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Biology and Ecology of Crapemyrtle Bark Scale, Acanthococcus Lagerstroemiae (Kuwana) (Hemiptera: Eriococcidae)

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BIOLOGY AND ECOLOGY OF CRAPEMYRTLE BARK SCALE, *ACANTHOCOCCUS LAGERSTROEMIAE* (KUWANA) (HEMIPTERA: ERIOCOCCIDAE)

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ABSTRACT

The crapemyrtle bark scale, *Acanthococcus lagerstroemiae* (Kuwana) (Hemiptera: Eriococcidae), is an exotic pest on crapemyrtles, *Lagerstroemia* spp. (Myrtales: Lythraceae). Because of its recent arrival in the US, little is known about its biology and ecology. The purpose of my thesis was to improve the knowledge about *A. lagerstroemiae* in four aspects involving its thermal tolerance, physiological adaptations to cold temperatures, temperature-dependent development and host range.

Thermal tolerance was determined to understand how temperature extremes constrain the distribution of *A. lagerstroemiae* in the US. Results suggested that *A. lagerstroemiae* can tolerant high heat, but its potential distribution to the northern US may be limited by cold temperatures. Based on laboratory experiments and local temperatures from reported infestations, *A. lagerstroemiae* can establish in areas south of 43 °N, which is similar to the northern distribution limit of crapemyrtles. Therefore, the temperature extremes cannot limit its distribution on crapemyrtles in the US.

To adapt to winter, cold tolerance of *A. lagerstroemiae* nymphs was observed to increase since November. The mechanisms of this increase were investigated by measuring seasonal changes of biochemical variables. From November to February, *A. lagerstroemiae* had 20% less water and higher energy reserves, which could have contributed to the increased cold tolerance. A restructuring of fatty acid composition in the body fat of overwintering nymphs was reported indicating accumulation of fatty acids in shorter chains

(C6:0, C8:0 and C10:0), resulting in lower melting points that can help maintain lipid fluidity for energy conversion.

The development and host range of *A. lagerstroemiae* were also studied. Developmental time and survival of *A. lagerstroemiae* eggs and nymphs were assessed under different temperatures, and results can help IPM practitioners improve field sampling strategies and timing of control measures. *Callicarpa americana* L. (Lamiales: Lamiaceae), *Heimia salicifolia* Link, *Lawsonia inermis* L., *Lythrum alatum* Pursh, and *Punica granatum* L. (Myrtales: Lythraceae) supported life cycle development and reproduction of *A. lagerstroemiae* and thus determined as suitable hosts other than *Lagerstroemia* spp. Scouting is recommended on these host species, following immediate responses to avoid additional spread, economic loss, and ecological disturbance of this pest.

CHAPTER 1. INTRODUCTION[1]

1.1 Crapemyrtle Bark Scale

The crapemyrtle bark scale, *Acanthococcus lagerstroemiae* (Kuwana) (Hemiptera: Eriococcidae), is a newly introduced insect pest of crapemyrtles in the US. Native to Asia, *A. lagerstroemiae* was first reported in 2004 in a nursery in Richardson, TX (Dallas County) (Merchant et al. 2014). The wide distribution of crapemyrtles in the US may facilitate the rapid spread of *A. lagerstroemiae*. Associated with accumulation of black sooty mold (Fig. 1.1), *A. lagerstroemiae* infestations could cause aesthetic damage to crapemyrtle (Gu et al. 2014, Wang et al. 2015). Because of this plant damage, *A. lagerstroemiae* was recognized as one of the top nine pests in 2015 by the Greenhouse Grower magazine (Miller 2015a).

2.2 Crapemyrtle

Crapemyrtles, *Lagerstroemia* spp. L. (Myrtales: Lythraceae), are popular flowering shrubs and small trees around the world. Native to Southeast Asia and Australia, for example, China, Japan, India, Australia and Oceania (Egolf and Andrick 1978), crapemyrtles have been introduced into the US as ornamentals for 180 years (Chappell et al. 2012). Crapemyrtles have become a dominant landscape tree in the southern US with an annual wholesale value of approximately $66 million in 2014 (USDA-NASS 2014). Breeding programs over the last 35 years have produced superior varieties in a wide range of plant

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sizes and growing habits with improved flowering, new flower and foliage colors, ornamental bark, increased vigor and adaptability to a wide range of soil types (Chappell et al. 2012, Gu et al. 2014). In the US, crapemyrtle is hardy from USDA Plant Hardiness Zone 6 to 10 (temperature ranging from -23.3°C to -1.1°C), while its roots are believed to be winter hardy in Zone 5 (temperature ranging from -28.9°C to -23.3°C) (Chappell et al. 2012).

Figure 1.1. Branch dieback and accumulation of black sooty mold on a crapemyrtle tree infested with *Acanthococcus lagerstroemiae*.

Crapemyrtles are valued for their relatively easy maintenance and limited pest problems (Chappell et al. 2012, Gu et al. 2014). The main diseases of crapemyrtle are powdery mildew caused by the fungus *Erysiphe australiana* (= *lagerstroemiae*) (McAlpine) U. Braun & S. Takamatsu (Erysiphales: Erysiphaceae), and Cercospora leaf spot caused by *Pseudocercospora lythracearum* (Heald & Wolf) Liu & Guo (Capnodiales: Mycosphaerellaceae) (Chappell et al. 2012). Until the discovery of *A. lagerstroemiae*, the

primary insect pests of crapemyrtle were the crapemyrtle aphid, *Sarucallis* (= *Tinocallis*) *kahawaluokalani* (Kirkaldy) (Hemiptera: Aphididae) and the Japanese beetle, *Popillia japonica* Newman (Coleoptera: Scarabaeidae), followed by flea beetles, *Altica* spp. Geoffroy (Coleoptera: Chrysomelidae), and the granulate ambrosia beetle, *Xylosandrus crassiusculus* (Motschulsky) (Coleoptera: Curculionidae) (Chappell et al. 2012). However, these pests on crapemyrtles can largely be managed with resistant cultivars, landscape planning including plant placement in sunny locations with good ventilation, and environmentally friendly insecticides, such as insecticidal soaps or horticultural oils (Knox 2003, Chappell et al. 2012, Gu et al. 2014).

1.3 Taxonomy

Acanthococcus lagerstroemiae (Kuwana), formerly *Eriococcus lagerstroemiae* Kuwana (Hemiptera: Eriococcidae), was combined into the genus *Acanthococcus* (Acanthococcidae) in 2013 along with other 345 species (Kozar et al. 2013). The family Eriococcidae *sensu lato* contained about 80 species in 10 genera in the US (Miller 2005), with some important ornamental plant scale pests. Kozar et al. (2013) placed most of these scales into Acanthococcidae (Group Family), for example, *Acanthococcus* (=*Eriococcus*) *azalea* (Comstock) on azaleas and *Gossyparia spuria* (Modeer) on elms (Miller et al. 2005, Kozar et al. 2013). The definition and borderlines of the family Eriococcidae (or Acanthococcidae) is still debated among coccidologists due to its diverse morphology and behavior (Miller 2005, Kozar et al. 2013). Molecular analyses using the nuclear small subunit

ribosomal RNA gene (SSU rRNA or 18S) also suggested the polyphyletic relationships within genus *Eriococcus sensu lato* (Gullan and Cook 2007). Here I refer to the crapemyrtle bark scale as *A. lagerstroemiae* based on a latest review of the genus (Kozar et al. 2013).

1.4 Biology

Acanthococcus lagerstroemiae has the same incomplete metamorphosis as other species in the superfamily Coccoidea (Kondo et al. 2008). The female is paedomorphic, meaning that its form resembles that of a nymph (Gu et al. 2014, Wang et al. 2015). The male turns into an alate without mouthparts after the prepupal and the pupal stage (Wang et al. 2015). Eggs are 0.35 ± 0.05 mm (mean ± standard error) long, 0.15 ± 0.05 mm wide (n=20), pink, and surrounded with white filaments (Fig. 1.2a). Eggs are laid inside the white felt-like covering secreted by the female.

Nymphs are pink and mobile (Fig. 1.2b). The first instars or crawlers are 0.5 ± 0.1 mm long and 0.15 ± 0.05 mm wide (n=20). After hatching, crawlers settle on the woody parts of the stem and new growth. Three nymphal stages were observed (Wang et al. 2015). Nymphs and females secrete honeydew as a result of feeding.

Male pre-pupae and pupae are pink, non-feeding, immobile, and completely enclosed by white sacs (Fig. 1.2c). Male pre-pupae are 0.9 ± 0.1 mm long, 0.4 ± 0.1 mm wide (n=20), and male pupae are 1.2 ± 0.1 mm long and 0.5 ± 0.1 mm wide (n = 20) (Fig. 1.2c-1). The blackish eyes and wing pads in the pupal stage are distinct from the prepupae (Fig. 1.2c-2). Males are pink, alate, and have two long white filaments at the tip of the abdomen (Fig. 1.2e).

The mesothoracic wings have reduced venation, and the metathoracic wings have been lost along with the mouthparts. There are two pairs of ocelli each on dorsal and ventral side of the head, and a pair of smaller lateral ocelli. The filaments and extra ocelli have also been observed on other scales in Coccoidea which might function to stabilize the flight (Gullan and Kosztarab 1997).

Females are 2.0 ± 0.9 mm long, 1.2 ± 0.6 mm wide (n=20), wingless, pink, and sessile (Fig. 1.2d). Female shape and size varies according to the location of settling and presence of eggs inside the abdomen, but in general the size is much larger than the male. After production of the white ovisac, all eggs are laid, the female decreases in size and dies. The female white ovisac likely functions as a barrier against natural enemies and a mechanism to maintain humidity (Fig. 1.2f).

Acanthococcus lagerstroemiae has high fecundity and populations can grow rapidly. Females lay from 114 to 320 eggs during their lifetime (Jiang and Xu 1998). After hatching, the first instars or crawlers disperse along the branches for one to two days, after which they molt and become sessile again on the woody part of stems (Wang et al. 2015). Scales colonize the leaves, branches, twigs, trunk, stems and fruits. Some empirical evidence suggests that females and males have three and five nymphal stages, respectively (Wang et al. 2015). Number of generations per year ranges from two to four depending on the climate in Asia (Jiang and Xu 1998, Luo et al. 2000, He et al. 2008, Ma 2011) and is thought to be two to four in the US (Gu et al. 2014). In Anhui, China (31° 81’ N, 117° 21’ E), two generations

each year were observed (He et al. 2008), and the life cycle from egg to adult varied from 56 to 83 days (Jiang and Xu 1998). In Guiyang, China (26° 41' N, 106° 68' E) and Sichuan, China (27° 95' N, 102° 21' E), four generations were recorded (Luo et al. 2000, Ma 2011). In Asia, *A. lagerstroemiae* overwinters as egg, nymph, prepupa and pupa (Kwon et al. 1995, Luo et al. 2000, He et al. 2008), while in the US, it has been reported to overwinter as nymphs (Gu et al. 2014).

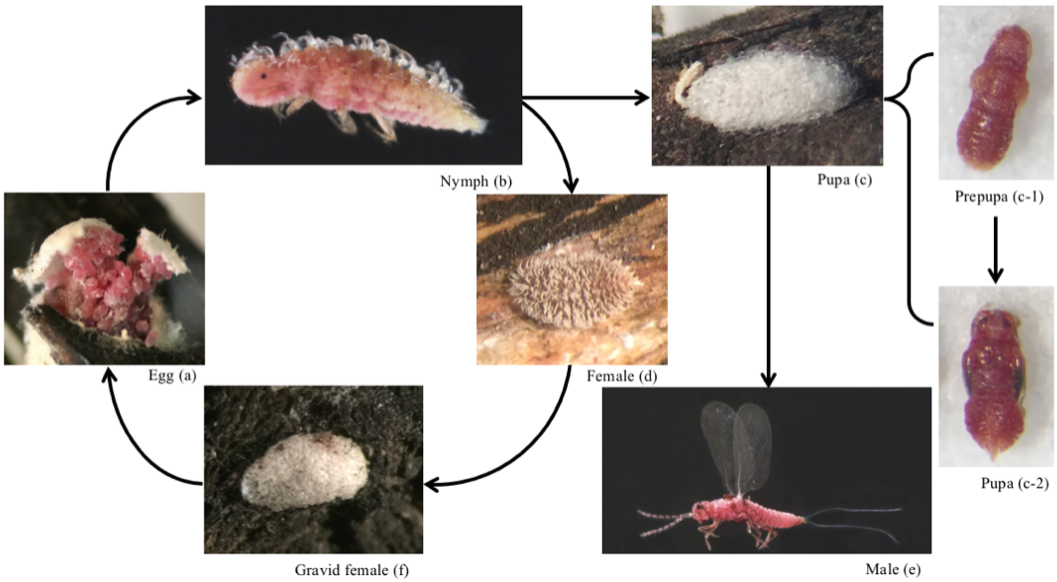

Figure 1.2. Life cycle of *Acanthococcus lagerstroemiae*. (a) Egg; (b) Nymph; (c) Pupa covered with white sac; (c-1) Prepupa; (c-2) Pupa; (d) Adult female; (e) Adult male; and (f) Ovisac containing the gravid adult female.

1.5 Host Range in Native Range

Host records revealed that *A. lagerstroemiae* not only attacks crapemyrtle but also other plant species in different families. In China, Japan, and Korea, this pest has been reported on thirteen other plants of ecological and economic importance (Table 1.1). For example, *A. lagerstroemiae* was reported to be a problem to pomegranate, *Punica granatum*

Table 1.1 Host plants of *Acanthococcus lagerstroemiae* in Asia and Hungary (except for *Lagerstroemia* spp.).

Scientific Name	Common Name	Order	Family	Country	Reference
Anogeissus latifolia (Roxb.ex DC.) Wall. ex Guill. & Perr.	Axlewood	Myrtales	Combretaceae	Korea	Hoy 1963
Anogeissus sp.	–	Myrtales	Combretaceae	China	Wang 2001
Buxus microphylla Sieb. et Zucc.	Korean boxwood	Buxales	Buxaceae	Korea	Park et al. 1993
Celtis sinensis Pers.	Chinese hackberry	Rosales	Cannabaceae	Korea	Park et al. 1993
Dalbergia eremicola Polhill	Indian rosewood	Fabales	Fabaceae	Korea	Hoy 1963
Diospyros kaki Thunb.	Japanese persimmon	Ericales	Ebenaceae	Korea	Park et al. 1993, Son and Park 2008
Ficus carica L.	Edible fig	Rosales	Moraceae	Korea	Park et al. 1993
Glochidion puberum (L.) Hutch	Needlebush	Malpighiales	Euphorbiaceae	China	Hua 2000
Glycine max (L.) Merr.	Soybean	Fabales	Fabaceae	China	Hua 2000
Ligustrum obtusifolium Sieb. et. Zucc.	Border privet	Lamiales	Oleaceae	–	Kozar et al. 2013
Malus pumila Mill.	Paradise apple	Rosales	Rosaceae	China	Hua 2000
Mallotus japonicus Muell. Arg.	Food wrapper	Malpighiales	Euphorbiaceae	Korea	Park et al. 1993, Kwon and Park 2002
Myrtus sp.	Myrtle	Myrtales	Myrtus	Hungary	Kozar et al. 2013
Punica granatum L.	Pomegranate	Myrtales	Lythraceae	China and Korea	Park et al. 1993, Hua 2000, Kwon and Park 2002
Pseudocydonia sinensis Schneid.	Chinese quince	Rosales	Rosaceae	Korea	Son and Park 2008
Rubus sp.	Brambles	Rosales	Rosaceae	Hungary	Kozar et al. 2013

L. (Myrtales: Lythraceae) in Pan Xi District, Sichuan, China (27°02' N, 101°44' E), due to sooty mold accumulation (Ma 2011). Despite being present in the US for more than ten years, *A. lagerstroemiae* has only been reported feeding on crapemyrtle (Gu et al. 2014) and American beautyberry (*Callicarpa americana* L.) (Gates 2015). Understanding the impact of *A. lagerstroemiae* to other plant species in the US could help predict the potential economic damage and prevent its spread to other plant species.

1.6 Distribution and Dispersal

Acanthococcus lagerstroemiae is widely distributed in Asia. The most northern and southern locations reported in Asia are Beijing, China (40°12' N, 116°21' E) (Knox 2014) and Tamil Nadu, India (10 °77' N, 78 ° 71'E) (Varshney 1992), respectively. It was reported in England (Hoy 1963) in 1915 in a nursery but has not been reported since then (Williams 1985). Since its first detection in 2004 (Gu et al. 2014), *A. lagerstroemiae* has been reported in the US states of Alabama, Arkansas, Georgia, Louisiana, Mississippi, New Mexico, North Carolina, Oklahoma, Tennessee, Texas, Virginia (EDDMapS 2017), and Washington (Carol 2016), as of August, 2016. To predict the potential geographic distribution of the scale, I performed a niche modelling exercise using worldwide locations (MaxEnt version 3.3.2; http://www.cs.prince-ton.edu/~schapire/maxent/) (Phillips et al. 2006). Eighty-two confirmed locations were used in the model, including 57 locations in the US, 22 locations in China, and one location each in Japan, Korea, and India, respectively (Table A2.1). I used altitude and 19 bioclimatic (bioclim) variables related to temperature and precipitation from the WORLDCLIM database (http://www.worldclim.org) to predict the climatic suitability in the

US and Asia. The prediction suggested that *A. lagerstroemiae* has established in different climates, and perhaps has reached the upper geographical limit in the US (Fig. 1.3).

Short distance dispersal of *A. lagerstroemiae* occurs by nymphs, and long-distance dispersal could be attributed to wind, birds, and human activities (Gu et al. 2014). Morphological characters of crawlers could facilitate its dispersal by wind including flat and small body, relatively long legs, and lateral wax filaments on the body fringe (Gullan and Kosztarab 1997). Under experimental conditions, birds were capable of transferring nymphs of the hemlock woolly adelgid, *Adelges tsugae* (Annand) (Hemiptera: Adelgidae) by touching infested branches (Russo et al. 2016). Crawlers of four armored scales, including *Aspidiotus nerii* Bouche (Hemiptera: Diaspididae), *Abgrallaspis aguacatae* Evans, Watson & Miller, *Hemiberlesia lataniae* (Signoret), and *Diaspidiotus perniciosus* (Comstock), were found possessing a suction cup-like structure on hairs at the end of each leg, which can help them latch on larger insects to disperse (Magsig-Castillo et al. 2010). I suspect *A. lagerstroemiae* could use larger animals to disperse. The discontinuous reports of *A. lagerstroemiae* in the US (Fig. 1.3. Survey points) suggested that human activities, trade and transportation of infested crapemyrtles could have facilitated the pest's long-distance movement. Measures should be taken to prevent further dispersal, for example, sales-stop restriction in reported area (Miller 2015b). Potential distribution range estimated by climatic suitability and host range can help early detection and timely management.

1.7 Plant Damage and Economic Impact

Acanthococcus lagerstroemiae could damage its host plant. Several instances suggested heavy infestation of *A. lagerstroemiae* could cause branch dieback (Fig. 2) and stunt growth (Jiang and Xu 1998, Luo et al. 2000, Ma 2011). Limited empirical evidence has suggested a reduction in blossoms as a result of infestation with *A. lagerstroemiae* (Merchant 2014). The scale secretes honeydew, which facilitates the growth of black sooty mold (Jiang and Xu 1998, Luo et al. 2000, Ma 2011, Gu et al. 2014, Robbins 2014) and could interfere with plant photosynthesis. Extensive honeydew deposits and sooty mold can turn branches and trunks to an unappealing black color, significantly reducing landscape aesthetic value of infested plants (Gu et al. 2014). However, relationship between population density of *A. lagerstroemiae* and different aspects of plant damage is still unclear. Research on this relationship may provide decision-making guidance on management options.

The economic impact of *A. lagerstroemiae* has not been quantified. However, failure to manage this exotic pest could lead to serious economic loss for wholesale and retail nurseries, landscape professionals, and consumers. To manage *A. lagerstroemiae*, nurseries would have to increase labor and insecticides which could result in greater costs (Gu et al. 2014). This scale could also potentially decrease the production and market value of crapemyrtle because of reduced sales. In states such as Arkansas, Louisiana, Oklahoma, Tennessee, and Texas, the stop-sale restriction of crapemyrtle has been enacted in nurseries

with *A. lagerstroemiae* infestation (Miller 2015b). Because some of the potential hosts of *A. lagerstroemiae* are fruit crops of economic importance, for example, paradise apple, Japanese persimmon, pomegranate, fig, and blackberry (Table 1.1), research to confirm host status of *A. lagerstroemiae* on these crops in the US is critical for establishing preventive management measures.

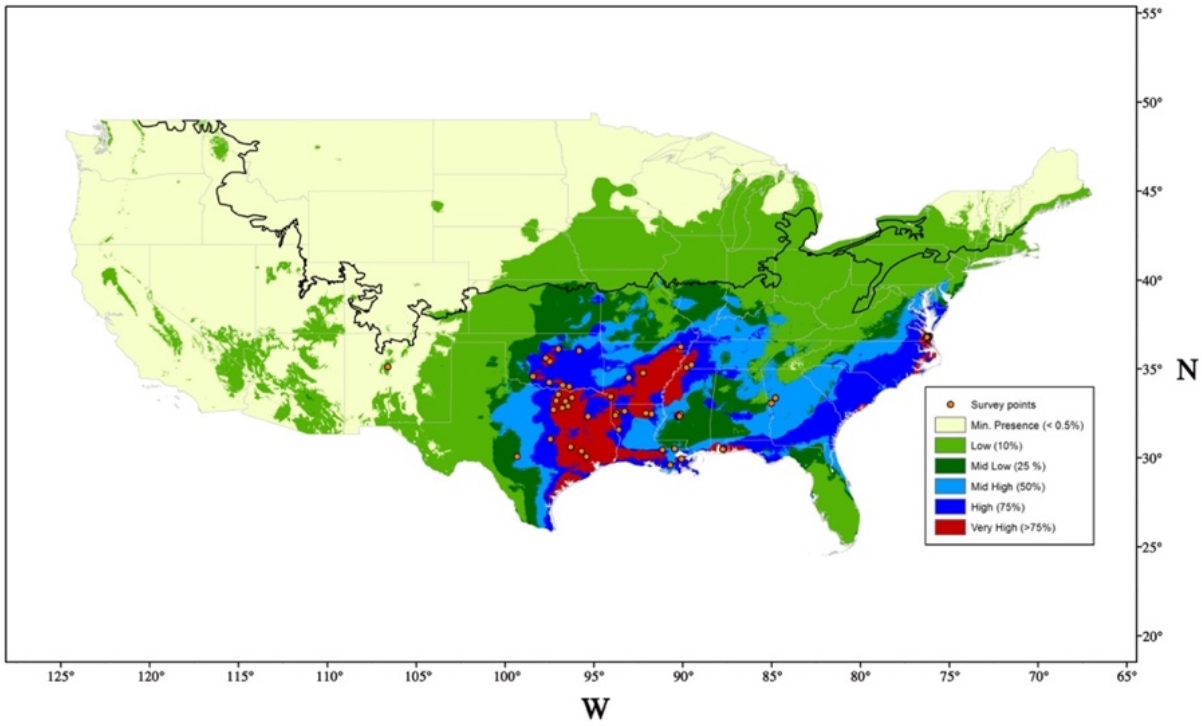

Figure 1.3. Projected distribution of *Acanthococcus lagerstroemiae* in the US. Various depths of grey color indicate changes in climatic suitability from minimum presence (< 0.5% presence) to very high (> 75% presence). Points indicate the location of reported infestation. The black line indicates the upper limit of USDA Plant Hardiness Zone 6.

