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# Population Dynamics, Surveys for Natural Enemies, and the Developmental Biology of Cotton Seed Bug, *Oxycarenus hyalinipennis* (Costa) (Hemiptera: Oxycarenidae), on Cow-Itch Trees, *Lagunaria patersonia* (Andrews) G. Don (Malvales: Malvaceae)

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ABSTRACT OF THE THESIS

Population Dynamics, Surveys for Natural Enemies, and the Developmental Biology of Cotton Seed Bug, *Oxycarenus hyalinipennis* (Costa) (Hemiptera: Oxycarenidae), on Cow-Itch Trees, *Lagunaria patersonia* (Andrews) G. Don (Malvales: Malvaceae)

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The cotton seed bug (CSB), *Oxycarenus hyalinipennis* (Costa) (Hemiptera: Oxycarenidae), is seed-feeding insect that may become a significant pest of cotton, valued at $135.2 million in California. CSB feeding can reduce the cotton seed weight by up to 15 percent, germination rates by as much as 88 percent, and cotton lint may be stained if CSB are crushed during the ginning process. While CSB is not yet found on cotton in California, CSB is found on ornamental plants such as cow-itch trees, *Lagunaria patersonia* (Andrews) G. Don (Malvales: Malvaceae). *Lagunaria patersonia* is a good host plant because seed pods present year-round provide food and shelter for CSB feeding swarms. However, minimal studies have analyzed the population dynamics of CSB on *L. patersonia*, documented natural enemies, or described the developmental biology of CSB.

Phenological sampling found that *L. patersonia* blooms once per year around May/June and pods reach maturity in early September. Pod sampling determined that CSB populations reached their peak in November and December. Population pyramids

demonstrated that adult CSB made up most of the population and were significantly more abundant that nymphs. Generalized Linear Mixed-effects Models (GLMM) found that for Adult CSB in 2024 pods, month was a significant predictor, pod width, length, and number of seeds were positively associated with number of CSB adults, and temperature was negatively associated. For Adult CSB in 2025 pods, month was a significant predictor, pod width, and number of seeds were positively associated with number of CSB adults, and pod length was negatively associated. Month and number of seeds per pod were significant predictors, the latter of which had a positive effect on CSB nymphs in 2024 and 2025 pods.

Sentinel egg cards placed on *L. patesonia* and *Sphaeralcea ambigua* (A. Gray) (Malvales: Malvaceae) did not detect parasitoids but detected a whirligig mite, *Anystis* sp. (von Heyden) (Trombidiformes: Anystidae), and Argentine ants, *Linepithema humile* (Mayr) (Hymenoptera: Formicidae). Retrieval and hatch rates on *L. patersonia* were significantly greater than on *S. ambigua*. A significant annual effect, where the retrieval rate for eggs in 2025 was consistently lower than the retrieval rate in 2024, was observed on both plants. There was no significant annual effect on hatch rates for either plant. Predator bioassays revealed that long-legged sac-spiders, *Cheiracanthium* sp. (C. L. Koch) (Araneae: Cheiricanthiidae buttonhook leafbeetle jumping spiders, *Sassacus vitis* (Cockerell), (Araneae: Salticidae), green lacewing larvae, *Chrysoperla Comanche* (Banks) (Neuroptera: Chrysopidae), pirate bugs, *Buchananiella continua* (White), (Hemiptera: Anthocoridae), leafhopper assassin bug, *Zelus renardii* (Kolenati), (Hemiptera: Reduviidae), ground sac spider, *Trachelas* sp. (L. Koch) (Araneae:

Trachelidae), and plant bug nymphs, *Phytocoris* sp. (Fallén) (Hemiptera: Miridae) all fed on at least one stage of CSB. However, this feeding provided little to no natural control of CSB.

At a fluctuating 27 °C, CSB reared on *L. patersonia* seeds developed faster, had a higher probability of surviving to adulthood, and lived longer as adults than CSB reared on upland cotton seeds. Fecundity of females on a diet of *L. patersonia* or upland cotton seeds was not significantly different. Furthermore, females on both seeds exhibited two peaks for egg production. On *L. patersonia* seeds, unmated males and females lived significantly longer than their mated counter parts of the same sex and the opposite result was found on upland cotton seeds. Life table analysis predicted that CSB populations on a diet of both seeds would increase. Generation and doubling times were much lower for CSB on a diet of *L. patersonia* seeds.

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## Chapter 1: Introduction

The cotton seed bug (CSB), *Oxycarenus hyalinipennis* (Costa) (Hemiptera: Oxycarenidae), is a small seed-feeding insect that has the potential to become a significant pest of cotton in California. CSB is native to Africa, but it has successfully invaded parts of Asia, the Middle East, Europe, South America, and the Caribbean (Dueñas-López 2022). In 2010, CSB populations were detected in the Florida Keys, which was the first time this pest was documented in the US (Halbert and Dobbs 2010). CSB populations in the Florida Keys were declared successfully eradicated in 2014 by detecting CSB populations early, quarantining potential Malvales host plants, and destroying infested host plants (USDA-APHIS 2024). In April-May 2019, CSB was detected on mallow, *Abutilon palmeri* (Gray) (Malvales: Malvaceae), plants in urban areas of Los Angeles, California (CDFA 2019). Furthermore, the California Department of Food and Agriculture (CDFA) confirmed populations of CSB in Orange, San Diego, and Riverside counties in 2021 (Hoddle and Hoddle 2023; Hoddle 2024). As of January 2026, iNaturalist records have documented CSB in San Bernardino, Santa Barbara, Santa Clara, and Ventura counties (Hoddle et al. 2026). Currently, CSB has not been detected in commercial cotton production areas in California or other major cotton producing states in the US.

CSB has a hemimetabolous life cycle, including an egg stage, five nymphal instars, and an adult stage. Each motile stage of CSB is polyphagous and primarily feeds on plants in the Malvaceae or mallow family within the order Malvales (USDA 2010). Additionally, CSB may also feed on plants in the families Sterculiaceae and Tiliaceae,

both of which are Malvales (Slater and Baranowski 1994). Regardless, it is widely accepted that nymphs and adults require seeds from plants in the Malvales to successfully complete development (CAPS 2023). Nymph and adult CSB feed on almost mature or mature seeds by piercing the seed coat with their needle-like mouthparts, injecting saliva, and then ingesting the liquefied food (Halbert and Dobbs 2010). CSB commonly form "feeding swarms," where nymphs and adults conspicuously aggregate on host plants (USDA 2021). The resulting high numbers of swarming CSB increases the amount of inflicted on seeds of host plants.

In California, the host plant of primary economic concern is cotton, *Gossypium hirsutum* (L.) (Malvales: Malvaceae). CSB feeding can damage cotton seeds in a variety of ways. For example, CSB feeding can reduce the cotton seed weight by up to 15 percent (USDA 2010), and germination rates by as much as 88 percent (Kirkpatrick 1923). Moreover, cotton lint may be stained if any CSB stages are crushed during the ginning process (Henry 1983). Therefore, even small numbers of CSB in cotton crops have the potential to have significant regulatory and economic consequences.

In California, cotton is primarily grown in San Joaquin Valley, including the counties of Fresno, Madera, Merced, Kings, Kern, and Tulare (CDFA 2022). Furthermore, in Southern California, cotton is grown in Riverside and Imperial counties. The economic value of cotton grown in California is difficult to determine because there appears to be no current CDFA or USDA statistics on the cash value of this crop. However, using online production statistics and estimated bale values, the cotton crop grown in California in 2024 was worth approximately $135.2 million (Hoddle et al.

2026). If the current CSB populations infesting urban and wilderness areas are not controlled, it is very likely that CSB will invade commercial cotton fields (CDFA 2022). Such a CSB invasion will certainly result in significant economic losses.

While CSB is not yet found on cotton in California, CSB is found on ornamental plants such as cow-itch trees, *Lagunaria patersonia* (Andrews) G. Don (Malvales: Malvaceae), growing in urban areas. *Lagunaria patersonia* is native to Australia (i.e., Queensland and New South Wales) and some Pacific Islands (i.e., Lord Howe Island, Norfolk Island) (Iamonico and Ridha El Mokni 2020). These trees are evergreen and are commonly planted as ornamental trees in urban landscapes. (Esmaiel et al. 2014, Roy et al. 2025). *Lagunaria patersonia* produce pink flowers from which seed pods, around 2-4 cm in length and diameter, will develop (Iamonico and Ridha El Mokni 2020). *Lagunaria patersonia* seed pods are an excellent host for CSB as seeds provide food and shelter for cryptic feeding swarms (USDA 2021). Because seed pods are present year-round, *L. patersonia* trees are good host plants to use in studies investigating the population dynamics of CSB in California.

