

BIOLOGY AND MANAGEMENT OF
ALLOKERMES KINGII (HEMIPTERA: KERMESIDAE)
ON OAK TREES

By

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by

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I dedicate this thesis to my wonderful family who has continuously told me how proud they are of me. I want to give a special thank you to my husband, Neal; without his support and encouragement I would never have made it this far. I want to thank my children, Crystal and Nickolus, for all of their patience with me and never giving up on their mom. I also want to thank my brothers, James and Christopher, for the support that they have given me and for always being there.

SCALES

By Albert A. Grigarick

Scales are soft and scales are hard.

They are in the orchard and in the yard.

The soft ones are attached to their outer skin,

While the hard ones live free within.

Soft scales produce honeydew,

But hard scales find it impossible to do.

It's just as well it works that way-

Legless, the hard ones can't move away.

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Abstract of Thesis Presented to the Graduate School
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BIOLOGY AND MANAGEMENT OF *ALLOKERMES KINGII* ON OAK TREES

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Chair: Dr. Eileen E. Buss

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Kermesid scales (*Allokermes* spp.), which resemble galls or buds, typically infest oak trees, *Quercus* spp. Their feeding causes branch dieback, flagging, reduced growth rates, and occasionally tree death. We sought to determine their life history and management in Florida, which was thought to differ from northern states. Shoot samples were collected biweekly, and the number and life stages of scales, and presence of natural enemies were recorded. We also conducted an insecticide trial in May 2003, corresponding to the presence of first and second instar *A. kingii*. Shoot samples were collected biweekly, and nymphal survival was determined. Hydrocarbons extracted from male tests were collected and analyzed with the use of a gas chromatography. The chemical composition of the fatty acids and methyl esters from the male test were also analyzed.

CHAPTER 1 GENERAL LITERATURE REVIEW

Scale insects are best known as plant pests, but historically they have proven more useful than has any other insect group of comparable physical size (Morrison 1926, Kosztarab 1987). Since 1200 BC humans have been using products produced by scale insects. It has been suggested that in biblical times the manna which sustained the Israelites' migration was the product of *Trabutina mannipara* (Hemerich & Ehrenburg) and *Trabutina serpentina* (Green) (Morrison 1926, Kosztarab 1987). The cochineal scale, which means scarlet-colored, is famous as a dye in both textile and food industries (Olson 2002). The Aztecs were the first to cultivate the cochineal scale insects *Datylopius coccus* Costa in 1500 (Morrison 1926). Ancient writers used kermesid scales for their royal purple ink. Red dye from Kermesidae, Kerriidae, Margarodidae and Dactylopiidae were used in Michelangelo's paintings, the British redcoats, the Canadian Mounted Police coats, Hungarian Hussars pants, Turks' Fez (the brimless hat they wore) and the caps of the Greeks (Morrison 1926, Kosztarab 1987, Olson 2002).

The wax produced by lac scales is used to make resin, the precursor of shellac, in India (Varshney 1970, Qin 1997), and candles in China (Boratynski 1970, Qin 1997). Natives of Mexico and Central America used the "fat" from adult female "nig" (Margarodidae) bodies to make lacquer for waterproofing wood and gourds and also as a base for medicines and cosmetics (Jenkins 1970, Qin 1997). Ground pearls have been strung as beads for necklaces in the Mediterranean region (Kosztarab 1987).

In addition to product usefulness, coccoids provided the first historical breakthrough in biological control (1988) with the use of the Vedalia beetle, *Rodolia cardinalis* Mulsant (Coccinellidae) (Kosztarab 1987). The Vedalia beetle was used to control cottony cushion scale *Icerya purchasi* Maskell (Margarodidae). The success of this biological control project paved the way for integrated control and pest management (Kosztarab 1987).

Integrated control and pest management was much needed in 1880 when the San Jose scale, *Quadraspidiotus perniciosus* Comstock, made history by becoming the first insect to become resistant to the pesticide lime sulfur (Marlatt 1953, McKenzie 1956, Kosztarab 1987). Because of this resistance, Dr. Comstock established an Advisory Board of Horticulture in 1881 that was later changed (1883) to the State Horticulture Commission. This commission was able to pass the Federal Plant Quarantine Bill of 1912, which is still enforced today (Marlatt 1953, McKenzie 1956).

Boitard in 1828 proposed the genus *Kermes* for some insects resembling galls (Bullington and Kosztarab 1985). Riley in 1881 described the first species of *Kermes*, *K. galliformis* from North America. In 1898 Cockerell named and described *K. kingii* after his good friend Dr. King. In 1890, King presented the first morphological synopsis for 15 species of *Kermes* and Cockerell prepared a key to 13 species by studying the external morphology of post-reproductive females (Bullington and Kosztarab 1985). Ferris began to use slide-mounted specimens in 1920 to illustrate *K. cockerelli* Ehrhorn, and by 1955 illustrated two new species of *Kermes* from southwestern United States (McConnell and Davidson 1959).

Kermesites by Signaret 1875 was the first family group name since the original designation of genus *Kermes*, it was placed in many different families including: Coccidae, Kermidae, Leconidae, Dactylopiidae, Kermococcidae and Eriococcidae. Lobdell (1929) designated the name Kermesidae in place of the Eriococcidae based on the Law of Priority citation with the type-genera (Bullington and Kosztarab 1985). However, this was never accepted and in 1969 Williams stated that the family name should be Kermesidae based on the type-genus *Kermes* (Boitard). McConnell and Davidson (1959) described the slide-mounted adult male, newly-molted adult female, and several preliminary stages of *Kermes pubescens* Bogue (McConnell and Davidson 1959). Based on microscopic characters of slide-mounted first instars several *Kermes* species were revised (Bullington and Kosztarab 1985). Bullington and Kosztarab separated the genus *Allokermes* from *Kermes* in 1985 based on the slide-mounted pre-reproductive females.

The Kermesidae are one of the least-studied families among the scale insects (Kosztarab 1996). Kermesid scales are gall-like insects that infest oak trees (*Quercus* spp.) throughout the world. These scales are often mistaken for small galls or buds, which allows populations to increase to damaging levels (Solomon et al. 1980). Their feeding causes branch dieback, flagging, reduced growth rates and occasionally tree death (Hamon 1977, Vranjic 1997, Futch et al. 2001). All species appear to be univoltine (McConnell and Davidson 1959, Hamon et al. 1976). Scales may be found in bark crevices, forks between twigs and buds, on branches, or in tree wounds (Ben-Dov 1997). The size, shape, and color pattern of post-reproductive females vary considerably within the same species (Baer and Kosztarab 1985, Kosztarab 1987). However, the adult female

is globular and heavily sclerotized, which may protect it from the adverse conditions. Weather can change the color pattern on the cuticle of the adult female scale (Hodgson 1997). Because of the scale's color and globular form, it is often mistaken for a gall on oak trees. Oaks are commonly planted street trees in the United States (Harms 1990), and uncontrolled scale infestations may decrease the tree's economic and aesthetic value.

In North America there are 32 species of Kermesidae in five genera, but in northeastern North America there are nine species in four genera (*Eriokermes*, *Nanokermes*, *Allokermes*, and *Kermes*) (Kosztarab 1996). Kermesid scales are located in 32 states (Fig. 1-1). All records of infestation are reported from *Quercus* spp. except for one record on *Castanopsis* in California (Ferris 1955) and *Eriokermes gillettei* on *Juniperus* sp. The *Allokermes* spp. that are of economic importance in Florida are *A. cueroensis* (Cockerell), *A. galliformis* (Riley), and *A. kingii* (Cockerell) (Kosztarab 1996).

A. cueroensis is often called the live-oak kermes. It specifically attacks *Q. virginiana* (Miller) and possibly also *Q. alba* (L). Post-reproductive females are approximately 8 mm wide, convex, with no median constriction. The *A. cueroensis* adult female is brownish-white, slightly marbled with very pale gray and somewhat wavy brown bands. The surface of the female is speckled with brown spots. *Allokermes cueroensis* can be distinguished from the other two species in Florida by two characteristics: 1) pre-anal row of multilocular disc pores extending dorsally to anal ring only, median lobe of false venter without disc pores, and 2) the spinescent 8-shaped pores are broader than long and with well-developed pits (Baer and Kosztarab 1985, Kosztarab 1996).

The common name for *A. galliformis* is gall-like kermes scale. This scale infests at least 40 *Quercus* spp. (Kosztarab 1996). *Allokermes galliformis* has one generation per year. Post-reproductive females are about 5 mm in diameter. The body is usually

