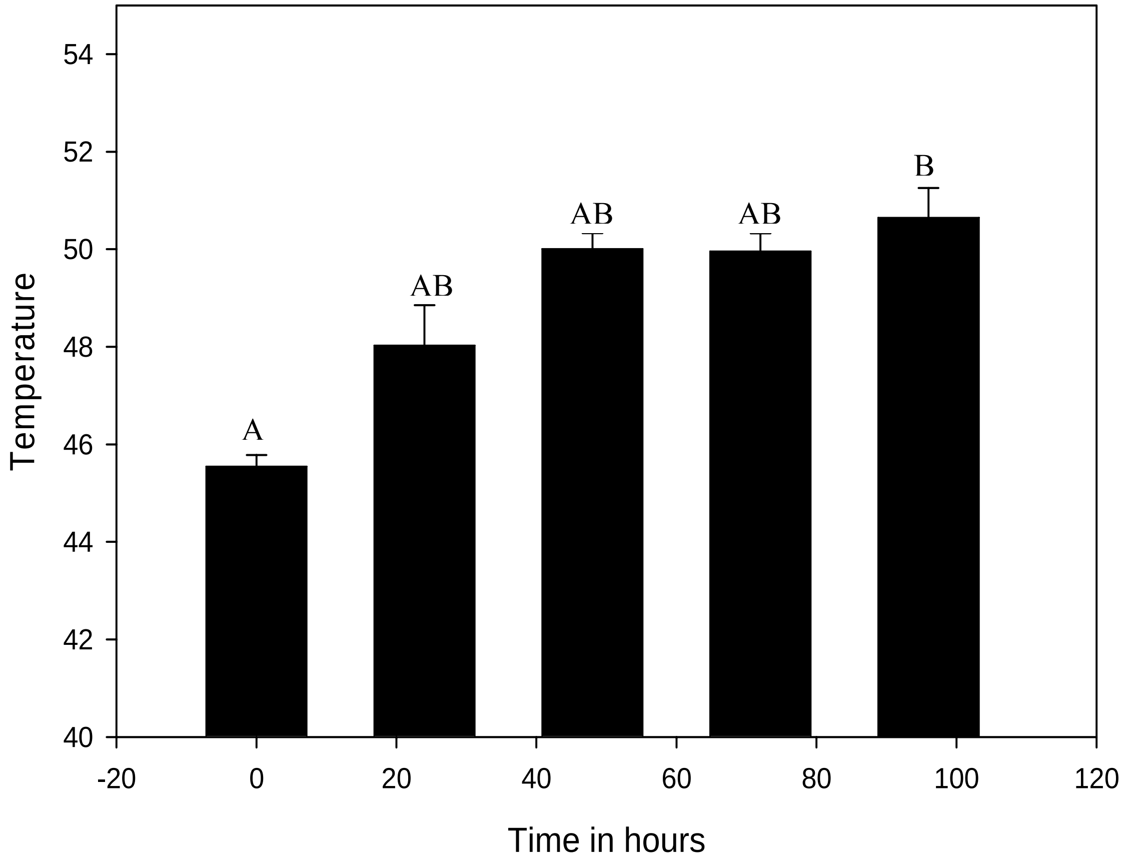

Figure 16. *B. asahinai* 35°C acclimation experiment CTMax mean temperatures for large nymphs.