In the first research chapter (Chapter 2), I documented the population dynamics of CSB on *L. patersonia*. This work analyzed the sex ratio of adult CSB in seed pods, the total number of nymphal and adult CSB, compared the sex ratios of nymph and adult stages, and created population structure pyramids. These data were analyzed for temporal patterns. Tree phenology data was also collected to assess if climate change affected the phenology (i.e., flower budding, flowering, or pod development stages) of the tree. Effects of time (i.e., week, month, or season), covariates that captured pod characteristics

(i.e., length, width, number of seeds), and covariates related to climate characteristics (i.e., temperature, humidity) were analyzed to determine which factors best predicted patterns of CSB activity. Tree phenology was found to not be affected by climate change and seed abundance best predicted patterns of CSB activity.

The next chapter (Chapter 3) investigated the results of CSB egg cards placed on both *L. patersonia* and desert mallows, *Sphaeralcea ambigua* (A. Gray) (Malvales: Malvaceae). Sentinel egg cards were deployed on CSB-infested to determine the presence of egg parasitoids or predators of CSB eggs. The goal of egg card deployments was to identify potential biological control agents. Recovery, hatch rate, parasitism, and predation rates of eggs on egg cards were recorded and compared between the two host plants. Because egg cards were deployed outside year-round, the effects seasonal and temperature on CSB eggs were analyzed. Collectively, data from egg card deployment studies were used to estimate the percentage of CSB eggs that hatch in the field over time. Additionally, predators found inside *L. patersonia* pods sampled for CSB were collected, identified, and used bioassays to determine if collected predators would consume different instar nymphs and adults. Bioassay data were used to determine the likelihood of predators feeding on CSB in the field and to identify the most and least vulnerable CSB stages to predation. Predators, but not egg parasitoids, were found on sentinel egg cards and all predators from bioassays fed on at least one stage of CSB.

The last chapter (Chapter 4) explored the development and survival of CSB on *L. patersonia* and upland cotton (*G. hirsutum*) seeds at a fluctuating temperature that averaged 27ºC over a 24 h period. These data provide an estimate for total development

time, the percentage of CSB that reach adulthood, and the mean longevity of CSB adults when reared on either seed. Mating pairs of CSB reared on either seed were set up to estimate daily and lifetime fecundity. These data were used to develop a vertical lifetable for CSB reared on either seed at 27$^{o}$C. Developmental data for CSB reared on either seed was compared. On a diet of *L. patersonia* seeds, CSB was found to develop faster, have a greater survivorship to adulthood, and have a similar lifetime fecundity.

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# Chapter 2

## Population dynamics of cotton seed bug, *Oxycarenus hyalinipennis* (Costa) (Hemiptera: Oxycarenidae) on cow-itch trees, *Lagunaria patersonia* (Andrews) G. Don (Malvales: Malvaceae)

## Introduction

Effective pest management relies on a comprehensive understanding of the life cycle of a pest species in conjunction with its phenology (Pedigo and Rice 2006). Furthermore, phenological studies provide valuable insights into time periods when pest populations increase and decrease, allowing growers to implement targeted control measures (i.e. the action threshold) as population densities increase towards levels that may cause economic damage (i.e. economic injury level) (Pedigo and Rice 2006). Knowing when pest populations are at low densities and when they are likely to increase assists in the timing of prevention strategies, allowing for proactive rather than reactive control strategies.

Cotton seed bug (CSB), *Oxycarenus hyalinipennis* (Costa) (Hemiptera: Oxycarenidae), is a seed feeding pest that requires seeds from plants in the Malvales order to successfully complete development (CAPS 2023). One plant species of economic concern is cotton, *Gossypium hirsutum* (L.) (Malvales: Malvaceae). CSB feeding can damage cotton seeds by reducing the cotton seed weight by up to 15 percent (USDA 2010), and germination rates by as much as 88 percent (Kirkpatrick 1923). Moreover, cotton lint may be stained when any CSB stage is crushed during the ginning process such discoloration reduces the economic value of the commodity (Henry 1983). All damage is exacerbated by the CSB's propensity to form "feeding swarms," where nymphs and adults conspicuously cluster together on host plants, especially within cotton bolls (USDA 2021). The resulting high numbers of CSB increase damage levels inflicted on seeds of host plants.

CSB is native to Africa and has successfully invaded Asia, the Middle East, Europe, South America, and the Caribbean (Dueñas-López 2022). The first time this pest was documented in the US was in the Florida Keys in 2010 (Halbert and Dobbs 2010), but CSB was declared successfully eradicated in 2014 (USDA-APHIS 2024). Eradication was achieved by detecting incipient CSB populations when they were small and localized, quarantining potential Malvales host plants, and destroying infested host plants (USDA-APHIS 2024). The second time this pest was confirmed in the US was in 2019 when CSB was detected on Indian mallows, *Abutilon palmeri* (Mill) (Malvales: Malvaceae), in Los Angeles, California (CDFA 2019). Subsequently, the California Department of Food and Agriculture (CDFA) confirmed populations of CSB in Orange, San Diego, and Riverside counties in 2021 (Hoddle and Hoddle 2023; Hoddle 2024). As of January 2026, iNaturalist records have documented CSB in the California counties of San Bernardino, Santa Barbara, Santa Clara, and Ventura (Hoddle et al 2026). In Riverside County, CSB has been detected on cow-itch trees, *Lagunaria patersonia* (Andrews) G. Don (Malvales: Malvaceae) and native California mallows (e.g., *Abutilon* sp.) (Hoddle et al 2026). To our knowledge, CSB has not been detected yet in commercial cotton production areas in California or other major cotton producing states in the US.

In California, upland and pima cotton are primarily grown in San Joaquin Valley, including the counties of Fresno, Kern, Kings, Madera, Merced, and Tulare and is also grown in Riverside and Imperial counties (CDFA 2022). Using online production statistics and estimated bale values, the cotton crop grown in California in 2024 was

worth approximately $135.2 million (Hoddle et al. 2026). If the current CSB populations infesting urban and wilderness areas are not controlled, it is likely that CSB will invade commercial cotton fields in Riverside and may establish in the Central Valley (CDFA 2022). Therefore, proactive research is needed to develop integrated pest management (IPM) programs for CSB before it invades and establishes in cotton production areas.

Many studies on CSB have been completed under controlled laboratory environments on a diet of cotton. One such study examined the reproductive behavior and basic life history of CSB maintained at 26 °C ± 1 °C, 75% relative humitity (RH), and 16 h light/8 h dark cycle (Saveer et al. 2024). Additionally, many controlled laboratory studies have examined the effects of insecticides on CSB. For instance, Zilnik et al. (2025) tested the efficacy of insecticides, such as imidacloprid, on CSB in laboratory under controlled conditions at 26°C, 25% RH, and 16 h light/8 h dark cycle.

Few published studies have examined the population dynamics of CSB in outdoor settings, but none of these studies on commercial cotton fields were completed in the US. For example, in cotton grown in Faisalabad, Pakistan, CSB appeared in the second week of September, with populations peaking in the first week of October, before declining to undetectable levels after the second week of November (Khan & Naveed 2017). Another study in cotton grown in Bahawalpur, Pakistan, examining the population dynamics of CSB from July to November, found that CSB began to appear in the third week of July and peaked in October and November (Iqbal et al. 2018). In Adana, Turkey, adult CSB appeared in mid-June and peaked in early August, while nymphal CSB appeared in late July and peaked in mid to late September (Atakan et. al 2021).

Additionally, there has not been much research on the population dynamics of CSB on other Malvaceae plants. On okra, *Abelmoschus esculentus* (L.) Moench (Malvales: Malvaceae), grown in Assiut Governorate, Egypt, CSB populations peaked twice in the summer: the third week of July and mid-August, suggesting that there were two generations of CSB (El-Rahim et al. 2015). However, data was only collected in the two months of July and August. Furthermore, a one-year study (October 1974 - October 1975) in southern Ghana on *Abutilon mauritianum* (Jacq.) Medik. (Malvales: Malvaceae), found that CSB exhibited a large peak in January and a smaller peak in August-September (Adu-Mensah & Kumar 1977). CSB peaks coincided with dry seasons/minimal rainfall and the appearance of mature seeds on host plants (Adu-Mensah & Kumar 1977).