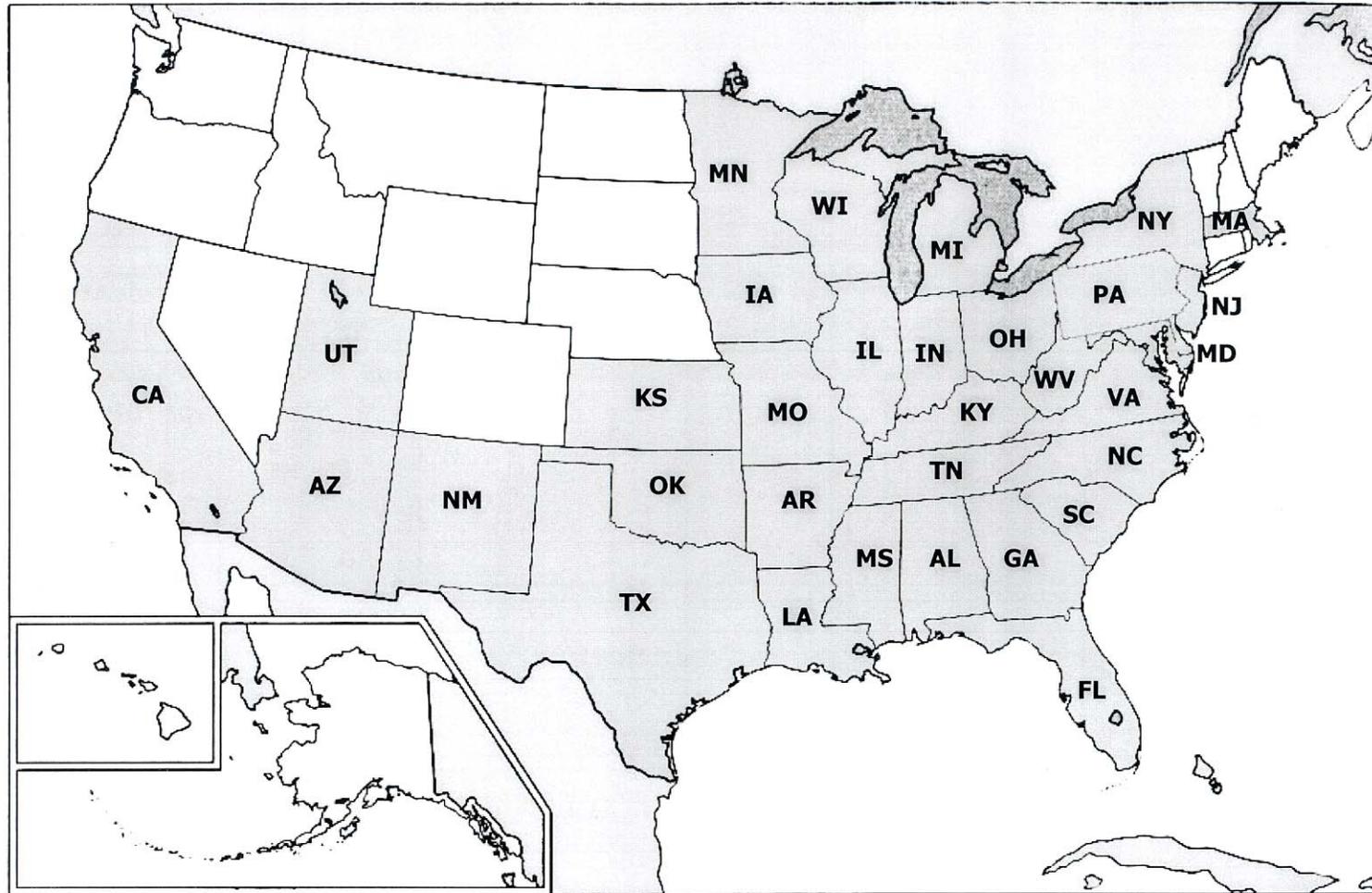


Figure 1-1. Kermes scales are distributed in the states labeled on the U.S. map (Scalenet).

somewhat broader than long and smooth. The outer covering of *A. galliformis* is pale yellow with brown specks, more or less mottled with gray or brown. The body has about seven rows of black dots running across it, often connected by an irregular black line.

Allokermes galliformis can be distinguished from the other species by: 1) pre-anal row of multilocular disc pores with a few pores extending medially onto median lobe of false venter, and also with pores extending dorsally to the area adjacent to anal lobes but not above them; 2) lateral multilocular disc pores lightly in wide row; 3) spinescent 8-shaped pores are longer than broad, with teeth and pits; and 4) anal lobe setae 54µm to 91µm long (Baer and Kosztarab 1985, Kosztarab 1996).

Allokermes kingii, which is one of the most damaging species in Florida (D. Miller, pers. comm.), is also known as the northern red-oak kermes. *Allokermes kingii* has been recorded on eight oak species, but primarily infests *Q. borealis* (Michx.) and *Q. velutina* (Lamarck). All records of infestation are reported from *Quercus* spp. except for one record on *Castanopsis* in California (Ferris 1955). *Allokermes kingii* is recorded from five counties (Alachua, Gilchrist, Hendry, Pinellas and Polk), but likely has a broader distribution (Greg Hodges, pers. comm.). This scale is very convex with the sides barely bulging. Adult females measure 5 mm long, 4.3 mm wide, and about 3.5 mm high. The color is pale brownish-yellow with small black spots covering the entire surface. This species can be distinguished from other species by: 1) pre-anal row of quinquelocular disc pores not extending medially onto median lobe of false venter, also with pores extending dorsally to above anal lobes, encircling them; 2) lateral row of quinquelocular disc pores present, extending dorsally into sparsely distributed quinquelocular disc pores

on mid-dorsum; and 3) spinescent 8-shaped pores with small teeth that are subequal to length of pits (Baer and Kosztarab 1985, Kosztarab 1996).

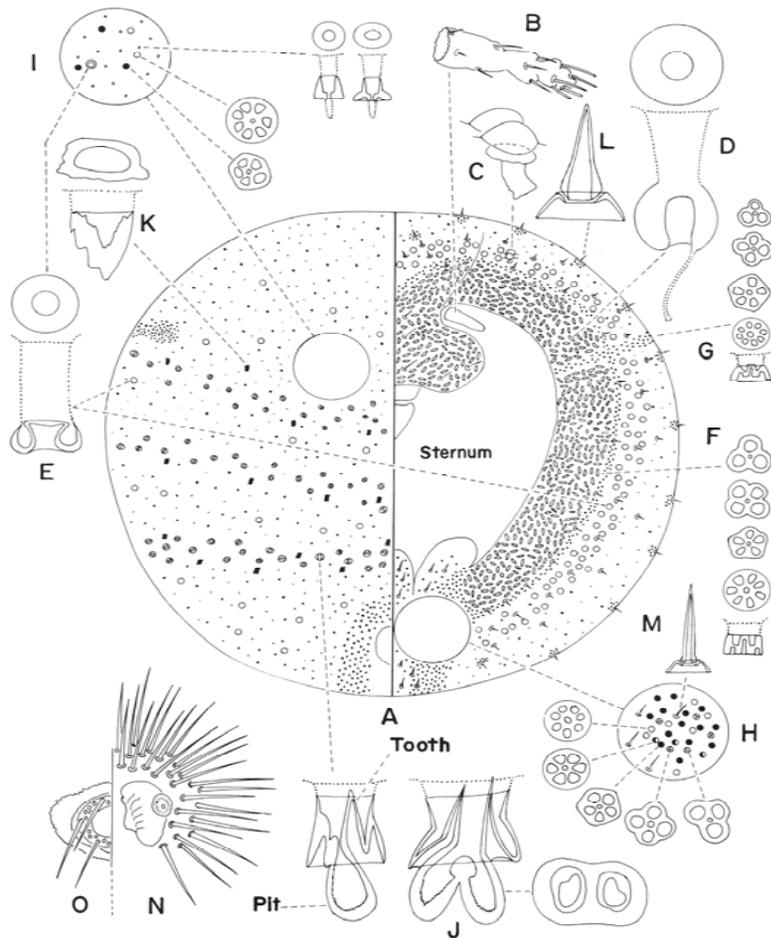
Female *A.kingii* develop with a simple (paurometabolous) metamorphosis; males develop with complete (holometabolous) metamorphosis (Hamon et al. 1976, Kosztarab 1987, Ben-Dov 1997, Marotta 1997, Daly et al. 1998). The terms prereproductive or teneral females (individuals soon after final molt) and postreproductive females (individuals after eggs are produced) are used to describe scale insects (Kosztarab 1987). Adult females are neotenic, which is a prolonged larval form in a sexually mature organism. Females reach the adult stage after two to three molts. The female life cycle consists of three nymphal instars and then an adult stage. General morphology of adult female tergum is shown in Fig. 1-2. The tergum normally applies in insects to the dorsal or upper surface of any body segment (Kosztarab 1996). Male Kermesids are holometabolous endopterygotes that delay the development of external wings until the prepupal and pupal stage (Daly et al. 1998). The male life cycle includes two nymphal instars, sessile prepupal and pupal stages, and then a winged adult (see Ch. 2). To properly identify kermes scales, prereproductive teneral females must be slide mounted (Baer 1980, Kosztarab 1987).

After hatching an *Allokermes* spp. first instar or crawler remains beneath the parental brood chamber until environmental conditions favor its dispersal, possibly up to several days (Baer 1980). The most important factors affecting these population redistributions are light, gravity, temperature, humidity or a combination of these (Greathead 1997, Marotta and Tranfaglia 1997). Crawlers generally settle within a meter

from the mother, insert their stylets into the host plant, and consume phloem sap (Raven 1983, Baer and Kosztarab 1985, Vranjic 1997).

Allokermes spp. crawlers are the most active, dispersal stage (Marotta 1997, Williams 1997). The body of the first instar *A. kingii* is salmon-colored, oblong, widest at the mesothorax, and tapers posteriorly. Antennae are six-segmented with slender setae. The legs are well developed with a single curved claw at the base of each. The anal lobes are partially or entirely sclerotized with numerous setae. Tubular ducts are always absent on both dorsal and ventral derm (Baer and Kosztarab 1985). There are no sexual differences at this stage (Williams 1997). Sexual dimorphism becomes apparent in second instar females and males, although very similar morphologically, the female lacks the tubular ducts which are present dorsally in the male (Baer and Kosztarab 1985, Gullan and Kosztarab 1997, Williams 1997). When preparing to molt to the second instar, two phases are visible: (i) an initial change in body color, particularly around the body margins, followed by (ii) contractible motions and the gradual extrusion of the exuviae (Annecke 1966). Because crawlers lack a waxy exterior, this stage is most vulnerable to adverse conditions and insecticides (Marotta 1997).

Second instar males often congregate on branches, twigs or in bark crevices on the tree trunk. Dorsal tubular ducts are present at this stage (Williams 1997, Hamon et al. 1976). Once a male settles it secretes a “test”, a thin glossy wax over its body. The male test represents an adaptation not only to dry conditions but also to wet conditions by protecting the insects against bacteria and fungi (Boratynski et al. 1982). At the end of the pupal stage the male’s body is elongated, has wing pads, eye pigmentation, short legs, and seven-segmented antennae. The male lacks functional mouthparts so feeding stops



- A. Body shape
- B. Legs
- C. Eyes
- D. Tubular duct
- E. Tubular duct
- F. Disc pore
- G. Disc pore
- H. Pre-anal enlargement
- I. Mid-dorsal enlargement
- J. Spinescent 8-shaped pores
- K. Amorphous pores
- L. Marginal setae
- M. Pre-anal setae
- N. Anal lobes
- O. Anal ring

Figure 1-2. General morphology of adult Kermesidae female, tergum (Bullington and Kosztarab 1985).

(Gullan and Kosztarab 1997). After emerging the adult male will live from a few hours to a week (Marotta 1997).

Second and third instar female bodies are oblong to oval, antennae are six-segmented, and the dorsal tubular ducts are usually absent. First or early second instar females will migrate and settle to the new growth on the branch of the tree and become stationary. The second instar will molt into a short third instar and later become an adult female. The female secretes a wax coat and increases in size (Gullan and Kosztarab 1997). Occurrence of a third instar is debatable; the life cycle has only been studied in a few species (Williams 1997). The third instar differs from second instar and adult by the number of dermal structures, having more than the second instar and less than the adult (Williams 1997). If present, the third instar maybe very short (2 to 4 days) and its occurrence may have been overlooked in some species (Ben-Dov and Hodgson 1997).

In most species, after the female's last molt and before oviposition, the scale's body will increase its size, mainly length. The dorsum becomes sclerotized and the female body color darkens. The dorsum becomes convex, and the female develops ovaries, ovarian eggs, and the brood chamber. The brood chamber is located beneath the female's venter where she will lay eggs. The female's abdomen shrinks after the eggs are laid. Dead females may remain on the host for a year or more after first instar emergence (Baer 1980).