1.8 Natural Enemies

Natural enemies of *A. lagerstroemiae* found in Asia and North America include predators and parasitoids. In Asia, the scale is attacked by the parasitoids *Grandiclavula spatulata* Zhang & Huang (Zhang and Huang 2001), *Metaphycus eriococci* (Timberlake),

Metaphycus cylindricus Wang, Li & Zhang (Wang et al. 2014), *Comperiella* sp., *Clausenia* sp. (Jiang and Xu 1998), *Metaphycus maculatus* Agarwal (Zeya and Hayat 1993) and *Adelencyrtus longiclavatus* Hayat, Alam and Agarwal (Hayat et al. 1975) (Hymenoptera: Encyrtidae); and the predators *Chilocorus kuwanae* (Silvestri), *Chilocorus rubidus* Hope, *Rodolia limbata* Motschinsky, *Propylaea japonica* (Thunberg), *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae), *Chrysopa septempunctata* Wesmael, and *Chrysopa sinica* (Tjeder) (Neuroptera: Chrysopidae) (Jiang and Xu 1998), and *Cybocephalus nipponicus* Endrody-Younga (Coleoptera: Coccinellidae) (Yu 2016). *Chilocorus kuwanae* was introduced to the US from Korea in 1984 to help control euonymus scale, *Unaspis euonymi* (Comstock) (Hemiptera: Diaspididae), and was established in temperate regions (USDA Zone 7 or colder) of the US after multiple releases (Hendrickson et al. 1991).

In Louisiana, four ladybeetles (Coleoptera: Coccinellidae) were found associated with the infestation of *A. lagerstroemiae*, including two species of twice-stabbed lady beetle, *Chilocorus cacti* L. (Fig. 1.4A, B) and *Chilocorus stigma* (Say), *Hyperaspis bigeminata* (Randall) (Fig. 1.4C), and multicolored Asian ladybeetle, *Harmonia axyridis* (Pallas) (Wang et al. 2015). In Texas, the ladybeetle, *Hyperaspis lateralis* Mulsant (Coleoptera: Coccinellidae) were observed in association with *A. lagerstroemiae* (Vafaie 2014). Field and laboratory observations in Louisiana further confirmed the predation of the cactus lady beetle,

C. cacti and *H. bigeminata* on *A. lagerstroemiae* (Wang 2015) (Fig. 1.4B, D). I collected field samples of *A. lagerstroemiae* nymphs in several locations in Beijing, China, during the summer and also in Louisiana during the fall of 2015. Three species of unidentified Hymenopteran parasitoids were reared from females in Beijing (Fig. 1.5A-D), and one species from nymphs in Louisiana (Fig. 1.5E). As these parasitoids have potential to be used in classical or augmentative biological control, their morphological and molecular identification need to be confirmed. In addition, a small predacious beetle *C. nipponicus* was reared from the colony of *A. lagerstroemiae* in Beijing, China in summer 2015 (Fig. 1.5F).

Chilocorus cacti is a predator of eggs and crawlers of *A. lagerstroemiae* in Louisiana and Texas (Fig. 1.4B) (Wang 2015). In the laboratory, fourth instar of *C. cacti* can feed on about 400 scale eggs over 24h (Wang 2015). *Chilocorus cacti* has been used as a biological control agent for several scale pests. In 1966, this predator was introduced into South Africa from Texas to control the California red scale, *Aonidiella aurantii* (Maskell) (Hemiptera: Diaspididae) (DeBach and Rosen 1976). Despite high predation levels and widespread releases, *C. cacti* established only in southwestern South Africa and failed to control *A. aurantii*, probably because of the extensive parasitism of *C. cacti* (Hattinghl and Samways 1994). From 1987 to 1992, hundreds of *C. cacti* were released with other predators to control *H. lataniae* on kiwifruits, *Actinidia deliciosa* (A. Chev.) Liang et Ferguson (Ericales:

Actinidiaceae) in New Zealand (Hill et al. 1993). However, it failed to establish probably due to habitat destruction and pesticide use (Charles et al. 1995). More research is needed to determine the potential of *C. cacti* as biological control agent for *A. lagerstroemiae*.

1.9 Current Management

Acanthococcus lagerstroemiae is currently managed using chemical and/or mechanical methods in the US. The protective covering secreted by *A. lagerstroemiae* and its feeding behavior under bark crevices make control by contact insecticides difficult (Gu et al. 2014). In China, lime sulphur, imidacloprid, cypermethrin, methidathion, dimethoate, abamectin, triazophos, and acetamiprid have been evaluated for controlling nymphs over one generation (He et al. 2008, Zhang 2010, Zhang 2011, Chen and Zhang 2012). However, there is no information on the efficacy of these chemicals over more generations or subsequent years. Physical methods

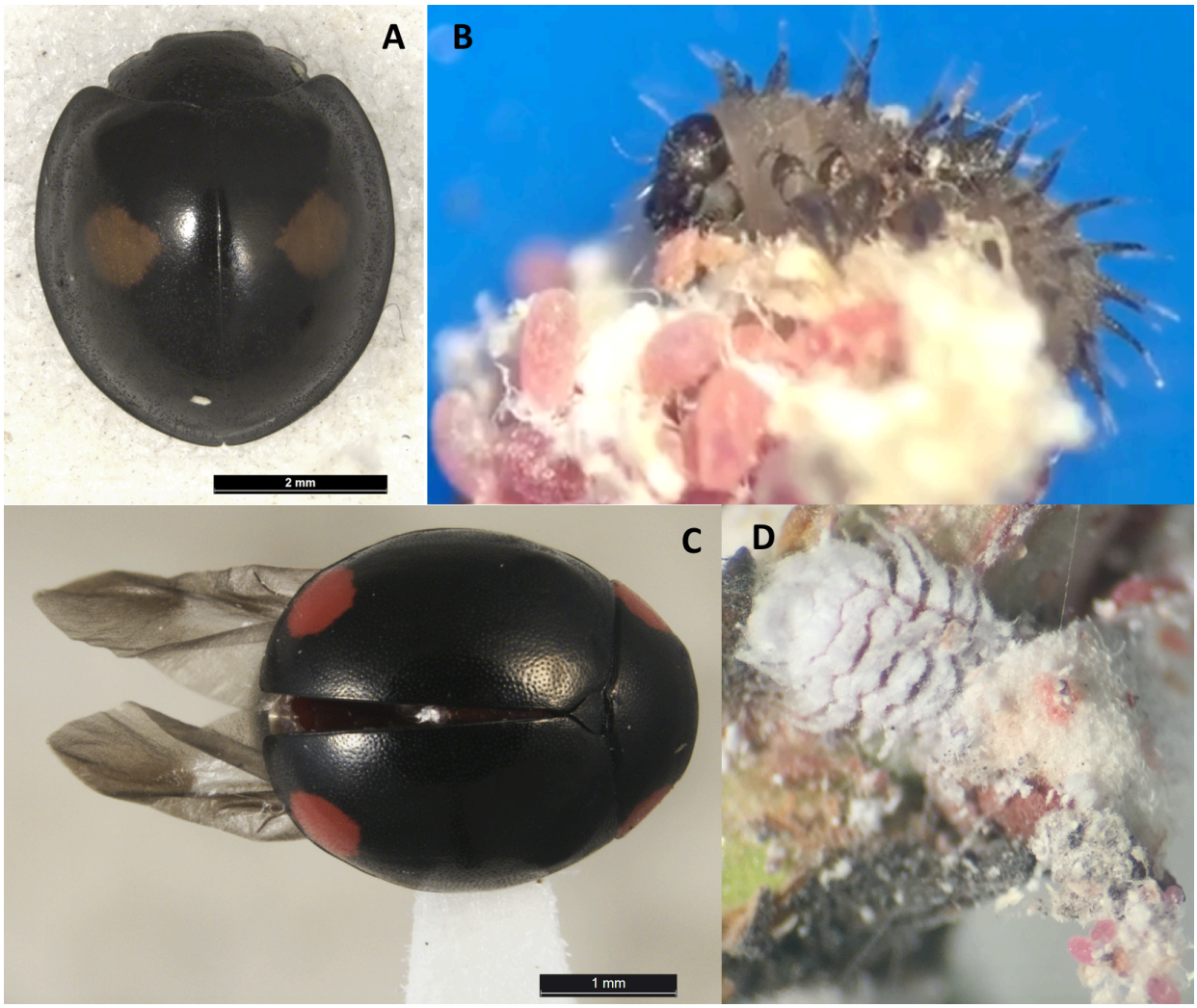

Figure 1.4. Predators of *Acanthococcus lagerstroemiae* found in Louisiana. (A) Adult of *Chilocorus cacti*; (B) Larva of *Chilocorus cacti* feeding eggs of *A. lagerstroemiae*; (C) Adult of *Hyperaspis bigeminata*; (D) Larva of *Hyperaspis bigeminata* feeding eggs of *A. lagerstroemiae*. Voucher specimen of these two ladybeetles were deposited in Louisiana State Arthropod Museum at Louisiana State University.

to reduce *A. lagerstroemiae* populations include brushing infested trunks with mild dishwashing solution, and removing scales and sooty mold with high water pressure washes (Gu et al. 2014, Kilpatrick 2014, Merchant et al. 2014, Robbins 2014). Chemical control with soil-applied

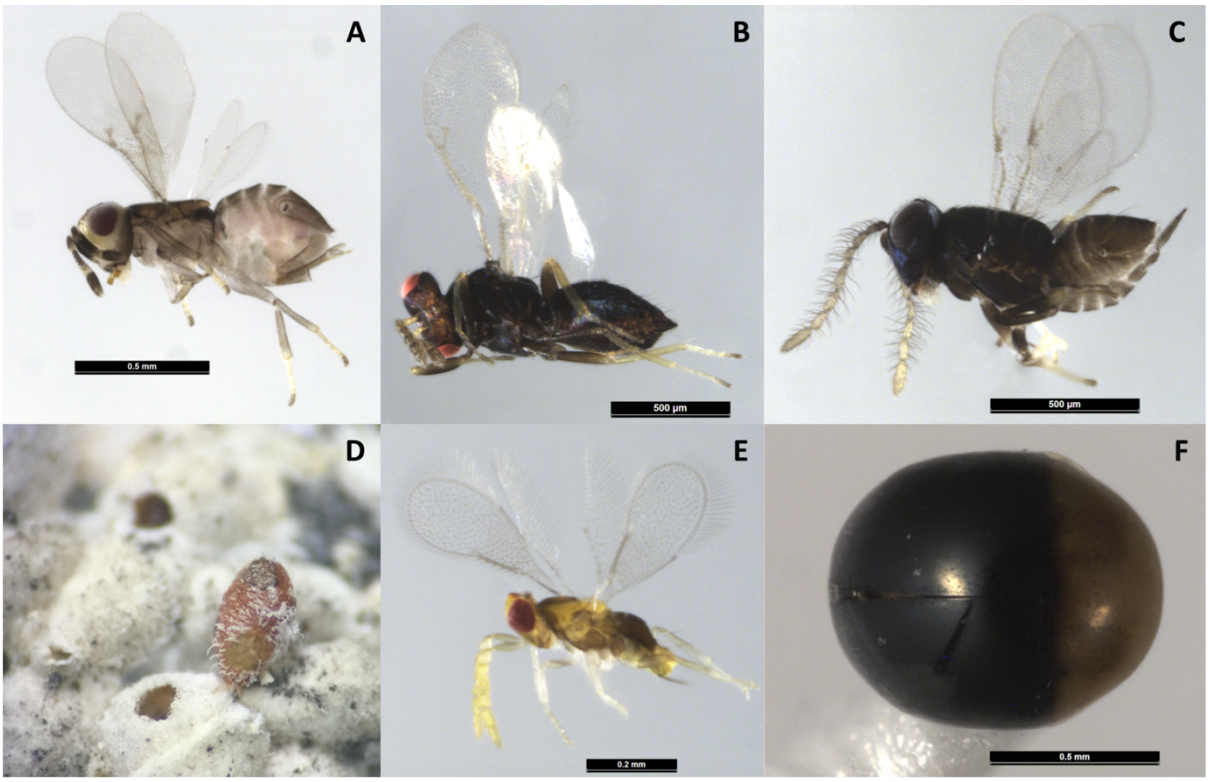

Figure 1.5. Parasitoids reared from *Acanthococcus lagerstroemiae* (A-C) and caused damage (D) in Beijing, China, and the parasitoid reared in Louisiana, US (E). The predator, *Cybocephalus nipponicus* Endrody-Younga, reared from *A. lagerstroemiae* in China (F). Voucher specimen of these natural enemies were deposited in Louisiana State Arthropod Museum at Louisiana State University.

systemic neonicotinoids, such as dinotefuran and imidacloprid, are most effective (Gu et al. 2014). Adding insect growth regulator or ultrafine oils as tank-mix or rotation partners may help with long-term control. Cost of chemical control is about $10 per 10-foot-tall tree using a rotation between two neonicotinoid insecticides as estimated by Bruce Nelms, ground manager of Louisiana State University Shreveport campus, who has been treating >100 infested crapemyrtles from 2013 to 2015 (Nelms 2015). Side-effects to pollinators and natural enemies may be a concern when applying these insecticides.

1.10 Objectives

a. To assess the thermal tolerance of *A. lagerstroemiae* nymphs over various seasons and predict its potential thermal limits in the US;

b. To assess the physiological changes of *A. lagerstroemiae* nymphs associated with seasonal changes in its cold tolerance;

c. To determine the host range of *A. lagerstroemiae* in the US and assess the temperature-dependent development of the immature *A. lagerstroemiae*;

d. To determine physiological and aesthetic impact of *A. lagerstroemiae* on crapemyrtles.

1.11 References

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CHAPTER 2. THERMAL TOLERANCE AND PREDICTION OF NORTHERN DISTRIBUTION OF *ACANTHOCOCCOUS LAGERSTROEMIAE* (KUWANA) (HEMIPTERA: ERIOCOCCIDAE)

2.1 Introduction

Understanding the potential distribution of exotic species in the adventive area is important for developing management strategies (Simpson et al. 2009). Physiological limits of species to environmental factors can be studied under laboratory conditions, which then can be incorporated into Species Distribution Modeling (SDMs) for the prediction of the distribution (Kearney and Porter 2009, Peterson 2011), for example, exposing individuals under thermal extremes and/or certain desiccations (Burgi and Mills 2010, Arnan and Blüthgen 2015). For ectotherms like insects, climatic events with extreme temperatures, such as cold and heat fronts, could cause mortality and reduced fitness (Chown and Nicolson 2004, Sinclair et al. 2015), therefore, limiting the colonization and establishment of exotic species in the introduced areas (Sinclair et al. 2003, Bale and Hayward 2010, Gallien et al. 2010). Studying the development and survival under thermal extremes is an initial step to predict exotic species distribution (Arbogast et al. 1998, Lapointe et al. 2007, Manrique et al. 2012). Based on the prediction, preventive measures including early detection and rapid response can be developed (Simpson et al. 2009, Liebhold et al. 2016), and risk analyses can help allocate limited resources more efficiently and

make informed decisions on pest control (Venette et al. 2010, Sciarretta and Trematerra 2014, da Silva et al. 2016).

Variables closely related to cold tolerance of insects include Supercooling Points (SCP), Low Lethal Temperature (LLT), Lethal Time at Low Temperature (Lt) (Andersen et al. 2015), and Upper Limit of the Cold Injury Zone (ULCIZ) (Nedved et al. 1998). Detected by thermocouples as latent heat of crystallization (Sinclair et al. 2015), SCP is the temperature of insect body being spontaneously frozen when cooled below the melting point (Zachariassen 1985). The LLT and Lt are quantitative indicators of insects' performance under cold temperatures for certain time periods (Kimura 2004, Lapointe et al. 2007), usually determined in cold exposure experiments as the temperature and time when certain mortality of the population occurs (Sinclair et al. 2015). Chill-susceptible, freeze-avoidant and freeze-tolerant are three main strategies for insects to overwinter and are defined by survival from chilling temperature and internal ice formation (Salt 1961, Bale and Hayward 2010). The specific strategy can be determined by interpreting the SCPs and results of cold exposure experiments (Sinclair et al. 2015). Another important variable is the Upper Limit of the Cold Injury Zone (ULCIZ) defined as the highest temperature at which the insect starts accumulating cold injuries (Nedved et al. 1998). In the northern hemisphere, the southern distribution of an insect can be inferred by their

performance under heat exposures using similar temperature- or time-dependent variables (Kimura 2004, Sunday et al. 2011).

Methods for studying cold tolerance and distributions of ectotherms have different assumptions, and can be classified into two major groups: (a) using temperature-dependent variables, including LLT and/or ULCIZ, to fit the average of minimum temperatures at different time scales ranging from days to years, and use isothermal lines to delimit geographical limits (Chen and Kang 2005, Cramer and Maywright 2008, Zhao et al. 2015); and (b) using time-dependent variables such as lethal time under specific temperature to fit the frequency of days per year under that temperature (Matsuura and Naito 1997, Lapointe et al. 2007, Takano et al. 2012). However, performance of ectotherms at low temperatures is affected by the cumulative effects of both temperatures and sustained time periods at those temperatures, as modeled under constant temperatures (Nedved et al. 1998) and fluctuating temperatures (Colinet et al. 2011). Currently, there is no method modelling both temperatures and sustained time periods for the prediction of geographical limits of insects.

Native to Asia, the crapemyrtle bark scale, *Acanthococcus lagerstroemiae* (Kuwana) (Hemiptera: Eriococcidae) is an exotic insect pest on crapemyrtles, *Lagerstroemia* spp. L. (Myrtales: Lythraceae). *Acanthococcus lagerstroemiae* is a scale pest which is sessile on its host

plant for most of its life cycle (Wang et al. 2016). Heavy infestations of *A. lagerstroemiae* cause branch dieback, accumulate sooty mold, and reduce aesthetic value of the crapemyrtle (Wang et al. 2016). Since its first report in Texas, US in 2004 (Merchant et al. 2014), *A. lagerstroemiae* has spread to 10 states including Alabama, Arkansas, Georgia, Louisiana, Mississippi, New Mexico, North Carolina, Oklahoma, Tennessee, and Virginia (EDDMapS 2017). The main overwintering stage of *A. lagerstroemiae* in the US is the nymph, which is also a mobile stage (Wang et al. 2016). Considering *A. lagerstroemiae* is still spreading (EDDMapS 2017), there is a critical need to determine the northern and southern distribution limits of this pest in the US. By assessing the mortality of *A. lagerstroemiae* at different temperatures, its potential distribution in the US can be predicted and used for risk analyses and preventive managements.

The goal of this study was to understand the thermal tolerance of *A. lagerstroemiae* and predict its potential distribution in the US. The specific objectives were (1) to assess the thermal tolerance of *A. lagerstroemiae* using SCP and cold and heat limits, and (2) to develop a model for predicting the northern limit of *A. lagerstroemiae* in the US. Mortalities at low temperatures were used to build models to describe the potential distribution of *A. lagerstroemiae* by fitting historical temperature data of winter cold fronts in the US. Preventive measures for controlling *A. lagerstroemiae* are then discussed based on its thermal tolerance and potential distribution.

2.2 Method and Materials

2.2.1 Insect sources

Acanthococcus lagerstroemiae were collected from infested branches of crapemyrtles ranging from 0.4 to 0.8 cm in diameter and located in Shreveport, LA (32.55°N, 93.78°W) every other month from July, 2015 to June, 2016. Upon arrival at the laboratory, branches with nymphs were immediately placed in a growth chamber at 25 °C. All experiments were conducted two days after the field collection to ensure insects were alive after transportation. Because nymphs of *A. lagerstroemiae* in 2nd and 3rd instar are difficult to distinguish, a mixture of both was used in all experiments in this study. All experiments were conducted at the Department of Entomology at Louisiana State University in Baton Rouge, LA.

2.2.2 Heat tolerance

The objective of this assay was to determine the heat tolerance of *A. lagerstroemiae*. Nymphs were exposed under five different exposure time periods for each of the three constant temperatures, including 5, 12, 24, 36, and 48h under 40°C; 2, 5, 12, 24, and 36h under 45°C; and 1, 2, 5, 12, and 24h under 48°C. The heat exposure experiments were conducted in July, 2015, and fifty and ninety percent lethal times (Lt_{50} and Lt_{90}) were calculated. Each temperature-time combination was one treatment, and each treatment had four replicates. Each replicate was a vial

containing one branch segment with 20 or more nymphs. The control treatment was placed under room temperature (23-25°C) until the end of other treatments to account for mortality caused by factors other than heat. Four vials were randomly assigned to each treatment and were placed in one single paper bag and maintained in the dark. All paper bags with vials were gradually acclimated at 25, 30, and 35°C with each for 24h in incubators (Series 101, Percival Scientific®, Perry, IA), and then transferred to incubators set with 40, 45, and 48°C for exposures. After the exposures, paper bags with vials were placed under room temperature for 24h allowing nymphs to recover. The mortality of *A. lagerstroemiae* nymphs on each branch segment was checked by the leg movements after gently flipping them over using a pin and observed under a dissecting scope.

2.2.3 Cold Tolerance

The objective of this assay was to assess the cold tolerance using supercooling points (SCP) and through cold exposure experiments under 5, 0, and -5 °C with *A. lagerstroemiae* nymphs collected every other month from July 2015 to June 2016.

The SCPs of *A. lagerstroemiae* were estimated using surface-contact thermometry (Sinclair et al. 2015). Second or third instars of *A. lagerstroemiae* (> 0.8mm) were gently separated from the branches using a pin, and attached to the Type J thermocouples (DATAQ®

Instruments, Akron, OH) using high vacuum grease (Dow Corning®, Midland, MI). Before all SCP measurements, the living status of nymphs was confirmed with leg movements. Insect-thermocouple arrangements, placed into 2.0 ml CryoTubes (Thermo Fisher Scientific™, Sugar Land, TX) and fixed with cotton, were inserted into a Mr. Frosty™ Cryo 1 °C Freezing Container (Nalgene® Thermo Fisher Scientific™, Sugar Land, TX). Freezing container was placed inside a freezer set at -70 °C (HS-129C, Midea®, Beijing, China), with temperature lowered at the rate of 1.0 °C /min to -50 °C. Temperatures were recorded by DI-1000TC-8 isolated thermocouple instrument (DATAQ® Instruments, Akron, OH) transferring data at 5s intervals into a computer and data were read using WinDaq Data Acquisition and Playback Software (DATAQ® Instruments, Akron, OH). To make sure the same starting condition was met for each test, thermocouples were placed in ice-water mixture and dried with paper towel after each one. Temperature readings were transformed into Excel 2013 (Microsoft®, Redmond, WA) and viewed as line chart. The SCP was identified as the temperature when the body started releasing heat, which can be observed as a small jump on the temperature curve in the line chart. The SCPs of at least twenty *A. lagerstroemiae* nymphs were measured every other month from July, 2015 to June, 2016.

Vials containing branch segments infested with *A. lagerstroemiae* nymphs were put in paper bags, gradually acclimated at 25, 20, and 15°C for 24h, and exposed under 5, 0, and -5°C in incubators and refrigerated in a bath circulator (A28, Thermo Fisher Scientific™, Sugar Land, TX). To understand the scale's survival over various seasons, different exposure time periods were tested (Table 2.1). Because the winter population of nymphs was hypothesized to have the highest cold tolerance, four low temperatures were added to the treatments in the study conducted in January, 2016 (Table 2.1). For all thermal exposure experiments, temperatures inside incubators and the refrigerated bath circulator were confirmed using external Lascar® data loggers (EL-USB-2, DATAQ® Instruments, Akron, OH) and HOBO® data loggers (Pendant® Temp/Light,8K, Onset®, Bourne, MA). After the exposures, paper bags with branches were placed under room temperatures for 24h allowing nymphs to recover. The mortality of *A. lagerstroemiae* nymphs on each branch segment was recorded (same as 2.2.2).

2.2.4 Statistics

For each temperature treatment in cold or heat exposure experiments, mortality of *A. lagerstroemiae* after each exposure time period was analyzed using logistic regression (PROC LOGISTICS, SAS Institute 2016). No mortality was found in control treatments, and therefore, no corrections were made to heat or cold treatments. Lt_{50} and Lt_{90} for all cold or heat

Table 2.1. Temperatures and exposure time periods in six cold exposure experiments conducted from July, 2015 to June, 2016.