While their limited publications documenting the population dynamics of CSB on host plants (e.g., *Gossypium* spp., *A. esculentus*, and *A. mauritianum*) under field conditions, only one of these studies was conducted for a full year. There are no studies documenting the population dynamics of CSB on *L. patersonia*. *Lagunaria patersonia* is a Malvaceae host of interest because this species is a good host plant for use in studies investigating the population dynamics of CSB in California. *Lagunaria patersonia* trees grow to a height of 10 m (a large host plant that can support a large CSB population), they flower once per year over the period May – June in California, and subsequently produce seed pods, approximately 2-4 cm in diameter that provide year round shelter for cryptic CSB feeding swarms, each of which contains 9-10 seeds (Iamonico and Ridha El Mokni 2020, USDA 2021).

In addition to location, host plant species, temperature, and daylength, temporal fluctuations of insect population densities depend on host plant phenology (Ekholm et al. 2020). There is limited information on the phenology of *L. patersonia*. For example, the only known reference, Hill (1831) observed that *L. patersonia* flowers in late spring (May) to early summer (June) in San Diego, California. Climate change has been demonstrated to affect flowering time, with estimates that every 1°C increase in temperature may cause flowering to occur 2-3 days earlier (Geissler et al. 2023). This means *L. patersonia* flowering periods may no longer align with Hill's (1831) observations. Thus, documenting the flowering period of *L. patersonia* may provide insight into the effects of climate change on the blooming of this tree in California, which may determine CSB population dynamics.

In this study, we determined the population dynamics and structure of CSB, and flowering and seed pod phenology of *L. patersonia* over a two-year period. These data were used to determine which factors (e.g., time of year, seed pod dimensions, or climate covariates) best predicted patterns of CSB activity.

## Materials and Methods

### *Lagunaria patersonia* phenology

Phenology of eight *L. patersonia* on Citrus Drive adjacent the Genomics building on the University of California, Riverside campus (33° 97′ 15″ N 117° 32′ 65″ W) was monitored biweekly (i.e. Mondays and Thursdays) over the period July 2024 through January 2026. To reduce possible directional bias due to sunlight effects on tree

phenology, growth stages of trees were monitored on north and south sides only (Milosavljević et al. 2018). To standardize sampling area, a 39-cm-diameter steel hoop that enclosed $0.12m^2$ of foliage was placed 2 meters above the ground on the north and south side of *L. patersonia*. Within the circumscribed area, the following data were recorded: presence and number of flower buds, flowers, senescing flowers, mature 2023 pods, mature 2024 pods, mature 2025 pods, developing 2024 pods, and developing 2025 pods. Flower buds had a very light white/pink coloration, flowers were a vibrant pink color with exposed reproductive structures, senescing flowers were wilted with a dark purple color, mature 2023 pods had a black colored shell, mature 2024 pods had a shell that was black on the upper side and yellow on the underside, mature 2025 pods had a yellow shell, developing 2024 pods were green and developed from flowers that bloomed in 2024, and developing 2025 pods were green and developed from flowers that bloomed in 2025 (Fig. 2.1).

**Seed Pod Collection**

On the same days tree phenological stages were recorded, seed pods were collected from only the west side of *L. pateronsia*. This sampling technique was employed to not affect tree phenology data. From July 2024 to June 2025, four pods per tree were collected (mature 2023 pods and developing/mature 2024 pods) for a total of 32 pods per sampling day. Beginning in May 2025, four to six pods per tree were collected, for a total of 40 pods per sampling day, and from August 2025 onward, due to a lower prevalence of pods on most trees, a total of approximately 36 pods were collected per sampling day. This sampling resulted in a total of 5676 pods collected.

To collect pods, Fiskars 1-⅞ inch micro-tip pruning snips (Middleton, WI, USA) were used to cut the pedicels to which pods were attached. This approach was necessary because *L. patersonia* branches are fragile and tugging pods from trees often caused the entire branch to be pulled from the tree. Pods were individually collected into 26.67 cm x 27.94 cm Ziploc® bags (S.C. Johnson & Son, Inc., Bay City, MI, USA) labeled by tree, date and pod ID to ensure that the pod of origin was recorded for CSB and other arthropods that left the pod. The bags were placed in a Kenmore 253.11301101 chest freezer (56.1 cm x 77.0 cm x 85.1 cm, (Gibson/Frigidaire, Greenville, MI, USA) at -18°C to kill all the CSB and other pod inhabiting arthropods, which made counts easier to complete.

**Pod Sorting**

After sufficient freezing time (> 2 hours) had elapsed, bags were removed from the freezer, and pods were examined for CSB and other species inhabiting pods. Pods were removed from bags, placed on a Petri dish, and the bag the pod came from was examined under the microscope to look for any organisms (e.g., CSB, psocids, spiders, etc.) that exited the pod and were trapped inside the bag during freezing. The length and width of the pods from the developing/mature 2024 and 2025 pods were measured using a digital caliper (NEIKO®, Wenzhou, Zhejiang, China). Mature 2023 pods were not measured because many pods from these bags were fragile. The number of seeds in pods from the developing/mature 2024 and 2025 labeled pods were counted and count data were used as a potential predictor of CSB abundance. Seeds in the mature 2023 pods were not measured because many of these pods were not in good condition and due to

probable dehiscence, likely had fewer seeds than they initially had prior to collection. Anvil Single Edge Razor Blades (0.23mm, carbon steel, Exeter, NH, USA) were used to open developing pods (i.e. 2024 and 2025 developing pods). Mature pods (i.e. 2024 and 2025 mature pods) usually had an opening, two pairs of 5-SA tweezers (Rubis Switzerland, Stabio, Ticino, Switzerland) were used to force the pod sutures open so internal contents could be accessed. Once pods were opened, a paintbrush was used to transfer CSB onto Petri dishes labeled by stage (i.e., individual nymphal instars 1 through 5, males, and females). Male and female count data were used to calculate sex ratios for each sampling event. Additionally, all other arthropods (e.g., psocids, spiders, beetles) found in opened pods that were not CSB were stored in labeled 95% ethanol vials for later identification.

**Temperature and Humidity**

A HOBO Pro V2 External Temperature/RH loggers part number U23-001 (Onset Computer, Pocasset, Massachusetts, USA) was placed on tree 5 at the study site (33° 97′ 15″ N 117° 32′ 65″ W) and programmed to record temperature and humidity at 60-min intervals. These data were averaged weekly and used to assess seasonal variations in temperature and humidity where CSB were collected. Because the climate in Riverside California is classified as Mediterranean, seasons were broken down in the following officially recognized ranges: winter (24 Nov. – 20 Mar.), spring (21 Mar.- 12 Jun.), summer (13 Jun. – 7 Sep.), and fall (8 Sep. –23 Nov.) (Kotsias et al. 2021).

**Population Pyramids**

Population pyramids were constructed from CSB counts within the days of the previously mentioned seasons. Because eggs were not observed in the pods, the number of eggs in a season was determined by dividing the number of first instars by the hatch rate (79%) of CSB eggs estimated in Chapter 3. Afterward, the proportion of each stage was determined by dividing the number of CSB counted in a stage by the total number of counted CSB with all seven stages combined.

**Statistical Analyses**

The following statistical analyses were run in IBM SPSS Version (31.0.0.0). One-sample t-tests were used to compare the sex-ratio of males to females observed in pods each month to the expected population sex ratio of 1:1 (male: female; Fisher 1999, Edwards 2000). Independent sample t-tests were used to compare the mean number of adults and nymphs observed in pods each month and the mean number of seeds found in pods that developed over September – January for the 2024 or 2025 pod development periods.

Finally, CSB counts over time were analyzed using a set of generalized linear mixed-effects models (GLMM), with four separate analyses of adult versus nymphal abundance in 2024 versus 2025 pods. Initial versions of each analysis included a fixed effect of time (i.e., week, month, or season), covariates that captured pod characteristics (i.e., length, width, number of seeds), covariates related to climate characteristics (i.e., temperature, humidity), non-Gaussian error distributions to appropriately capture the count nature of the data and potential zero-inflation, and random effects including both

temporal effects and tree identity to account appropriately for autocorrelation in CSB counts stemming from repeated measurements made on each tree. All analyses were conducted in R (version 4.5.2; R Core Team 2025), using the package glmmTMB (version 1.1.9; Brooks et al. 2017) in R (version 4.5.2; R Core Team 2025). An example of how this was done for adult CSB in 2024 pods is described below.