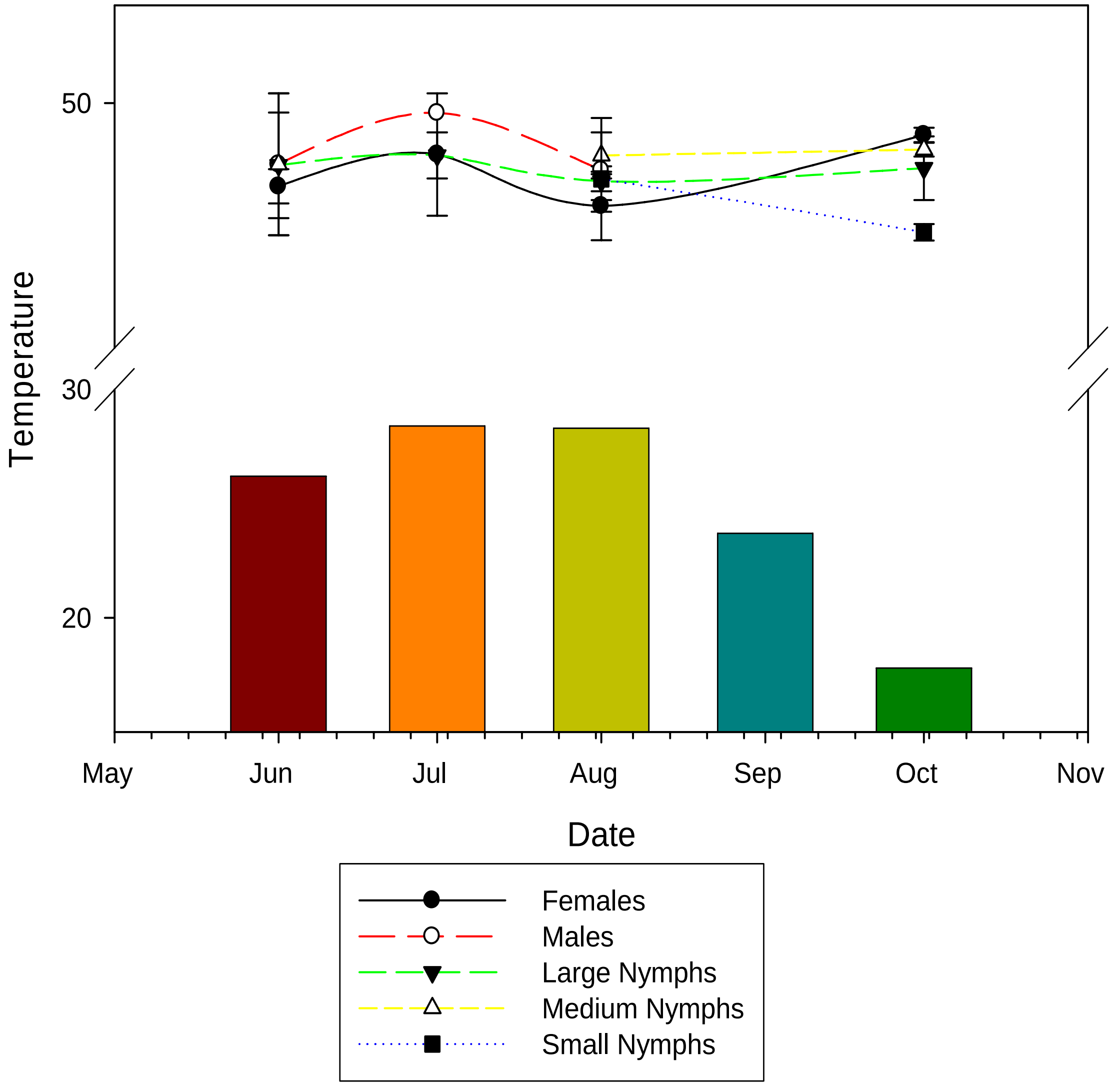

Figure 17. 2006 Field populations of *B. asahinai* CTMax temperature means for all stages.

