Temperature-Dependent Development and Host Range of Crapemyrtle Bark Scale, Acanthococcus lagerstroemiae (Kuwana) (Hemiptera: Eriococcidae)

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Temperature-dependent development and host range 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 invasive pest of crapemyrtles, *Lagerstroemia* spp. L. (Lythraceae) in the southeastern USA. Information about its temperature-dependent development and host range is limited. The objectives of this study were to evaluate the effects of temperature on the immature development of *A. lagerstroemiae*, and to determine plant species suitable for immature development and reproduction. Developmental time and survival of eggs were evaluated at 7 constant temperatures from 17.5 to 32 °C, and of nymphs at 20, 25, and 30 °C. Results suggested that 27.5 °C was the optimum temperature for egg hatching with the shortest time (10 d) and the highest hatching rate (95%). The developmental time for *A. lagerstroemiae* from nymph to prepupa and gravid female was 56 d and 68 d at 30 °C, respectively. Five plant species besides crapemyrtle (*Lagerstroemia indica × fauriei* L.) were able to support the immature development and reproduction of *A. lagerstroemiae* under no-choice conditions, including *Lawsonia inermis* L., *Heimia salicifolia* Link, *Punica granatum* L., *Lythrum alatum* Pursh (all Lythraceae), and *Callicarpa americana* L. (Lamiaceae). At wk 12 from inoculation, the density of gravid females was 482 ± 92 (mean ± standard error) on *L. indica × fauriei*, 200 ± 70 on *C. americana*, and < 150 on other species. Using data from developmental time and host range, integrated pest management (IPM) practitioners can implement preventive strategies for *A. lagerstroemiae*.

Key Words: alternative hosts; developmental time; immature development; temperature effects; no-choice test

Resumen

La escama de la corteza del crespón, *Acanthococcus lagerstroemiae* (Kuwana) (Hemiptera: Eriococcidae), es una plaga invasiva del crespón, *Lagerstroemia* spp. L. (Lythraceae) en el suereste de EE. UU. Hay muy poca información sobre el desarrollo de la escama a diferentes temperaturas o sobre su rango de hospederos. Los objetivos de este estudio fueron evaluar los efectos de la temperatura en el desarrollo de inmaduros de *A. lagerstroemiae*, y determinar las plantas capaces de sostener desarrollo de inmaduros y reproducción. El tiempo de desarrollo y sobrevivencia de huevos fueron evaluados a 7 temperaturas constantes desde 17.5 a 32 °C, y de ninfas a 20, 25, 30 °C. Los resultados sugieren que 27.5 °C es la temperatura óptima para la eclosión de huevos debido a su corto tiempo (10 d) y alta tasa de eclosión (95%). El tiempo de desarrollo de *A. lagerstroemiae* de ninfa a prepupa y a hembra gestante fue 56 d y 68 d a 30 °C, respectivamente. Cinco especies de plantas adicionales al crespón (*Lagerstroemiae indica × fauriei* L.) pudieron sostener el desarrollo de inmaduros y reproducción de *A. lagerstroemiae* bajo condiciones de no-elección, incluyendo *Lawsonia inermis* L., *Heimia salicifolia* Link, *Punica granatum* L., *Lythrum alatum* Pursh (Lythraceae), y *Callicarpa americana* L. (Lamiaceae). En la semana 12 desde la inoculación, la densidad de hembras gestantes fue 482 ± 92 (promedio y error estándar) en *L. indica × fauriei*, 200 ± 70 en *C. americana*, y < 150 en otras especies. Usando datos del tiempo desarrollo y rango de hospederos, trabajadores en (MIP) pueden implementar estrategias de prevención de *A. lagerstroemiae*.

Palabras Clave: hospederos alternativos; desarrollo de inmaduros; efectos de temperatura; test de no-elección; tiempo de desarrollo.