Adult CSB counts in 2024 pods were heavily right skewed, with many pods recording zero or near-zero adults. To identify the appropriate error distribution, six candidate generalized linear mixed-effects models (GLMM) were compared across combinations of error distribution (i.e., negative binomial type-1, negative binomial type 2, Poisson) and zero-inflation specification (zero-inflated or not). Models were ranked using Akaike Information Criterion (AIC) to identify the preferred model structure (Table 2.5). None of the potential covariates in the model (pod width, pod length, number of seeds, temperature, and humidity) were strongly correlated ($|r| > 0.50$) with one another (Table 2.4). In step two, three candidate time terms were compared (i.e., month, week, and season as factors), of which month performed the best based on AIC rankings (Table 2.6). In step three, a random effect that included a random slope of week and random intercept of tree identity (Week|Tree) was compared to a random intercept of tree only and found to perform better based on AIC rankings (Table 2.7). The same steps were completed for nymphs in 2024 pods, nymphs in 2025 pods, and adults in 2025 pods.

## Results

### Tree Phenology

At the UCR study site, *L. patersonia* blooms once per year around May/June, and pods begin to form around late June to early July and reach maturity in early September (Fig. 2.2). The low number of pods that were sampled in 2025 can be attributed to four out of the eight trees blooming and two of these blooming trees only produced about 36 total pods in 2025 (Fig. 2.2). Pods can hang on trees for a minimum of three years before dropping off (Fig. 2.2).

### Number of Seeds Per Pod

Pods that formed in 2024, had on average, nearly nine seeds per pod (8.953 ± 0.173) vs pods that formed in 2025 that had approximately 1.5 seeds per pod (1.458 ± 0.237, Table 2.3). This difference in seed number per pod between 2024 and 2025 was significant ($t = 25.516$, $df = 386.028$, $P < 0.001$). This trend was consistent with each month sampled: September, October, November, December, and January (Table 2.3).

### Number of Total CSB

The abundance of CSB was generally low during the months of July 2024 to September 2024, quickly increased in the month of October, remained high in the months of November and December of 2024, and steadily declined from January 2025 to January of 2026 (Fig. 2.3).

### Sex Ratio

Thirteen out of nineteen months exhibited a sex ratio that was significantly skewed towards males (Table 2.1). Five months had nonsignificant sex ratios suggesting

approximately equal numbers of males and females were recovered from pods. Overall, sex ratio ranged from greater than 2:1 in August 2024 to less than 1:1 in April 2025 (Table 2.1).

**Nymphs and Adults**

Twelve out of the nineteen months had significantly more adults than nymphs (Table 2.3). Two months had significantly more nymphs than adults: October 2024 and November 2024. All other months had statistically similar numbers of nymphs and adults, with two months having more nymphs: July 2024 and September 2024, and three months having more adults: August 2024, December 2024, and June 2025 (Table 2.3).

**Population Pyramids**

Overall, adult CSB made up the majority of the population (55% of insects counted), followed by second instars (8.44%), third instars (8.33%), eggs (7.76%), fifth instars (7.45%), fourth instars (6.79%), and first instars (6.24%, Fig. 2.4). Adults were most abundant, albeit plurality, in Fall 2024 (24.65% of the CSB sampled) and Summer 2024 (46.34%). In all other seasons, adults made up most of the population (> 50%) with the highest percentages in Winter 2025/2026 (83.56%), Fall 2025 (78.37%), and Spring 2025 (64.78%, Fig. 2.4). Fifth instars were the second most common stage in Winter 2024/2025 (8.15%), third instars were the second most common stage in Summer 2024 (12.85%, Fig. 2.4). Second instars were the second most common stage in Spring 2025 (8.14%) and Summer 2025 (7.96%, Fig. 2.4). Eggs were the second most common stage in Fall 2024 (13.36%), Fall 2025 (5.96%), and Winter 2025/2026 (3.26%, Fig. 2.4).

**Characteristics that Affected Adult CSB Numbers in 2024 Pods**

The final model included the fixed effects of month (as a factor), pod width, pod length, number of seeds, temperature, and humidity as covariates, a random slope and intercept of week and tree, respectively, and negative binomial with zero inflation. Type II Wald $X^2$ Tests indicated that month, pod width, pod length, number of seeds, and temperature were significant predictors of adult CSB (Table 2.8). Pod width, length, and number of seeds were positively associated with number of CSB adults, whereas temperature had a negative effect (Table 2.8). Humidity was not a significant predictor of adult CSB densities (Table 2.8).

**Characteristics that Affected Nymph CSB Numbers in 2024 Pods**

The final model for CSB nymphs in 2024 pods included fixed effects of month, pod width, pod length, number of seeds, temperature, and humidity, a random slope and intercept of week and tree, respectively, and negative binomial error with zero inflation. Analyses indicated that month and number of seeds per pod were significant predictors, the latter of which had a positive effect on CSB nymphs (Table 2.8). Pod width, pod length, temperature, and humidity were not significant predictors of CSB nymph densities (Table 2.8).

**Characteristics that Affected Adult CSB Numbers in 2025 Pods**

The final model included the fixed effects of month, pod width, pod length, number of seeds, temperature, and humidity, and the random slope and intercept were week and tree, respectively. Results indicated that the number of seeds per pod, pod length, and pod width were significant predictors (Table 2.8). The number of seeds per

pod and pod width had a positive effect, whereas pod length had a negative effect (Table 2.8). Month, temperature, and humidity were not significant predictors of adult CSB densities (Table 2.8).

**Characteristics that Affected Nymph CSB Numbers in 2025 Pods**

The final model included the fixed effects of month, pod width, pod length, number of seeds, temperature, and humidity, and the intercept of tree. Again, results indicated that month and number of seeds per pod were significant predictors, the latter of which had a positive effect on CSB nymphs (Table 2.8). Pod width, pod length, temperature, and humidity were not significant predictors of CSB nymph densities (Table 2.8).

## Discussion

The purpose of this study was to describe the phenology of *L. patersonia* and population dynamics of CSB infesting *L. patersonia* pods. Bi-weekly count data were used to characterize the population structure of CSB, and factors (e.g., seed number, time of year) affecting adult and nymph CSB abundance on *L. patersonia* in southern California.

Here, we demonstrate that the phenology of *L. patersonia* matches what was reported for San Diego in the literature 195 years ago; *L. patersonia* flowers once per year in late spring to early summer (Hill, 1831). Therefore, climate change has likely had little to no effect on the phenology of *L. patersonia* almost two centuries after this observation was reported. This is likely because irrigated urban trees have been shown to

exhibit greater functional trait plasticity, allowing the trees to cool their leaves in extreme heat environments and combat climate change (Ibsen et al. 2023). The smaller bloom in 2025 vs 2024 may not be caused by alternate bearing, as *L. patersonia* has been reported to consistently bloom each year (Bracewell 2005). While watering differences may affect plant phenology, the watering regime was kept consistent between 2024 and 2025 (Ibsen et al. 2023). *Lagunaria patersonia* can be propagated by seed, producing genetically distinct offspring, or semi-hard wood cuttings, producing genetically identical "clonal" offspring (Ivan and Hill 1989). If trees on the UCR campus were grown from seeds, it is possible that variable genetic makeup may account for the difference in blooming intensity between trees (Wickson 1898). However, the high infestation of CSB in 2024 seed pods may have caused induced effects that caused reduced flowering which resulted in reduced seed availability in 2025 (Ouaarous et al. 2025).

Based on the first year of this study (2024), when all eight *L. patersonia* trees were in full bloom, CSB populations peaked at different months compared to *Gossypium* spp., *A. esculentus*, and *A. mauritianum*, growing in Pakistan and Turkey (Khan & Naveed 2017, Iqbal et al. 2018, Atakan et. al 2021), in Egypt (El-Rahim et al. 2015), and in southern Ghana (Adu-Mensah & Kumar 1977), respectively. In 2024, CSB populations on the UCR campus peaked in November-December. This is later than the CSB population peaks reported in August-October on *Gossypium* spp. reported from Pakistan and Turkey (Khan & Naveed 2017, Iqbal et al. 2018, Atakan et. al 2021), July-August on *A. esculentus* in Egypt (El-Rahim et al. 2015), and August-September on *A. mauritianum*, in southern Ghana but before a second peak reported in January on *A. mauritianum* (Adu-

Mensah & Kumar 1977). These peaks may be related to the seed phenology of the host plant which is driven by season. For example, CSB are commonly found on cotton toward the end of boll formation and boll maturation, which usually occurs in late August-early September in Turkey (Atakan et al. 2021). Dry seed pods of *A. esculentus* appeared in July, corresponding to the population peak of CSB in Egypt (El-Rahim et al. 2015). *Abutilon mauritianum* produced mature seeds in January-February and August-October, with both periods of seed production coinciding with the population peaks of CSB in Southern Ghana (Adu-Mensah & Kumar 1977). For *L. patersonia* growing in Riverside, California in 2024, mature pods with seeds began to form in late September-early October, the period when CSB populations began to increase (Fig. 2.1, Fig. 2.2). Thus, knowing the phenology of seed production for a crop or host plant of interest may assist growers when predicting CSB population outbreaks.