Experiments	Temp (°C)	Exposure time period (h)				
Jul., 2015	5	2	5	12	24	36
	0	0.5	1	2	5	12
	-5	0.5	0.67	1	1.5	5.5
Sep., 2015	5	2	5	12	24	36
	0	1	2	5	12	24
	-5	0.17	0.5	1	2	3
Nov., 2015	5	5	12	25	31	48
	0	2	5	12	24	30
	-5	0.5	1	3	5	7
Jan., 2016	5	11	24	31	48	60
	2.5	5	11	24	31	48
	0	2	5	12	24	30
	-2.5	1.75	5	13	18	24.5
	-5	1	3	5	11	19.5
	-7.5	0.5	1	3	4.5	8.3
	-10	0.2	0.33	1	3	5
Mar., 2016	5	6	12	24	36	48
	0	3	6	12	24	36
	-5	1	3	6	12	24
May, 2016	5	6	12	24	36	48
	0	3	6	12	24	36
	-5	1	3	6	12	24

temperatures were estimated using logistic models, as well as corresponding 95% confidence intervals (CI). Because the Shapiro-Wilk test suggested that the distribution of SCPs of *A. lagerstroemiae* was not normal ($P< 0.0001$), the Kruskal-Wallis (KW) Analysis of Variance was used to compare the SCPs among sampling dates (PROC NPAR1WAY, SAS version 9.4, SAS

Institute 2016, Cary, NC) and *post hoc* analyses were then conducted if significant differences were found (Elliott and Hynan 2011).

To understand the cumulative impacts of cold temperatures and sustained time periods on *A. lagerstroemiae* during winter, two models of surface responses were built fitting cold exposure data measured in January, 2016 using logistic regression in PROC LOGISTIC (SAS software Version 9.4 (SAS® Institute Inc., Cary, NC). In the first model, the linear and quadratic terms of temperatures, exposure time periods, and their interactions were tested with multisource logistic regression and stepwise selection, both of which had entry and stay thresholds set at 0.15. Following the equation proposed by Nedved et al. (1998), the second model was pre-specified only with the linear term of exposure time period and interactions between temperatures and exposure time periods. Data model was then transformed to the format below;

$$\mathrm{S(T,t)} = \frac{e^{a+bt(T-c)}}{1+e^{a+bt(T-c)}} \qquad \text{Equation 2.1}$$

where, T is exposure temperature (°C), t is the time of exposure (h), S(T, t) is the survival percent (0 – 100%) after exposure to T °C for t h, and a, b, and c are estimated parameters. In this equation, parameter c was interpreted as ULCIZ, and a/b as the ratio describing the exposure time-temperature relationship in cold injury (Nedved et al. 1998).

The goodness-of-fit of the two models were compared using the Akaike information criterion (AIC) and likelihood ratio test (Agresti and Kateri 2011). Because of the predictive purpose, K-fold cross validation (where K is the number of subsets of original data) was used to validate the predictive ability of these two models, which training models in K-1 subsets and then compared with the remaining subset using certain statistics (Shtatland et al. 2004). K = 10 was chosen for the model validation considering the optimum fold number and our sample size (Nadeau and Bengio 2003, Witten and Frank 2005), and c-statistics, specifically, the Receiver Operating Characteristic (ROC) curve and the Area Under the ROC Curve (AUC), was used to evaluate the predictive accuracy of logistic regression models (Austin and Steyerberg 2012, Harrell Jr 2015). The ROC curve illustrates the model performance for prediction by comparing values predicted from models with the sampled values and plotting the true positive rate (TPR) against the false positive rate (FPR) at various threshold settings (Austin and Steyerberg 2012). The AUC can be calculated as quantitative indicators of the model's predictive power, which was calculated using trapezoid rules and reported as Mean and 95% Confidence Interval (CI) in this study.

2.2.5 Distribution prediction in the US

The objective of this analysis was to predict the potential distribution of *A. lagerstroemiae* in the US using mortality estimated by selected model to historical temperature data of cold fronts from 2000 to 2016. This procedure included three main steps. First, the raw temperature data with one-minute resolution were downloaded from the National Oceanic and Atmosphere Administration (NOAA) (ftp://ftp.ncdc.noaa.gov/pub/data/asos-onemin/), and then rearranged by deleting invalid data and extracting air temperature data of three coldest fronts within periods of 24 h. Then the mortality of each cold front was calculated by fitting temperature data of each extracted cold front to Model 2. To account for the assumption of constant temperature used in cold exposure experiments and modelling, continuous temperature data from each cold front were transformed by calculating combinations of constant temperatures and sustained periods of time for all temperatures lower than the constant temperature. After fitting all combinations of temperature and sustained periods into Model 2, the maximum mortality in each cold front was used to represent the potential mortality that could be caused by such cold front. Mortalities caused by all cold fronts from 2000 to 2016 were calculated, sorted by weather station, and reported as median ± median absolute deviation (MAD) (Hampel 1974). Operations in these two steps were finished using Python programming language (Python

Software Foundation, https://www.python.org/) and Visual Basic for Applications (VBA) in Excel 2013 (Microsoft Corp., Redmond, WA).

The last step was to map the northern limit of *A. lagerstroemiae* using predicted mortality of climatic stations using ArcGIS® Software (ESRI 2011, Redlands, CA). The Inverse Distance Weighed (IDW) interpolation in spatial analyst function was used to generate weighed averages from locations of known mortalities and then used to predict the mortality of *A. lagerstroemiae* on the US map (Childs 2004). The generated raster was classified as four ranges of mortality including 0% – 50%, 50% – 75%, 75% – 90%, and 90% – 100%. The line of 90% mortality was chosen as the potential northern distribution limit of *A. lagerstroemiae* in the US, and upper and lower thresholds of this northern limit were determined using MAD. Details of the predictive procedure were further described in the Appendix A2.

2.3 Results

2.3.1 Heat tolerance

Mortality of *A. lagerstroemiae* nymphs increased as heat temperatures increased and exposure time periods prolonged (Fig. 2.1). Lt_{50} under 40, 45, and 48°C were 37.2, 8.7, and 1.3h, and Lt_{90} were 73.2, 16.4, and 2.1h, respectively (Table 2.2). Because the observed mortality at

48h of exposure to 40°C was about 60%, the Lt_{90} values predicted by the logistic model might not be accurate.

2.3.2 Cold tolerance

There was a significant difference in the SCPs of *A. lagerstroemiae* nymphs among all sampling dates ($\chi^2_{(5)} = 66.6$, $P < 0.0001$), with mean rank scores of 120.4, 67.9, 51.8, 27.2, 80.8, and 70.4 for every other month from July, 2015 to June, 2016, respectively. *Post-hoc* comparisons suggested that the SCP of *A. lagerstroemiae* nymphs reduced from July, 2015 to Jan, 2016, and then increased in May, 2016 (Fig. 2.2).

The mortality of *A. lagerstroemiae* nymphs increased at lower temperatures and longer exposure periods for all sampling dates, and the logistic models were able to describe the relationships between mortality and exposure time periods at treatment temperatures (Table 2.2). Among sampling dates, *A. lagerstroemiae* nymphs collected in the winter survived better than those collected in the summer when exposed to the same temperatures (Table 2.2). For example, Lt_{90} at 0 °C was 50h for nymphs collected in the winter (January, 2016), but 8h for those collected in the summer (July, 2015) (Table 2.2, Fig. 2.3).

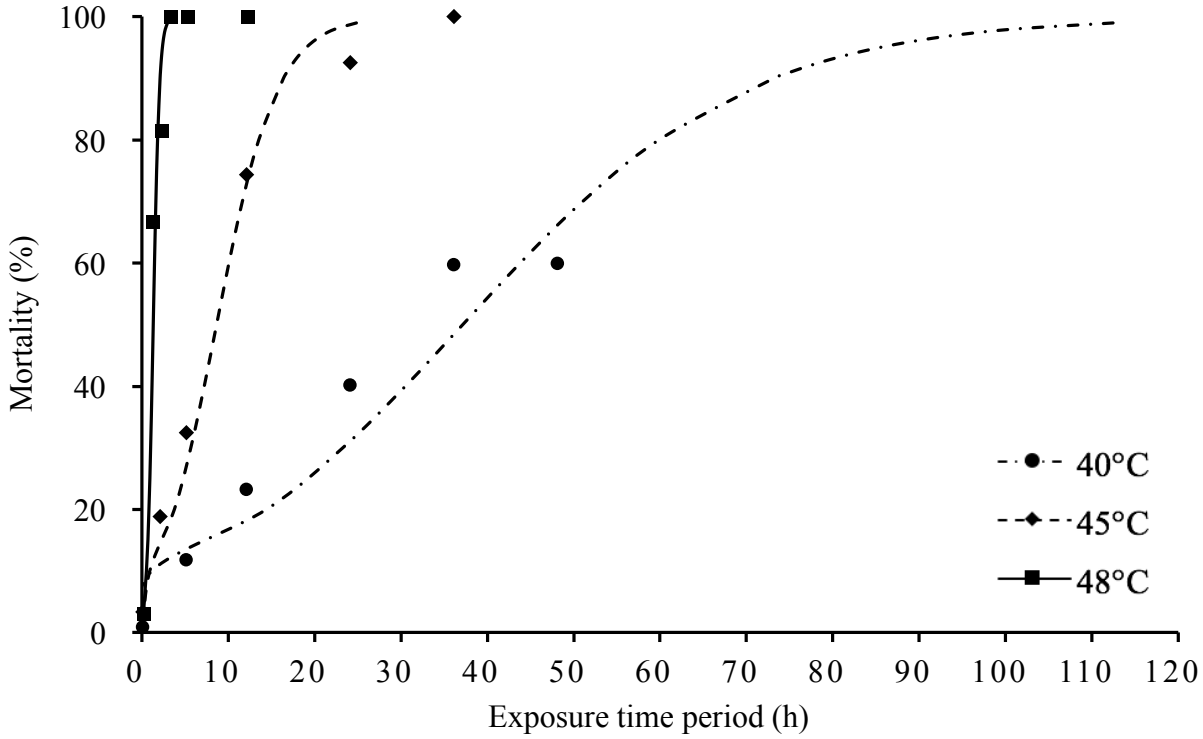

Figure 2.1. Mortality of *Acanthococcus lagerstroemiae* nymphs after various exposure time periods at 40, 45, and 48°C. Lines are expected values from the logistic regressions and symbols are observed values. The logistic models describing the relationships between mortality and exposure time periods at treatment temperatures are presented in Table 2.2.

To describe the accumulative effects of cold temperatures and sustained time period to the winter population of *A. lagerstroemiae* nymphs, two models were built and compared (Table 2.3, Fig. 2.4). In the first model, the stepwise selection procedure removed the quadratic terms of the temperature and time (Table 2.3, Model 1). In the second model, only linear term of time and the interaction were included (Table 2.3, Model 2). Model 1 had better goodness-of-fit than Model 2 because of the lower AIC value (Table 2.3) and a significant likelihood ratio test ($\chi^2_{(1)}$ =

144.4, $P < 0.0001$). Compared with Model 2, Model 1 fits the experimental data better at temperatures 2.5 and 5°C, but is biased when the exposure time period is 0h (Fig. 2.4). Although they are different in terms of goodness-of-fit, both models have the same AUC, indicating similar predictive power (Table 2.3). Considering the simplicity and biological meaning of estimated parameters, Model 2 was chosen to represent the cumulative impact of low temperature and sustained time period on *A. lagerstroemiae* nymphs, and thus used for predicting the potential distribution of this pest. The equation for Model 2 is presented below, where ULCIZ for *A. lagerstroemiae* is determined to be 8.5 °C and the ratio describing the exposure time-temperature relationship in cold injury is 161.

$$S(T, t) = \frac{e^{1.24+0.0077*t*(T-8.51)}}{1+e^{1.24+0.0077*t*(T-8.51)}} \qquad \text{Equation 2.2}$$

2.3.4 Potential distributions

Mortality of *A. lagerstroemiae* nymphs increased latitudinally in the US as predicted by historical cold fronts (Fig. 2.5). All known infestations of *A. lagerstroemiae* are limited up to north Oklahoma, Arkansas, and Tennessee within the range < 75% mortality (or the latitude < about 38° N). The isothermal lines on Fig 2.5 indicate the northern limit of *A. lagerstroemiae* with upper and lower thresholds, predicting 90% mortality caused by the cold fronts along the latitude of about 43° N.

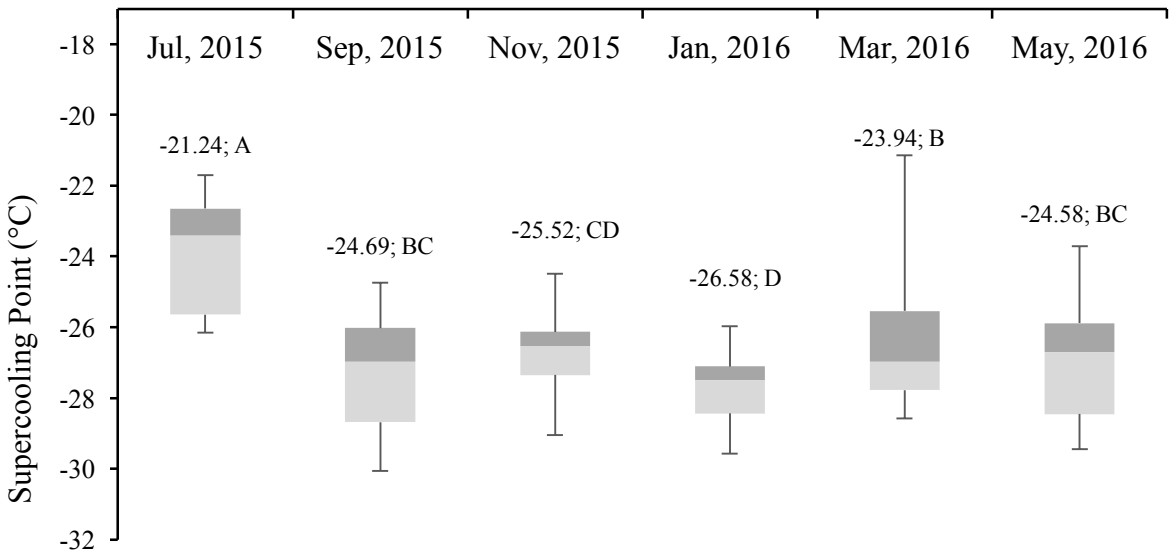

Figure 2.2. Box plot of supercooling points (°C) of *Acanthococcus lagerstroemiae* nymphs every other month from July, 2015 to June, 2016. The lines within each box plot corresponds to the median value, the box length to the interquartile range, and the lines emanating from the box (whiskers) extend to the smallest and largest observations. Values labeled on the top of the box are the means of SCPs, and dates sharing different letters are significantly different.

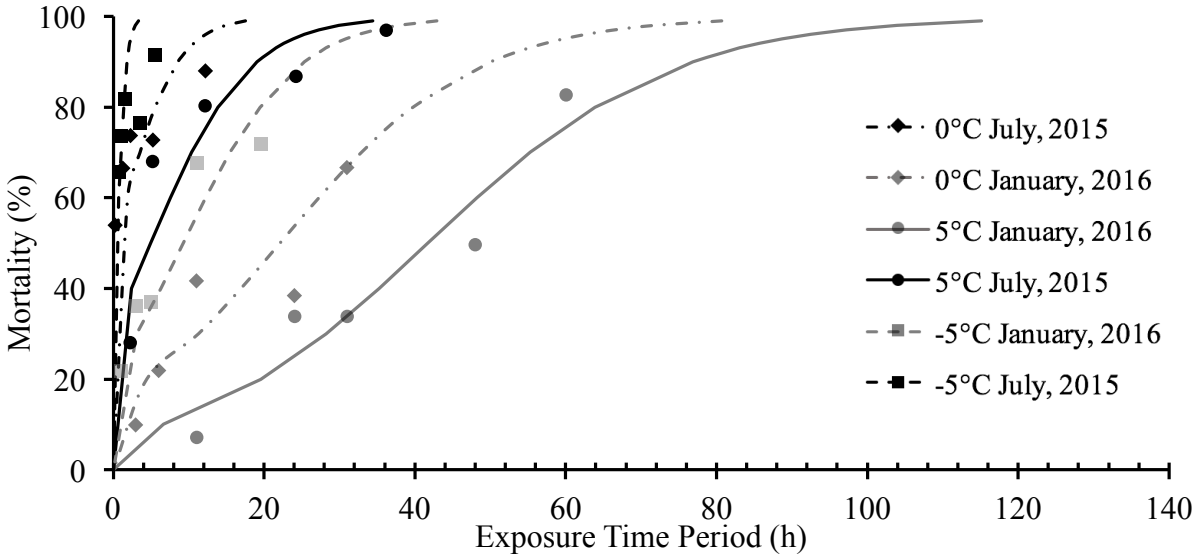

Figure 2.3. Mortality of *Acanthococcus lagerstroemiae* nymphs at 5, 0, and -5°C evaluated in July, 2015 and January, 2016. Lines are expected values from the logistic regressions, and symbols are observed values.

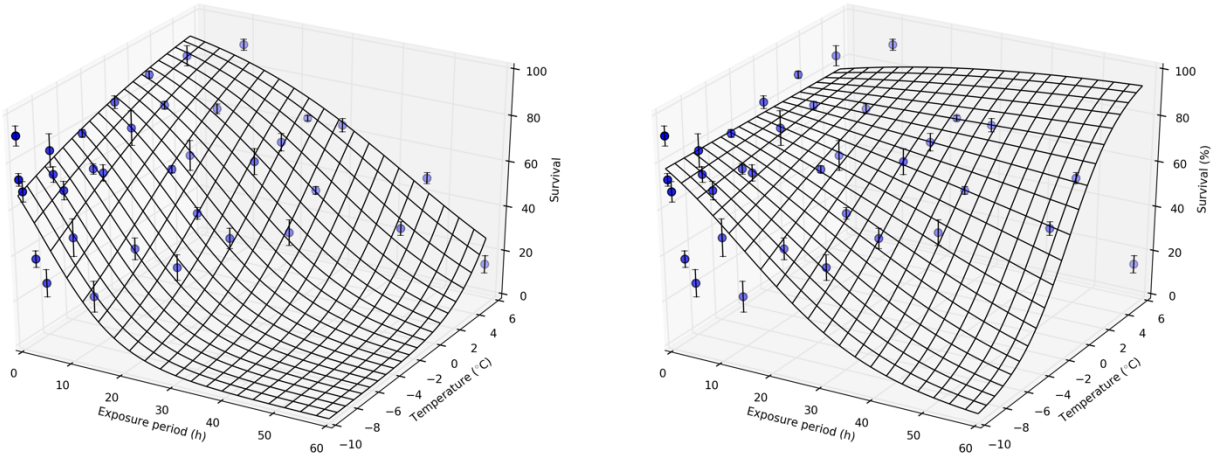

Figure 2.4. Percentage survival of *Acanthococcus lagerstroemiae* nymphs in response to cold temperatures (°C) and exposure periods (h) evaluated in January, 2016 using Model 1 (left) and Model 2 (right). Model 1 represents the logistic responses of the survival of nymphs to the temperature, exposure time, and their interactions, while Model 2 represents the logistic responses of the survival of nymphs to exposure time and the interaction between temperature and exposure time. Points are observed values and bars are standard errors.

2.4. Discussion

In this study, the thermal tolerance of *A. lagerstroemiae* nymphs collected in the US was assessed, and a model for predicting its potential distribution was developed. As indicated by the model, the potential distribution of this newly introduced species would be limited by cold temperatures, and probably would not be limited by warm temperatures. During the winter months, the cold tolerance of *A. lagerstroemiae* becomes greater compared with individuals collected in the summer. Based on predictive models developed from this study, the potential northern distribution limit of *A. lagerstroemiae* is about 43° N, which suggests that this pest can survive in most areas where crapemyrtles are being planted in the US.

Table 2.2. Lethal time observed to kill 50% or 90% (Lt_{50} or Lt_{90}) of *Acanthococcus lagerstroemiae* nymphs exposed to various heat and cold temperatures.

Experiments	Temperature (°C)	Number of observation	Slope ± SE	Lt_{50} (h) (95% CI[a])	Lt_{90} (h) (95% CI)	χ^2
July 2015	40	1443	0.1 (0.004)	37.2 (35.0 - 39.6)	73.2 (68.0 - 79.5)	338.8
	45	1117	0.3 (0.02)	8.7 (8.0 - 9.4)	16.4 (15.1 - 18.0)	696.3
	48	1129	2.8 (0.2)	1.3 (1.2 - 1.4)	2.1 (2.0 - 2.3)	894.8
	5	489	0.2 (0.02)	5.0 (3.6 - 6.4)	19.1 (16.1 - 23.8)	177.2
	0	467	0.3 (0.04)	NA[b]	8.6 (6.8 - 12.0)	57.2
	-5	458	1.6 (0.2)	NA	1.9 (1.6 - 2.3)	111.0
September 2015	5	301	0.1 (0.01)	11.1 (7.9 – 14.0)	35.6 (30.0 - 45.0)	69.8
	0	744	0.1 (0.02)	4.2 (2.2 - 5.6)	22.7 (19.4 - 28.0)	72.1
	-5	385	0.9 (0.2)	NA	2.1 (1.7 - 2.9)	43.6
November 2015	5	874	0.1 (0.01)	29.6 (27.0 - 32.7)	63.5 (56.8 - 72.9)	147.2
	0	640	0.1 (0.01)	12.6 (10.7 - 14.4)	35.8 (31.8 - 41.4)	131.2
	-5	1000	0.1 (0.03)	3.0 (1. 8 - 3.9)	18.7 (15.3 - 28.8)	28.9
January 2016	5	618	0.1 (0.01)	41.8 (38.9 - 44.6)	76.8 (70.6 - 85.7)	130.5
	2.5	766	0.1 (0.01)	32.5 (29.5 - 36.3)	71.8 (63.2 - 84.7)	102.7
	0	730	0.1 (0.01)	22.1 (20.0 - 24.6)	50.1 (44.6 - 58.1)	119.6
	-2.5	769	0.1 (0.01)	18.0 (16.0 - 20.4)	44.7 (38.8 - 53.6)	89.0
	-5	758	0.1 (0.02)	9.3 (8.1 - 10.9)	25.4 (21.4 – 32.0)	71.1
	-7.5	723	0.3 (0.04)	3.8 (3.3 - 4.5)	10.8 (9.2 - 13.4)	85.4
	-10	550	0.6 (0.1)	3.2 (2.8 - 3.6)	7.0 (6.1 - 8.3)	112.0

(Table 2.2. Continued)

Experiments	Temperature (°C)	Number of observation	Slope ± SE	Lt_{50} (h) (95% CI[a])	Lt_{90} (h) (95% CI)	χ^2
March 2016	5	950	0.1 (0.01)	33.8 (31.2 - 36.7)	73.4 (66.4 - 83.1)	147.7
	0	1037	0.1 (0.01)	18.4 (16.0 - 20.9)	58.7 (51.7 - 68.9)	112.3
	-5	840	0.1 (0.01)	3.1 (1.3 - 4.5)	23.2 (19.8 - 28.4)	95.5
May 2016	5	959	0.1 (0.01)	24.3 (21.8 - 26.6)	61.3 (55.5 - 69.5)	134.9
	0	1111	0.1 (0.01)	12.3 (10.0 - 14.2)	41.6 (37.8 - 46.7)	140.2
	-5	1468	0.2 (0.01)	4.5 (3.5 - 5.3)	18.5 (16.9 - 20.7)	244.8

[a] Confidence interval.

[b] Unable to obtain a lethal time based on experimental data collected.

Table 2.3. Summary of the two models constructed using different terms based on the results generated from the cold exposure experiments conducted in January, 2016

Model	Model description	AIC	DF[a]	Log-Lik[b]	AUC (95% CI[c])
1	Survival ~ Temperature + Time + Temperature*Time	821.62	3	-813.62	0.70 (0.68 - 0.72)
2	Survival ~ Time + Temperature*Time	963.99	2	-957.99	0.69 (0.67 - 0.70)

[a] Degree of freedom.