The crapemyrtle bark scale, *Acanthococcus lagerstroemiae* (Kuwana) (Hemiptera: Eriococcidae), is an invasive pest of crapemyrtles, *Lagerstroemia* spp. L. (Lythraceae) (Wang et al. 2016). Native to Asia, this pest was first reported in Richardson, Texas, USA, in 2004 (Merchant et al. 2014), and it is currently present in 11 other states including Louisiana (EDDMapS 2017). *Acanthococcus lagerstroemiae* is a sexual dimorphic with the 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 producing 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 a 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 problems (Knox 2003; Chappell et al. 2012). Current management of *A. lagerstroemiae* 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 bee-attractive plants including crapemyrtle (Riddle & Mizell 2016).

Temperature is one of the most important abiotic factors influencing the survival and development of insects and consequently population growth (Ratte 1984; Amarasekare & Savage 2011; Régnière et al.

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2012). For *A. lagerstroemiae*, most of its phenology comes from field observations both in the native range and the US (Gu et al. 2014). In China, the number of generations of *A. lagerstroemiae* increased latitudinally from 2 to 4 generations from 32 to 26 °N (Jiang & Xu 1998; Luo et al. 2000; He et al. 2008; Ma 2011). Despite the importance for the development of phenological models, there is no information on the immature survival and developmental times of *A. lagerstroemiae* at constant temperatures. By understanding the developmental 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 13 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. (Lythraceae), persimmon, *Diospyros kaki* Thunb. (Ebenaceae), and edible fig, *Ficus carica* L. (Moraceae) (USDA NASS 2012). In addition, polyphagous pests including *A. lagerstroemiae* may expand or shift the host range in the adventive area (Strong 1979). These changes in the host range have been reported for invasive scales in different regions (Hemiptera: Coccoidea) (Cham et al. 2011; Culik et al. 2013; Silva et al. 2017). However, there are no studies in the US of the host range of the population of *A. lagerstroemiae*.

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; and (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 7 families were tested under no-choice conditions. Preventive strategies and improvement of IPM plans for this scale are discussed.

Materials and Methods

INSECT AND PLANT COLONIES

Branches of crapemyrtles infested with different stages of *A. lagerstroemiae* were collected in Shreveport (32.5500°N, 93.7800°W), Louisiana, USA, from Apr 2016 to Jul 2016. Upon arrival at the laboratory, infested branches were immediately placed in a growth chamber at 25 ± 1 °C with a photoperiod of 12:12 h (L:D). Experiments were conducted 1 or 2 d after the field collection to ensure that the insects were alive.

Crapemyrtles, *Lagerstroemiae indica* × *fauriei* 'Natchez White' (Lythraceae) in 1 L pots were purchased from local nurseries in Baton Rouge, Louisiana. Other plant species were purchased from local nurseries or were obtained from the Louisiana State University Agricultural Center (LSUAC) Hammond Research Station, Hammond, Louisiana, with container sizes ranging from 1 to 3.8 L. All plants were placed under full sun, fertilized every 3 mo with 14 g of a controlled release fertilizer (OsmocotePlus®, 15N-9P-12K; The Scotts Miracle-Gro Company, Marysville, Ohio, USA), and watered daily.

TEMPERATURE-DEPENDENT DEVELOPMENT

The immature development and survival of *A. lagerstroemiae* were examined at 7 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, Iowa, USA) set at 12:12 h (L:D) photoperiod. Short branches (< 5 cm) containing gravid females were placed inside Petri dishes (9 cm diam) and monitored for the presence of eggs. Recently deposited eggs (< 1 d old) 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 3 females were pooled at each temperature. A single egg was considered a replicate. All Petri dishes were examined daily under a microscope, and the numbers of crawlers were counted and recorded until all eggs had hatched or died.

For nymphal development, 50 newly hatched crawlers (< 1 d old) were inoculated on a potted crapemyrtle plant, and 4 plants (replicates) were used per temperature (20, 25, and 30 ± 1 °C; photoperiod 12:12 h [L:D]). Each infested plant was kept inside a 49 L plastic wastebasket (20 × 30 × 45 cm; Mainstays™, Kenmore, Virginia, USA) that was modified by removing the plastic material from each of the 4 sides and the bottom, then covering with fine mesh. The fine mesh served to maintain air ventilation and humidity inside the container and prevented the crawlers from escaping. The top of the basket was covered with transparent plastic wrap. Because of the minute size and similar morphology among different *A. lagerstroemiae* instars (Wang et al. 2016), it was difficult to differentiate each molting during the nymphal stages. However, the presence of white waxy coverings of male prepupa and gravid female was considered in this study to be the end of the 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 the presence of white coverings were recorded and marked with a permanent marker on the bark. Because most nymphs cannot finish their development at 20 °C, we harvested all plants at 7 mo. For the nymphs that were left on the plant, we confirmed the mortality under a microscope by leg movement. Developmental time and survival per observed life stage were compared among temperatures using 1-way analysis of the variance (ANOVA) in PROC MIXED (SAS Version 9.3; SAS Institute 2011), and the LSMEANS were compared using Tukey's Honestly Significant Difference (HSD) test at $\alpha = 0.05$.