In addition to host plant phenology, the sex ratio of an insect may predict population dynamics. The theoretically ideal sex ratio of males to females should be 1:1 (Fisher 1999, Edwards 2000) because individuals in the rarer sex will have a greater reproductive opportunity than individuals of the more common sex, causing an equilibrium to establish (Wishart et al. 2018). However, this study found that the sex ratio was significantly skewed towards males (Table 2.1). This is not likely due to females biasing the sex ratio during oviposition given results from the laboratory that showed a sex ratio from mating pairs was 1.102 and 0.836 on a diet of *L. patersonia* and upland cotton, respectively (Chapter 4). Furthermore, consistent sampling throughout the year prevented possible protandry (i.e. males mature, emerge, or become reproductively active

earlier than females of the same species) from biasing estimates of sex ratio. Because CSB does not exhibit protandry, and both sexes are equally likely to be sampled in pods because of their cryptic behavior. (Chapter 4, Kirkpatrick 1932). One explanation for the observed male biased sex ratio is that mated male CSB survive longer than mated females. However, based on life table studies in the lab, differences in longevity between males and females are not significant (Chapter 4). Sex ratio bias may result from sub-optimal nutrient conditions for females, as the larger body size (4.3 mm vs 3.8 mm body length of females and males, respectively) and fecundity of females could require more energy, leading to higher female mortality rates and reduced longevity (Kirkpatrick 1923, Teder and Kaasik 2023). Another potential factor skewing sex ratio in pods is male mate-searching behavior. Males may be attracted to pods containing females and possible release of attractants as the male population increases draws in more males. This volatile-induced aggregation response can lead to an apparent male-biased field sex ratio even though the true population sex ratio is 1:1 (Saveer et al. 2026).

CSB nymphs were more common than adults in the months prior (September, October and November 2024) to the highest population density peak of CSB (November and December 2024). This is consistent with previous literature showing a large number of younger individuals can promote population increase as they mature into fertile adults (Singh 2024). As adults become more common than nymphs, beginning in December 2024, the total population of CSB continued to decrease. Again, previous literature reports that a population mainly comprised of older individuals will experience a decline in the

population as adults senesce due to old age (Singh 2024). This is also seen in CSB as older adults have lower fecundity (Chapter 4).

The temporal dynamics of CSB nymphs likely has to do with the seasonality of egg laying by female CSB. Kirkpatrick (1923) reported that CSB breeds and lays eggs when host plants have available seeds (usually over a period of three months) and enter a "quiescent" phase (where no reproduction occurs) when seeds are unavailable. Thus, for this study, it is likely that CSB females laid most of their eggs over September – November 2024, when the number of mature pods with mature seeds increased, leading to increases in numbers of nymphs over that period. Afterward, CSB adults would have entered a "quiescent stage," leading to the decline in the number of nymphs as seed availability declined. This pattern has been observed for other hemipterans, such as bed bugs, *Cimex lectularius* Linnaeus, (Hemiptera: Cimicidae), where field sampling showed that adults usually comprise 5-7% of the population, but can increase to higher proportions of 45-100% when overwintering (Johnson 1941, Schaafsma et al. 2012).

Seed availability has also been shown to influence the population dynamics of another seed feeder, the black-and-red bug, *Lygaeus equestris* L., (Hemiptera: Lygaeidae), where an increase in the number of seeds the previous summer caused the population to increase the following summer (Solbreck and Knape 2025). Therefore, reduced availability of mature seeds in 2025 may explain, in part, why densities of CSB nymphs failed to rebound in Oct. 2025 when populations would be expected to increase (Table 2.3).

Population pyramids showed that the proportion of adult CSB was the smallest during seasons of population growth (i.e. Summer and Fall 2024). This pattern is seen in

other hemipterans, such as the black-and-red bug, where the population grows because of an increase in the number of nymphs during the summer months (Solbreck and Knape 2025). Adult CSB were the most common life stage observed because adults are long lived, and when seeds are scarce or temperatures are low, reproduction declines so that the number of nymphs fails to exceed the number of adults (Chapter 4). This has been observed with other hemipterans, where overwintering populations (i.e. populations where adults do not lay eggs) have a population pyramid dominated by adults (Johnson 1941, Solbreck and Knape 2025).

The number of seeds in a pod was a positive predictor for adult CSB abundance in 2024 and 2025 (Table 2.8). This is likely because adult seed-feeding hemipterans search for hosts with nutrients conducive for survival and reproduction (Ralph 1976). Consequently, adult CSB are more likely to be found in pods with higher numbers of seeds (Panizzi 1997). Survey month was a significant predictor of adult CSB in 2024 (Table 2.8). Because *L. patersonia* only blooms once per year, adult CSB are more likely to enter pods, feed on seeds, and oviposit in mature pods when seeds are fresh (Hill 1831, USDA 2010). Consequently, adult CSB are known to search for alternative hosts when seeds of a current host have been depleted of nutrients (Kirkpatrick 1923). Thus, adult CSB are likely to be leaving older pods as seed quality deteriorates. Females of another species of seed-feeding hemipteran, *Oncopeltus fasciatus* Dallas (Hemiptera: Lygaeidae), are known to oviposit where their offspring will have the greatest chance of surviving, such as areas with a high pod density (Ralph 1976). Thus, in years when pods may have an abundant number of seeds (i.e. 2024 pods), adult CSB may aggregate in larger pods

where their offspring have increased probabilities of surviving (Kirkpatrick 1923; Table 2.3). Conversely, in years when pods have a low number of seeds (i.e. 2025 pods), pod characteristics may not be as important because adult CSB are searching for pods with seeds regardless of quality (Table 2.3). This may explain why in 2024, pod length and pod width were positive predictors, but in 2025, pod length and pod width were negative predictors and positive predictors, respectively (Table 2.8). Pod length and width were weakly to moderately positively correlated with the number of seeds, but were not a significant contributor to adult CSB abundance in 2025 (Table 2.4). High temperatures negatively affect insects by altering respiration, metabolism, and nervous system function, which collectively reduces survivorship rates (Neven 2000). This may explain why higher temperatures over summer predicted lower adult CSB densities whereas lower temperatures over the winter predicted higher adult CSB densities in 2024. Ultimately, seed abundance was a consistent predictor of adult CSB abundance, and fewer seeds in 2025 pods may explain why all other covariates except for pod length were non-significant predictors for CSB densities. Further analysis could examine the effects of flower and pod phenology on the abundance of CSB.

In conclusion, this is the first study to document the phenology, population abundance, and population characteristics of CSB under field conditions in southern California. Population sampling indicated a male-biased sex ratio and adult-biased population structure. CSB populations peaked on *L. patersonia* during the months of October - December due to pod development after flowering was observed over May – June. This time period of CSB abundance was different from that observed for CSB on

other species of Malvaceae hosts, which is likely related to host plant phenology and subsequent timing of seed production. In southern California, the best predictors of nymph and adult CSB abundance on *L. patersonia* are month (i.e. Sep. – Oct.) and the number of seeds in pods. However, the significance of these findings, especially time of year for maximum CSB abundance, cannot be translated to other malvaceous host plant species in California because CSB dynamics are strongly driven by seed production, the periodicity of which varies across species of host plants. Thus, monitoring programs needed to guide control measures for CSB will need to be developed for specific host plant species, as findings on the timings of population peaks from phenological studies on *L. patersonia* are unlikely to translate accurately to non-*L. patersonia* host-plant species, such as cotton.