[b] The log-likelihood of the model.

[c] Confidence interval.

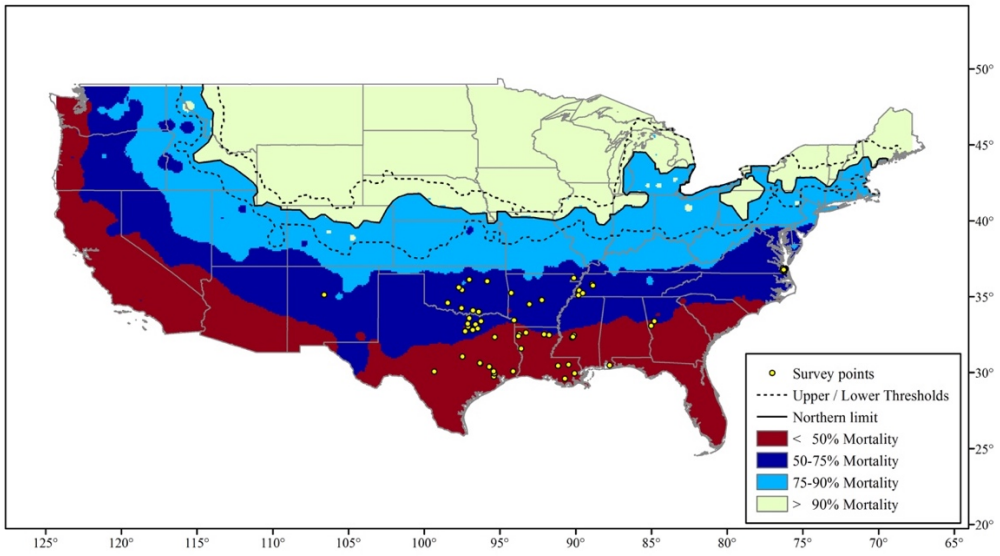

Figure 2.5. Potential distribution of *Acanthococcus lagerstroemiae* predicted by the nymphal mortality caused by historical cold fronts. Lighter colors indicate higher nymphal mortality.

Acanthococcus lagerstroemiae can tolerant high temperatures as indicated by their ability to survive at 45°C and 48°C (Lt_{50} = 8.7h and 1.3h, respectively) under laboratory conditions. Though the highest historical temperature in Louisiana can get to 46°C, usually it is less likely for this temperature to last for 8h or longer. Therefore, the ambient high temperatures most likely would not cause high mortality of *A. lagerstroemiae* and consequently may not affect its population growth. However, in certain urban areas such as parking lots, heat reflection from the ground may result in extremely high local temperatures (Oke 1973) within a short period of time and cause some mortality of *A. lagerstroemiae*. This type of mortality was noted in field observation of *A. lagerstroemiae* on crapemyrtles planted near parking lots. Few studies have evaluated heat tolerance in scale insects. For example, high heat tolerance was observed in the

California red scale, *Aonidiella aurantii* (Mask.) (Hemiptera: Diaspididae) that 50% of tested nymphs survived under fluctuated temperatures with maximums ranged 47 to 48°C (Abdelrahman 1974), and in the San Jose scale, *Quadraspidiotus perniciosus* Comstock (Hemiptera: Diaspididae) that 42% survival of mixed stages was reported when exposed to 46°C for 5h (Lurie et al. 1998). In contrast, low heat tolerance was reported for mango mealy bug, *Drosicha mangiferae* Stebbins (Hemiptera: Margarodidae), with 100% mortality when exposed to 40°C for 1.5h (Nandi and Chakraborty 2015).

According to this study, cold temperatures are more critical than heat in delimiting the potential distribution of *A. lagerstroemiae*, which is in accordance to other scale species in temperate regions (Abdelrahman 1974, Chong et al. 2008, Preisser et al. 2008). In the winter, the SCP of *A. lagerstroemiae* nymphs can get as low as -27°C. In the present study, combining SCPs with results from the cold exposure experiments, it is concluded that the overwintering strategy of *A. lagerstroemiae* is chill susceptible (Sinclair et al., 2015). According to Sinclair et al. (2015), chill susceptible is not a strategy *per se;* but to successfully overwinter, *A. lagerstroemiae* has to avoid freezing or reduce chilling injuries. To adapt to the cold in the winter, the cold tolerance of *A. lagerstroemiae* increased, as found with lower SCPs and longer Lt_{50}s at low temperatures. Similar seasonal variation in cold tolerance was reported for the pine armored scale,

Hemiberlesia pitysophila Takagi (Hemiptera: Diaspididae) (Zhang et al. 2010). This enhanced cold tolerance can help *A. lagerstroemiae* overwinter in most areas where crapemyrtles are planted (USDA Hardiness Zone 6 to 11). Field observations showed that a certain amount of overwintering nymphs stayed under crevices of bark tissues protecting them from the chilling air, which may suggest behavioral strategy of avoiding cold exposure.

The incorporation of both temperature- and time-dependent variables into the prediction model was able to compensate for the disadvantages of methods that only use one of these variables. For example, Zhao et al. (2015) determined the northern limits of *Agasicles hygrophila* Selman and Vogt (Coleoptera: Chrysomelidae) by directly comparing the temperature thresholds, including ULCIZ and SCP, with the historical average low temperature in the winter. This prediction could have overestimated by neglecting mortality caused by cold fronts, which could be major cause of mortality during winter. For methods only using time-dependent variables, for example, using the frequency of days per year when daily average temperature reached pre-specified values to estimate mortality (Lapointe et al. 2007), the estimation and prediction may change due to different pre-specified temperature. When modelling both variables, the criteria of model selection is important. Although Model 2 had less goodness-of-fit to the experimental data compared with Model 1, it was chosen for the prediction of distribution due to its equal

predictive power as Model 1 and its biologically meaningful parameters. Model 2 was initially proposed in Nedved et al. (1998) without considering the predictive power, possibly due to a relatively small sample size. However, fortunately, the abundant availability of *A. lagerstroemiae* nymphs in the field allowed ample sample sizes (near 5000) for the use of 10-fold cross validation in this study. Despite a large sample size, this method has other limitations, for example, the negative impact of prolonged exposure to cold temperatures (longer than experimented exposure time periods) may have been ignored. In addition, the use of constant temperatures in the evaluation of thermal tolerance may bring bias, as demonstrated in other studies where fluctuating temperatures enhanced insect performance under extreme temperatures (Colinet et al. 2006, Jeffs and Leather 2014, Colinet et al. 2015).

In summary, based on the results from the cold tolerance study, *A. lagerstroemiae* has a potential to establish in areas south from the latitude of 43° N. The predicted distribution and models developed in this study can help manage this pest, in particular in the development of effective prevention strategies within the distribution of its major host plants, crapemyrtles. Currently *A. lagerstroemiae* is found in areas as north as 40° N, but further expansion to more northern areas where crapemyrtles have been planted can occur. More research is needed about

the biology of this pest including population growth, host range and dispersal rates, which can help generate a risk map and implement IPM strategies.

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CHAPTER 3. COLD TOLERANCE PHYSIOLOGY OF *ACANTHOCOCCOUS LAGERSTROEMIAE* (KUWANA) (HEMIPTERA: ERIOCOCCIDAE)

1.1 Introduction

The crapemyrtle bark scale, *Acanthococcus lagerstroemiae* (Kuwana) (Hemiptera: Eriococcidae) is a newly introduced pest of crapemyrtles, *Lagerstroemia* spp. (Myrtales: Lythraceae) (Wang et al. 2016), one of the most popular summer-flowering ornamental shrubs and trees in the southern US (Chappell et al. 2012, USDA-NASS 2014). *Acanthococcus lagerstroemiae* has sexual dimorphism: male is movable with wings and female is wingless and sessile on the bark. Nymphs are the main overwintering stages observed in the field, and gender is hard to differentiate among nymphs. Crapemyrtles heavily infested with *A. lagerstroemiae* have been reported to be stressed resulting in growth reduction (Luo et al. 2000, Ma 2011) or fewer blossoms in the field (Merchant 2014). Similar to other scale pests (Gullan and Kosztarab 1997), *A. lagerstroemiae* secretes honeydew which causes accumulation of sooty mold and turns plant parts beneath the infested branches into an unsightly black color, reducing the aesthetic value of the plant (Gu et al. 2014, Wang et al. 2016).

Our previous experiments revealed that cold tolerance of *A. lagerstroemiae* nymph

changes over the seasons (Chapter 2). The supercooling points (SCP) of *A. lagerstroemiae*, defined as the temperature when the insect body spontaneous gets frozen during gradually cooling environment (Sinclair et al. 2015), decreased from -21.2°C in July 2015 to -26.6°C in January 2016. Mortality of nymphs collected in different seasons responded in a similar pattern when exposed to various cold temperatures. At 0°C, the exposure time needed to cause 90% mortality increased from 9 h from nymphs sampled in summer to 50 h from nymphs sampled in winter, with similar trends observed at other low temperatures. Seasonal changes in cold tolerance has also been observed and reported in other scales, for example, *Hemiberlesia pitysophila* Takagi (Hemiptera: Diaspididae) (Zhang et al. 2010). However, cold tolerance mechanisms have not been investigated. In the winter, most *A. lagerstroemiae* nymphs were observed exposed to the chilling air, suggesting that physiological changes may play important roles under cold temperatures.

Diverse and complex physiological mechanisms have evolved to help insects adapt to seasonal changes in temperature and prevent potential cold injuries (Denlinger and Lee Jr 2010). Identify and quantify biochemical composition may help reveal mechanisms associated with cold tolerance such as seasonal changes in water content and lipids levels (Slachta et al. 2002, Clark and Worland 2008, Rozsypal et al. 2014). Water plays an important role in cold tolerance of

insects, especially in maintaining osmotic stasis. For example, loss of water increases the concentration of cryoprotectants that keeps the body in an unfrozen state (or supercooled). Lipids of different fractions (such as triacylglycerols and phospholipids) are also important for insects to overwinter, as well as their fatty acid compositions (Clark and Worland 2008, Rozsypal et al. 2014). Triacylglycerols (TAGs) function as energy reserves and are accumulated during the winter by many insects, which directly affects their overwintering capacity (Ohtsu et al. 1992, Ohtsu et al. 1995, Buckner et al. 2004). Phospholipids (PLs) are basic structure of cell membranes (Lodish and Zipursky 2000). Adaptations have evolved to alter fatty acid compositions in lipids when facing changing temperatures, generally referred to as "homeoviscous adaptation" (hereafter referred to as HVA) (Sinensky 1974). For example, a remodeling of lipid structure occurs to maintain membrane fluidity with more unsaturated fatty acids under cold temperatures. First demonstrated in bacteria, HVA was reported in several insects in temperate areas (Michaud and Denlinger 2006, Kayukawa et al. 2007, Rozsypal et al. 2014). Associations between lipids or fatty acid alteration and cold tolerance, however, have not been studied in *A. lagerstroemiae* or any other scale insects.

The goal of this study was to describe the biochemical and physiological changes in *A. lagerstroemiae* associated with seasonally altered cold tolerance, specifically, the seasonal

changes of water content, ratios of TAGs to PLs, and fatty acid compositions in both TAGs and PLs. Results obtained from this study provide a better understanding of the overwintering strategies of *A. lagerstroemiae* and reveal insights into the cold physiology of scale insects.

3.2 Methods and Materials

3.2.1 Insect source

Branches with length ranging from 0.4 to 0.8 cm were collected from crapemyrtle trees infested with *A. lagerstroemiae* nymphs in Shreveport, LA (32.55°N, 93.78°W) every other month from November, 2015 to October, 2016. Once arrived in the laboratory, branches were placed into a plastic bag and kept under 25°C in an incubator (Series 101, Percival Scientific®, Perry, IA). Branches were examined under a microscope within 24 h, and nymphs were gently flipped over without injuring any appendages using a needle and gathered onto a Petri dish containing a dry filter paper (10 cm diameter). Because it is almost impossible to differentiate between the second and third instars (Wang *et al.* 2016), a mixture of both instars was used in this study. Six branches (replicates) were used for each collection time, with each replicate being a 1.5ml Eppendorf vial containing multiple live individuals of *A. lagerstroemiae* nymphs collected from a branch. Vials with *A. lagerstroemiae* nymphs were stored in a freezer at -75°C until experiments.

3.2.2 Materials for the lab assay

Tricosanoic acid was purchased from NuCheck Prep®, Inc. (Elysian, MN). HPLC grade chloroform, methanol, hexane, ethyl ether, and distilled water were ordered from Fisher Scientific™, Houston, TX.

3.2.3 Water content

Water content of *A. lagerstroemiae* nymphs was measured as the difference in body mass before and after the lyophilization process using a lyophilizer (FreeZone® Plus 6, Labconco®, Kansas, MO). Fresh weight of nymphs of each replicate was recorded using Delta Range MicroBalance (Mettler-Toledo® Series AX26). The amount of water of each replicate was calculated as the weight loss after lyophilization, and water content of the replicate was calculated as the percentage of water amount to the replicate's fresh weight.

3.2.4 Lipid and fatty acid composition

To determine the energy storage levels of *A. lagerstroemiae* nymphs, total lipids were extracted from nymphs collected in different seasons and separated into different fractions to quantify various fatty acids in each lipid fraction. In addition, the ratio of TAGs to PLs was also calculated.

Folch's method was used to extract lipids of *A. lagerstroemiae* nymphs (Folch *et al*. 1957). Lyophilized nymphs were homogenized by hand using a glass/glass Dounce homogenizer in 1.5 ml chloroform/methanol (2:1 v/v). Then 0.6 ml water was added to the homogenization, after which homogenate separated into two layers. After centrifuging at 10,000 g for 5 min, the lower layer containing lipids was transferred into glass pipettes and stored in a 4 ml Teflon-lined bottle. To extract most of the lipids, remaining material in the homogenizer was diluted with another 1.5 ml chloroform/methanol (2:1 v/v) and 0.6 ml distilled water, then homogenized and pooled with the first extraction. The final lipids were evaporated under a slow flow of nitrogen gas and then reconstituted in 50µl chloroform/methanol (2:1 v/v).

The energy storage levels of *A. lagerstroemiae* nymphs collected at different times were estimated by the ratio of TAGs to PLs. The TAG and PL for each replicate were first separated by thin layer chromatography (TLC) on normal phase silica gel plates (10 by 2.5 cm; 250 microns thick, Uniplate^{TM}, Analtech, Newark, DE) (Chen and Laine 2015), and the ratio of relative amounts of TAG to PL was estimated as the peak color intensity of each band using ImageJ (https://imagej.nih.gov/ij/) (Saunders et al. 2006, Schneider et al. 2012). Extracted lipid from one replicate and the standards [sesame oil and phosphatidylcholine each with 4 µg dissolved in chloroform/methanol (2:1 v/v)] were individually spotted on one plate. After the

samples dried, the plate was inserted into a Teflon-lined TLC tank (3 cm × 3 cm × 10 cm) prewashed by and containing 1 ml chromatography solvent (hexane: ether: acetic acid; 80:20:1, v/v/v). After development, different lipid fractions were visualized by exposing the plate to iodine vapor for 5 min and photographed. The TAG and PL fractions were located by comparing their positions to the sample lane of standards. Pictures of TLC iodine staining results were imported to ImageJ, and the TLC lane was selected using "Select First Lane" function with its color density plotted by "Plot Lane" Function. The TAG/PL ratio was determined as the ratio of peak areas of TAG to PL.

For each replicate, TAG and PL were re-extracted from TLC plate, methylated to fatty acid methyl esters (FAME), and then analyzed on a GC/MS instrument. Before iodine vaporized from the TLC plates, TLGs and PLs located previously were marked with pencil. To re-extract them from the TLC plate, each marked spot of silica gel was collected to a glass vial by a razor blade after addition of 5 μg tricosanoic acid [C23:0; 1 μg/μl dissolved in chloroform/methanol (2:1 v/v)] as internal standard, and 1 ml chloroform/methanol (2:1 v/v) was added as solvent into each vial. To detect potential contamination, the controls were set as two circular spots (in diameter of 0.5 cm) from area without lipids on one of developed TLC plates, with one spot spiked with 5 μg C23:0. All glass vials with silica gels were sonicated for 12.5 min using an

ultrasonic cleaner (Chicago Electric®, Carol Stream, IL), and solvents were transferred to 1.5 ml Eppendorf vials for centrifuging. The supernatants including TAGs and PLs were then transferred into new glass vials and dried under nitrogen gas. After adding 200 µl 1N hydrogen chloride methanol solution, vials containing lipids were heated at 100°C for 1h, following the addition of 200 µl hexane. After centrifuging, the hexane supernatant fractions containing FAME were transferred and analyzed by the GC (7890B, Agilent Technologies, Santa Clara, CA) coupled with a DB-5 column 30m by 0.25 mm (i.d.) with a 0.25 µm film thickness (J&W Scientific, Folsom, CA). The mass spectra were acquired in Electron Ionization (EI) mode (70 eV) with Total Ion Mode (TIM) using the MS (5977A, Agilent Technologies, Santa Clara, CA). The peak areas were recorded by MassHunter software (Agilent Technologies, Santa Clara, CA). Ultrahelium was the carrier gas at 1.5 ml/min and the GC thermal program was set as follows: 60°C for 4 min, 15°C/min to 200°C, 5°C/min to 260°C, then held for 10 min. Peaks were identified by comparing retention times and mass spectra with authentic FAMEs (NuCheck Prep®, Inc., Elysian, MN). Not all fatty acids were detectable in all samples due to some being at very low concentrations. Fatty acids with less than three detected values were considered as "trace" value. Two pairs of fatty acids, the pair of 13-methyl C15:0 and 12-methyl C15:0 and the pair of C18:1n9 and C18:2n6, overlapped and were integrated together. To estimate the

comparable peak areas of overlapped fatty acids, the peak heights were used to calculate the peak area for each pair, assuming the width at half-height to be nearly identical. Each FAME was quantified based on its comparison with the quantity of spiked internal standard (C23:0). Small amounts of C16:0 and C18:0 were found in the controls, and this was corrected for all samples.

To understand the seasonal adaptations of *A. lagerstroemiae* nymphs, the total amount of fatty acids, proportions of single fatty acid and total unsaturated fatty acids were calculated for both TAG and PL. Normality of each variable was tested using Shapiro-Milk test. Data of water content ($P = 0.37$) and TAG/PL ratio ($P = 0.12$) were normally distributed and analyzed using one-way analysis of variance (ANOVA) in PROC MIXED (SAS Version 9.3; SAS Institute, Cary, NC), and the LSMEANS were separated using Tukey's HSD test at alpha = 0.05. Because of the non-normal distributions of the amounts of single fatty acids and calculated dependent variables in both TAG and PL (results of Shapiro-Milk test not shown), a log transformation was applied before the one-way ANOVA.

3.3 Results

3.3.1 Water content

The water content of *A. lagerstroemiae* nymphs was lower in winter and early spring compared with those in summer and early fall (40.8% vs. 63.3%) ($F_{(5, 30)} = 14.0$, $P < 0.0001$; Fig. 3.1).

3.3.2 Lipid and Fatty Acid Composition

The ratio of TAGs/PLs of *A. lagerstroemiae* nymphs differed between summer and winter seasons ($F_{(5, 30)}=7.1$, $P = 0.0002$). The highest ratio was recorded in March/April (1.7) and the lowest in July/August (0.5) (Fig. 3.2). The ratios from November to April were slightly higher than those from May to October, but no significant differences were detected (Fig. 3.2). The ratio in March had a high variation (Fig. 3.2).

A total of twenty fatty acids with 6 to 30 carbon atoms were found present in *Acanthococcus lagerstroemiae* nymphs (Fig. 3.3, Table 3.1 and 3.2). Most fatty acid types were found similar between PLs and TAGs, except that only short chain fatty acids (C6:0 and C8:0) were found in TAG, and long chain fatty acids (C24:0, C26:0, C28:0, and C30:0) and odd chain fatty acid (C17:0) were found in PL (Fig. 3.3, Table 3.1 and 3.2). Although total amount of fatty acids in PL was increased about three times from November, 2015 (33 ± 3 nmol/mg) to May,

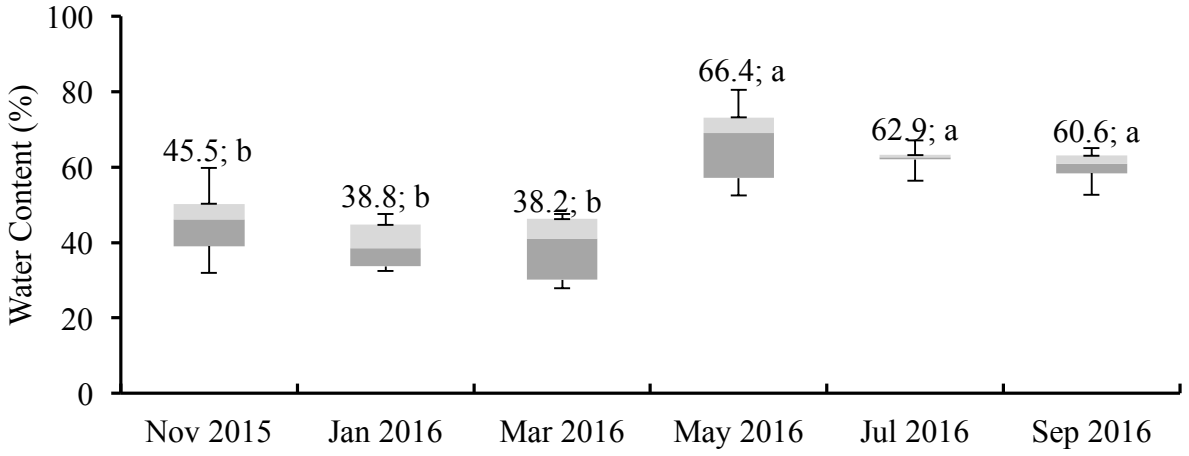

Figure 3.1. Box plot of water content (%) of *Acanthococcus lagerstroemiae* nymphs sampled every other month from November 2015 to October 2016. The lines within each box plot corresponds to the median value, the box length to the interquartile range, and the lines emanating from the box (whiskers) extend to the smallest and largest observations. Values labeled on the top of the box are the means of SCPs, and dates sharing different letters are significantly different.

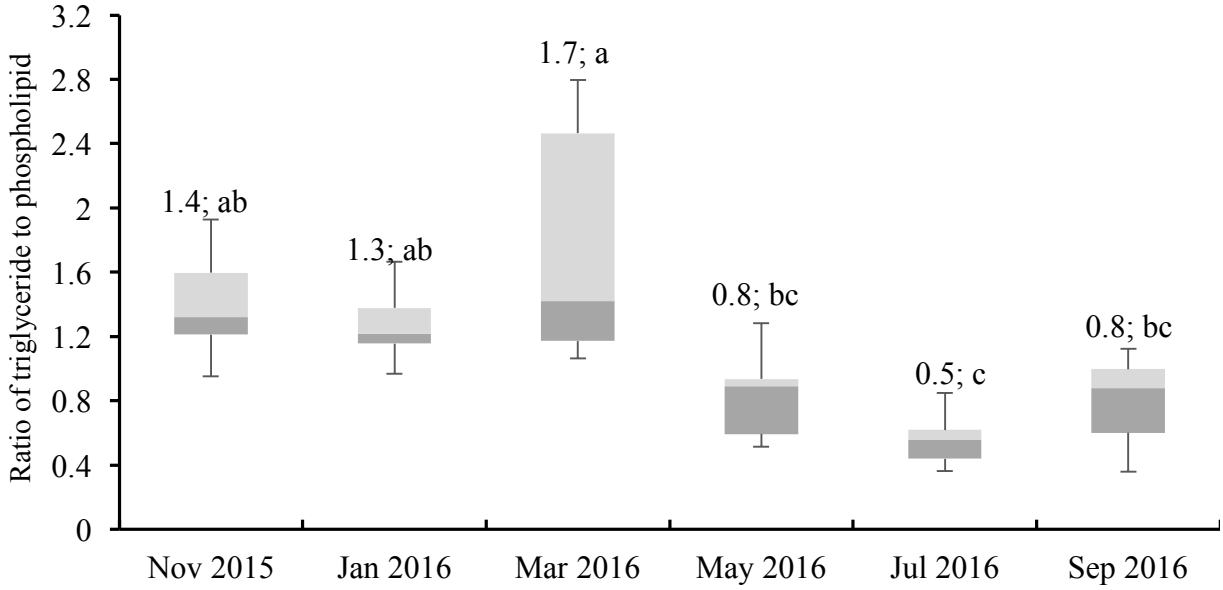

Figure 3.2. Box plot of the ratio of triglyceride (TAG) to phospholipid (PL) of *Acanthococcus lagerstroemiae* nymphs sampled every other month from November 2015 to October 2016. The lines within each box plot corresponds to the median value, the box length to the interquartile range, and the lines emanating from the box (whiskers) extend to the smallest and largest observations. Values labeled on the top of the box are the means of TAG/PL ratios, and dates sharing different letters are significantly different.