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 13 plant species were selected based on 3 criteria: (1) plants previously were 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. (Lamiaceae) that was observed infested with *A. lagerstroemiae* in the field (Wang et al. 2016) (Table 1). Plant species reported as hosts in Asia and found in the United States were *Buxus microphylla* Siebold & Zucc. (Buxaceae), *Celtis laevigata* Willdenow (Combretaceae), *Diospyros kaki* Thunb. (Ebenaceae), *Ficus carica* L. (Moraceae), *Punica granatum* L. (Lythraceae), and *Rubus fruticosus* L. 'Kiowa' (Rosaceae). According to the phylogenetic analysis of Lythraceae (Myrtales) (Graham et al. 2005), another 4 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 (8–10 cm in length) to the main stem of test plants for 1 wk. Then each plant was placed inside a cage (61 × 61 × 91 cm) (BioQuip® Compton, California, USA) and allowed to grow under greenhouse conditions.

Table 1. Plant species as host candidates of *Acanthococcus lagerstroemiae* used in no-choice tests.

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

Gravid females, recognized by the white ovisacs found on each plant, were counted and recorded every wk for a total of 14 wk. The experiment was conducted from Apr to Oct 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 4 wk of inoculation, plants were re-infested using the same protocol to confirm the non-host status. The total number of gravid females by wk 12 were compared among host plants using 1-way ANOVA in PROC MIXED (SAS Version 9.3; SAS Institute 2011), and the LSMEANS were separated using Tukey's HSD test at $\alpha = 0.05$.

Results

TEMPERATURE-DEPENDENT DEVELOPMENT

Developmental time differed among temperatures for eggs ($F = 1076.0$; df = 5,159; $P < 0.001$; Fig. 1), male nymphs ($F = 84.9$; df = 2,48; $P < 0.001$), and female nymphs ($F = 350.2$; df = 1,65; $P < 0.001$; Table 2). Mean developmental time for eggs decreased from 36 d at 17.5 °C to 10 d at 27.5 °C, and then increased to 11 d at 30 °C (Fig. 1). Development 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. 1) and nymphs at different temperatures ($F = 7.4$; df = 2,9; $P < 0.01$; Table 2). Lower egg survival (≤ 55%) was recorded for temperatures lower than 25 °C, and most eggs hatched (≥ 90%) when the temperature ranged from 25 to 30 °C (Fig. 1). No eggs hatched at 32 °C. For nymphs, the highest survival rate (30%) was found at 25 °C and the lowest (16%) at 20 °C (Table 2).