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**Fig. 2.1** Phenology of *Lagunaria patersonia* on UC Riverside Campus. (A) Phenology sampling on south side of tree (B) Flower buds (C) Flowers (D) Senescing flowers (E) Developing pods (F) Mature 2025 pods (G) Mature 2024 pods (H) Mature 2023.

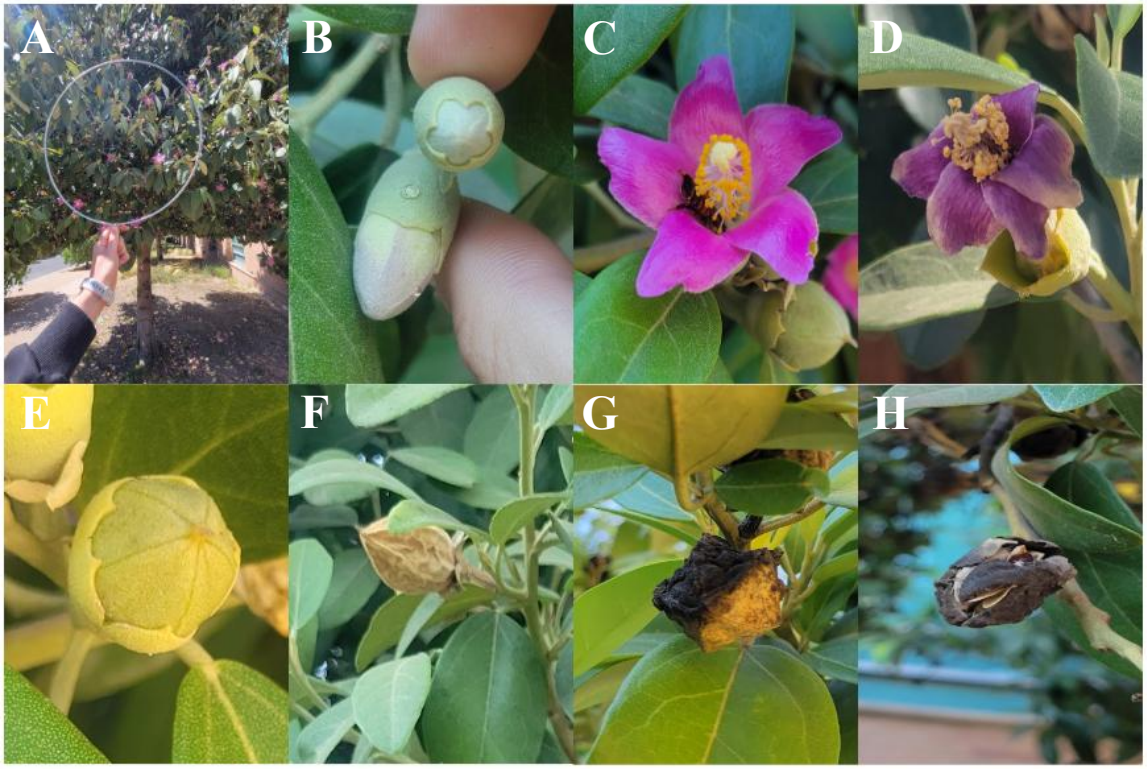


**Fig 2.2** Phenology of *Lagunaria patersonia* on the University of California, Riverside campus over the period July 2024 to January 2026.

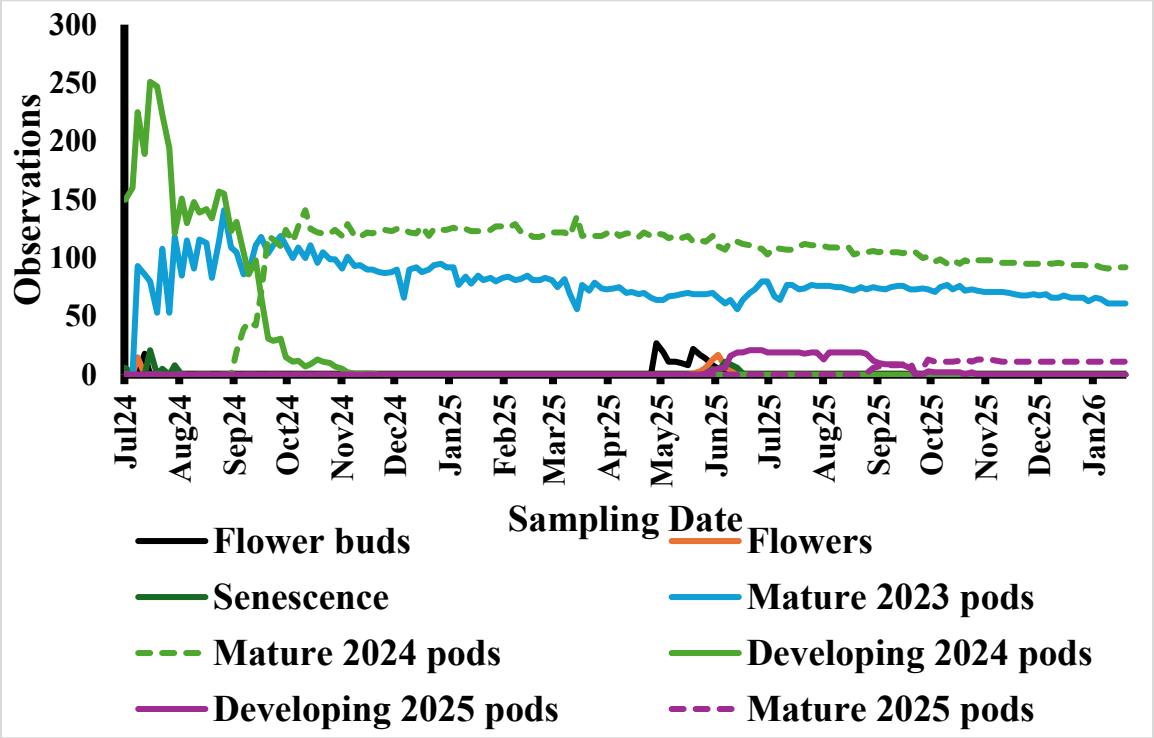


**Fig. 2.3** CSB counted in *Lagunaria patersonia* pods, 2024 and 2025 pods, from July 2024 to January 2026. Number of nymph and adult CSB ± SE.