2016 (103 ± 23 nmol/mg) (Table 1), the types and proportions of fatty acids in PL were not significantly different among samples collected over the season (data not shown), as well as the proportion of total unsaturated fatty acids in PL (35 ± 2%; $P = 0.173$).

The composition and amounts of fatty acids in TAGs differed over time. Higher amounts of shorter chain fatty acids such as C8:0 and C10:0 were found in colder seasons compared with summer (Table 3.2). Small amounts of C6:0 (< 5 nmol/mg) were detected in November, 2015 and January, 2016 (Table 3.2). Amount of C8:0 increased about 30 fold comparing September, 2016 (3 ± 0.3 nmol/mg) to January, 2016 (91 ± 13 nmol/mg), but absent in May and July, 2016 (Table 2). The total amount of TAG fatty acids reached a maximum at the sample collection time in November, 2015 (639 ± 160 nmol/mg) and a minimum in July, 2016 (166 ± 45 nmol/mg) (Table 2). The percentage of C8:0 and C10:0 increased 32 and 2.6 times comparing September, 2016 to January, 2016, respectively (Fig. 3.4). For fatty acids with more than 12 carbon atoms, their percentages in May, July, and September, 2016 were significantly higher than those in November, 2015, January and March, 2016 (Fig. 3.4). The percentages of total unsaturated fatty acids in TAGs were relatively low (≤ 1%) with marginal differences over time ($F_{(5, 31)} = 2.6$, $P = 0.047$).

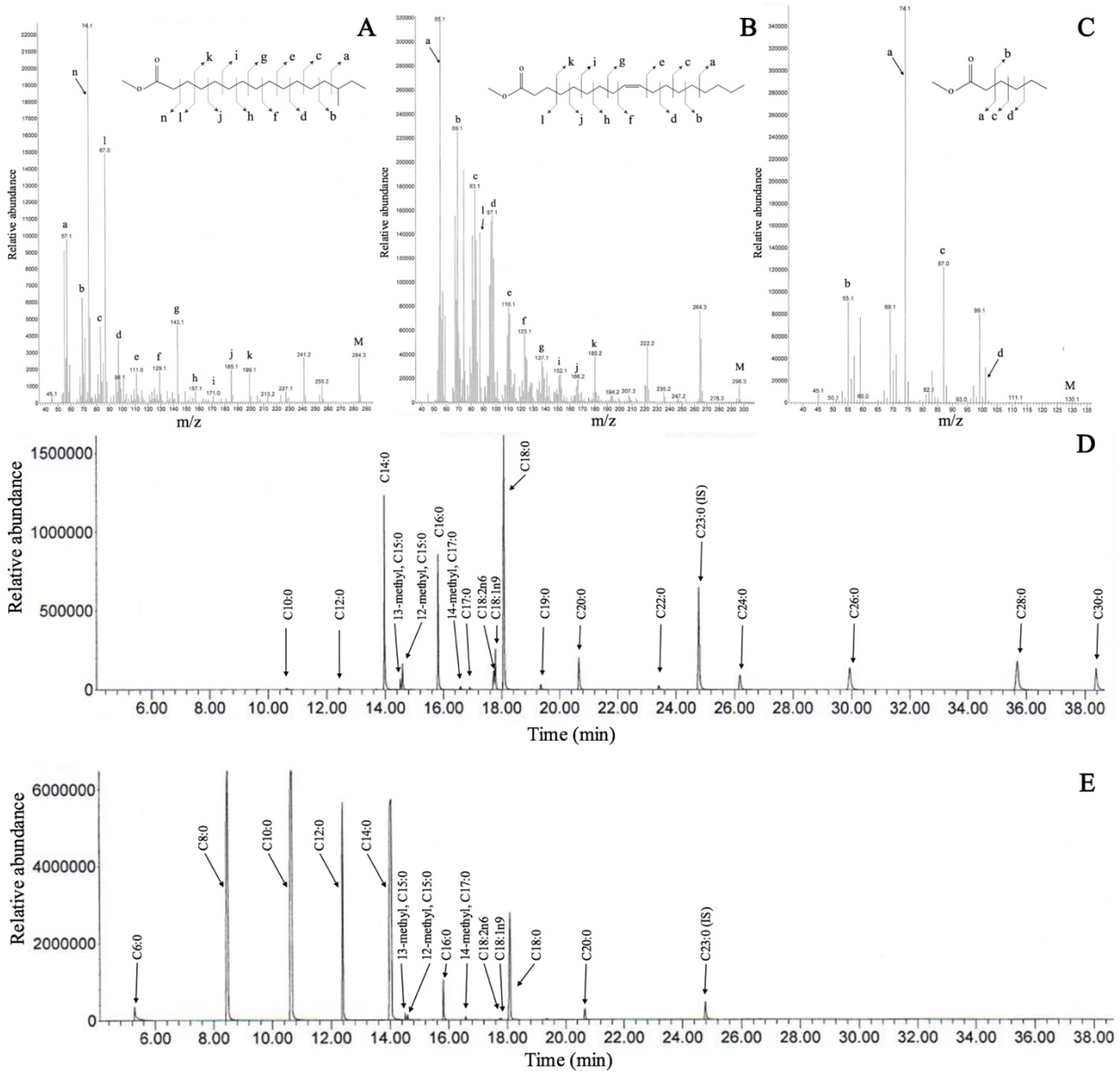

Figure 3.3. Identification of various types of fatty acids in *Acanthococcus lagerstroemiae* nymphs. (A) Mass spectra of 14-methyl C17:0 methyl ester; (B) Mass spectra of C18:1n9; (C) Mass spectra of C6:0; (D) Total ion chromatography of fatty acids in phospholipid for A. *lagerstroemiae* nymphs collected in September, 2016; (E) Total ion chromatography of fatty acids in triacylglycerol for A. *lagerstroemiae* nymphs collected in January, 2016.

3.4 Discussion

Insect adaptations to cold temperatures are critical for winter survival and population growth (Chown and Nicolson 2004, Sinclair et al. 2015). Cold tolerances of insects are often measured to understand their overwintering strategies and to predict their potential distribution, which can help design management strategies (Lapointe et al. 2007, Sunday et al. 2012). Seasonally altered cold tolerance is one common overwintering strategy for insects in temperate region, which is usually associated with complex physiological changes (Teets and Denlinger 2013).

One of these changes is the reduction in body water content (Salt 1961). In this study, a 20% reduction in water content of *A. lagerstroemiae* nymphs was found in winter compared with the water contents in nymphs during summer. Reduction in water, a universal metabolite in living organisms, can concentrate body fluids containing important cryoprotectants, and/or decrease the supercooling and freezing points by reducing amount of freezable water (Ring and Danks 1994, Elnitsky et al. 2008). Our previous studies showed a reduction of 5 °C in the supercooling points of *A. lagerstroemiae* nymphs comparing winter samples with summer samples (Chapter 2, Figure 2.2). As indicated by Ring and Danks (1994), the desiccation and cold tolerance are overlapping adaptations in the field. The water reduction in *A. lagerstroemiae*

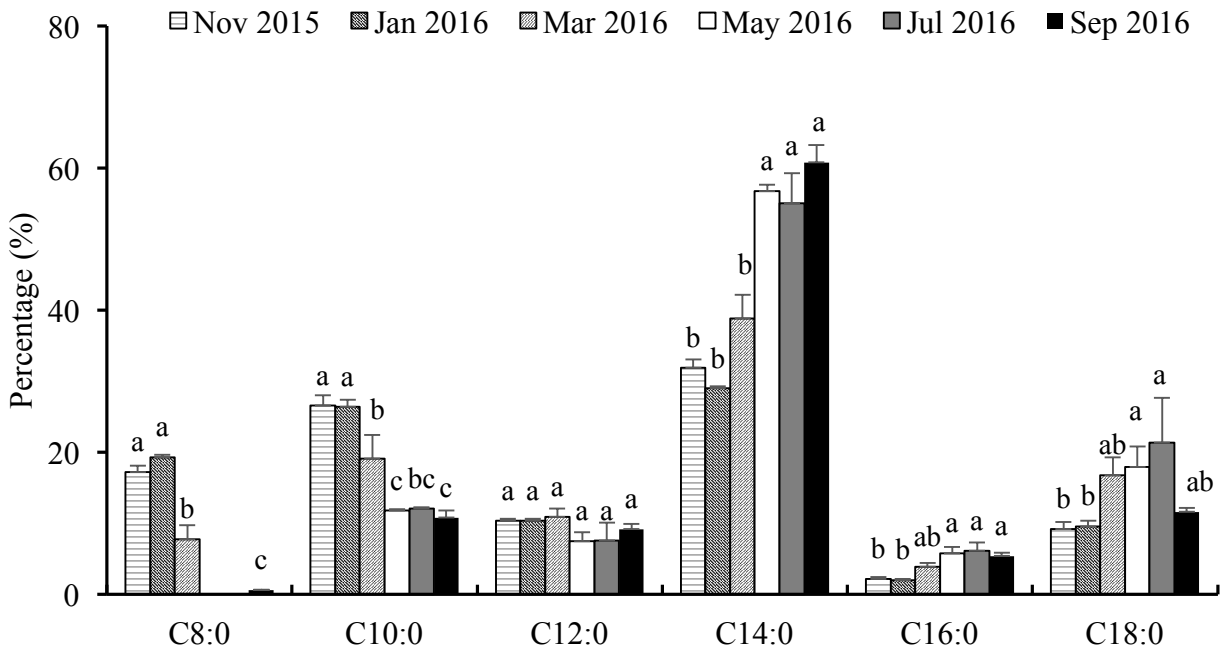

Figure 3.4. The percentages of molar concentrations of the six most abundant fatty acids in triacylglycerol of *Acanthococcus lagerstroemiae* nymphs, including C8:0, C10:0, C12:0, C14:0, C16:0 and C18:0. The GC/MS analyses were conducted every other month from November 2015 to October 2016. Bars are standard errors. Groups labeled with different letters within each type of fatty acid indicate significant difference among sample collection time.

nymphs during winter months found in this study may have contributed to their enhanced cold tolerance. However, it is possible that multiple mechanisms were involved.

Energy reserves of *A. lagerstroemiae* nymphs increased from summer to winter as indicated by increased amount of TAGs and PLs. The relatively low TAG/PL ratio in the summer indicate that storage of TAGs at a time when food (plant saps) is readily availability may not be needed by *A. lagerstroemiae*

Table 3.1. Amounts of fatty acids (mean ± standard error; nmol/mg) in phospholipids of *Acanthococcus lagerstroemiae* nymphs sampled every other month from July 2015 to June 2016. Groups labeled with different letters within each row indicate significant differences among sample collection dates.

Fatty acid / Variable	Nov 2015	Jan 2016	Mar 2016	May 2016	Jul 2016	Sep 2016
C10:0	Trace	Trace	0.4 ± 0.2	Trace	Trace	Trace
C12:0	0.6 ± 0.1 a	0.4 ± 0.1 ab	0.4 ± 0.1 ab	0.1 ± 0.04 b	0.3 ± 0.1 ab	Trace
C14:0	2.9 ± 0.4 a	2.5 ± 0.2 a	2.5 ± 0.8 a	4.9 ± 1.4 a	3.8 ± 0.5 a	4.5 ± 0.9 a
13-methyl ,C15:0	0.2 ± 0.03 a	Trace	0.3 ± 0.01 a	0.7 ± 0.2 a	Trace	0.4 ± 0.03 a
12-methyl, C15:0	0.4 ± 0.05 b	0.3 ± 0.04 b	1.0 ± 0.4 ab	1.9 ± 0.5 a	1.3 ± 0.2 a	1.3 ± 0.2 a
C16:0	3.6 ± 0.9 a	4.8 ± 2.2 a	5.0 ± 1.7 a	8.3 ± 1.9 a	5.4 ± 0.6 a	10.0 ± 4.4 a
14-methyl, C16:0	0.3 ± 0.05 a	0.2 ± 0.04 a	0.4 ± 0.1 a	0.5 ± 0.1 a	0.4 ± 0.06 a	0.6 ± 0.2 a
C17:0	0.2 ± 0.1 a	0.2 ± 0.03 a	0.2 ± 0.1 a	0.3 ± 0.1 a	Trace	0.6 ± 0.4 a
C18:2n6	6.7 ± 1.7 a	9.6 ± 1.9 a	21.6 ± 9.3 a	33.8 ± 8.4 a	17.8 ± 1.3 a	23.9 ± 6.4 a
C18:1n9	3.7 ± 0.7 b	5.3 ± 0.7 ab	8.3 ± 2.7 ab	16.6 ± 4.1 a	11.3 ± 0.8 ab	13.0 ± 3.8 ab
C18:0	10.5 ± 1.7 a	11.7 ± 1.1 a	15.0 ± 5.5 a	26.3 ± 5.8 a	17.9 ± 1.9 a	32.8 ± 17.4 a
C19:0	0.4 ± 0.1 a	0.5 ± 0.1 a	0.4 ± 0.1 a	0.6 ± 0.1 a	0.6 ± 0.1 a	0.7 ± 0.3 a
C20:0	1.3 ± 0.1 b	1.6 ± 0.2 ab	2.2 ± 0.7 ab	3.4 ± 0.7 a	2.1 ± 0.4 ab	4.3 ± 1.2 a
C22:0	Trace	0.3 ± 0.1 a	Trace	0.3 ± 0.1 a	0.3 ± 0.1 a	0.4 ± 0.1 a
C24:0	0.6 ± 0.1 a	1.0 ± 0.4 a	0.7 ± 0.3 a	1.1 ± 0.3 a	0.6 ± 0.08 a	2.8 ± 0.9 a
C26:0	0.8 ± 0.2 a	0.9 ± 0.4 a	0.9 ± 0.4 a	2.2 ± 0.8 a	1.1 ± 0.3 a	4.3 ± 2.0 a
C28:0	0.9 ± 0.2 a	0.9 ± 0.4 a	0.8 ± 0.3 a	1.8 ± 1.4 a	1.2 ± 0.5 a	5.9 ± 2.4 a
C30:0	Trace	Trace	0.3 ± 0.2 a	1.0 ± 0.9 a	1.2 ± 0.6 a	3.0 ± 1.2 a
Total amount	33.2 ± 2.7 b	39.4 ± 5.0 ab	59.0 ± 21.5 ab	102.9 ± 22.8 a	64.2 ± 3.0 ab	108 ± 39.6 a

Table 3.2. Amount of fatty acids (mean ± standard error; nmol/mg) in triacylglycerol of *Acanthococcus lagerstroemiae* nymphs sampled every other month from July 2015 to June 2016. Groups labeled with different letters within each row indicate significant differences among sample collection dates.

Fatty acid / Variable	Nov 2015	Jan 2016	Mar 2016	May 2016	Jul 2016	Sep 2016
C6:0	4.4 ± 1.1 a	2.8 ± 0.4 a	Trace	Trace	Trace	Trace
C8:0	108 ± 25.6 a	91.4 ± 13.2 a	18.6 ± 6.1 b	Trace	Trace	1.9 ± 0.3 b
C10:0	178.5 ± 54.1 a	126.5 ± 20.8 a	43.9 ± 11.6 b	30.3 ± 3.3 b	27.9 ± 3.8 b	34.5 ± 2.9 b
C12:0	65.2 ± 15.1 a	48.1 ± 5.1 a	24.2 ± 5.3 ab	19.0 ± 4.1 ab	16.5 ± 6.6 b	29.3 ± 2.1 ab
C14:0	209.1 ± 57.5 a	136.5 ± 17.9 a	81.1 ± 10.8 a	131.1 ± 22.3 a	95.4 ± 26.8 a	196.0 ± 14.3 a
13-methyl, C15:0	0.8 ± 0.1 ab	0.9 ± 0.2 a	0.5 ± 0.1 ab	0.3 ± 0.1 b	0.3 ± 0.1 ab	0.5 ± 0.1 ab
12-methyl, C15:0	1.0 ± 0.3 a	1.6 ± 0.5 a	0.7 ± 0.1 a	0.5 ± 0.1 a	0.5 ± 0.3 a	1.2 ± 0.1 a
C16:0	12.7 ± 1.6 ab	9.1 ± 1.1 b	7.9 ± 0.9 b	12.0 ± 1.2 ab	8.1 ± 1.2 b	17.1 ± 0.1 a
14-methyl, C17:0	1.2 ± 0.1 ab	1.1 ± 0.1 a	0.6 ± 0.1 ab	0.6 ± 0.1 ab	0.6 ± 0.1 b	0.5 ± 0.1 b
C18:2n6	1.0 ± 0.7 ab	2.8 ± 0.7 a	0.7 ± 0.1 b	0.7 ± 0.2 b	0.2 ± 0.1 b	0.4 ± 0.2 b
C18:1n9	1.3 ± 0.5 ab	1.7 ± 0.4 a	0.6 ± 0.2 ab	0.9 ± 0.2 ab	0.2 ± 0.1 b	0.6 ± 0.2 ab
C18:0	52.6 ± 5.7 ab	42.9 ± 2.4 a	34.2 ± 4.2 bc	36.6 ± 3.2 bc	26.0 ± 3.3 c	37.3 ± 2.7 abc
C19:0	Trace	Trace	0.2 ± 0.04	Trace	Trace	Trace
C20:0	2.8 ± 0.2 a	3.5 ± 0.4 a	2.3 ± 0.3 ab	1.6 ± 0.3 ab	1.2 ± 0.3 b	1.6 ± 0.3 ab
C22:0	Trace	Trace	0.2 ± 0.02 b	0.2 ± 0.05 b	0.3 ± 0.05 ab	0.5 ± 0.03 a
Total amount	639.0 ± 159.5 a	470.1 ± 59.6 ab	215.9 ± 36.1 bc	229.3 ± 36.7 bc	166.2 ± 45.0 c	320.9 ± 14.9 abc

Storing TAGs as an energy reserve is a universal solution to limited availability of food when plants are in dormancy in the winter (Arrese and Soulages 2010), as reported for *A. lagerstroemiae* and other hemipterans, for example, *Biprorulus bibax* (Breddin) (Hemiptera: Pentatomidae) (James et al. 1990) and *Agonoscena pistaciae* Burckhardt & Lauterer (Hemiptera: Psyllidae) (Sadeghi et al. 2012). A high variance of the TAG/PL ratio was found among individuals of *A. lagerstroemiae* in March, 2016, when most stored TAGs were supposed to be consumed. The high TAG/PL ratio for some nymphs in March could be result from reduced PLs, as less total amounts of fatty acids in PL were found in March.

The GC/MS analyses detected 20 different fatty acids in *A. lagerstroemiae* nymphs, with 13 fatty acids overlapping between TAG and PL. The most abundant fatty acids for PL are C18:0, C18:1n9, and C18:2n6, and C10:0, C12:0, C14:0 and C18:0 for TAG. Few studies have been conducted on fatty acid identification in scale insects. C14:0, C18:0, C18:1n9, and C18:2n6 were reported in the total lipid of three species in *Ceroplastes* (Hemiptera: Coccidae) (Strong 1963) and the citrus mealybug, *Planococcus citri* Risso (Hemiptera: Pseudococcidae) (Cripps et al. 1986), which are also common fatty acids reported in other hemipterans (Thompson 1973, Hodkova et al. 1999, Buckner and Hagen 2003). The unsaturated fatty acids of *A. lagerstroemiae* only consisted of C18:1n9 and C18:2n6, but C16:1 and C18:3, which are often reported in other hemipterans such as the silverleaf whitefly [*Bemisia tabaci* (Gennadius) (Hemiptera:

Aleyrodidae)] (Buckner and Hagen 2003) and the pea aphid [*Acyrthosiphon pisum* Harris (Hemiptera: Aphididae)] (Chen et al. 2005), were not found in the current study. In addition, several methyl-branched fatty acids, including 12-methyl C15:0, 13-methyl C15:0, and 14-methyl C17:0, were detected in small amounts in both TAGs and PLs of *A. lagerstroemiae*, and very long chain fatty acids, including C24:0, C26:0, C28:0, and C30:0, were detected in PLs. For scale insects, methyl-branched fatty acids are often used as pheromones (Millar et al. 2012, Burger et al. 2017), and very long chain fatty acids are important compositions of waxes (Faurot-Bouchet and Michel 1964, Hashimoto and Kitaoka 1982). However, the functions of these fatty acids in *A. lagerstroemiae* are unknown.

Cold adaptations of *A. lagerstroemiae* were demonstrated by the TAG accumulation of shorter chain fatty acids including C6:0, C8:0, and C10:0, of which C6:0 and C8:0 were found only during colder season. High percentage of C8:0 and C10:0, each about 20% of total TAG fatty acids were found in the samples collected during winter months at the cost of reductions in longer chain fatty acids including C14:0, C16:0, and C18:0. The increased cold tolerance of *A. lagerstroemiae* nymphs collected in the winter months (Chapter 2) correlated with seasonal restructuring of TAGs reported here. The accumulation of shorter chain fatty acids in TAGs which have lower melting points can be explained by HVA theory of maintaining the lipid

fluidity in the winter, although not in membrane lipids. Similar adaptations were only reported for the membrane lipid of *Sarcophaga crassipalpis* Macquart (Diptera: Sarcophagidae) (Michaud and Denlinger 2006), but only for the increased amount of C16:0 comparing to C18:0, instead of fatty acids with carbon atoms less than 12. No studies have been conducted on the seasonal changes of fatty acids in scale insects, and it is still unknown if this is a common adaptation among scale insects. In TAGs, a slight increase of unsaturated fatty acids was also observed comparing samples collected during summer to those in the winter. As all percentages of unsaturated fatty acids throughout the season were less than 1%, however, it is unlikely that they could have major contribution to maintaining fluidity. The reduction of total fatty acids in PL from summer to winter suggests a low metabolic rate at lower temperatures. Nevertheless, none of any adaptive changes was found in membrane lipids based on the same percentages for major fatty acids and total unsaturated fatty acids. Fatty acids adaptation in membrane lipids of *A. lagerstroemiae* is found different from what have been reported in other insects accumulating more unsaturated fatty acids as membrane lipids in winter (Bennett et al. 1997, van Dooremalen et al. 2011).

This study is the first to investigate cold tolerance mechanisms in the superfamily Coccoidea. In summary, the water content and energy reserve of *A. lagerstroemiae* nymph

changed over time, potentially contributing to their overwintering survival. The reduced water content during the winter allows the organism to concentrate body fluids containing important cryoprotectants, and/or decrease the supercooling and freezing points by reducing freezable water. In addition, the restructuring of fatty acid composition in triacylglycerol with accumulation of shorter chain fatty acids (C6:0, C8:0 and C10:0) in winter results in lower melting points that can help the scales to maintain lipid fluidity for energy conservation.