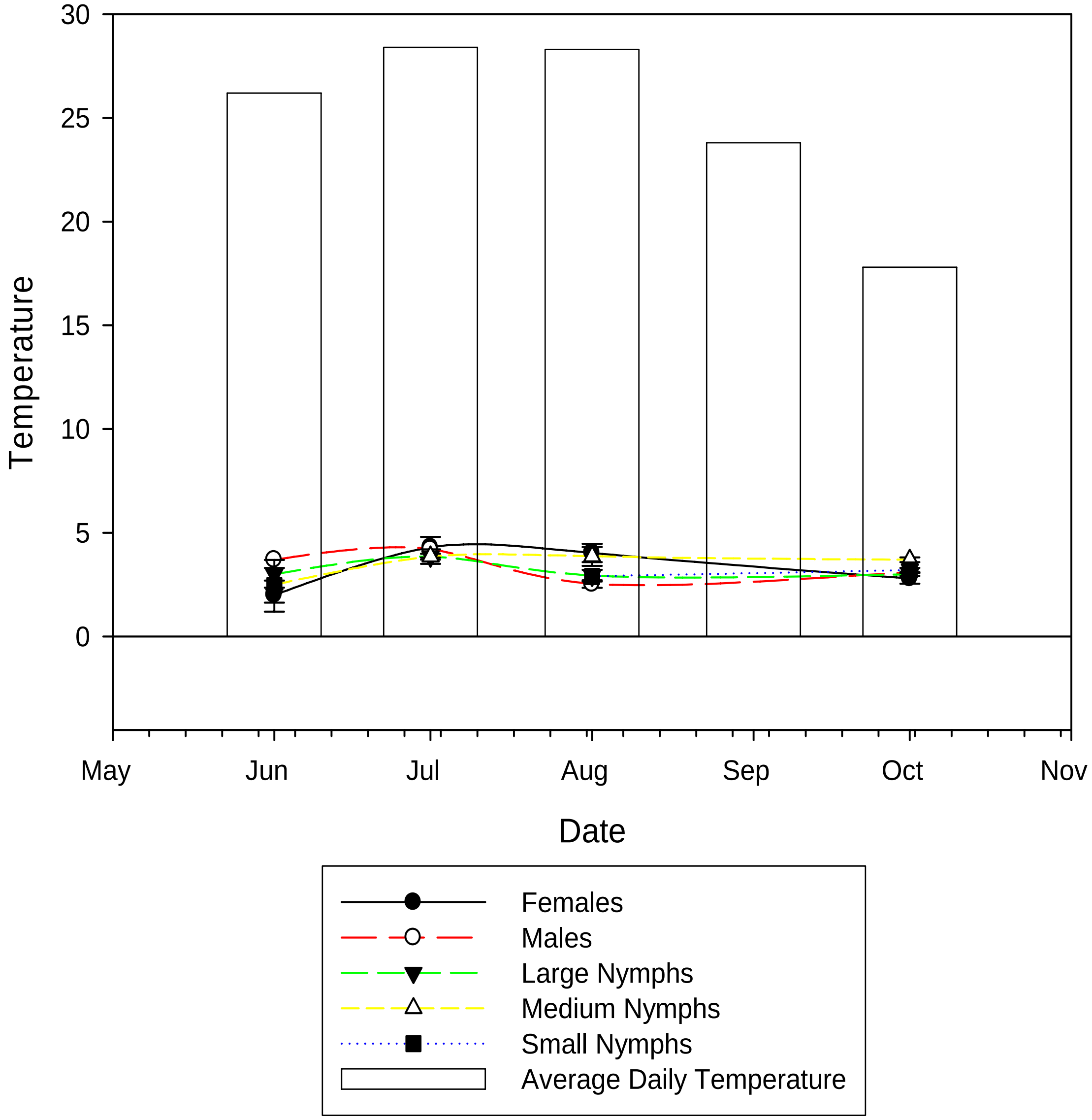

Figure 18. 2006 Field populations of *B. asahinai* CTMin temperature means for all stages.