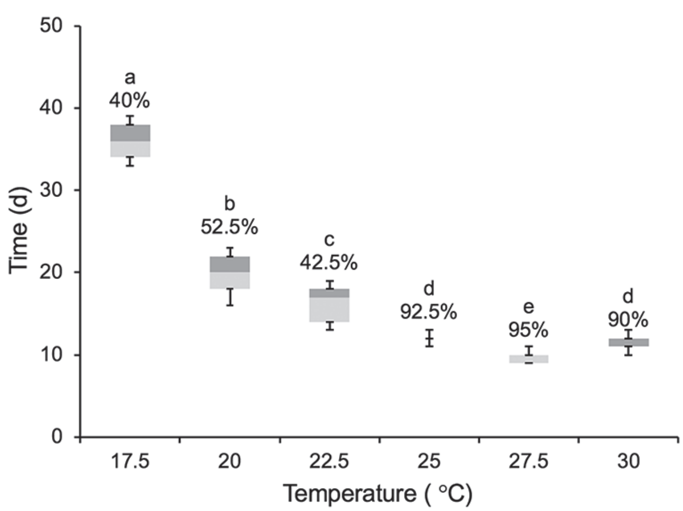

Fig. 1. Box plot of the developmental time in d of *Acanthococcus lagerstroemiae* eggs at 6 constant temperatures. The lines within each box plot corresponds to the median value, the box length to the interquartile range, and the lines emanating from the box (whiskers) extend to the smallest and largest observations. Different letters indicate significantly different developmental times among temperatures at Type I error = 0.05. The number above the box plot is the percent survival of eggs.

HOST RANGE

Results under no-choice conditions indicated that *La. inermis*, *H. salicifolia*, *P. granatum*, *Ly. alatum*, and *C. americana* supported nymphal development and reproduction of *A. lagerstroemiae*. The number of females on all hosts was lower than 100 after the first 6 wk, then increased to different levels (Fig. 2). The number of gravid females at wk 12 also differed among species ($F = 8.5$; df = 5,19; $P < 0.001$; Fig. 2). Crapemyrtle (*L. indica × fauriei*) had the highest number of gravid females (482 ± 92; mean ± SE), followed by *C. americana* (200 ± 70), and lower numbers (< 150) were obtained on the other 4 plants (Fig. 2). Sooty mold accumulated on all these plant species, and the amount of accumulation varied with the density of *A. lagerstroemiae*. Branch dieback was reported for *L. indica × fauriei* and *C. americana*.

Discussion

The developmental time and survival for *A. lagerstroemiae* eggs and nymphs varied among temperatures. The optimum temperature

Table 2. Mean (± SE) developmental time (d) and nymphal survival (%) of *Acanthococcus lagerstroemiae* at 3 constant temperatures. Means within each row followed by different letters are significantly different ($P < 0.05$; Tukey's HSD).

	Temperature (°C)		
Stage/variable	20	25	30
Nymph to male prepupa (d)	154.0 ± 6.6 a	122.0 ± 3.8 b	55.5 ± 5.1 c
Nymph to gravid female (d)	NA	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

NA indicates no gravid female developed successfully.

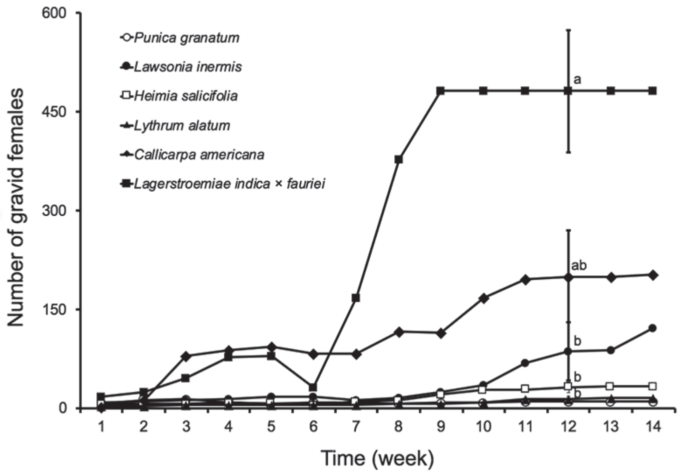

Fig. 2. Number of *Acanthococcus lagerstroemiae* gravid females on 6 host plant species for 14 wk. Different letters represent statistically different means at wk 12 adjusted by Tukey's HSD method at Type I error = 0.05, and bars are standard errors for comparisons at wk 12.