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CHAPTER 4. TEMPERATURE-DEPENDENT DEVELOPMENT AND HOST RANGE OF *ACANTHOCOCCOUS LAGERSTROEMIAE* (KUWANA) (HEMIPTERA: ERIOCOCCIDAE)

4.1 Introduction

The crapemyrtle bark scale, *Acanthococcus lagerstroemiae* (Kuwana) (Hemiptera: Eriococcidae), is an invasive pest of crapemyrtles, *Lagerstroemia* spp. (Myrtales: Lythraceae) (Wang et al. 2016). Native to Asia, this pest was first reported in Richardson, TX in 2004 (Merchant et al. 2014), and it is currently present in eleven other US states including Louisiana (EDDMapS 2017). *Acanthococcus lagerstroemiae* is sexual dimorphic with adult female being sessile on the bark for most of her lifetime (Wang et al. 2016). Honeydew secreted by this scale facilitates sooty mold accumulation on the crapemyrtles thus reducing aesthetic values as well as limited photosynthesis (Gu et al. 2014). Crapemyrtles are ornamentals with the highest economic value in the southeastern US (USDA-NASS 2014). With more than 130 cultivars, *Lagerstroemia* spp. have wide range of plant size, flower, foliage, and bark color (Chappell et al. 2012). Before the arrival of *A. lagerstroemiae*, crapemyrtles were valued as an ornamental with low pest problem (Knox 2003, Chappell et al. 2012). Current management of *A. lagerstroemiae* typically relies on insecticides such as imidacloprid, cypermethrin, and dinotefuran both in China (He et al.

2008, Zhang 2011) and the US (Gu et al. 2014, Robbins 2014), though most of these chemicals have been prohibited on pollinator-attractive plants – including crapemyrtles (Riddle and Mizell III 2016).

Temperature is one of the most important abiotic factors influencing insects' survival and development, and consequently population growth (Ratte 1984, Amarasekare and Savage 2011, Régnière et al. 2012). For *A. lagerstroemiae*, most of its phenology comes from field observations both in the native area (Jiang and Xu 1998, Luo et al. 2000, He et al. 2008, Ma 2011) and the US (Gu et al. 2014), and these studies showed that the number of generations of *A. lagerstroemiae* increased latitudinally from two to four generations from 32 to 26°N in China. Despite the importance for the development of phonological models, there is no information on the immature survival and developmental times at constant temperatures of *A. lagerstroemiae.* By understanding the development time of a pest, effective management plans can be developed such as better timing of insecticide applications, delivering preventive strategies or releasing biological control agents (Waage et al. 1985, May et al. 1988, Tang et al. 2010).

Understanding the host range of exotic pests is critical to determine potential risks and economic losses (Venette et al. 2010, Zalucki et al. 2012). In Asia and Hungary, *A. lagerstroemiae* was reported to attack thirteen species of ecological and economic importance

(Hoy 1963, Hua 2000, Kozar et al. 2013). Some of the reported hosts of this scale are also important crops in the US, including pomegranate, *Punica granatum* L. (Myrtales: Lythraceae), persimmon, *Diospyros kaki* Thunb. (Ericales: Ebenaceae), and edible fig, *Ficus carica* L. (Rosales: Moraceae) (USDA-NASS 2012). In addition, polyphagous pests including *A. lagerstroemiae* may expand or shift the host range in the adventive area (Strong Jr 1979). These changes in host range in different regions has been reported for invasive scales (Hemiptera: Coccoidea) (Karar 2008, Cham et al. 2011, Culik et al. 2013). However, the plant species at risk of *A. lagerstroemiae* in the US are unknown.

The purpose of this study was to understand the temperature-dependent development and host range of *A. lagerstroemiae*. The specific objectives were: 1) to assess the effects of temperature on the development and survival of immature stages; 2) to determine the host range of the scale under no-choice conditions. Temperature-dependent development was evaluated at constant temperatures in the laboratory. Thirteen plant species from seven families were tested under no-choice conditions. Preventive strategies and improvement of IPM plans against this scale are discussed.

4.2. Method and Materials

4.2.1 Insect and Plant Colonies

Branches of crapemyrtles infested with different stages of *A. lagerstroemiae* were collected in Shreveport, Louisiana (32.55°N, 93.78°W) from April, 2016 to July, 2016. Upon arrival at the laboratory, infested branches were immediately placed in a growth chamber at 25 ± 1°C with photoperiod 12:12 (L:D). Experiments were conducted 1 or 2 days after the field collection to ensure insects were alive.

Crapemyrtles, *Lagerstroemiae indica* × *fauriei* "Natchez White" (Myrtales: Lythraceae) in trade-gallon pots (1 L) were purchased from local nurseries in Baton Rouge, LA. Other plant species were purchased either from local nurseries or obtained from Louisiana State University Agricultural Center (LSUAC) Hammond Research Station, Hammond, LA with container sizes ranging from one to 3.8 L. All plants were placed under full sun, fertilized every three months with 14 g of a controlled release fertilizer (OsmocotePlus®, 15N-9P-12K; The Scotts Miracle-Gro Company, Marysville, OH) and watered daily.

4.2.2 Temperature-Dependent Development

The immature development and survival of *A. lagerstroemiae* were examined at seven constant temperatures (17.5, 20, 22.5, 25, 27.5, 30, and 32.5 ± 1°C) in environmental growth

chambers (Series 101, Percival Scientific®, Perry, IA) set at 12:12h L:D photoperiod. Short branches (< 5 cm) containing gravid females were placed inside Petri dishes (9-cm diameter) and monitored for the presence of eggs. Fresh eggs (< 1 d old) laid from gravid females were gently removed using a pin and transferred to new Petri dishes containing dry filter paper. One Petri dish was assigned to each temperature, and 40 eggs laid by at least three females were pooled at each temperature. A single egg was considered a replicate. All Petri dishes were examined under a dissecting scope daily and numbers of crawlers were counted and recorded until all eggs had hatched or died.

For nymphal development, fifty newly hatched crawlers (<1d old) were inoculated on a potted crapemyrtle plant, and four plants (replicates) were used per temperature (20, 25, and 30 ± 1°C; photoperiod 12:12 D:L). Each infested plant was kept inside a 49 L plastic wastebasket (20 × 30 × 45 cm; Mainstays™, Kenmore, VA) that was modified by removing the plastic material from each of the four sides and the bottom and then covering with fine mesh. The fine mesh served to maintain air ventilation and humidity inside the container and prevent escaping of crawlers. The top of the basket was covered with transparent plastic wrap. Because of the tiny size and similar morphism among different *A. lagerstroemiae* instars (Wang et al. 2016), it was difficult to differentiate each molting during the nymphal stages. Therefore, the presence of

white coverings of male prepupa and gravid female was determined as the end of nymphal stage for male and female, respectively. Because females produce a white covering when they are ready to lay eggs, the developmental time for female nymphs measured in this study could be overestimated. All plants were examined daily, and individuals with presence of white coverings were recorded and marked with a sharpie on the bark. Since most nymphs could not finish their development for seven months at 20°C, at that point all plants were harvested, and nymph mortality was confirmed under a microscope by leg movement. Developmental time and survival per observed life stage were compared among temperatures using one-way analysis of the variance (ANOVA) in PROC MIXED (SAS Version 9.3; SAS Institute, Cary, NC), and the LSMEANS were compared using Tukey's Honestly Significant Difference (HSD) test at alpha = 0.05.

4.2.3 Host Range Test

The immature development and reproduction of *A. lagerstroemiae* reared on different plant species were examined under no-choice conditions. A total of thirteen plant species were selected based on three criteria: 1) plants were previously reported as hosts (reviewed in Wang et al. 2016), 2) plants are closely related as determined by the centrifugal phylogenetic method (Wapshere 1989), and 3) *Callicarpa americana* L. (Lamiales: Lamiaceae) that was observed

infested with *A. lagerstroemiae* in the field (Wang et al. 2016) (Table 4.1). Plant species reported as hosts in Asia that found in the US were *Buxus microphylla* Siebold & Zucc. (Buxales: Buxaceae), *Celtis laevigata* Willdenow (Myrtales: Combretaceae), *Diospyros kaki* Thunb. (Ericales: Ebenaceae), *Ficus carica* L. (Rosales: Moraceae), *Punica granatum* L. (Myrtales: Lythraceae), and *Rubus fruticosus* L. "Kiowa" (Rosales: Rosaceae). According to the phylogenic analysis of Lythraceae (Myrtales) (Graham et al. 2005), another four plant species were selected including *Cuphea ignea* A. DC., *Heimia salicifolia* Link, *Lawsonia inermis* L., and *Lythrum alatum* Pursh. Four plants (replicates) of each species were used in this study, and *Lagerstroemiae indica* × *fauriei* "Natchez White" was considered the control. Plants were inoculated by tying infested branches (8-10cm in length) to the main stem of test plants for one week. Then each plant was placed inside a cages (61 × 61 × 91 cm) (BioQuip® Compton, CA) and allowed to grow under the greenhouse conditions. Gravid females, recognized by the white ovisacs found on each plant were counted and recorded every week for a total of fourteen weeks. The experiment was conducted from April to October, 2016. Plant species that supported complete life cycle development from egg to adult and the reproduction of adults were defined as host plants of *A. lagerstroemiae* (Heard 1997). When no gravid females were found after four weeks of inoculation, plants were re-infested using same protocol to confirm the non-host status.

Table 4.1. Plant species as host candidates of *Acanthococcus lagerstroemiae* used in the no-choice test

Scientific name	Variety	Common name	Order	Family
Buxus microphylla Siebold & Zucc.	"Japonica"	Japanese Boxwood	Buxales	Buxaceae
Diospyros kaki Thunb.	Wild variety	Japanese persimmon	Ericales	Ebenaceae
Callicarpa americana L.	-	Beautyberry	Lamiales	Lamiaceae
Celtis laevigata Willdenow	-	Sugarberry	Myrtales	Combretaceae
Cuphea ignea A. DC.	"Strybing Sunset"	Cigar flower	Myrtales	Lythraceae
Cuphea ignea A. DC.	"Dynamite"	Cigar flower	Myrtales	Lythraceae
Cuphea ignea A. DC.	"Vermillionaire"	Cigar flower	Myrtales	Lythraceae
Heimia salicifolia Link	-	Sinicuichi	Myrtales	Lythraceae
Lagerstroemia indica × *fauriei*	"Natchez White"	Crapemyrtle	Myrtales	Lythraceae
Lawsonia inermis L.	-	Henna	Myrtales	Lythraceae
Lythrum alatum Pursh	-	Winged loosestrife	Myrtales	Lythraceae
Punica granatum L.	"Wonderful"	Pomegranate	Myrtales	Lythraceae
Ficus carica L.	"Tiger"	Edible fig	Rosales	Moraceae
Rubus fruticosus L.	"Kiowa"	Blackberry	Rosales	Rosaceae

The total number of gravid females by week 12 were compared among host plants using one-way ANOVA in PROC MIXED (SAS Version 9.3; SAS Institute, Cary, NC), and the LSMEANS were separated using Tukey's HSD test at alpha = 0.05.

4.3 Results

4.3.1 Temperature-dependent development

Developmental time differed among temperatures for egg ($F_{(5, 159)} = 1076.0$, $P < 0.0001$; Fig. 4.1), and nymphs of male ($F_{(2, 48)} = 84.9$, $P < 0.0001$) and female ($F_{(1, 65)} = 350.2$, $P < 0.0001$; Table 4.1). Mean developmental time for eggs decreased from 36 d at 17.5°C (36 d) to 10 d at 27.5°C, and then increased to 11 d at 30°C (Fig. 4.1). Developmental time from nymph to male prepupa increased from 56 d at 30°C to 154 d at 20°C, and the time from nymph to gravid female was 68 and 137 d at 30 and 25°C, respectively.

Survival was different for eggs (Fig. 4.1) and nymphs at different temperatures ($F_{(2, 9)} = 7.4$, $P < 0.013$; Table 4.2). Lower egg survival (≤ 55%) was recorded for temperatures lower than 25°C, and most eggs hatched (≥ 90%) when temperature ranged from 25 to 30°C (Fig. 4.1). No eggs hatched at 32°C. For nymphs, the highest survival (30%) was found at 25°C and the lowest (16%) was found at 20°C (Table 4.2).

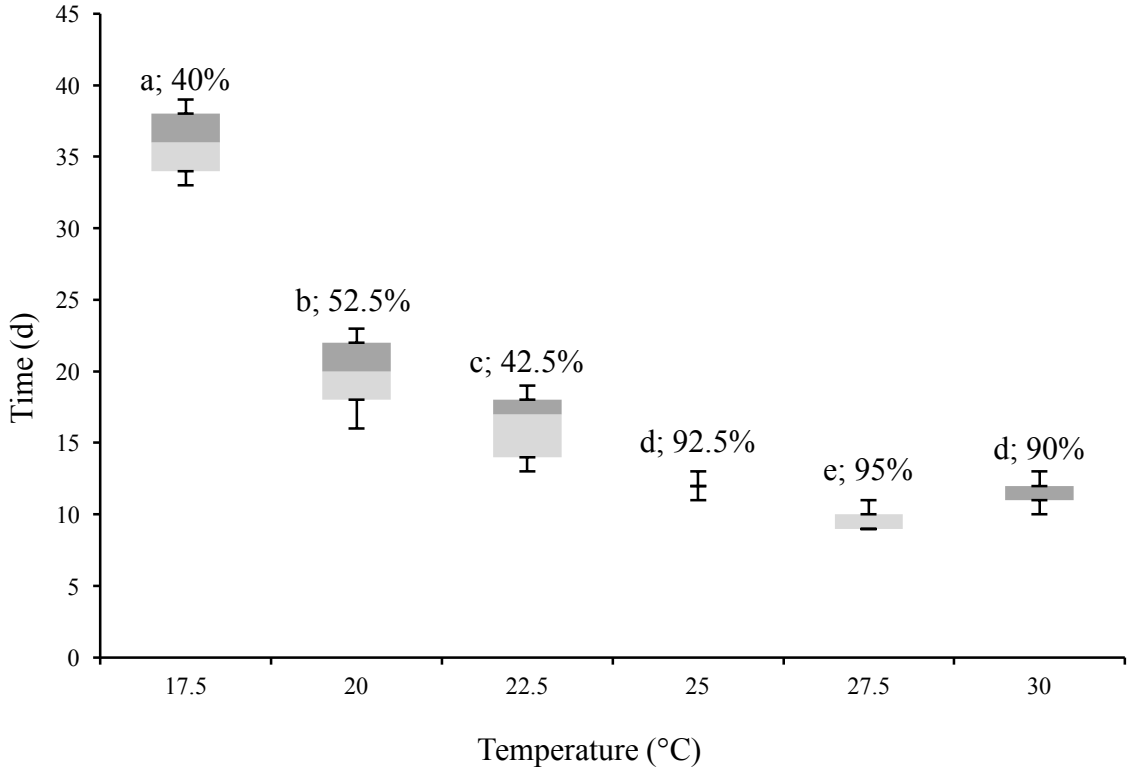

Figure 4.1. Box plot of the developmental time in days of *Acanthococcus lagerstroemiae* eggs at six constant temperatures. The lines within each box plot corresponds to the median value, the box length to the interquartile range, and the lines emanating from the box (whiskers) extend to the smallest and largest observations. Different letters indicate significantly different developmental times among temperatures. Values are egg survivals at each temperature.

Table 4.2. Mean (± SE) developmental time in days and nymphal survival of *Acanthococcus lagerstroemiae* at three constant temperatures. Means within each row followed by different letters are significantly different ($P < 0.05$; Tukey's HSD).

Stage/Variable	Temperature (°C)		
	20	25	30
Nymph to Prepupa	154.0 ± 6.6 a	122.0 ± 3.8 b	55.5 ± 5.1 c
Nymph to Gravid Female	NA[a]	136.7 ± 2.4 a	68.3 ± 2.7 b
Nymphal Survival (%)	16.0 ± 0.8 b	30.0 ± 4.7 a	22.5 ± 1.8 ab

[a] NA indicates no gravid female successfully developed.

4.3.2 Host range

Results from the no-choice tests indicated that *La. inermis*, *H. salicifolia*, *Punica granatum*, *Ly. alatum*, and *C. americana* supported nymphal development and reproduction of *A. lagerstroemiae*. Numbers of females on all hosts were lower than 100 after the first 6 weeks, then increased to different levels (Fig. 4.2). The number of gravid females at week 12 also differed among species ($F_{(5, 19)} = 8.5$, $P = 0.0002$; Fig. 4.2). The crapemyrtle (*L. indica* × *fauriei*) had the highest number of gravid females (482 ± 92), followed by *C. americana* (200 ± 70), and lower numbers (< 100) were obtained on the other four species (Fig. 4.2).

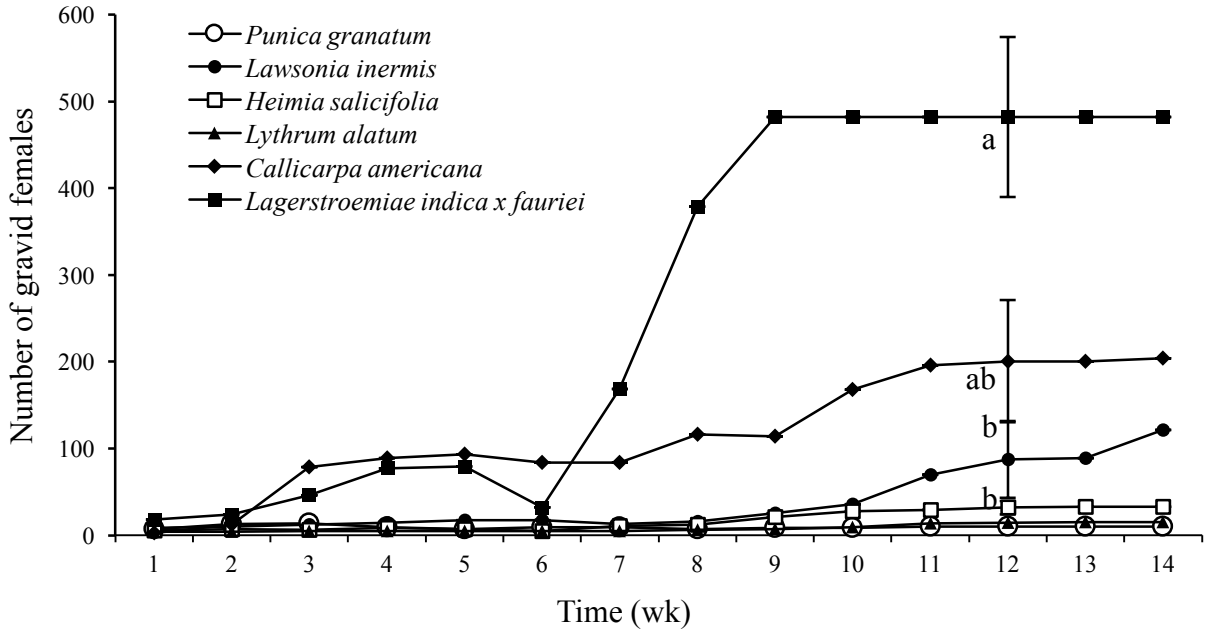

Figure 4.2. Number of *Acanthococcus lagerstroemiae* gravid females on six host plant species for 14 weeks. Different letters represent statistically different means at 12th week adjusted by Tukey's HSD method, and bars are standard errors for comparisons at 12th week.

4.4 Discussion

The developmental time and survival of *A. lagerstroemiae* egg and nymph varied among temperatures. Unlike other scales with a linear relationship between the development time and temperature (Blank et al. 2000, Amarasekare et al. 2008), there was an optimum temperature (27.5°C) for the egg hatching because of the shortest time and the highest hatching rate. Constant temperatures below 25°C resulted in lower egg hatching rate while temperatures above 32°C resulted in complete mortality. However, as mentioned in Gullan and Kosztarab (1997) that the ovisacs of scales could prevent heat and moisture exchanges and maintain a relatively stable microenvironment inside, thus ambient temperature maintained constant in the lab may not represent good simulation for egg hatching in the field. Nymphs of *A. lagerstroemiae* have much slower growth than other scales. The development from crawler to gravid female was 137d at 25°C for *A. lagerstroemiae*, but 54 d for *Pseudaulacaspis pentagona* (Targioni-Tozzetti) (Erkiliç and Uygun 1997) (Hemiptera: Diaspididae), 65 d for *Hemiberlesia rapax* (Comstock) (Blank et al. 2000) (Hemiptera: Diaspididae), and 24 d for *Phenacoccus solani* Ferris (Nakahira and Arakawa 2006) (Hemiptera: Pseudococcidae). Nymphal survival of *A. lagerstroemiae* was lower (<35%) compared to other scales (70-90%), including *P. solani* (Nakahira and Arakawa 2006), *Paracoccus marginatus* (Hemiptera: Pseudococcidae) (Amarasekare et al. 2008), and

Phenacoccus solenopsis Tinsley (Hemiptera: Pseudococcidae) (Prasad et al. 2012). The slower development and less survival in this study could be caused by poor plant quality under the less favorable conditions (for example, artificial light and constant temperatures inside the growth chamber), and may have adversely impacted an accurate estimation on the developmental time.

The developmental time of *A. lagerstroemiae* estimated in this study can still be used to better understand the phenology of this pest in the field. The time for *A. lagerstroemiae* to completely develop one generation is about 4 months at 25°C and 3.5 months at 30°C, and could be shorter at relatively warmer temperatures. Nymphs of *A. lagerstroemiae* stayed quiescent and cannot reach reproductive stage at constant 20°C. According to the National Climatic Data Center (NCDC) (https://www.ncdc.noaa.gov/), the daily average temperatures in subtropical areas such as Louisiana and Texas get higher than 20°C since mid-April and lower than 20°C since later October for each year, suggesting the potential time of crawler emergence and starting overwintering for *A. lagerstroemiae*. Therefore, *A. lagerstroemiae* should have more than two generations per year in Louisiana and Texas. The information obtained from this study could help build models combined with data collected by our collaborators and predict the population dynamics in different locations (Schwartz 1999, Yurk and Powell 2010), as demonstrated for the

population growth of *P. solenopsis* (Fand et al. 2014), and for the crawler emergence of *Unaspis yanonensis* Kuwana (Hemiptera: Diaspididae) (Kim and Kim 2013).

Acanthococcus lagerstroemiae is polyphagous, and it can develop and reproduce on at least five species from different genera and families. Four out of the five plant species are phylogenetically related to the crapemyrtle (Lythraceae), but the American beautyberry (*C. americana*; Lamiaceae: Lamiales) is relatively distant from Lythraceae in phylogeny (AGPII 2003). Reasons for the polyphagy of *A. lagerstroemiae* are unknown but one speculation is that these plant species could share somewhat similar plant chemistry (Ehrlich and Murphy 1988, Erbilgin et al. 2014), or simply that *A. lagerstroemiae* has the adaptations to overcome the chemical defense of plants in multiple families and orders (Dicke 2000, Harrison et al. 2016). The phylogenetic relationship of scales in *Acanthococcus* (= *Eriococcus*; Eriococcidae) is still ambiguous (Cook et al. 2002, Kozar et al. 2013), and the host ranges for these scales are poorly investigated. However, several phylogenetically related species to *A. lagerstroemiae* recorded in Kozar et al. (2013) were collected from plants species from multiple families and orders including *Acanthococcus* (= *Eriococcus*) *macedoniensis* Fetyko & Kaydan, *Acanthococcus* (= *Eriococcus*) *melnikinensis* (Kuwana), *Acanthococcus* (= *Eriococcus*) *onukii* (Kuwana). Furthermore, except for the pomegranate (*P. granatum*), the host species of *A. lagerstroemiae*

found in this study are different from what have been reported in Asia (Wang et al. 2016). To determine the full host range of this invasive pest in the US, more plant species having been reported in Asia or phylogenetically related to confirmed hosts should be tested.