The waxy test provides a shield for the eggs and first instars (Kosztarab 1987, Gullan and Kosztarab 1997, Marotta 1997). The eggs are uniformly covered with wax filaments secreted from the ventral tubular ducts and multilocular disc-pores (Tamaki et al. 1969, Hamon et al. 1975). The wax filaments prevent the eggs from drying and

sticking together (Gullan and Kosztarab 1997). The number of eggs per female varies within each species. *Allokermes kingii* has been reported to have an average of 3,000 eggs per female (Hamon et al. 1975). The size of the female, the position on the host plant, the health of the host plant, and weather are all factors that affect fecundity (Hodgson 1997, Marotta 1997).

CHAPTER 2 BIOLOGY OF *ALLOKERMES KINGII* IN FLORIDA

Trees are an important part of everyday life. They improve environmental quality, provide shade, windbreaks, regulate temperature, and are aesthetically pleasing (Dwyer et al. 2000). Trees afford wildlife refuge in both urban and rural areas. People benefit from trees through an increase in property value and wood products that are produced (Dwyer et al. 2000). In urban areas within the 48 continental states, approximately 3.8 billion trees cover 27.1% of the land, with a tree canopy cover of 2.8%. In Florida alone the estimated portion of the state covered by trees is 10.8%, which amounts to about 169,587,000 trees (Dwyer et al. 2000). Oaks (*Quercus* spp.) are commonly used street trees in the United States, and live oaks (*Q. virginiana* Mill.) have been frequently planted throughout Florida. Live oaks are fast growing, easily transplanted when young, and can provide abundant shade (Harms 1990).

Scale insects are frequent pests of trees, and at least 81 species are known to infest oaks in North America (Scalenet 2001). Although soft (Coccidae) and armored (Diaspididae) scales are typically the most abundant and damaging, ornate pit scales (Lecanodiaspididae), pit scales (Asterolecaniidae), and gall-like scales (Kermesidae) scales have also periodically reached outbreak levels (Solomon et al. 1980). Gall-like scales (*Allokermes* spp.) in particular are difficult to detect because they have a mottled appearance and often resemble the buds of their host tree or galls of cynipid wasps on oaks (Gullan and Kosztarab 1997). These native scales are known to occur on *Q. borealis* (L.), *Q. coccinea* (Muenchhausen), *Q. ilicifolia* (Wangenheimd), *Q. imbricaria*

(Michaux), *Q. laurifolia* (Michaux), *Q. phellos* (L.), *Q. rubra* (L.), and *Q. velutina* (Lamarck) (Kosztarab 1996). Kermesids directly affect plant growth by their feeding, which involves the penetration of their stylets into the phloem and the uptake of sap as food (Raven 1983, Vranjic 1997). The feeding results in branch dieback, reduced tree growth rates, and sooty mold, which grow on the honeydew secreted by the scales (Vranjic 1997). There are twelve *Allokermes* spp. in the world, and eleven are distributed throughout North America (Baer and Kosztarab 1985). The North American species are *A. branigani* (King), *A. cueroensis* (Cockerell), *A. dubius* (Bullington and Kosztarab), *A. essigi* (King), *A. ferrisi* (Bullington and Kosztarab), *A. galliformis* (Riley), *A. gillettei* (Cockerell), *A. grandis* (Cockerell), *A. kingii* (Cockerell), *A. nivalis* (King and Cockerell), and *A. rattani* (Ehrhorn) (Baer and Kosztarab 1985). However, the most damaging to live oak is *A. kingii* (D. Miller, pers. comm.).

Hamon described the taxonomy and biology of *A. kingii* in 1976 at Virginia Polytechnic Institute in Blacksburg, Virginia (Hamon et al. 1976, Baer and Kosztarab 1985). Adult female *A. kingii* are convex in shape, measure 5 mm long, 4.3 mm wide, and about 3.5 mm high. The color is pale brownish-yellow with small black spots covering the entire surface. Each adult female may lay on average 2,820 eggs during her lifetime (Hamon et al. 1975). Adult males are minute with fragile wings, lack functional mouthparts, and live only for a few hours to a week. *Allokermes kingii* has been reported to have one generation per year in Virginia (Hamon et al. 1976).

The geographic and climatic conditions in Virginia, where Hamon et al. (1976) conducted his study, and those in central Florida are different, which may account for variances in *A. kingii*'s life cycle. The research site used by Hamon et al. was located

within the Appalachian Mountain chain, approximately 304 miles from the Atlantic coast (Rand McNally 1998). Virginia has a cold-winter climate with hardy zones of 6 and 7, average minimum temperatures of -23 to -18° and -18 to -12° C, respectively (Dolezal 2000). The average yearly precipitation in Virginia is 109 cm (Tunnell and Woodward 2003). Blacksburg, Virginia, is located within hardy zone 6 and the annual precipitation in 1976, when Hamon et al. did their research, was <102 cm. In contrast, Florida has subtropical and tropical climates (Almanac 2004), with hardy zones of 9 and 10, average minimum temperatures of -1 to 4° and 4 to 10° C, respectively (Kramer and Paukowits 2003). The average yearly precipitation for Florida in 2003 was 155 cm. We conducted our study in Clearwater, FL, which is subtropical with a hardy zone of 10 and an average yearly precipitation of 132 to 142 cm (Muller and Solomon 2003). Clearwater is located on the western coast of Florida, next to the Gulf of Mexico, and is subjected to tropical storms and hurricanes from June to November. During the storm season high winds, increased rainfall, and warm temperatures make conditions conducive to increased scale survival. We sought to determine if more than one generation of *A. kingii* existed in Florida.

Materials and Methods

Study Site

Eight oak trees (two sand live oaks, *Q. geminata* Small, and six live oaks, *Q. virginiana*) with moderate infestations of *A. kingii* in Clearwater, FL (Pinellas Co.), were selected for this study. The infested area in Clearwater was approximately 300 m from the Gulf Coast. Tree height and diameter (diameter at breast height, DBH) were measured on 10 May 2002 and again on 29 August 2003. Tree height was measured with a clinometer and the DBH was measured with a centimeter cloth measuring tape.

Scale Life Cycle

To determine the life cycle of *A. kingii*, five branches (20 - 26 cm long) were cut from each tree, every 2-3 wk, approximately 1.8 - 3.7 m off the ground from 6 June 2002 to 29 August 2003. Branches were transported to the laboratory in a cooler, frozen, and examined with a dissecting binocular microscope (10-20X). The number of healthy first and second instars, healthy female adults, and dead female adults, on each branch was counted and totals for each five-branch tree sample were recorded. The total number of *A. kingii* per main branch and lateral branches, the total length (cm) of each main branch (five per tree), total number of lateral branches per main branch, and their lengths (cm) were recorded. Any arthropods or potential natural enemies found within or in association with adult female *A. kingii* were preserved in 80% EtOH, and identified by taxonomists at the Department of Agricultural and Consumer Services, Division of Plant Industry and/or University of Florida. Voucher specimens will be placed at The Museum of Entomology FSCA, Gainesville, FL.

To examine male *A. kingii*, five bark samples (1.3 cm²) with male tests were taken from each of the eight trees at three heights (0.61, 1.22, 1.83 m from the ground) every 2-3 wk from 23 May to 10 October 2002, and transported to the laboratory in scintillation vials in a cooler. Tests were first observed with a dissecting binocular microscope (10-20X), and then removed from the male scales with a minuten pin, male scales were slide-mounted, and examined with a compound microscope (10-40X) to determine the life stages (e.g., prepupa, pupa, or adult). However, density and distribution of tests on stems were not determined.

Results

Study Site

This study was conducted on *Q. geminata* and *Q. virginiana* trees, which represent new host records for *A. kingii*. The mean height of the eight oak trees at the beginning of the study on 10 May 2002 was 5.9 ± 0.7 m, mean DBH of 51.7 ± 14.3 cm, and mean canopy radius of 9.4 ± 1.3 m. The mean height of the eight oak trees at the end of the study on 29 August 2003 was 6.4 ± 1.0 m, mean DBH of 52.4 ± 14.8 cm, and mean canopy radius of 10.3 ± 2.2 cm.

Scale Life Cycle

Allokermes kingii appears to complete a full generation and a partial second generation each year in Clearwater, FL (Fig.2-1). Salmon-colored crawlers (Fig.2-2A) emerged from late May to the first week of August in 2002 and females migrated to the larger branches while males went onto the tree stem. Crawlers began to molt into second instars by mid-July. At this time, second instar females (Fig.2-2B) migrated to tree wounds or new growth, often near new leaf petioles, became sessile, and secreted a hard, waxy covering over themselves. Second instar males migrated further down on the tree stems, became sessile, and covered themselves with a white, felt-like waxy pupal case (test). Second instar females go through a short third instar (Fig.2-2C), which lasts approximately 2-4 d (Marotta 1997), but this was not observed during the study. Second instar females molted into adults from late August to mid-December. Gravid females occurred in early September to mid-December and laid eggs (Fig.2-2D) under their venter, in the brood chamber. Average length of all branches collected from 6 June 2002 to 29 August 2003 was 198.4 ± 66.9 cm. We found 728.6 ± 399.1 (range 166-1,755) *A.*

kingii, all life stages combined, on 1,120 main branches (22.9 ± 2.3 cm) and 781.6 ± 309.2 (range 371-1,586) on lateral shoots (4.2 ± 1.0 cm).

Male tests (Fig.2-2E) were found in crevices or wounds on tree stems. Male prepupae (Fig.2-2F) were present on the bark from early June to mid-July, and again in mid-September to mid-October. Pupae (Fig.2-2G) occurred throughout the 6-month collection period, with higher numbers at the beginning of June to mid-July and in October. Adult males (Fig.2-2H) were present in late May to early June, early July to mid-August, and throughout October.

Second generation crawlers began emerging in mid-September and molted into second instars by mid-October. The nymphs overwintered as first and second instars. Scale development appeared to slow or stop until the first molt in mid-February 2003. By late April-early May, second instar female *A. kingii* molted into mature adults. Second-generation adult female *A. kingii* produced eggs until mid-June.