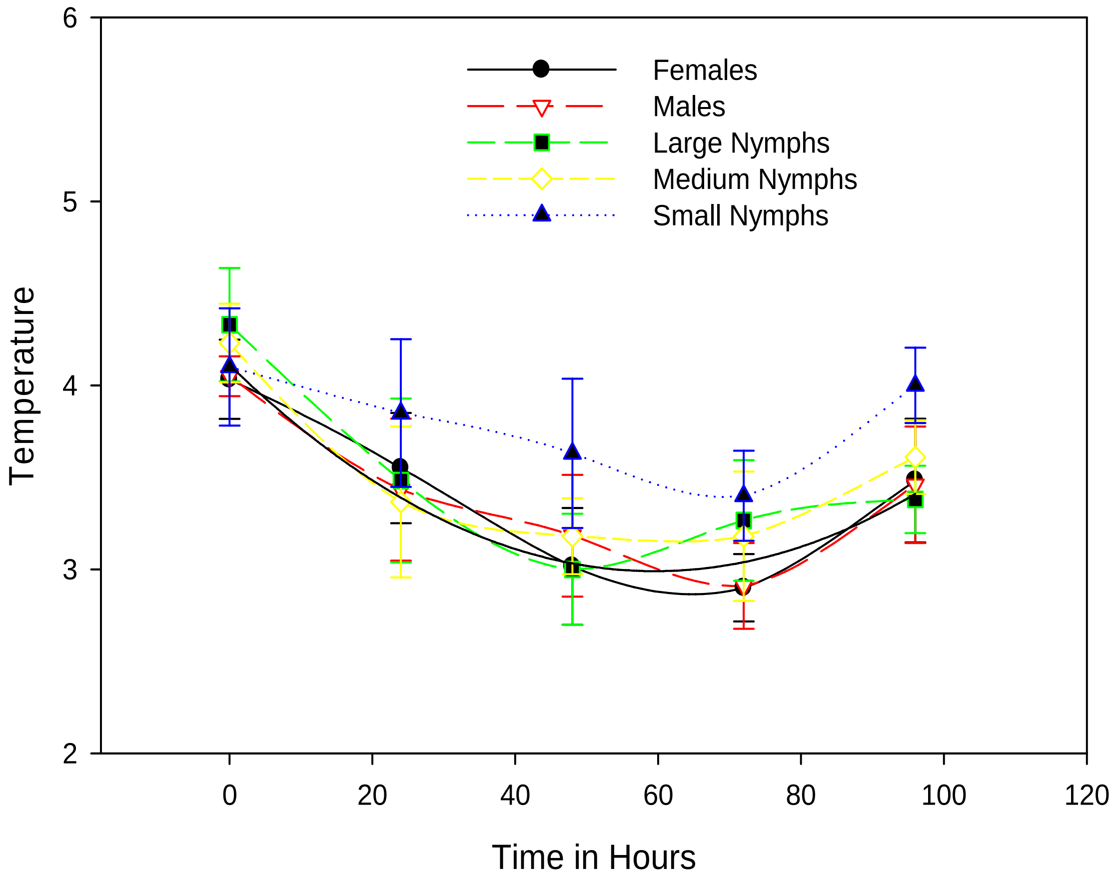

Figure 19. *B. asahinai* 10° C acclimation experiment CTMin temperature means for all stages.