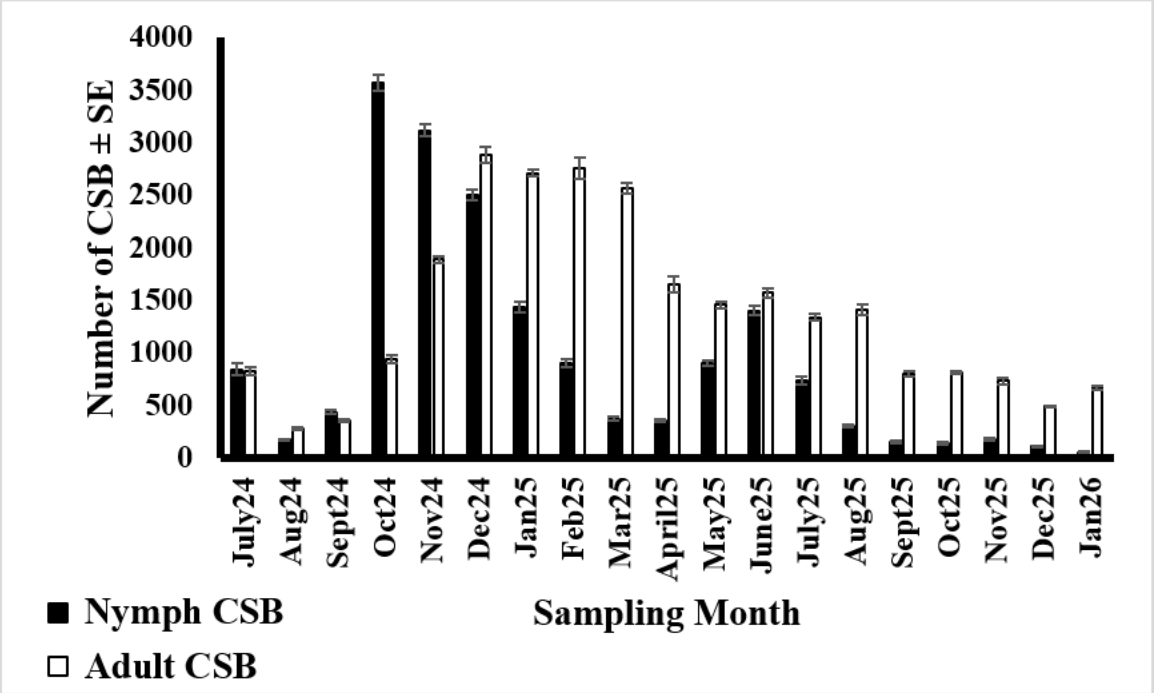
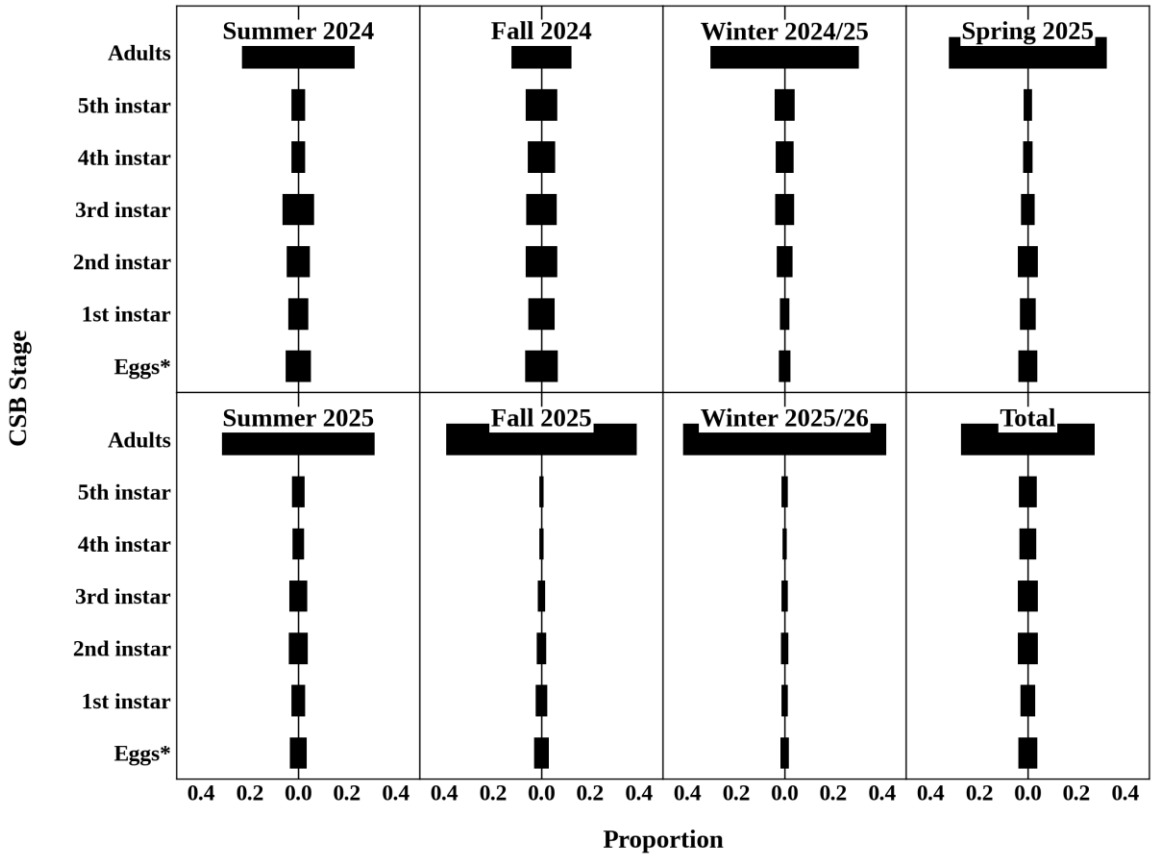


**Fig 2.4** Population pyramids of CSB for each season




*The number of eggs was determined by dividing the number of first instars by the hatch rate (79%) found in Chapter 3.

**Table 2.1** Mean (± SE) sex ratio (M/F) per month for CSB adults collected on *Lagunaria patersonia* compared to the expected sex ratio of 1:1. Overall mean (± SE) includes the sex ratio (M/F) for each sampling date.

| Month | Sex ratio (M/F) | *t* | *df* | *P* |
|---|---|---|---|---|
| July 2024 | 1.549 ± 0.171 | 3.221* | 7 | 0.015 |
| Aug 2024 | 2.044 ± 0.446 | 2.341* | 8 | 0.047 |
| Sept 2024 | 1.795 ± 0.214 | 3.712** | 8 | 0.006 |
| Oct 2024 | 1.738 ± 0.116 | 6.378*** | 8 | <0.001 |
| Nov 2024 | 1.334 ± 0.060 | 5.529*** | 7 | <0.001 |
| Dec 2024 | 1.266 ± 0.032 | 8.395*** | 8 | <0.001 |
| Jan 2025 | 1.215 ± 0.058 | 3.683** | 8 | 0.006 |
| Feb 2025 | 1.114 ± 0.074 | 1.546 | 7 | 0.166 |
| Mar 2025 | 1.146 ± 0.063 | 2.326* | 8 | 0.048 |
| April 2025 | 0.978 ± 0.049 | -0.455 | 7 | 0.663 |
| May 2025 | 1.098 ± 0.092 | 1.063 | 8 | 0.319 |
| June 2025 | 1.186 ± 0.087 | 2.143 | 8 | 0.064 |
| July 2025 | 1.117 ± 0.071 | 1.639 | 8 | 0.140 |
| Aug 2025 | 1.241 ± 0.078 | 3.073* | 7 | 0.018 |
| Sept 2025 | 1.344 ± 0.111 | 3.097* | 8 | 0.015 |
| Oct 2025 | 1.436 ± 0.097 | 1.503 | 7 | 0.177 |
| Nov 2025 | 1.218 ± 0.145 | 3.221* | 8 | 0.015 |
| Dec2025 | 1.394 ± 0.126 | 3.117* | 8 | 0.014 |
| Jan 2026 | 1.511 ± 0.154 | 3.312* | 8 | 0.011 |
| Overall | 1.358 ± 0.038 | 9.230**** | 165 | <0.001 |

Means within columns followed by the same letter are not significantly different ($p > 0.05$) as determined by t-tests. *$p$<0.05, **$p$<.01, ***p<.001.

**Table 2.2** Mean (± SE) number of nymph and adult CSB per month collected on *Lagunaria patersonia*. Overall mean (± SE) includes the number of nymphs and adults for each sampling date.

| Month | Nymphs (Mean± SE) | Adults (Mean ± SE) | *t* | *df* | *P* |
|---|---|---|---|---|---|
| July 2024 | 105.000 ± 29.290 | 103.375 ± 18.442 | 0.047 | 14 | 0.963 |
| Aug 2024 | 19.000 ± 4.830 | 31.444 ± 6.153 | -1.591 | 16 | 0.131 |
| Sept 2024 | 48.444 ± 11.451 | 39.889 ± 5.858 | 0.665 | 16 | 0.515 |
| Oct 2024 | 397.222 ± 39.536 | 104.333 ± 16.607 | 6.830*** | 10.738 | <0.001 |
| Nov 2024 | 389.750 ± 31.238 | 236.250 ± 16.500 | 4.345*** | 14 | <0.001 |
| Dec 2024 | 278.556 ± 26.618 | 320.222 ± 40.587 | -0.858 | 16 | 0.403 |
| Jan 2025 | 159.333 ± 26.348 | 301.222 ± 15.957 | -4.606*** | 16 | <0.001 |
| Feb 2025 | 112.750 ± 19.575 | 344.750 ± 52.659 | -4.130** | 8.898 | 0.003 |
| Mar 2025 | 41.222 ± 10.460 | 284.556 ± 26.295 | -8.599*** | 10.470 | <0.001 |
| April 2025 | 44.250 ± 5.640 | 206.375 ± 41.172 | -3.901** | 7.263 | 0.005 |
| May 2025 | 100.111 ± 15.205 | 161.778 ± 16.676 | -2.732* | 16 | 0.015 |
| June 2025 | 155.556 ± 23.054 | 174.556 ± 21.777 | -0.599 | 16 | 0.557 |
| July 2025 | 62.000 ± 18.480 | 148.667 ± 17.329 | -2.632* | 16 | 0.019 |
| Aug 2025 | 37.375 ± 6.027 | 175.750 ± 27.461 | -4.922** | 7.673 | 0.001 |
| Sept 2025 | 17.000 ± 5.951 | 88.778 ± 14.102 | -4.690*** | 10.762 | <0.001 |
| Oct 2025 | 15.667 ± 5.104 | 89.889 ± 6.917 | -8.688*** | 16 | <0.001 |
| Nov 2025 | 22.000 ± 6.132 | 91.500 ± 16.606 | -3.926** | 14 | 0.002 |
| Dec2025 | 12.444 ± 3.367 | 54.222 ± 3.829 | -8.194*** | 16 | <0.001 |
| Jan 2026 | 5.889 ± 2.098 | 74.111 ± 7.426 | -8.840*** | 16 | <0.001 |
| Overall | 107.158 ± 10.082 | 158.346 ± 8.957 | -3.796*** | 328 | <0.001 |

Means within columns followed by the same letter are not significantly different ($p > 0.05$) as determined by t-tests. *$p$<0.05, **$p$<.01, ***p<.001.