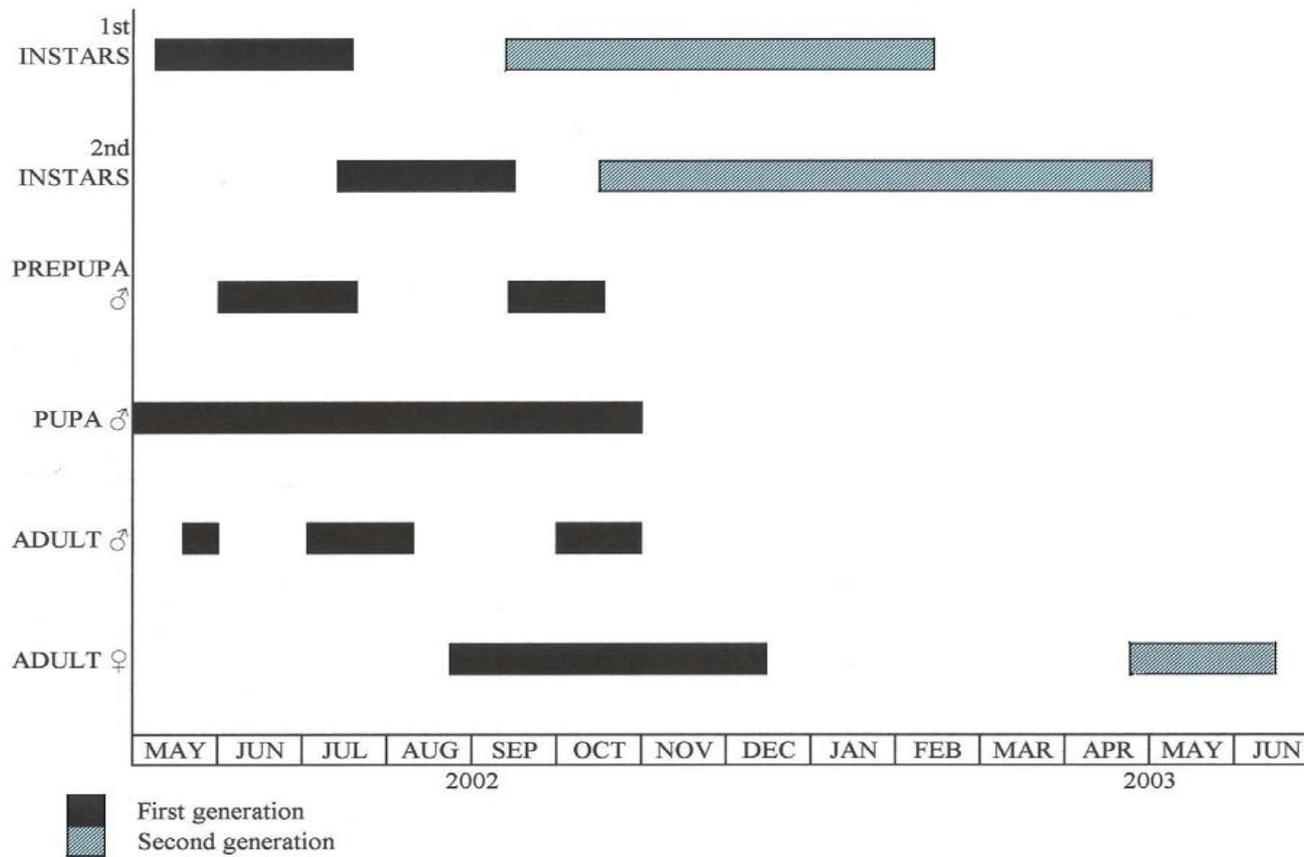


Figure 2-1. Seasonal history of *Allokermes kingii* on *Quercus geminata* and *Q. virginiana* in Clearwater, FL.

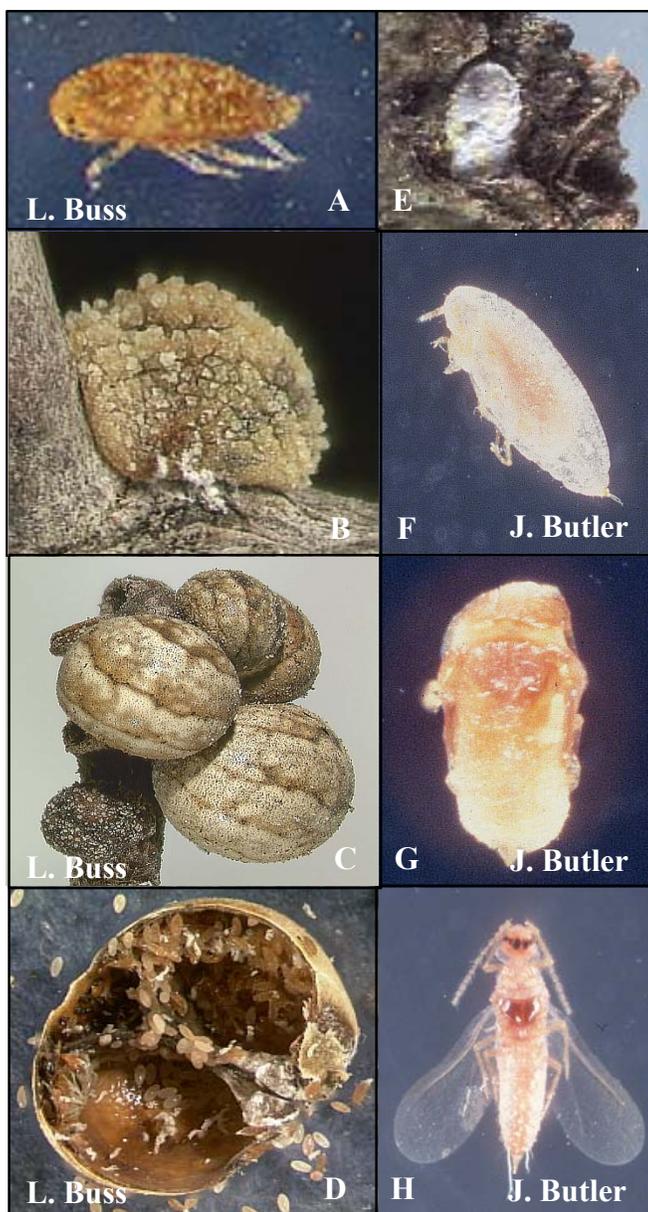


Figure 2-2. Flow chart of life stages for *A. kingii*. A. First instar. B. Second instar female. C. Third instar female. D. Adult female with eggs. E. Male test. F. Male prepupa. G. Male pupa. H. Adult male.

Natural Enemies

Several insects were found either feeding directly on or in association with *A. kingii* in this study (Table 2-1), but none appeared abundant enough to regulate *A. kingii* populations. Several different lepidopteran larvae were found inside adult female *A. kingii* (Fig. 2-3A). These larvae may have been feeding on the eggs, because only a few of the adult females had both eggs and lepidopteran larvae in their brood chambers, but this behavior was not observed. There were several mites (*Tuckerella pavoniformi* (Ewing)), found in association with *A. kingii* throughout the collection period (Fig. 2-3B). These mites are plant-parasites that feed on the cambium. Several hymenopteran eggs were laid on a leaf near *A. kingii* scales from which one ichneumonid wasp (*Trachaner* pr. n. sp.) emerged. A total of 717 adult female *A. kingii* appeared to be parasitized, with exit holes on their bodies (Fig. 2-3C). Formicid ants, *Pheidole dentata* (Mayr), were tending *A. kingii* throughout the collection period. These ants collect and feed on the honeydew that is secreted by *A. kingii*. With decreasing temperatures the activity of the ants decreased. This could indicate that *A. kingii* ceased honeydew production and became dormant. Two different ladybird beetle species were found in association with *A. kingii*. Several lacewings (*Chrysoperla* sp.) eggs were found on leaves and one was on top of an adult female *A. kingii* (Fig. 2-3D). Other insects in the orders Psocoptera and Thysanoptera were also found in association with *A. kingii* (Table 2-1). Dipteran larvae were found inside adult females (Fig. 2-3E), but were easily damaged and could not be identified.

Table 2-1. Arthropods found in association with *A. kingii*.

Order	Family	Species	Date	Number found
Acariforme	Tuckerellidae	<i>Tuckerella pavoniformi</i> (Ewing)	6 June 2002	1
Coleoptera	Attelabidae	<i>Homoelabus analis</i> (Illiger)	7 July 2002	1
Coleoptera	Coccinellidae	<i>Chilocorus cacti</i> (L)	6 June 2002	1
Coleoptera	Coccinellidae	<i>Harmonia axyridis</i> (Pallas)	6 June 2002	1
Hemiptera	Diaspididae	<i>Pseudaulacaspis pentagona</i> (Targ.-Tozz.)	29 August 2003	6
Hymenoptera	Encyrtidae	<i>Metaphycus</i> sp. (Howard)	6 June 2002	1
Hymenoptera	Formicidae	<i>Pheidole dentata</i> (Mayr)	16 July 2003	50
Hymenoptera	Ichneumonidae	<i>Trachaner</i> pr. n. sp. (Townes)	23 May 2002	1
Lepidoptera	Blactobasidae	<i>Holcocera coccivorella</i> (Chambers)	5 June 2003 19 June 2003 19 July 2003	1 2 1
Lepidoptera	Cosmopterigidae	<i>Euclementia bassettella</i> (Clemens)	20 June 2002 5 June 2003	2 1

Table 2-1. Continued.

Order	Family	Species	Date	Number found
Lepidoptera	Cosmopterigidae	<i>Pyroderces</i> sp. (Herrich-Schäffer)	6 June 2002	2
Lepidoptera	Pyralidae	<i>Laetilia coccidivora</i> (Comstock)	22 May 2003	1
Lepidoptera	Pyralidae	<i>Laetilia</i> sp. (Ragonot)	5 June 2003 19 June 2003	1 2
Neuroptera	Chrysopidae	<i>Chrysoperla</i> sp.	20 June 2002	1
Neuroptera	Chrysopidae	—	5 May 2002 23 May 2002 20 June 2002	1 2 1
Psocoptera	Psocidae	—	2 Aug 2002 2 Aug 2002	2 1
Thysanoptera	Phlaeothripidae	—	6 June 2002	2
Thysanoptera	—	—	20 June 2002	1