for egg hatching is 27.5 °C, which was determined by the shortest hatching time and highest hatching rate. Constant temperatures below 25 °C resulted in lower egg hatching whereas temperatures above 32 °C led to complete mortality. However, the ovisacs of scales could prevent heat and moisture exchanges and maintain a relatively stable microenvironment inside (Gullan & Kosztarab 1997); thus, air temperature may not represent the best predictor for temperature inside the ovisac. Nymphs of *A. lagerstroemiae* have a much slower growth rate than other scales. The development from crawler to gravid female was 137 d at 25 °C for *A. lagerstroemiae*, but 54 d for *Pseudaulacaspis pentagona* (Targioni-Tozzetti) (Hemiptera: Diaspididae) (Erkiliç & Uygun 1997), 65 d for *Hemiberlesia rapax* (Comstock) (Hemiptera: Diaspididae) (Blank et al. 2000), and 24 d for *Phenacoccus solani* Ferris (Hemiptera: Pseudococcidae) (Nakahira & Arakawa 2006). Nymphal survival of *A. lagerstroemiae* was lower (< 35%) compared to other scales (70–90%), including *P. solani* (Nakahira & Arakawa 2006), *Paracoccus marginatus* (Hemiptera: Pseudococcidae) (Amarasekare et al. 2008), and *Phenacoccus solenopsis* Tinsley (Hemiptera: Pseudococcidae) (Prasad et al. 2012). Another factor that may lead to the slower development and lower survival in this study could be less favorable conditions with artificial light and constant temperatures inside the growth chamber (Colinet et al. 2015).

The developmental time of *A. lagerstroemiae* estimated in this study can be used to understand the phenology of this pest in the field. The time for *A. lagerstroemiae* to complete 1 generation is about 4 mo at 25 °C and 3.5 mo at 30 °C, and could be shorter at relatively warmer temperatures. Nymphs of *A. lagerstroemiae* stayed quiescent and did not reach the reproductive stage at constant 20 °C. According to the National Climatic Data Center (https://www.ncdc.noaa.gov/), the daily average temperatures in subtropical areas such as Louisiana and Texas increase above 20 °C from mid-Apr to Oct and decrease to lower than 20 °C from Oct to mid-Apr, suggesting the potential time of crawler emergence and beginning of overwintering for *A. lagerstroemiae*. Therefore, *A. lagerstroemiae* should have more than 2 generations per yr in Louisiana and Texas. The information obtained from this study could help build models combined with data collected by our collaborators to predict the population dynamics in different locations (Yurk & Powell 2010), as demonstrated for the population growth of *P. solenopsis* (Fand et al. 2014), and for crawler emergence of *Unaspis yanonensis* Kuwana (Hemiptera: Diaspididae) (Kim & Kim 2013).

Acanthococcus lagerstroemiae is polyphagous, and can develop and reproduce on at least 5 species from different genera and families. Four out of the 5 plant species are phylogenetically related to the crapemyrtle (Lythraceae), but the American beautyberry (*C. americana*; Lamiales) is relatively distant to Lythraceae phylogenetically (AGP II 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 & 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* including *Acanthococcus* (= *Eriococcus*) *macedoniensis* Fetyko & Kaydan, *Acanthococcus* (= *Eriococcus*) *melnikinensis* (Kuwana), and *Acanthococcus* (= *Eriococcus*) *onukii* (Kuwana) were collected from several families and orders of plants (Kozar et al. 2013). Furthermore, the host species of *A. lagerstroemiae* found in this study are different from reports in Asia, except for pomegranate (*P. granatum*) (Wang et al. 2016). Considering the potential of a wider host range, additional plant species having been reported to be suitable in the native range, or phylogenetically related to confirmed hosts, should be evaluated.

Prevention should be the primary approach to manage *A. lagerstroemiae* in nurseries growing potential host plants. Host species of *A. lagerstroemiae* found in this study are economically and ecologically important. Pomegranate (*P. granatum*) is a fruit crop produced in 13,309 ha in the US as recorded in 2012 (USDA NASS 2014), with a value of about US $184 million reported in California alone (CDFA 2016). American beautyberry (*C. americana*) (Wiersema & Leon 2016) and winged loosestrife (*L. alatum*) (Clute 1901) are important native plants that also are grown as ornamentals in 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). On all these host species the density of *A. lagerstroemiae* increased over time, with injuries appearing, including accumulation of black sooty mold and branch dieback. If not detected and controlled in time, *A. lagerstroemiae* could exert severe impacts on these plant species. Scouting is recommended for all plants in the host range of *A. lagerstroemiae*, and immediate responses, such as spraying 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-dependent development of *A. lagerstroemiae* can help to time the delivery of control tactics on development of population growth models. Five out of 13 plant species chosen from different genera and families were found as 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.

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