Prevention should be the primary approach to manage *A. lagerstroemiae* in nurseries growing the host plants. Host species of *A. lagerstroemiae* found in this study are economically and/or ecologically important. The pomegranate (*P. granatum*) is a fruit crop produced in 32,887 acres in the US (as recorded in 2012) (NASS 2014), with a value of about $184 million reported in California alone (CDFA 2016). American beautyberry (*C. americana*) (Wiersema and Leon 2016) and winged loosestrife (*L. alatum*) (Clute 1901) are important native plants that also grown as ornamentals in the nurseries. Sinicuichi (*H. salicifolia*) is valued for its medicinal traits (Baxter et al. 2001). Though not commercially planted in the US, Henna (*L. inermis*) is an economically important crop in India and several other countries for its medicinal and cosmetic uses (Kumar et al. 2005, Semwal et al. 2014). In this experiment, the density of *A. lagerstroemiae* increased over time on all these host species, although high density (> 150) occurred only on crapemyrtle and American beautyberry. Scouting is recommended for all plant species in the host range of *A. lagerstroemiae*, and immediate responses such as applying

insecticides or removing infested plants should be carried out to prevent further spread of this invasive scale (Kim et al. 2006, Zalucki et al. 2012).

In summary, temperature requirements for the immature *A. lagerstroemiae* to develop and the host range with selected plant species were evaluated in this study. The temperature-dependent development of *A. lagerstroemiae* can help better time its activities and corresponding control measures in the field. Five out of thirteen plant species chosen from different genera and families were found to be suitable host species of *A. lagerstroemiae*. Inspections in all potential host plants are recommended with appropriate treatments in order to prevent the spread of *A. lagerstroemiae* and potential economic losses caused by this pest. Information obtained from this study can be incorporated into the IPM of *A. lagerstroemiae* in the US.

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SUMMARY AND CONCLUSIONS

The crapemyrtle bark scale, *Acanthococcus lagerstroemiae* (Kuwana) (Hemiptera: Eriococcidae), is an exotic pest on crapemyrtles, *Lagerstroemia* spp. (Myrtales: Lythraceae). Because of its recent arrival to the US, little is known about its biology and ecology. The purpose of my thesis was to improve the knowledge about *A. lagerstroemiae* in four aspects involving its thermal tolerance, physiological adaptations to cold temperatures, temperature-dependent development, and host range.

This study first aimed to predict geographical limits of *A. lagerstroemiae* in the US by comparing its thermal tolerance with local temperatures. Laboratory experiments showed *A. lagerstroemiae* has high heat tolerance and can adapt well to southern US. However, *A. lagerstroemiae* could be limited by winter temperatures. To predict its northern limit, a model was developed to describe the relationship of the scale's survival at various constant cold temperatures after different exposure time periods. Comparing with temperature data from historical cold fronts, mortalities calculated by the model were used to plot the northern limit. Based on this prediction, *A. lagerstroemiae* is able to establish in areas south from the 43°N, which is similar to the northern limit of crapemyrtles. This study concludes that winter temperatures could cause mortality to *A. lagerstroemiae*, however, its predicted distribution in

US completely overlaps with the distribution limits of crapemyrtles based on the plant species' winter hardiness.

To adapt to colder temperatures in the winter, the cold tolerance of *A. lagerstroemiae* increased since November. The second objective of this study aimed to investigate the mechanisms of the altered cold tolerance by measuring seasonal changes of biochemical variables. From November to February, *A. lagerstroemiae* has less water and higher energy reserves, which are common strategies used by insects to increase cold tolerance. This study reported a restructuring of fatty acid composition in body fat of winter nymphs with accumulation of very short chain fatty acids (C6:0, C8:0 and C10:0), resulting in lower melting points that can help maintain lipid fluidity for energy conservation. Though the findings from this study can only partially explain their enhanced cold tolerance in winter, more biochemical variables, particularly the potential cryoprotectants (i.e., polyols and amino acids) should be investigated in the future.

The last objective of this study was to determine the temperature-dependent development and host range of *A. lagerstroemiae* in the US. Developmental time and survival of *A. lagerstroemiae* eggs and nymphs under different temperatures were assessed. At 27.5°C, *A. lagerstroemiae* eggs had highest survival (95%) and shortest developmental time (10.0 ± 0.1d).

At 30°C, nymphal development time was 55.5 ± 5.1 and 68.3 ± 3.7 d to prepupa and gravid female, respectively. *Callicarpa americana* L. (Lamiales: Lamiaceae), *Heimia salicifolia* Link, *Lawsonia inermis* L., *Lythrum alatum* Pursh, and *Punica granatum* L. (Myrtales: Lythraceae) supported development and reproduction of *A. lagerstroemiae*. Scouting is recommended on these host species in the field, following immediate responses to avoid economic loss and the spread of this pest. Considering the irregular phylogenetic relationship of host species, more tests should be conducted to reveal other potential host species and avoid further economic loss.

APPENDIX A1. THE PERMISSION FROM THE INSECTS JOURNAL TO REPRINT CHAPTER 1

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APPENDIX A2. OCCURRENCE DATA OF *ACANTHOCOCCUS LAGERSTROEMIAE* (KUWANA) (HEMIPTERA: ERIOCOCCIDAE) IN THE US AND CHINA

Table A2.1. Coordinates of 84 *Acanthococcus lagerstroemiae* occurrence reported in the US were retrieved from EDDMapS (Early Detection and Distribution Mapping System) (https://www.eddmaps.org/). Coordinates of 25 occurrences in Asia were retrieved from local reports and personal communications. Coordinates were obtained using Google Map (http://maps.google.com/) for reports with detailed addresses. For infestation reports only with vague description, a point in the center of the described polygon was chosen.

Reports in the US	Longitude	Latitude
1	-96.561323	33.218417
2	-97.528715	34.248939
3	-96.784898	32.80162
4	-96.464699	32.89801
5	-93.785576	32.400182
6	-97.018053	33.571722
7	-90.712452	29.595381
8	-93.718015	32.526937
9	-96.7753	34.0903
10	-84.798882	33.374325
11	-96.247443	33.379875
12	-89.807391	35.085743
13	-96.321863	30.623193
14	-94.034167	33.434928
15	-94.062628	33.440702
16	-96.612093	33.172243
17	-106.605068	35.118083
18	-96.3976	34.007
19	-91.754314	32.474734
20	-92.08057	32.51462
21	-93.28371	32.619825
22	-93.050627	34.49612

(Table A2.1 continued)

Reports in the US	Longitude	Latitude
23	-90.465941	30.516369
24	-95.416388	30.076222
25	-85.019812	33.075882
26	-90.072656	29.950249
27	-87.756827	30.476208
28	-76.26711	36.7762
29	-89.519472	35.225619
30	-95.337972	32.338723
31	-96.2987	30.5983
32	-90.228713	32.298431
33	-90.1148	36.2327
34	-97.284618	32.713096
35	-97.506663	35.43287
36	-92.237298	34.766021
37	-93.5957	31.583
38	-99.337492	30.070782
39	-95.698205	30.389004
40	-95.8386	36.0209
41	-97.470737	31.052155
42	-98.4454	34.5865
43	-97.129359	33.209732
44	-90.181246	32.35141
45	-97.7199	35.6058
46	-97.0168	36.116
47	-90.109288	32.456571
48	-97.0893	33.035
49	-88.863558	35.724356
50	-76.26711	36.7762
51	-76.294734	36.72543
52	-76.127973	36.843111
53	-76.26281	36.776564

(Table A2.1 continued)

Reports in the US	Longitude	Latitude
54	-76.225156	36.657395
55	-76.282046	36.719318
56	-76.295063	36.721198
57	-76.330052	36.727818
58	-76.300819	36.861877
59	-76.197416	36.811324
60	-76.193157	36.76864
61	-91.1565	30.4462
62	-89.766393	35.405055
63	-88.8983	35.8185
64	-94.1282	30.0798
65	-92.247699	34.781884
66	-95.3993	29.7435
67	-95.3679	29.9382
68	-94.252694	35.239457
69	-96.2513	30.5671
70	-90.623286	36.16777
71	-90.350459	35.285861
72	-92.660365	34.675087
73	-76.2002	36.9014
74	-95.359076	34.422772
75	-86.5334	36.1998
76	-94.6528	31.6285
77	-95.9696	36.1404
78	-92.30563	35.193854
79	-94.29767	35.60173
80	-93.024302	35.506821
81	-91.870499	35.222899
82	-91.775891	36.175564
83	-94.315113	33.693311
84	-9.621681	33.807435

(Table A2.1 continued)

Reports in Asia	Longitude	Latitude
1	78.718094	10.768851
2	135.77125	35.009334
3	128.610432	35.888726
4	121.652112	30.913046
5	116.347888	40.002954
6	102.209878	27.945995
7	117.271727	39.098239
8	115.786498	32.893185
9	117.294159	31.836276
10	116.005074	36.427981
11	115.445945	35.240412
12	118.416813	35.031681
13	119.180717	36.694145
14	120.473516	37.631029
15	113.846879	22.622914
16	120.150914	30.242408
17	119.898736	31.781352
18	113.68309	34.715524
19	113.697929	34.088935
20	121.545597	29.810677
21	111.985543	27.695953
22	105.912011	34.482543
23	107.752551	32.9364
24	106.677558	26.40761
25	107.196238	34.342516

APPENDIX A3. THE METHOD TO PREDICT THE NORTHERN LIMITS OF *ACANTHOCOCCUS LAGERSTROEMIAE*

This supplemental material shows details to predict potential distribution. The predictive procedure can be divided into three steps: (a) downloading and cleaning the temperature data with resolution of one-minute from National Oceanic and Atmosphere Administration (NOAA) (ftp://ftp.ncdc.noaa.gov/pub/data/asos-onemin/), and extracting air temperature data of three coldest fronts within period of 24h; (b) calculating mortality by fitting temperature data of each extracted cold front to the selected model; (c) mapping the potential distribution of *A. lagerstroemiae* using mortality in climatic stations in ArcGIS® Software (ESRI 2011, Redlands, CA).

In the first step, because the temperature data recorded in climatic stations from the US were found with different amounts and recording ways for missing data, it was necessary to screen and delete invalid data files that defined as containing more than one fifth missing values. For valid data files, columns of station name, recording time, and air temperature readings were extracted and saved as Comma-Separated-Value (CSV) format. Data cleaning were completed using Python 2.7 and further extractions of cold front data were conducted using Visual Basic for Applications (VBA) in Excel 2013 (Microsoft Corp., Redmond, WA). The cold front is defined

as the transition area with a cold air mass passes through when can cause reduction of the air temperature for at least 12h (Miller 1972). Here I identified the cold front as the lowest sum of continuous 720 readings (temperature × one min for 12h). For each identified cold front, the 720 readings in one-minute data files were then extracted, as well as 360 readings (temperature × one min for 6h) before and after the extracted 720 readings. Totally three data of 1440 readings (temperature × one min for 12h) for valid data files from 2000 to 2016 were extracted for calculating mortality.

The second step was to calculate the mortality caused by each cold front using the model 2. To account for the assumption of constant temperature used in cold exposure experiments and modeling, continuous temperature data from each cold front were first transformed by calculating combinations of constant temperatures and sustained periods of time, for all temperatures lower than the constant temperature. For example, if 600 readings were counted lower than 0°C, the sustained period for 0°C would be 10h. Because the estimated ULCIZ for *A. lagerstroemiae* was 8.5°C, I chose constant temperatures in integer from 8 to -20°C. Therefore, there were 29 combinations of temperature and sustained periods for each cold front, which generating 29 calculated mortalities. The maximum mortality in each cold front was used to represent the mortality caused by such cold front. The mortalities caused by all cold fronts from

2000 to 2016 were extracted, sorted by weather station, and reported as median ± median absolute deviation (MAD) which represented (Hampel 1974). All calculations were finished using Microsoft Excel VBA.

The distribution of *A. lagerstroemiae* was mapped using Inverse Distance Weighted (IDW) interpolation in ArcGIS. Average mortality for 750 climatic locations (Fig. A3.1) and the coordinates were converted to a shapefile using the ADD X-Y DATA function. A shapefile of the US with state boundary was obtained from the ArcGIS Database and inserted for the predictions. The IDW interpolation in spatial analyst function was used to generate weighted averages from locations of known mortalities and to predict the mortality of *A. lagerstroemiae* on the US map (Childs 2004). The generated raster was classified as four ranges of mortality including 0 – 50, 50 – 75, 75 – 90, and 90 – 100%. As the mortality caused by the coldest period, survived populations of *A. lagerstroemiae* from cold front can resurge on the rest of the year. I chose the line of 90% mortality as northern limit of *A. lagerstroemiae* in the US, and upper and lower threshold of the northern limit were drawn using MAD. Occurrence of *A. lagerstroemiae* confirmed in the US was added into the map as survey points (Table A1.1).

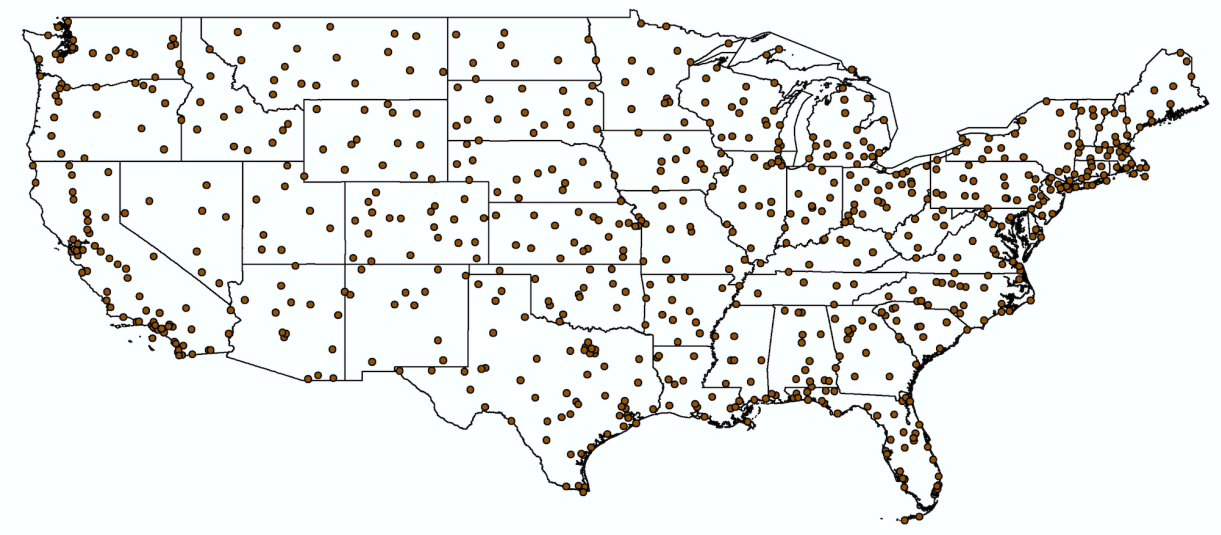

Figure A3.1. Locations of all 750 climatic stations of which the temperature data were used.

Reference:

Miller, R. C. 1972. Notes on analysis and severe-storm forecasting procedures of the Air Force Global Weather Central, pp. 184. Air Force Global Weather Central.

APPENDIX A4. PHYSIOLOGICAL AND AESTHETIC IMPACTS OF CRAPEMYRTLE BARK SCALE, ACANTHOCOCCUS LAGERSTROEMIAE (KUWANA) (HEMIPTERA: ERIOCOCCIDAE) TO "NATCHEZ WHITE" CRAPEMYRTLES

A4.1. Introduction

Ornamental trees are grown for their aesthetic and sustainable importance in the urban areas (Sæbø et al. 2005, De Groot et al. 2010). Invasive insect pests are a major problem causing plant injuries and economic loss (Pimentel et al. 2005, Aukema et al. 2011). Understanding effects caused by invasive pests is important for stakeholders to make informed decisions on pest control (Holmes et al. 2005, Flint 2012, Dhang 2014). For ornamental plants, a correct identification of injuries caused by a pest should focus on both physiological and aesthetic aspects (Raupp et al. 1992, Schumacher et al. 2006). Relationships among pest infestation, injuries, and marketability of ornamental plants have been used to improve IPM of several pests by (1) estimating economic risks (Poland and McCullough 2006, Fulcher et al. 2012), (2) optimizing surveillance and monitoring strategies (Augustin et al. 2004, Stone and Coops 2004, Wang et al. 2016a), (3) timing chemical and biological control treatments (Klingeman et al. 2001, Opit et al. 2005), and (4) assessing purchase intention of customers (Sadof and Raupp 1987, Yue et al. 2009).

Crapemyrtles, *Lagerstroemia* spp. L. (Myrtales: Lythraceae), are the most popular deciduous flowering trees in the southeastern US (Chappell et al. 2012), with an annual nursery wholesale value of approximately $66 million in 2014 (USDA-NASS 2014). Since the initiation of interspecific breeding between *L. indica* and *L. fauriei* in the early 1980s (Egolf 1981, 1987), many hybrids have been released and more than 130 varieties are currently available with improved ornamental traits, such as growth habit and plant size, flower color, foliage color, and sculptural trunk (Chappell et al. 2012). Crapemyrtles are also valued for their few pest problems and easy management. Varieties with pest resistance have been selected for major pests, including the crapemyrtle aphid, *Sarucallis* (=*Tinocallis*) *kahawaluokalani* (Kirkaldy) (Hemiptera: Aphididae) (Mizell III and Knox 1993, Herbert et al. 2009), the Japanese beetle, *Popillia japonica* Newman (Coleoptera: Scarabaeidae) (Held 2004, Pettis et al. 2004), and flea beetles, *Altica* spp. Geoffroy (Coleoptera: Chrysomelidae) (Pettis et al. 2004, Cabrera et al. 2008). Before the arrival of the crapemyrtle bark scale, *Acanthococcus lagerstroemiae* (Kuwana) (Hemiptera: Eriococcidae), these major pests of crapemyrtle can be easily managed (Knox 2003, Chappell et al. 2012).

Since its first discovery in Texas in 2004, *A. lagerstroemiae* has become a major pest of crapemyrtles (Wang et al. 2016b). Possibly facilitated by nursery trading and transportation, *A.*

lagerstroemiae has infested crapemyrtles in eleven US states and is still spreading (EDDMapS 2017). Injuries caused by this scale have been observed in the native and exotic ranges and included branch dieback, reduced growth, accumulation of black sooty mold (Jiang and Xu 1998, Luo et al. 2000, Ma 2011), and reduced blossom (Merchant 2014). However, due to the complex relationship with other organisms including *S. kahawaluokalani*, the reported symptoms from previous literature have not been experimentally demonstrated to be caused by *A. lagerstroemiae*. By feeding on phloem, *S. kahawaluokalani* is also able to secrete honeydew, and facilitate the occurrence of sooty mold and stress to the plant (Alverson and Allen 1991, 1992).

The purpose of this study was to determine physiological and aesthetic effects of *A. lagerstroemiae* to *Lagerstroemia indica* × *fauriei* var. "Natchez White" in a field study. Specifically, I quantified the impact of *A. lagerstroemiae* on plant growth, leaf greenness, photosynthesis, and plant aesthetic values over a six-month period. Information obtained from this experiment can help determine types of injuries caused by *A. lagerstroemiae* and better design monitoring and IPM strategies against this invasive insect pest.

A4.2 Methods and materials

Physiological and aesthetic effects of *A. lagerstroemiae* to the crapemyrtle, *L. indica* × *fauriei* var. "Natchez White" were quantified in a field experiment conducted at the Louisiana

State University Agricultural Center (LSUAC) Hammond Research Station, Hammond, LA (Lat. 30°32'N, Long. 91°9'W, US Department of Agriculture Hardiness Zone 8b) from April to November 2016. Multi-trunk crapemyrtles in 3.8L pots were provided by a local nursery and separated into individual plants each grown in a 11.4L pot in November 2015. Plants were then grown in an outdoor area under similar conditions as nursery container production areas.

A4.2.1 Field preparation.

Twenty-five crapemyrtle plants of similar size and trunk diameter were selected from the abovementioned group and transplanted on April 30, 2016. A Quonset hut, 6m wide (East-to-West) x 12m long (South-to-North) was built in an open field on native soil, of which the topsoil was a Cahaba sandy loam with 57% sand, 30% silt, 13% clay, and 1% organic matter. The top of the hut was covered by a 6 mm-thick clear polyethylene greenhouse film to provide protection from rain. The two long sides and the back side of the hut, up to 1.5m from the ground, were covered with Green-TEK® OptiNet Dual Thrips Control insect screen (Green-TEK, Janesville, WI) and connected with the polyethylene cover to facilitate air ventilation and prevent *A. lagerstroemiae* from escaping. Twenty plants were transplanted into the ground inside the hut, and five plants were transplanted into the open filed 1.5m away from the West side of the hut. All plants were planted at a spacing of 1.2m center-to-center. Therefore, inside the hut, there

were five rows running from South to North with four plants running from East to West within each row. Micro-sprinklers (0.004 m^3/h, Vari-Jet; Antelco Corp., Longwood, FL) were set to deliver 0.002 m^3 of water to each plant at each watering. An irrigation controller (ESP – 4STMe; Rain Bird, Azusa, CA) was programmed to manage watering at three times per week for the first four weeks after planting and then reduced to twice per week for the rest of the experiment.

A4.2.2 Treatments.

The experimental design was a factorial completely randomized design with two factors each at two levels and five replications. Of the twenty plants inside the hut, ten plants were infested with *A. lagerstroemiae* and another ten plants were left un-infested. Within each group, five plants were sprayed with insecticide, and the rest were left without insecticide spray. Therefore, a total of four treatment combinations were included: scale infested – insecticide sprayed (S-I), scale un-infested – insecticide sprayed (U-I), scale infested – insecticide un-sprayed (S-U), and un-infested un-sprayed (U-U), plus 5 plants that were planted outside the hut as an additional control to compare plant growth inside and outside the hut. Plants in the 'infested' group were inoculated with *A. lagerstroemiae* on May 5, 2016. Branches with *A. lagerstroemiae* nymphs were collected from heavily infested crapemyrtles in Shreveport LA. Once brought back to the laboratory, branches were cut into 10-cm-long segments, and predatory

insects were removed under microscope before branches being tied on the stems of crapemyrtles to be infested. The factor of insecticide was included due to the high densities of crapemyrtle aphids on all plants inside the hut by mid-May. Spinosad (Conserve® SC, The Dow Chemical Company, Midland, MI) at 76 liters was applied for a total of six applications during the experiment. CapSil® (Aquatrols®, Paulsboro, NJ) was added at 38 liters as an adjuvant to improve spray coverage. A thick cardboard was used to guard the un-sprayed plants during insecticide applications to avoid accidental spray to these plants.

A4.2.3 Data collection

Plant and insect variables were collected on May 7 (baseline), June 17, August 31, and October 31. Plant variables included plant size, leaf greenness, and aesthetic quality. The plant size was quantified by the size index (SI), which was the average of plant height measured from ground to the highest point, the widest width of plant canopy (width1), and the perpendicular width of the widest width (width2). Therefore, SI = (height + width1 + width2)/3. Size index was measured on four sample dates from May to October, 2016. Leaf greenness was assessed using a handheld reflectance colorimeter SPAD-502 (Minolta, Spectrum Technologies, Plainfield, IL). The SPAD meter determines the relative chlorophyll content in the leaf by measuring the transmittance of the leaf in wave bands 600 to 700 and 400 to 500 nm (Loh et al. 2002). Higher

readings in SPAD meter unit associates with darker green leaves (Loh et al. 2002). For each cardinal direction of each plant, SPAD meter readings of four youngest fully expanded (YFE) leaves were measured and averaged.

Photosynthesis rates (net CO_2 assimilation and A_{CO2}) of crapemyrtle plants were measured on six YFE leaves from 10:00 AM to 12:00 AM during June 25 to 27 using a portable photosynthesis system (LI-6400XT, LI-COR®, Lincoln, NE). The CO_2 mole fraction in the reference chamber was maintained at 400 μmol L^{-1}, and illumination inside the 2 x 3 cm leaf sample chamber was provided by a LED light source (6400-02B, LI-COR®, Lincoln, NE) attached to the top of the chamber. Light intensity was set at 1000 μmol photon m^{-2} s^{-1}. Leaf chamber temperature was maintained the same as air temperature by an external fan.