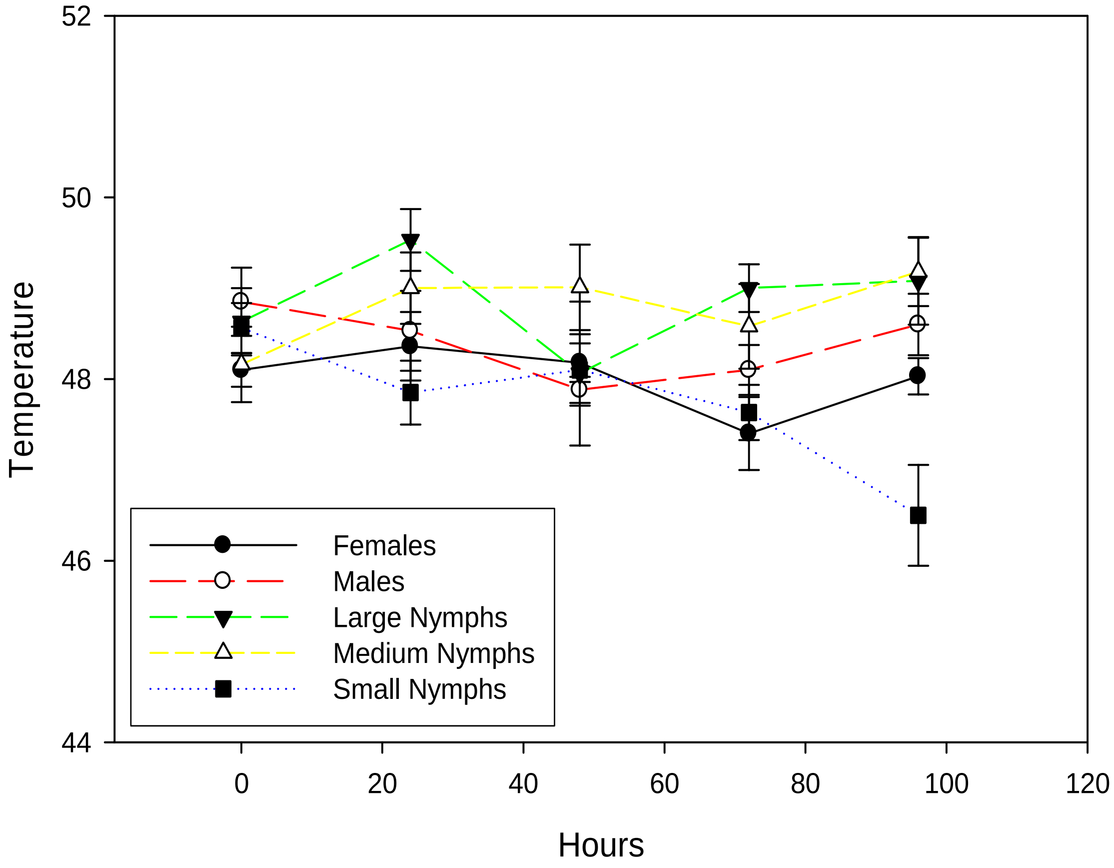

Figure 20. *B. asahinai* 10° C acclimation experiment CTMax temperature means for all stages.

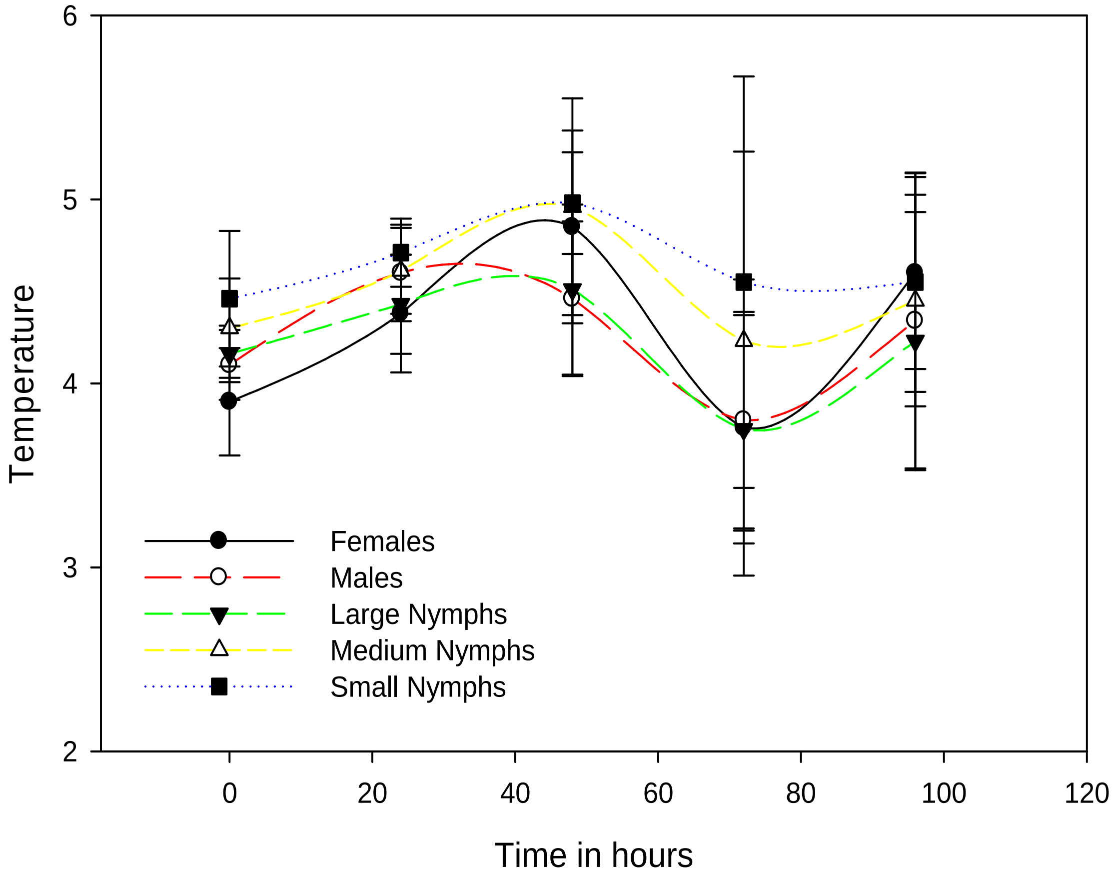

Fig. 21. *B. asahinai* 35° C acclimation experiment CTMin temperature means for all stages.

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