— = not determined

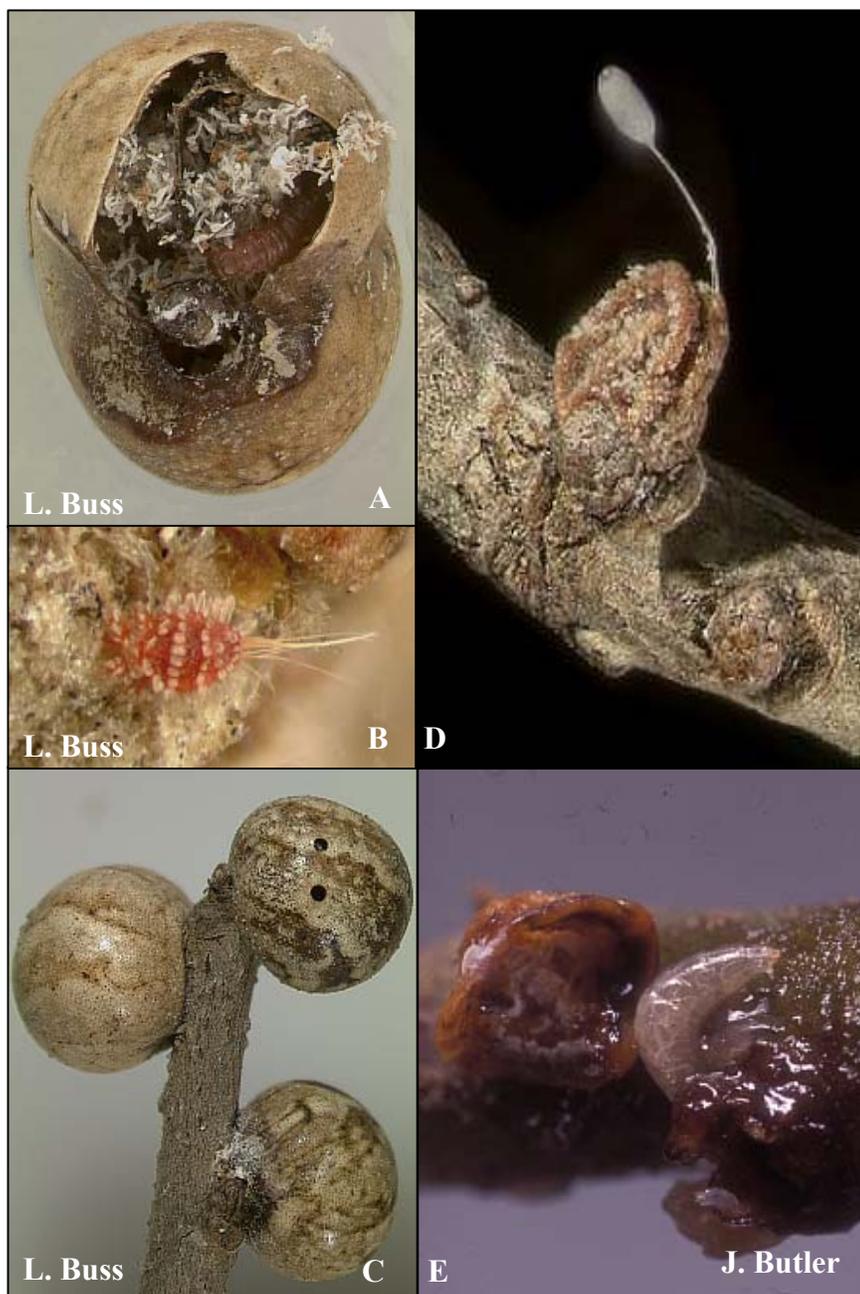


Figure 2-3. Potential natural enemies of *A. kingii*. A. Lepidopteran larva inside adult female. B. *Tuckerella pavoniformi*. C. Exit holes on adult female. D. Lacewing egg on second instar. E. Dipteran larva inside adult female.

Discussion

Allokermes kingii has a full generation and a partial generation, which overwinters as first and second instars in Clearwater, Florida. This seasonal phenology is different

from previously published life history data, which states that there is only one generation per year in Virginia (Hamon et. al. 1976). Several factors including insect phenology, tree species, location, or weather may contribute to the variance.

Allokermes kingii adult females are neotenuous and are able to mate with a male scale at a young age (Gullan and Kosztarab 1997). The adult male sperm is unique among insects. The lumen of the adult male *A. kingii* contains numerous sperm bundles in a liquid, each sperm bundle containing 8-12 spermatozoa surrounded by a sheath (Foldi 1997, Gullan and Kosztarab 1997). After mating with females, sperm bundles are stored within the female oviduct. If males mate with teneral females, then fertilization may need to be delayed for weeks or months until eggs are mature (Gullan and Kosztarab 1997). The longevity of sperm within the female scale suggests that fertilization of eggs may occur over a protracted oviposition period or a long time after copulation (Gullan and Kosztarab 1997). It is thought that neoteny shortens female development time, which caused the male dormant stages to evolved to synchronize reproductive maturity of the sexes (Danzig 1980).

After the adult female's last molt her size changes dramatically (McConnell and Davidson 1959, Matile-Ferrero 1997). The adult female *A. kingii* dorsum becomes heavily sclerotized at maturity, and a cavity under the moribund body acts as a secure brood chamber for the developing eggs (Bullington and Kosztarab 1985, Matile-Ferrero 1997). The brood chamber is formed by the development of a cavity beneath the abdomen (see Chap.1). By the time oviposition has been completed, the abdomen has become so shrunken through the loss of eggs that the venter may touch the dorsum, with the entire cavity beneath filled with eggs (Marotta 1997). *Allokermes kingii* secrete a

waxy cover that protects their eggs and hatching nymphs, which increase the survival of progeny (Kosztarab 1987, Gullan and Kosztarab 1997). Even after death *A. kingii* shelter their progeny with their body, protecting them against the environment, pesticides, and natural enemies (Kosztarab 1987, Gullan and Kosztarab 1997).

Allokermes kingii reportedly infests eight different oak species, *Q. borealis*, *Q. coccinea*, *Q. ilicifolia*, *Q. imbricaria*, *Q. laurifolia*, *Q. phellos*, *Q. rubra*, and *Q. velutina* (Kosztarab 1996), in addition to our new host records of *Q. geminata* and *Q. virginiana*. The geographic range of *A. kingii* in the United States overlaps with the distribution of these oaks (Bullington and Kosztarab 1985). All of the oaks infested by *A. kingii* are northern species, exception *Q. phellos* and *Q. velutina*, which extend from the northern United States into the western tip of Florida (Stein et al. 2003).

Hamon studied the biology of *A. kingii* on northern red oak (*Q. rubra*) and black oak (*Q. velutina*) in Virginia. The northern red oak's native range is in the northeastern part of the United States down to southern Alabama, Georgia and North Carolina (Sander 1990). Northern red oaks produce their first flush in April or May. Black oak is distributed from Maine west to Minnesota, and south to Texas northwestern Florida (Stein et al. 2003). Like the northern red oak it also flushes in April or May. The two oaks used in this study, sand live oak (*Q. geminata*) and live oak (*Q. virginiana*) are found along the lower Coastal Plains and throughout Florida (Stein 2003). The sand live oak trees in Clearwater, Florida, began to flush around mid-April and the live oak flushed in mid-March, with several additional flushes throughout the growing season.

By taking advantage of the plant/insect relationship, we can use plant cues (flush, flowering, petal fall, etc.) as indicators of insect development (Ascerno 1991). The

prolonged flush in Clearwater, one month longer than in Virginia, may enable *A. kingii* to increase its population by providing a food source that is available for a longer period of time. Weather may also indirectly influence plant feeding insects by causing stress in the host plant or causing excessive growth in the plant (Shetlar 1997).

Florida has a tropical storm and hurricane season that lasts for six months out of the year. On 1 September 2002, Hurricane Edouard was slightly north of Clearwater, FL. This hurricane lasted for 5 days with maximum winds of 65 mph. From 1 September to 14 September it rained a total 14.22 cm (FAWN 2004). The growth of scale insects depends upon the quality and quantity of the plant's sap. The plant sap contains a limited amount of nitrogen upon which the insect depends for growth, and quite small changes in the nitrogen content of the sap can have dramatic effects on population growth rates (Kunkel 1997). Water pushes nitrogen up from the soil into the plant, which will then be available to *A. kingii* in the sap. During September and October 2002 there was an increase in the number of first instars. The excessive amount of water caused by hurricane Edouard may have contributed to the outbreak of *A. kingii*.

It has been shown that parasitoid-host interactions become more frequent as the host matures (Blumberg 1997). Most of the mortality in this study can be attributed to parasitism or predation of adult females and second instars. Many females that are parasitized are able to oviposit, but the number of eggs they produce may be greatly reduced (Gordon and Potter 1988). During this study most of *A. kingii* with emergence holes were adult females, only a few were second instars.

The most abundant insect species associated with *A. kingii* was *Pheidole dentata* (Mayr). Ants commonly tend and defend scale insects. In return they feed off the

honeydew that *A. kingii* excretes (Gullan 1997). Scale insects are often attacked by caterpillars (Scoble 1995). All but one lepidopteran family collected during this study was found inside adult females. *Euclemensia bassettella* (Clemens) are known predators of *A. kingii* (Stehr 1987, Scoble 1995, and Scalenet 2001) and *Laetilia coccidivora* (Comstock) are known to prey on scale insects (Stehr 1987). Different coleopteran families are also common scale predators (Stehr 1987, Borror et al. 1989). A few thysanopteran species in the family Phlaeothripidae are known as predators of scale insects (Stehr 1987). Several psocopterans in the family Psocidae were also collected. A few psocid species are omnivorous feeding on insect eggs and possibly scale insects. Acariformes in the family Tuckerellidae: *Tuckerella pavoniformi* (Ewing) were found in abundance along with the scales. *Tuckerella pavoniformi* are obligant plant feeders and are not common in Florida.

The fecundity of an insect is affected by temperature, scale density, adult female size, and the species and edaphic conditions of the host plant (Salvatore 1997). The subtropical climate of Clearwater, Florida, and annual precipitation of 132-142 cm may make it conducive for adult female *A. kingii* to produce more eggs than are produced in Blacksburg, Virginia. With this increase in production of eggs the scale density on the trees is able to increase to a population that could be detrimental to the tree, causing branch die-back and even death of the tree.

CHAPTER 3
INSECTICIDAL MANAGEMENT OF *ALLOKERMES KINGII* ON SHADE TREES

Allokermes kingii Cockerell (Hemiptera: Kermesidae), is a native gall-like scale found on oak trees (*Quercus* spp.) throughout the eastern United States, west to Indiana and Tennessee. Damage from this scale results in flagging, branch dieback, reduced growth, and during heavy infestations, tree death (Hamon 1977). In addition, the honeydew that the kermes scale creates results in sooty mold problems, which may reduce a tree's ability to photosynthesize, and an increase in ant activity.

Populations of *A. kingii* have the potential to increase in numbers faster in Florida's subtropical climate than in more northern states. Previous research suggests that *A. kingii* is univoltine throughout most of its North American range (Hamon et al. 1976). However, *A. kingii* can produce one full generation and a partial second generation each year in Florida (see Chapter 2). First generation crawlers emerge in late May, become second instars by mid-July and reach the adult stage by late August. Second generation crawlers emerge in mid-September and become second instars by mid-October. Both the first and second instars overwinter and become adults in late April or early May of the following year. Control of this insect is difficult because of the extended egg hatch and crawler activity and unawareness of the partial second generation.

Several species of oak, especially live oak (*Quercus virginiana* Mill.), are frequently planted and maintained as street trees in Florida. Live oak trees are fast-growing and easily transported when young, which enables them to be widely used as ornamental trees (Harms 1990). The annual cost of tree installation and maintenance in

the City of Clearwater was \$234,000 in 2003 (A. Mayberry, pers. comm.). Because the Clearwater city arborist was concerned that hundreds of trees might die from kermes scale infestations, we conducted an insecticide trial to determine the efficacy of several insecticides against *A. kingii* nymphs.