Insect variables included densities of *A. lagerstroemiae* and *S. kahawaluokalani.* Number of gravid *A. lagerstroemiae* females, identified by the number of white ovisacs, was used as scale density. To estimate density of the aphid, one node on each cardinal direction was sampled, and the numbers of nymphs and adults were counted from two leaves in each direction.

Aesthetic quality was subjectively assessed for all plants including (1) plant size on a scale from 0 to 4, (2) foliage greenness on a scale from 0 to 3, and (3) severity of sooty mold on

a scale from 0 to 5 on upper 1/3 or lower 2/3 branches of the plant canopy. Both *S. kahawaluokalani* and *A. lagerstroemiae* secrete honey dew and cause accumulation of sooty mold. However, *S. kahawaluokalani* prefers feeding on young leaves and tender shoots (Alverson and Allen 1992) while *A. lagerstroemiae* only stays on the bark of woody branches (Wang et al. 2016b). Plants used in this study were about 2-year-old with a growth habit as a small shrub, and most of the sooty mold caused by *S. kahawaluokalani* were found on the upper 1/3 of the canopy. Contribution of *A. lagerstroemiae* to the sooty mold severity was more likely found on the lower 2/3 of the canopy. Sooty mold severity was rated by a scaled from 0 to 5, where 0 represented no sooty mold, 1 represented 1% to 20% of total foliage surfaces were covered by sooty mold, 2 represented 21% to 40%, 3 represented 41% to 60%, 4 represented 61 to 80%, and 5 represented 81% or more of foliage surfaces were covered by sooty mold.

In addition to the abovementioned individual quality assessment, plants were also rated for overall visual quality with a scale ranged from 0 to 15 considering management needs: dead plants are rated 0, plants rated 1 to 3 are considered poor quality with severe pest damage and stunted growth, and should be removed from the landscape; plants rated 4 to 6 are considered having below-average quality with moderate pest damage and would need managements such as insecticide or fertilizer applications; plants rated 7 to 9 are considered average quality and may

be benefited from fertilization or insecticide sprays; plants rated 10 to 12 are considered above-average quality and would be benefited from fertilization or insecticide sprays; plants rated 12 to 15 are considered premium quality and need no further input. All aesthetic quality ratings were independently conducted by two researchers, and then pooled for data analysis.

Temperatures and light intensities were recorded by temperature/light data logger (UA-002-08, HOBO® Pendant®, Bourne, MA). Two data loggers were attached at canopy height onto two polypropylene pipes installed inside and outside the hut. Data were recorded every two hours from May 23 to October 26, 2016. Temperatures inside and outside the hut were similar and ranged from 22 to 45°C. However, the maximum light intensity inside the hut was about half of the light intensity outside.

A4.2.4 Statistical analysis

All variables were analyzed as numerical data using PROC MIXED (SAS Version 9.3; SAS Institute, Cary, NC). Because I am more interested in the effects of *A. lagerstroemiae* infestation on plant growth and quality than the effects of the hut, data collected from the twenty plants inside the hut with a treatment structure of 2 levels of scale infestation × 2 levels of insecticide spray were analyzed for all variables. Data from plants outside the hut were present in Results when interested. The SI was analyzed using Repeated Measures with linear terms and

interactions of the two factors with time as fixed effects, and the baseline measurement of SI and its interaction with time as covariates (with formula: *SI ~ scale | insecticide | time + baseline + baseline * time*). The experimental site experienced historical flooding on August 19, 2016. All five plants in the treatment of scale infestation and insecticide unsprayed died (S-U). Therefore, analyses of data collected after the flood were conducted as a one-way involving only three treatments (S-I, U-I, and U-U), instead of factorial. Leaf greenness was analyzed as one-way Repeated Measures (with formula: *SI ~ treatment | time*). Pest densities and all aspects of aesthetic quality were analyzed in factorial analysis of variance (ANOVA) for measurements on June 17, 2016, and one-way ANOVA on August 31 and October 17, 2016. For treatments with significant effects, the *post-hoc* analyses were conducted using Tukey's HSD method at alpha = 0.05, and results were grouped using macro in Saxton (1998).

A4.3. Results

A4.3.1 Pest density

Both *A. lagerstroemiae* and *S. kahawaluokalani* acquired high densities at mid-June, 2016, which were then lowered at late-August and mid-October in 2016. At June 17, 2016, treatments with insecticide spray had pest densities about 2.5 times less than treatments without insecticide for both *A. lagerstroemiae* females (t=2.89, d.f.=8, $P = 0.0201$) and *S.*

kahawaluokalani nymphs ($F_{(1, 156)} = 23.31$, $P < 0.0001$; Table A4.1). Though only with three treatments, similar difference was also found at August 31, 2016 for both *S. kahawaluokalani* nymphs ($F_{(2, 57)} = 8.78$, $P = 0.0005$) and adults ($F_{(2, 57)} = 3.65$, $P = 0.0348$) (Table A4.1). All *A. lagerstroemiae* females, *S. kahawaluokalani* nymphs and adults were in low amount at October 17, 2016 (Table A4.1). Less than one *S. kahawaluokalani* nymph and adult in average were found on *L. indica* × *fauriei* outside the hut.

Table A4.1. Numbers (mean ± SE) of *Sarucallis kahawaluokalani* nymph and adult, and *Acanthococcus lagerstroemiae* female on *Lagerstroemia indica* × *fauriei* var. "Natchez White" counted three times from June to October, 2016. Means labeled with different letters are significantly different at each sample date.

Measured date	Treatment	Aphid nymph	Aphid adult	Scale female
Jun-17-2016	U-I	84 ± 11 b	19 ± 2 a	0
	U-U	232 ± 33 a	25 ± 3 a	0
	S-U	218 ± 31 a	24 ± 3 a	247 ± 48 a
	S-I	124 ± 21 b	20 ± 3 a	93 ± 23 b
Aug-31-2016	U-I	22 ± 3 b	6 ± 1 b	0
	U-U	49 ± 7 a	11 ± 2 a	0
	S-I	22 ± 4 b	7 ± 1 ab	77 ± 26
Oct-17-2016	U-I	9 ± 2 a	2 ± 1 a	0
	U-U	10 ± 2 a	2 ± 1 a	0
	S-I	7 ± 1 a	1 ± 1 a	8 ± 7

A4.3.2 Plant size

Responses of SI to the *A. lagerstroemiae* infestation and insecticide spray varied over time. Though the baseline SI of the four treatments was significantly different ($F_{(1, 17.1)} = 10.63$,

$P = 0.0046$), the interaction between the baseline and time was not significant ($F_{(2, 30.8)} = 0.70$, $P = 0.5020$), indicating the different baseline did not affect the treatment effects. Both scale infestation ($F_{(1, 17.1)} = 32.2$, $P < 0.0001$) and insecticide spray ($F_{(1, 17.1)} = 6.6$, $P = 0.0198$) significantly affected SI for crapemyrtles. All plants in treatments infested with *A. lagerstroemiae* had significantly lower SIs than treatments without *A. lagerstroemiae* (Fig. A4.1). In addition, interaction between the insecticide spray and time had a significant effect to the SI ($F_{(2, 30.8)} = 5.1$, $P = 0.0122$). The SIs in the four treatments inside the research structure were about 2 times lower than the control treatment (Fig. A4.1).

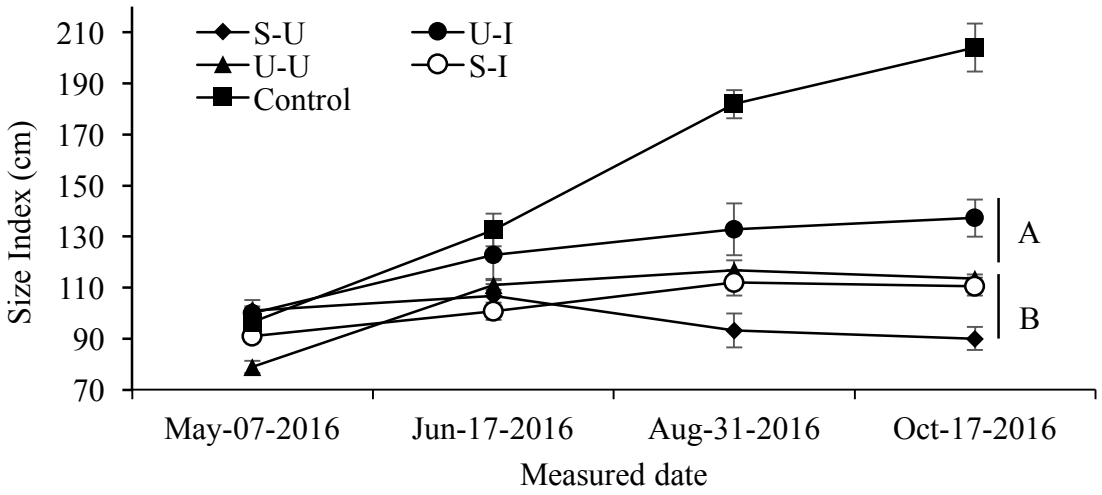

Figure A4.1. Size indices [(height + canopy width 1 + canopy width 2)/3] of *Lagerstroemia indica* × *fauriei* var. "Natchez White" measured from May to October, 2016. Four treatments were included and designed in a factorial structure, including scale infested – insecticide sprayed (S-I), scale infested – insecticide unsprayed (S-U), scale un-infested – insecticide sprayed (U-I), and scale un-infested – insecticide unsprayed (U-U). Control treatment was set outside and excluded from the statistical analysis. Different letters on the right side indicate significant difference between treatments with and without *A. lagerstroemiae* infestation. Bars are standard errors of data collected at each sampling date.

A4.3.3 Leaf greenness

There were significant effects of leaf greenness among treatments ($F_{(2, 201)} = 3.8$, $P = 0.0249$), time ($F_{(2, 201)} = 40.7$, $P = 0.0489$), and the interaction of the treatment and time ($F_{(4, 201)} = 5.2$, $P = 0.0002$). On June 17, 2016, the leaf greenness of plants in two treatments without *A. lagerstroemiae* infestation were both higher than plants with *A. lagerstroemiae* (Fig. A4.2). But no significant difference was found on August 31 and October 17, 2016 (Fig. A4.2). Over time, the leaf greenness of the treatments without *A. lagerstroemiae* infestation reduced to the same level of the treatment with *A. lagerstroemiae* infestation (Fig. A4.2).

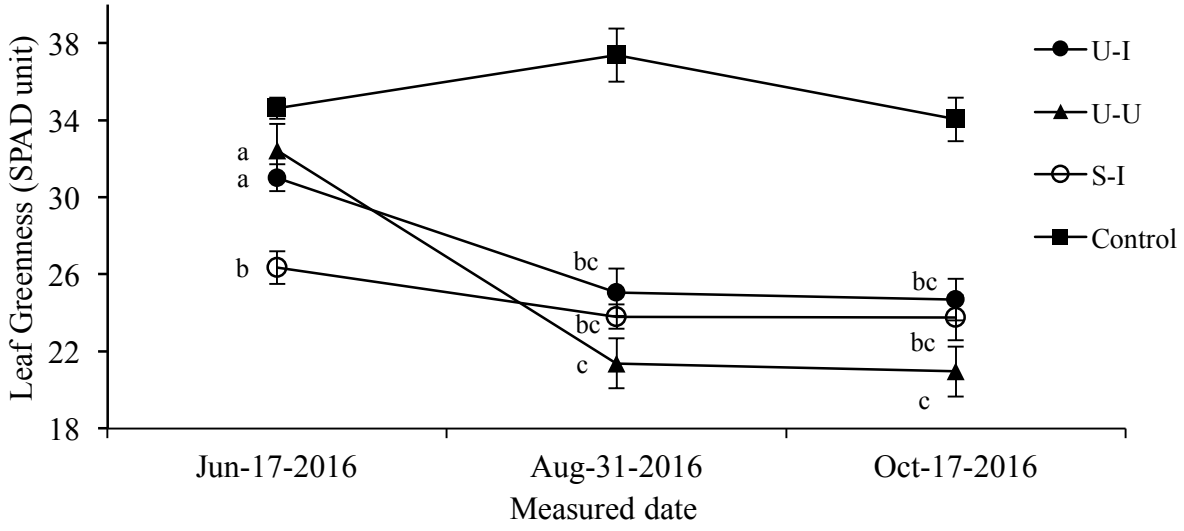

Figure A4.2. Leaf greenness (relative chlorophyll content; SPAD unit) of *Lagerstroemia indica* × *fauriei* var. "Natchez White" measured three times from Jun to Oct, 2016 with three treatments, including scale infested – insecticide sprayed (S-I), scale un-infested – insecticide sprayed (U-I), and scale un-infested – insecticide unsprayed (U-U). Control treatment was set outside and excluded from the statistical analysis. Significant differences for each measurement are indicated by different letters. Bars are standard errors of data collected at each sampling date.

A4.3.4 Photosynthesis

The A_{CO2} of *L. indica* × *fauriei* was only affected by the factor of *A. lagerstroemiae* infestation ($F_{(1, 104)} = 20.7$; $P < 0.0001$). Plants with *A. lagerstroemiae* (7.2 ± 0.5 μmol CO_2 m^{-2} s^{-1}) had lower A_{CO2} than plants without the scale (9.8 ± 0.6 μmol CO_2 m^{-2} s^{-1}), but all the four treatments inside the research structure had far less A_{CO2} than the control (16.8 ± 0.4 μmol CO_2 m^{-2} s^{-1}).

A4.3.5 Aesthetic values

The aesthetic quality of *L. indica* × *fauriei* was reduced by *A. lagerstroemiae* infestation over time. The plant size ratings assessed in June, August and October were all reduced by the *A. lagerstroemiae* infestation ($F_{(1, 16)} = 4.9$, $P = 0.0416$, $F_{(2, 12)} = 12.9$, $P = 0.001$, $F_{(2, 12)} = 13.9$, $P = 0.0007$, respectively; Fig. A4.3). The foliage greenness of plants infested with *A. lagerstroemiae* was lower than plants without *A. lagerstroemiae* at June and August, 2016 ($F_{(1, 16)} = 11.86$, $P = 0.0033$; $F_{(1, 16)} = 4.9$, $P = 0.0416$), but no difference were found in October, 2016 ($P = 0.1677$) (Fig. A4.3). Infestations of *A. lagerstroemiae* caused more accumulation of sooty mold in June and August ($F_{(1, 16)} = 12.75$, $P = 0.0026$; $F_{(2, 12)} = 20.28$, $P = 0.0001$; Fig. A4.3), but in October, the sooty mold accumulations among treatments were too high to be differentiated (Fig. A4.3). Though the ratings of the overall visual quality for all plants inside the hut were less than 12,

plants with *A. lagerstroemiae* had lower ratings over time ($F_{(1, 16)} = 11.14$, $P = 0.0042$, $F_{(2, 12)} = 13.72$, $P = 0.0008$, $F_{(2, 12)} = 8.26$, $P = 0.0055$, respectively; Fig. A4.3).

A4.4 Discussion

Infestation of *A. lagerstroemiae* adversely impacted growth and aesthetic value of newly planted "Natchez White" crapemyrtles. Plants infested with *A. lagerstroemiae* d were smaller in size, with pale yellow leaves and decreased photosynthesis rates. Aesthetically, plants infested with *A. lagerstroemiae* received lower overall visual quality ratings considering plant size, foliage greenness, and degree of sooty mold accumulation and compared with uninfested control plants. Severe infestation of *A. lagerstroemiae* can significantly stunt plant growth and render plants with unacceptable visual quality.

Plant growth and photosynthetic physiology are often adversely affected by sap-feeding scales (Zvereva et al. 2010). With large amounts of carbohydrates siphoned by *A. lagerstroemiae*, growth of crapemyrtles can simply be affected by carbohydrate depletion (Vranjic and Ash 1997, Smith and Schowalter 2001). Similar plant growth reduction has been demonstrated for other scale pests, for example, *Eriococcus coriaceus* Maskell (Hemiptera: Eriococcidae) (Vranjic and Gullan 1990) and *Toumeyella* sp. (Hemiptera: Coccidae) (Schaffer and Mason 1990). As found with *A. lagerstroemiae*, infestations are often associated with leaf chlorosis which indicates less

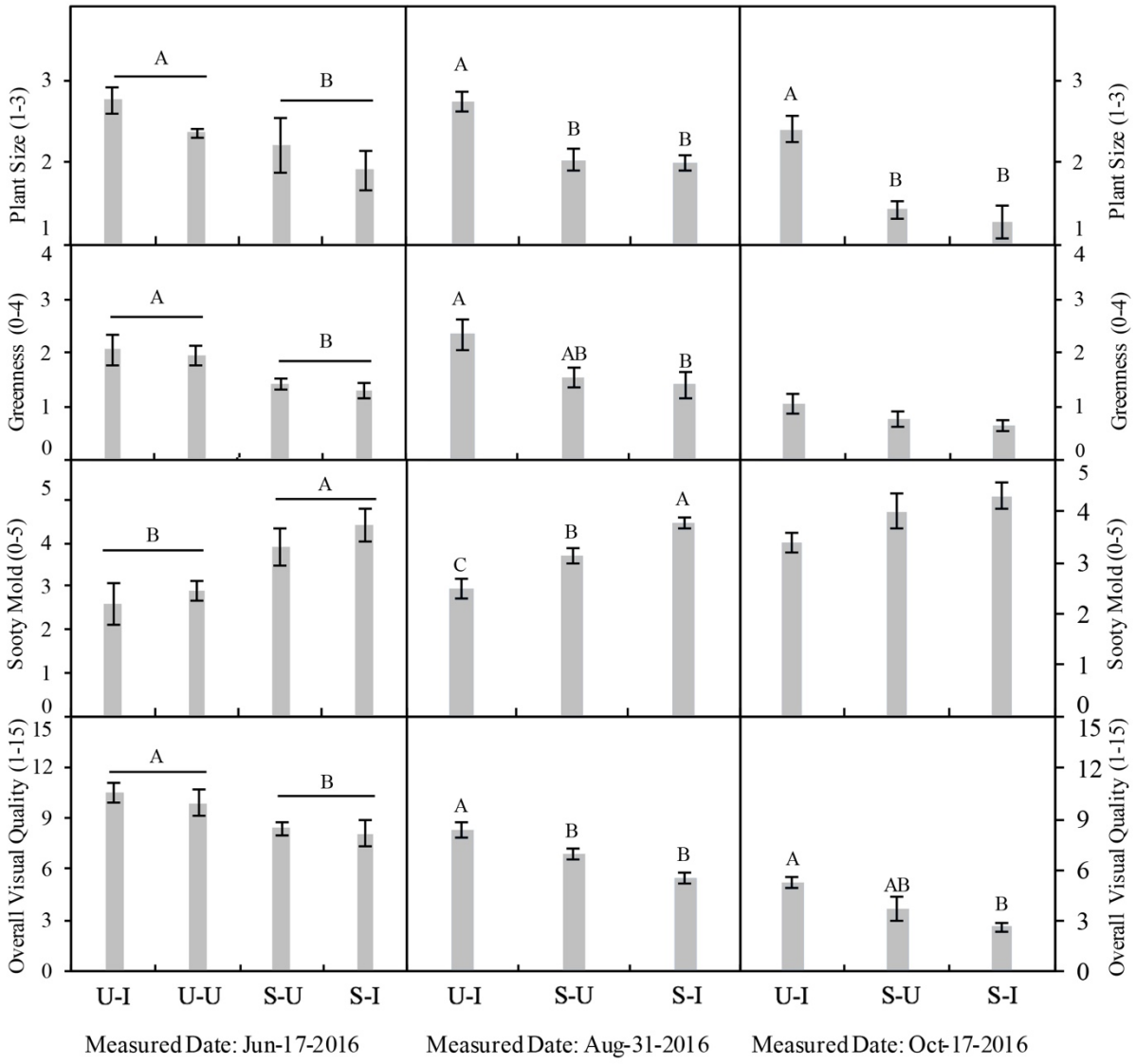

Figure A4.3. Aesthetic quality of *Lagerstroemia indica* × *fauriei* var. "Natchez White" assessed three times from June to October, 2016, including plant size, foliage greenness, sooty mold accumulation, and overall visual quality. Four treatments were included and designed in a factorial structure, including scale infested – insecticide sprayed (S-I), scale infested – insecticide unsprayed (S-U), scale un-infested – insecticide sprayed (U-I), and scale un-infested – insecticide unsprayed (U-U). Control treatment was set outside and excluded from the statistical analysis. Because all plants in treatment S-U died from historical flooding in August, 2016, this treatment was not rated for August and October, 2016. Significant differences within the same measured date are indicated by different letters over the bars. Bars are standard errors for data collected at each sampling date.

leaf chlorophyll content and potentially reduced photosynthesis in these leaves (Kumar and Sharma 2014, Golan et al. 2015). Grown on honeydew secreted by *A. lagerstroemiae* (and also by *S. kahawaluokalani* in this study), black sooty mold is one important factor that reduces photosynthesis by physically blocking leaves from sun light (Santos et al. 2013). Though high infestation of *A. lagerstroemiae* was not reported causing acute mortality to *L. indica* × *fauriei* (Wang et al. 2016b), it can stress plants and decrease their ability to tolerant climatic extremes. The number of *A. lagerstroemiae* females may be a poor indicator of total population density because the generation phenology of this pest is not completely understood yet. At the second and third sampling dates, number of female scales was found much lower than that at the first sampling date, but a high number of nymphs was observed, indicating a change of population composition due to the development of a new generation. Nevertheless, in addition to damages caused by *A. lagerstroemiae*, mortalities observed in the S-U treatment may be partially attributed to the natural infestation of *S. kahawaluokalani* and a less suitable environment (high ambient temperatures and less airflow) inside the research structure.

The infestation of *A. lagerstroemiae* can cause more aesthetic injuries with the presence of *S. kahawaluokalani* in terms of plant size, foliage greenness, sooty mold accumulation and overall visual quality. Differences of the SI and leaf greenness measurements among treatments

are not very large when comparing with healthy plants outside the hut, but the smaller plant size and less foliage greenness were still observed on plants with *A. lagerstroemiae* infestations. This indicates the subjective ratings on the plant growth and leaf greenness can be used to reflect the degree of injuries caused by *A. lagerstroemiae* infestation, while, in this study, I failed to build such connections. Similar connections were most reported for leaf-damage pests (Klingeman et al. 2001, Alatawi et al. 2007), but as far as I know, not for scales feeding on the bark. Sooty mold accumulation has been reported for sap-sucking pests with unpleasing black coating on the plants including scales, for example, *Eriococcus coriaceus* Maskell (Hemiptera: Eriococcidae) (Vranjic and Gullan 1990), and *Eulecanium cerasorum* (Cockerell) (Hemiptera: Coccidae) (Knox et al. 2012). For ornamentals with the bark color as one of their aesthetic traits (Chappell et al. 2012), black sooty mold on the bark caused by *A. lagerstroemiae* may cause additional loss in aesthetic quality of the plant to customers. All ratings of the overall visual quality in June, August, and October were lower than 12, 9, and 6, respectively, which are considered less favorable to customers. Though only rated by two people in this study, a preliminary conclusion can be drawn that *A. lagerstroemiae* infestation can reduce a customer's intention of purchasing *L. indica* × *fauriei* to certain extent.

Monitoring strategies can be designed based on the injuries caused by *A. lagerstroemiae* for a rapid detection of this invasive pest in urban areas. The foliage greenness and black sooty mold accumulation are handful indicators, which can be easily distinguished based on the color changes. Though the size index and plant size rating can be both reduced by *A. lagerstroemiae*, it could be difficult for consumers to notice these changes without a healthy crapemyrtle in vicinity to compare with. Action thresholds and timing of applying various treatments can be developed based on the visual damages described in this study. Early detection of *A. lagerstroemiae* infestation is critical and helps prevent further injuries and potential economic loss if control measures are carried out accordingly.

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VITA

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