Materials and Methods

Study Site

Thirty oak trees (*Q. geminata* Small and *Q. virginiana*) with moderate infestations of *A. kingii* in Clearwater, FL (Pinellas Co.), were selected for this study. The infested trees were located in a parking lot 300 m from the Gulf Coast. The mean height of the thirty trees was 5.9 ± 0.7 with a mean DBH of 14.3 ± 3.7 in May 2003. The height was measured with a clinometer and the DBH was measured with a centimeter cloth measuring tape. Trees were separated by at least 4.3 m and branches were not interconnected.

Field Test

Insecticide applications were timed to coincide with the emergence of first and early second instars of the first *A. kingii* generation. A certified arborist applied the insecticides on 1 and 2 May 2003. Treatments were assigned to trees using a randomized complete block design, with five replicates and six treatments. Treatments included label rates of acephate (Orthene TT&O[®], Valent USA Corp., Walnut Creek, CA), bifenthrin (Talstar Flowable[®], FMC, Philadelphia, PA), imidacloprid (Merit 75 WP[®], Bayer Environmental Science, Montvale, NJ), horticultural oil (Sunspray Ultra-Fine[®], Philadelphia, PA), horticultural oil plus acephate, and an untreated control. Adjuvant was not mixed with the insecticides. Acephate, bifenthrin, horticultural oil, and horticultural oil + acephate were applied as foliar sprays using a hydraulic sprayer (pressure: 25

ml/m²) with a 756 L tank. Trees were sprayed until run-off. The tank had an agitator with a single nozzle hand-held sprayer. Imidacloprid was applied under the tree canopy as a soil drench using an 18.9 L Solo backpack sprayer. Equipment was triple-rinsed between treatments to prevent contamination. Air temperature, soil temperature measured at 20.3 cm deep, relative humidity, wind speed, and cloud conditions were noted at application.

To determine product efficacy, the number of healthy and dead first and second instars of *A. kingii* were counted on four branches collected from each tree. One branch (20 - 26 cm long) was randomly cut from each of the four cardinal points of each tree, approximately 1.8 to 3.7 m up from the ground on 24 April (pretreatment), 9 May (1 week after treatment, WAT), 22 May (3 WAT), 5 June (5 WAT) and 19 June (7 WAT), 2003. The four branches per tree were put into a plastic bag, placed in a cooler, transported to the laboratory, frozen, and examined with a dissecting binocular microscope. First and early second instars that survived the treatments were salmon-colored. However, insecticide-killed nymphs were slightly brown and shriveled. A waxy secretion normally coats healthy second instars, but those affected by insecticides had black spots on the wax layer. Male *A. kingii* located on tree stems were not examined in this test.

Statistical Analysis.

The mean number of healthy first and second instar *A. kingii* per four-branch sample was calculated using a one-way analysis of variance (ANOVA) ($P < 0.05$) and treatments were compared to the control using a Dunnett's Test on each date (Jmp®, SAS Institute Inc. 2001). The proportion of scale mortality was calculated by dividing

the total number of dead nymphs by the total number of live and dead nymphs for each branch on each date. Proportions were arc-sine square root-transformed, analyzed using an ANOVA, and if statistically significant, treatments were compared to the control using a Dunnett's Test on each date (Jmp®, SAS Institute Inc. 2001).

Results

From the beginning to the end of the applications on 1 and 2 May 2003, the air temperature ranged from 26° to 34.2° C, soil temperature 20.3 cm deep had a range of 21.1° to 23.3° C, relative humidity ranged from 76 to 100%, and wind speed ranged from 1.2 to 4.3 kph. Cloud conditions ranged from partly cloudy to overcast. About 1.3 cm of rain fell lightly for 15 min after the horticultural oil, acephate, and oil plus acephate applications on 1 May 2003. Imidacloprid was applied as a soil drench after the rain. Bifenthrin was applied as a foliar spray the following day because the wind increased to >4 kph on 1 May. Trees measured from the trunk were 4.3 to 5.5 m apart with a mean DBH of 22.8 to 17.8 cm DBH and mean height of 5.5 ± 1.0 m. Trees were not irrigated.

Insecticidal treatments of *A. kingii* reduced nymphal survival by almost half 1 WAT, compared to the pretreatment sample (Table 3-1), but none of the treatments killed all of the nymphs. However, the number of healthy nymphs on control trees also declined over time. Significantly fewer nymphs survived 3 WAT on trees treated with mixed horticultural oil and acephate, compared to the control. The number of healthy nymphs markedly increased on trees treated with acephate or bifenthrin 5 WAT and significantly so on bifenthrin 7 WAT compared to the control. The population increase on 19 June in the bifenthrin treatment was largely from the crawler emergence from two

Table 3-1. Mean (\pm SEM) number of healthy first and second instar *A. kingii* per four-branch sample.

Treatment	Rate (prod./water)	24-April (pretreat.)	9-May (1 WAT)	22-May (3 WAT)	5-June (5 WAT)	19-June (7 WAT)
Control	—	69.0 \pm 32.1	66.6 \pm 24.0	41.2 \pm 8.4	43.2 \pm 13.3	33.2 \pm 10.9
Imidacloprid	5.65g/7.56L	117.8 \pm 49.6	71.6 \pm 22.5	56.0 \pm 14.2	52.4 \pm 37.1	57.6 \pm 27.3
Acephate	2.37L/378L	100.0 \pm 54.8	26.2 \pm 8.3	13.4 \pm 2.4	40.6 \pm 17.3	41.2 \pm 21.4
Horticultural oil	7.56L/378L	94.0 \pm 25.9	12.6 \pm 4.3	13.0 \pm 4.9	25.6 \pm 8.5	23.6 \pm 6.1
Oil + acephate	7.56L oil + 226.8g acephate/378L	49.0 \pm 27.5	5.2 \pm 2.8	7.8 \pm 3.0*	2.6 \pm 1.5	14.2 \pm 12.7
Bifenthrin	2.37L/378L	85.0 \pm 24.0	44.4 \pm 22.2	16.8 \pm 7.7	70.8 \pm 40.9	308.2 \pm 118.7*
		$F = 0.41$ $df = 5,24$ $P = 0.834$	$F = 2.77$ $df = 5,24$ $P = 0.041$	$F = 6.03$ $df = 5,24$ $P = 0.001$	$F = 0.91$ $df = 5,24$ $P = 0.494$	$F = 4.90$ $df = 5,24$ $P = 0.003$

Means within a column followed by an asterisk are significantly different from the control (Dunnett's test) at $P < 0.05$.

female *A. kingii*. No other crawler emergence was noted from any other treatments or dates.

The percentage of nymphal mortality was statistically greater on trees treated with acephate or horticultural oil 3 WAT (Table 3-2). The percentage of mortality 5 WAT was greatest on *A. kingii* treated with horticultural oil and acephate, statistically differing from the control.

Discussion

Scale insects on trees and shrubs can be difficult to control. To control scale insects effectively, their identification must be accurate and the crawler activity period should be known (Muegge and Merchant 2000). The first and early second instars of *A. kingii* are the stage that is controlled most effectively with insecticides (Muegge and Merchant 2000). Under most conditions, predators and parasites suppress scale populations to a level where chemical intervention is not needed (Futch et al. 2001). When scale populations are not controlled by biological or chemical means, high populations may damage leaves, fruit, twigs, branches, or tree trunks (Futch et al. 2001). Best practices for insecticides against scales would include proper timing of application and correctly labeled insecticides targeted against crawlers (Gilrein 2001).

Acephate is one of the more recent additions to systemic insecticides (Ware 1996). Acephate provides better long-term control through nymphal suffocation, systemic, or contact mortality. Acephate has a moderate persistence with 10 to 15 days of residual activity (Ware 1996, Syslo and Davy 1999). It is possible that the mixture of oil with acephate increases the adherence and dispersion on trees. Because of the short residual of acephate and lack of residual for the oil (Syslo and Davy 1999), as well as extended

Table 3-2. Mean (\pm SEM) percentage of dead first and second instar *A. kingii* per four-branch sample.

Treatment	9-May (1 WAT)	22-May (3 WAT)	5-June (5 WAT)	19-June (7 WAT)
Control	5.7 \pm 3.8	0	0	12.3 \pm 5.7
Imidacloprid	17.3 \pm 5.8	11.3 \pm 5.7	20.1 \pm 6.5	24.2 \pm 3.6
Acephate	31.7 \pm 4.1	32.7 \pm 5.2*	11.0 \pm 3.2	18.3 \pm 6.6
Horticultural oil	37.7 \pm 11.1	26.7 \pm 9.0*	15.8 \pm 3.0	21.7 \pm 9.2
Oil + acephate	23.6 \pm 10.7	13.2 \pm 8.9	41.7 \pm 19.3*	15.5 \pm 12.5
Bifenthrin	24.7 \pm 2.6	16.0 \pm 6.7	12.0 \pm 5.1	9.7 \pm 2.7
	$F = 2.33$ $df = 5,24$ $P = 0.074$	$F = 3.89$ $df = 5,24$ $P = 0.010$	$F = 2.62$ $df = 5,24$ $P = 0.050$	$F = 0.70$ $df = 5,24$ $P = 0.628$

Means within a column followed by an asterisk are significantly different from the control (Dunnett's test) at $P < 0.05$.

crawler emergence period (see Chapter 2), additional applications of an acephate-oil mixture or other insecticide may be needed.

Bifenthrin is a contact and stomach poison. Its mode of action is by paralyzing the nervous system of the insect. Degradation of bifenthrin can occur between 7 days to 8 months, depending on the oxidative microbial activity. Bifenthrin is a broad-spectrum insecticide (Dent 2000), and can kill natural enemies. It is possible that a bifenthrin application could cause *A. kingii* population to eventually rebound because of natural enemy mortality or increased plant growth (McClure 1977). However, natural enemies were never abundant during this study.

Soil drenches are useful in an urban environment because they reduce spray drift, thereby reducing nontarget impacts (Rebek and Sadof 2003). Imidacloprid is a frequently used soil drench used against scales. Imidacloprid is a systemic and contact insecticide, which has the potential for managing insects that have become insecticide resistant (Pedigo 1996). Systemic insecticides are taken up by the roots or leaves and translocated within the plant. Insects feeding on the plant digest the insecticide and are killed. Imidacloprid is in the new class of chloronicotinyls, which are synthetics of the natural product nicotine. Imidacloprid changes the behavior and mobility of an insect by affecting the insect's nervous system. It may, however, also negatively impact the behavior of natural enemies, such as diminish searching behavior and prey consumption of ladybird beetles (Smith and Krischik 1999). As a contact insecticide on the plant imidacloprid was shown to decrease parasitism of *Encarsia citrina* (Craw) on the Euonymus scale, *Unaspis euonymi* (Comstock), and resulted in an increase of that scale's population (Rebek and Sadof 2003).

Numerous researchers have recorded scale insect population outbreaks following pesticide applications. The brown soft scale, *Coccus hesperidum* L. (Bartlett and Ewart 1951), frosted scale, *Parthenolecanium prunosum* (Coq.) (Bartlett and Ortega 1952), Hemlock scale, *Fiorinia externa* Ferris (McClure 1977), California red scale *Aonidiella aurantii* (Maskell). (Stansly, et al 1999) all increased in population after the insecticide treatment. McClure (1977) stated several reasons for the resurgence of scale populations: 1) reduced numbers of natural enemies, 2) reduced competition among individuals, and 3) increased plant growth, which improved the nutritive quality of the host. The resurgence of healthy *A. kingii* nymphs may have been due to additional egg hatch, favorable weather conditions, or breakdown of insecticidal residues (Syslo and Davy 1999).

Lack of irrigation may have reduced the effectiveness of the application of imidacloprid. Grafton-Cardwell and Reagan (1999) indicated that there was a trend in greater efficacy of California red scale control when an imidacloprid treatment was preceded by 2 h of irrigation on fruit, leaves and soil. Thus pre-wetting of the soil appears to be important for the uptake of imidacloprid. There was no irrigation at the study site. Even though it rained about 1.3 cm, the lack of water and the amount of leaf debris under the trees' canopies may have reduced root uptake of imidacloprid, thus delaying the translocation of imidacloprid into the trees' phloem, which is where *A. kingii* feeds (Salvatore 1997).

In conclusion, this study has shown that horticultural oil and acephate mixed gave the fastest and longest lasting control of *A. kingii*. However, none of the products were highly efficacious against *A. kingii* nymphs at anytime in this test. Some other products

labeled for scale control in urban landscapes include Fish oil, Insecticidal soap, malathion (Malathion, Gowan, Yuma, AZ), pyriproxyfen (Distance, Valent USA Corp., Walnut Creek, CA), or thiamethoxam (Flagship, Syngenta USA, Greensboro, NC). Other control measures would include proper pruning and removal of scales by hand.

CHAPTER 4
HYDROCARBON AND FATTY ACID METHYL ESTERS ANALYSIS OF
ALLOKERMES KINGII

Allokermes kingii lives primarily on oak trees (*Quercus* spp.). The damage caused by *A. kingii* can be seen in branch die-back, reduced tree growth rates, and sooty mold, which grows on the honeydew that the kermes scales secrete (Hamon 1977, Vranjic 1997). Oak trees frequently planted along streets and in city parks. In order to protect the tree, one must first understand the insect that is affecting it. Knowledge of the composition of the wax secreted by insects is of interest, apart from the point of view of comparative biochemistry, because it may provide a clue to the best method of managing the insect (Hackman 1951).

Hydrocarbons and waxes serve many functions in insects. They prevent desiccation and are important in chemical communication (Nelson 1978, Howard 1982). The test or cover that is secreted by scale insects is believed to protect the scales from effects of weather, natural enemies, and possibly insecticidal sprays (Hackman 1951, Castner and Nation 1986, Stanley-Samuelson and Nelson 1993, Tamaki 1997). Sulc (1932) was the first to utilize characteristics of male tests as an aid for identifying species of soft scales (Miller and Williams 1990). This study was conducted to determine if a unique gas chromatography profile could be obtained from the male *A. kingii*'s test.

Materials and Methods

Study Site.

A. kingii tests were collected from eight oak trees (two sand live oaks, *Q. geminata* Small, and six live oaks, *Q. virginiana*) every 2-3 wk from 23 May to 10 October 2002, in Clearwater, FL (Pinellas Co). Tests were removed from each tree at three heights (0.61, 1.22, 1.83 m from the ground), and transported to the laboratory in scintillation vials in a cooler. Each bark sample measured approximately 2.54 cm. Five tests were collected from each tree and placed into a vial. The male tests were removed from the tree bark with a minuten pin in the laboratory and placed into a vial. The male scale was slide mounted and used to determine the male *A. kingii* biology (chapter 2).

Hydrocarbons.

Hydrocarbons and other lipids were extracted by immersing 231 tests in 5 ml of benzene, gently agitating, and saponifying over night. The solvent was gently evaporated with a stream of nitrogen. From 1-2 μ l of the concentrated extract was injected into a AT1 Heliflex Col from ALLTECH, cat. #932525, non-polar column. Data were collected and processed directly from the chromatograph by a Hewlett Packard 3390A integrator. Hydrocarbons were separated by a coiled glass column with an interior diameter of 25 mm by 25 m, with 0.2 μ m thickness. The carrier gas was helium at a flow of 21.8 cm per second. The injector port was set at 270° C with the out take valve set at 320° C. The glass column was at 200° C upon injection, and was immediately temperature programmed at 4° C per minute to 300° C and held for 20 minutes.

A standard was prepared from commercially available synthetic hydrocarbons (Sigma Chemical Company). The standards contained even and odd straight chain hydrocarbons from C20 to C30, plus C32 and C34.

Esters of Fatty Acids.

Esters of higher fatty acids were extracted from the same 231 tests. Tests were in a vial with a solution of 0.5 ml KOH and 0.5 ml Methyl alcohol (CH₃OH) and gently agitated for one minute. The solvent was evaporated with nitrogen and injected into the AT1 Heliflex Col from ALLTECH, cat. #932525. The methods from the hydrocarbon test were repeated for the esters of fatty acids test.

Results and Discussion**Hydrocarbons.**

Chromatogram profiles and relative quantities are proving to be characteristics for a wide variety of individual insect species. Temperature programmed gas chromatographic traces for hydrogen are shown in Fig. 4-1 with a corresponding linear retention graph in Fig. 4-2. Kovat Indices (KI) and hydrogen composition are shown in Table 4-1.

The main components of hydrocarbons in the test of *A. kingii* have calculated KI values 2000, 2200, 2300, 2400, 2500, 2600, 2700, 2800, 2900, 3000, 3200, 3400 were chromatographically identical to C₂₀, C₂₂, C₂₃, C₂₄, C₂₅, C₂₆, C₂₇, C₂₈, C₂₉, C₃₀, C₃₂ and C₃₄, respectively. Major peaks were recorded at 13.62, 20.40, 10.19, and 16.98 with a percentage of area covered 28.075, 15.651, 14.508, and 13.960 respectively.

Esters of Fatty Acids.

Temperature programmed gas chromatographic traces for fatty acids with methyl esters are shown in Fig. 4-3 with a corresponding linear retention graph in Fig. 4-4. Kovat Indices (KI) and fatty esters are shown in Table 4-2. The major constituents of fatty esters in the male test have calculated KI values of 1400, 1600, 1610, 1800, 18:1 and were chromatographically identical to C₁₄, C₁₆, C_{16:1}, C₁₈ and C_{18:1},

respectively. Major peaks were recorded at 24.02, 29.14, and 16.13 with percent area coverage of 54.993, 18.891, and 11.358, respectively.

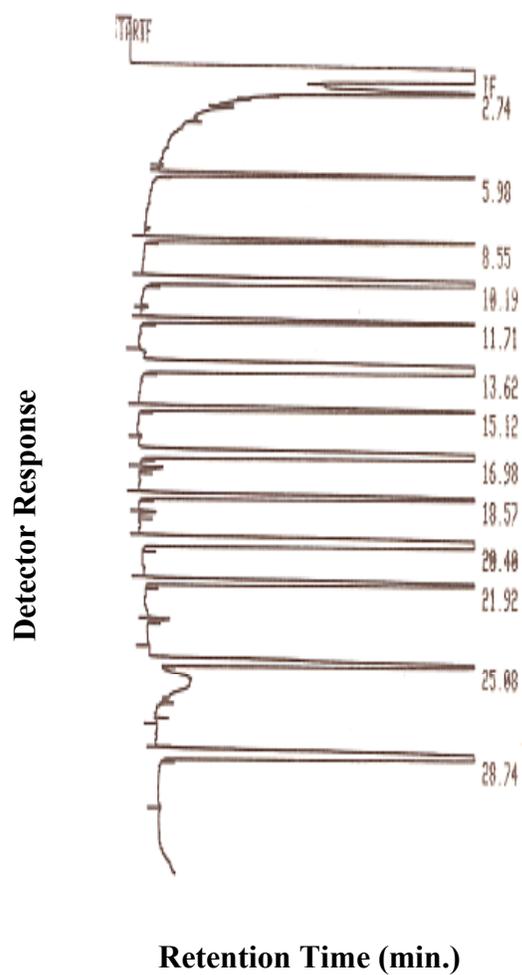


Figure 4-1. Temperature programmed gas chromatographic traces for hydrogen.

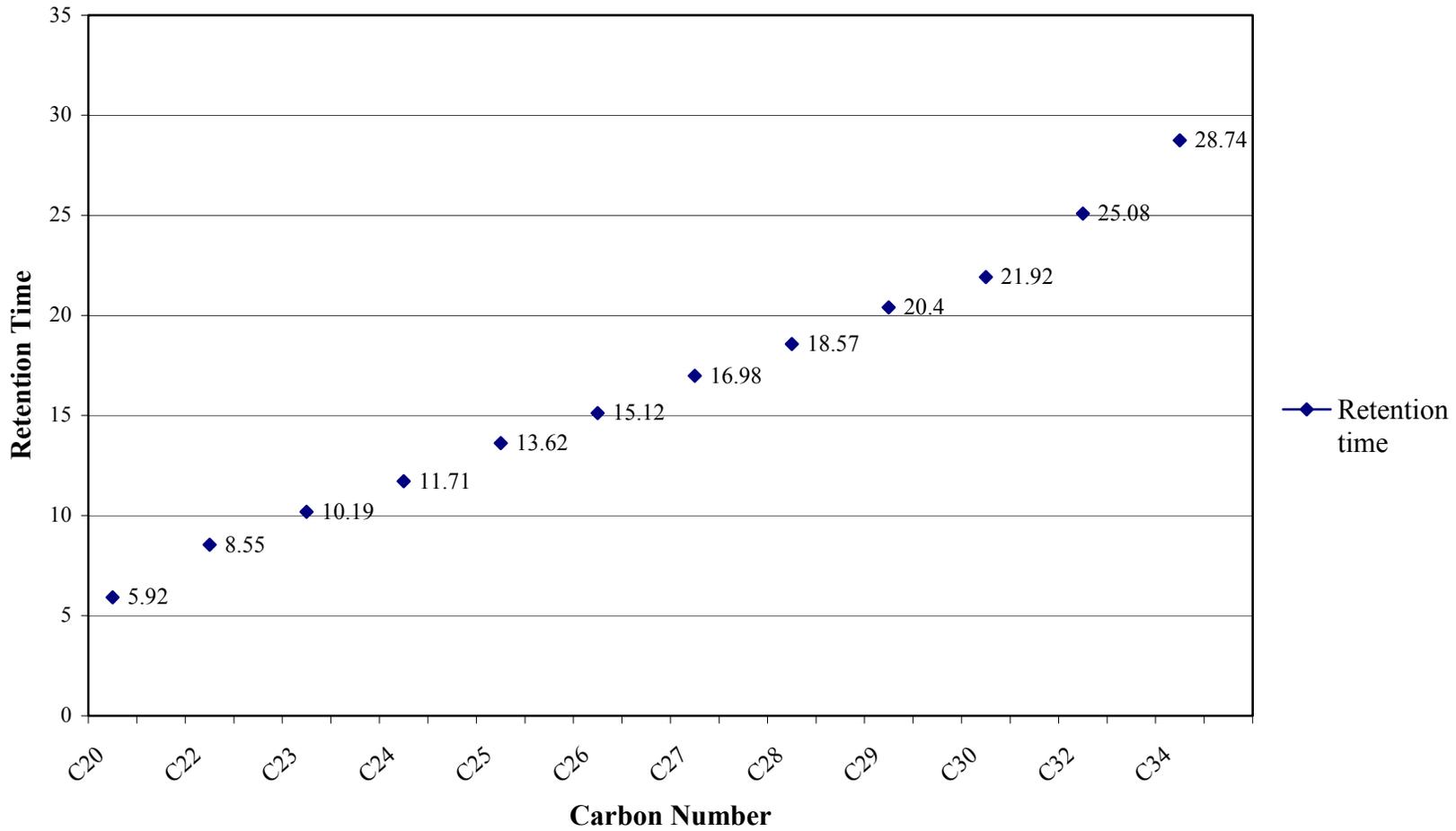


Figure 4-2. Linear retention time graph of hydrocarbons on male *A. kingii* tests.

Table 4-1. Composition of hydrocarbons from male *A. kingii* tests.

Hydrocarbon	Carbon number	Retention time	Area %	Calculated index value
Eicosane	C20	05.92	01.906	2000
Docosane	C22	08.55	01.671	2200
Tricosane	C23	10.19	14.508	2300
Tetracosane	C24	11.71	02.600	2400
Pentacosane	C25	13.62	28.075	2500
Hexacosane	C26	15.12	02.521	2600
Heptacosane	C27	16.98	13.960	2700
Octacosane	C28	18.57	03.763	2800
Nonacosane	C29	20.40	15.651	2900
Triacontane	C30	21.92	04.089	3000
Dotriacontane	C32	25.08	03.955	3200
Tethatriacontane	C34	28.74	05.417	3400

Table 4-2. Composition of methyl ester of the fatty acids from male *A. kingii* tests.

Hydrocarbon	Carbon number	Retention time	Area %	Calculated index value
Tetradecane	C14	06.63	00.369	1400
Hexadecane	C16	12.61	04.631	1600
	C16:1	16.13	11.358	1610
Octadecane	C18	24.02	54.993	1800
	C18:1	29.14	18.891	1810

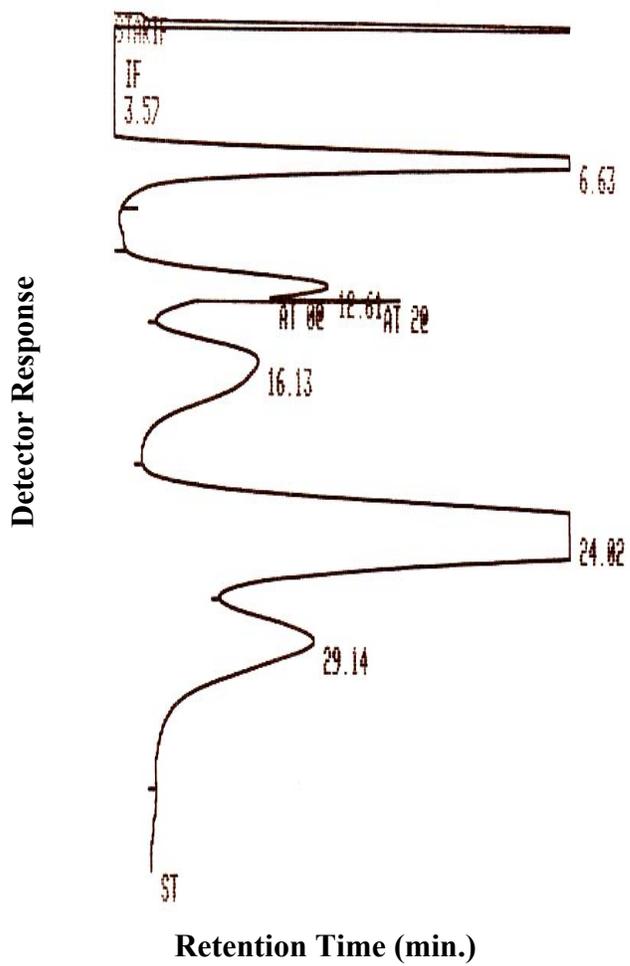


Figure 4-3. Temperature programmed gas chromatographic traces for fatty acids with methyl esters.

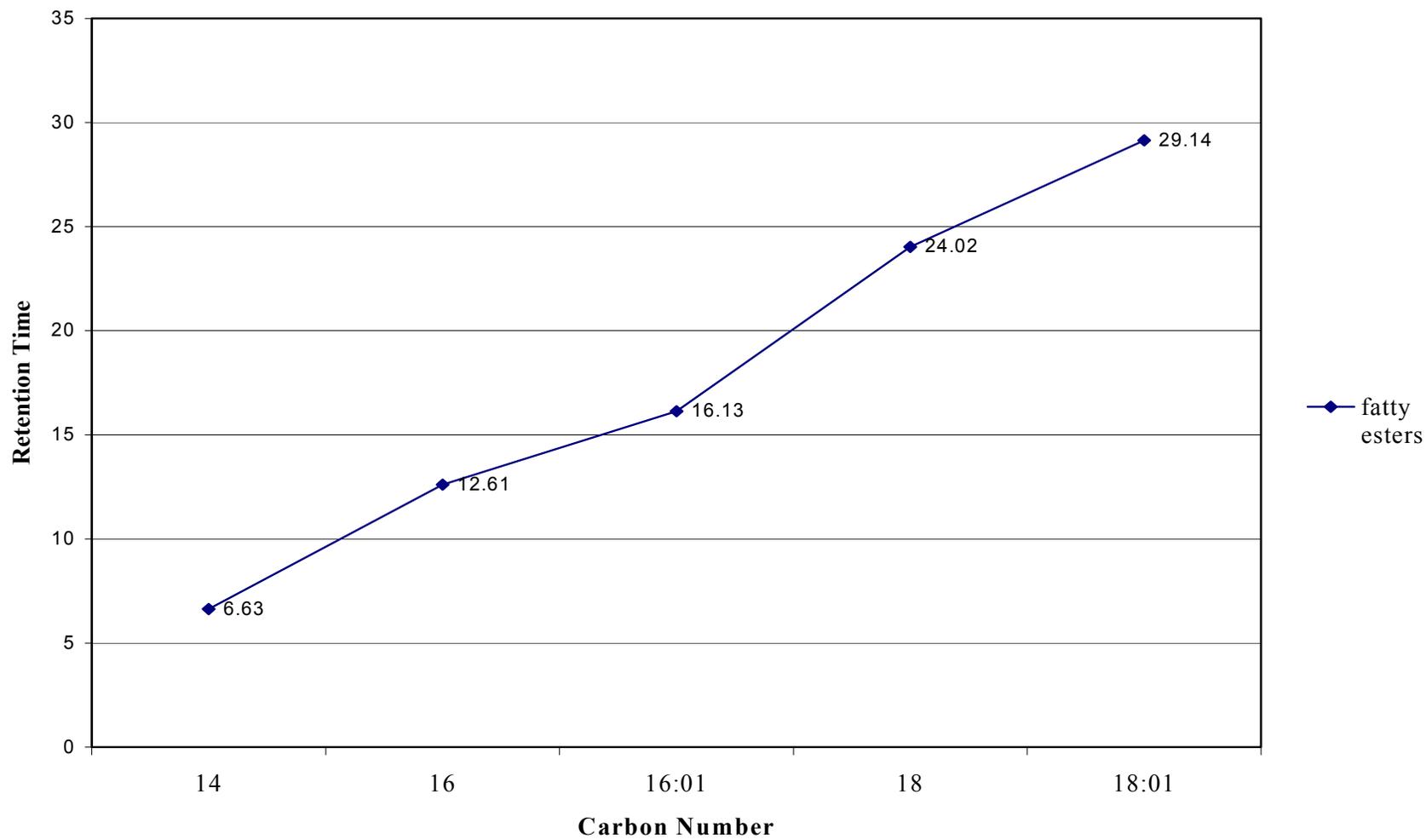


Figure 4-4. Linear retention time graph of hydrocarbons on male *A. kingii* tests.

Taxonomic descriptions of many scales are based primarily upon adult females, while the study of adult males and immature males has been largely neglected. The test, or pupal cover, that the second instar male secretes can also provide a means of identification (Miller and Williams 1990). The analysis of lipids by gas chromatography is one way of determining which hydrocarbons (or classes of lipids) and waxes it contains (Castner and Nation 1986). Hydrocarbons are the long-chain alkanes and alkenes, and the methyl-branched alkanes and alkenes (Nelson 1993). Fatty acids constitute parts of many parts of lipids. In simple lipids, esters form from fatty acids and alcohols. If the alcohol is a long-chain compound they are called waxes (Stenesh 1998).

Hydrocarbons are not major components of the cover of scale insects (Tamaki 1997). *Allokermes kingii* test is made up of mostly wax with almost 60% saturated fatty acid, and almost 30% as unsaturated fatty acid. Although a large but varying fraction of scale wax is composed of standard long-chain ester (C43-46), most waxes also seem to include a small amount of straight-chain hydrocarbons, containing from 15 to 33 or more carbon atoms (Brown 1975). Methyl-branched alkanes comprise a significant portion of hydrocarbon mixtures and serve as both pheromones and kairomones in many insects (Howard 1982). The chemical composition of the test differs between species, in the case of soft scales, waxy materials are an important component of the cover (Tamaki 1997).

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BIOGRAPHICAL SKETCH

Jay Cee Lynn Turner was born on 5 February 1963, in Pittsburgh, Pennsylvania. Upon graduation from high school, she entered Santa Fe Community College in Gainesville, Florida, where she obtained an Associate of Arts degree. Jay Cee owned and operated an upholstery business for 12 years. In 1999, while self-employed and raising two children, she enrolled in the University of Florida, Gainesville, Florida. Jay Cee closed her business and worked for Dr. Eileen Buss in the Department of Entomology and Nematology while completing her bachelors degree. In the summer of 2002, she began working on her master's degree under the guidance of Dr. Buss.