**Table 2.3** Mean (± SE) number of seeds per pod per month collected on *Lagunaria patersonia*. Overall mean (± SE) includes the number of seeds per pod for each pod sampled.

| | 2024 | 2025 | | | |
|---|---|---|---|---|---|
| Month | Seeds per pod (Mean± SE) | Seeds per pod (Mean ± SE) | *t* | *df* | *P* |
| Sept 2024 | 9.285 ± 0.402[a] | 0.865 ± 0.338[b] | 16.020*** | 139.621 | <0.001 |
| Oct 2024 | 8.826 ± 0.373[a] | 1.833 ± 0.558[b] | 10.413*** | 69.786 | <0.001 |
| Nov 2024 | 8.656 ± 0.401[a] | 1.875 ± 0.763[b] | 7.628*** | 158 | <0.001 |
| Dec 2024 | 9.160 ± 0.374[a] | 1.864 ± 0.545[b] | 11.300*** | 71.904 | <0.001 |
| Jan 2025 | 8.806 ± 0.388[a] | 1.083 ± 0.338[b] | 13.426*** | 100.9201 | <0.001 |
| Overall | 8.953 ± 0.173[a] | 1.458 ± 0.237[b] | 25.516*** | 386.028 | <0.001 |

Means within columns followed by the same letter are not significantly different ($p > 0.05$) as determined by t-tests. *$p$<0.05, **$p$<.01, ***p<.001.

**Table 2.4** Correlation coefficients between variables (pod diameter, pods width, number of seeds, temperature, and humidity) used in GLMM models

| Variables | Correlation Coefficient (*R*) |
|---|---|
| Pod width vs pod diameter | 0.372 |
| Pod width vs number of seeds | 0.370 |
| Pod length vs number of seeds | 0.375 |
| Temperature vs Humidity | -0.035 |

**Table 2.5** AIC comparison of models with candidate error distributions (NB1, NB2, and Poisson) and zero-inflation specifications for adult CSB sampled in 2024 pods, nymphs sampled in 2024 pods, adults sampled in 2025 pods, and nymphs sampled in 2025 pods. All models include the following fixed effects: month, pod width, pod length, number of seeds, humidity, and temperature, and the random intercept: tree.

| Adults in 2024 pods | | | |
|---|---|---|---|
| *Model* | *df* | *AIC* | *ΔAIC* |
| nbinom1, zi=~1 | 27 | 13026.57 | 0.0 |
| nbinom2, zi=~1 | 27 | 13344.68 | 318.11 |
| poisson, zi=~1 | 26 | 24833.54 | 11806.97 |
| nbinom1, no zi | 26 | 13037.66 | 11.09 |
| nbinom2, no zi | 26 | 13349.04 | 322.47 |
| poisson, no zi | 25 | 32437.90 | 19411.33 |
| Nymphs in 2024 pods | | | |
| nbinom1, zi=~1 | 27 | 9904.266 | 0.0 |
| nbinom2, zi=~1 | 27 | 9965.495 | 61.229 |
| poisson, zi=~1 | 26 | 22211.702 | 12307.436 |
| nbinom1, no zi | 26 | 9976.185 | 71.919 |
| nbinom2, no zi | 26 | 9963.495 | 59.229 |
| poisson, no zi | 25 | 29406.078 | 19501.812 |
| Adults in 2025 pods | | | |
| nbinom1, zi=~1 | 10 | 608.308 | 0.0 |
| nbinom2, zi=~1 | 10 | 611.912 | 3.604 |
| poisson, zi=~1 | 9 | 757.275 | 148.967 |
| nbinom1, no zi | 9 | 619.265 | 10.957 |
| nbinom2, no zi | 9 | 622.550 | 14.242 |
| poisson, no zi | 8 | 1795.060 | 1186.752 |
| Nymphs in 2025 pods | | | |
| nbinom1, zi=~1 | 10 | 431.828 | 14.182 |
| nbinom2, zi=~1 | 10 | 417.646 | 0.0 |
| poisson, zi=~1 | 9 | 460.445 | 42.617 |
| nbinom1, no zi | 9 | 426.109 | 8.281 |
| nbinom2, no zi | 9 | 423.003 | 5.175 |
| poisson, no zi | 8 | 947.463 | 529.635 |

ΔAIC is the difference in AIC compared to the model used, for which lower values indicate a preferred model structure

**Table 2.6** AIC comparison of models with candidate time scales month, week, and season. All models include the following fixed effects: pod width, pod length, number of seeds, humidity, and temperature, and random intercept: tree.

| Adults in 2024 pods | | | |
|---|---|---|---|
| *Model* | *df* | *AIC* | *ΔAIC* |
| Month | 27 | 13026.57 | 0.0 |
| Week | 89 | 13052.76 | 26.19 |
| Season | 15 | 13072.74 | 46.17 |
| Nymphs in 2024 pods | | | |
| Month | 27 | 9904.266 | 0.0 |
| Week | 89 | 9919.904 | 15.638 |
| Season | 15 | 10180.277 | 276.011 |
| Adults in 2024 pods | | | |
| Month | 10 | 608.308 | 0.0 |
| Week | 10 | 608.301 | -0.007 |
| Season | 11 | 608.337 | 0.029 |
| Nymphs in 2025 pods | | | |
| Month | 10 | 417.646 | 0.0 |
| Week | 10 | 418.598 | 0.952 |
| Season | 11 | 416.269 | -1.337 |

ΔAIC is the difference in AIC between models

**Table 2.7** $\chi^2$ tests comparing the model fit with a random slope (Week|Tree) or without a random slope (1|Tree). All models include the following fixed effects: month, pod width, pod length, number of seeds, humidity, and temperature, and the random intercept: tree.

| Adults in 2024 pods | | | |
|---|---|---|---|
| *Model* | $X^2$ | *df* | *P* |
| (1\|Tree) | - | - | - |
| (Week\|Tree) | 38.329*** | 2 | <0.001 |
| Nymphs in 2024 pods | | | |
| (1\|Tree) | - | - | - |
| (Week\|Tree) | 32.477*** | 2 | <0.001 |
| Adults in 2025 pods | | | |
| (1\|Tree) | - | - | - |
| (Week\|Tree) | 16.281*** | 2 | <0.001 |
| Nymphs in 2025 pods | | | |
| (1\|Tree) | - | - | - |
| (Week\|Tree) | 0.328 | 2 | 0.849 |

Means within each cell with * are significantly different ($p > 0.05$) as determined by $X^2$ tests. *p<0.05, **p<.01, ***p<.001.

**Table 2.8** Estimated fixed effects from the final GLMM for adult CSB sampled in 2024 pods, nymph CSB sampled in 2024 pods, adult CSB sampled in 2025 pods, and nymph CSB sampled in 2025 pods. Estimates are shown on the log scale.

| Predictor | *β* | *SE* | $\chi^2$ | *df* | *P* |
|---|---|---|---|---|---|
| **Adults in 2024 pods** | | | | | |
| Month | -[1] | - | 381.971*** | 18 | <0.001 |
| Pod width (mm) | 0.047 | 0.010 | 23.267*** | 1 | <0.001 |
| Pod length (mm) | 0.016 | 0.008 | 3.976* | 1 | 0.046 |
| No. seeds | 0.031 | 0.006 | 27.472*** | 1 | <0.001 |
| Humidity (%) | - | - | 1.953 | 1 | 0.162 |
| Temperature (°C) | -0.040 | 0.012 | 10.429** | 1 | 0.001 |
| **Nymphs in 2024 pods** | | | | | |
| Month | - | - | 706.930*** | 18 | <0.001 |
| Pod width (mm) | - | - | 2.516 | 1 | 0.113 |
| Pod length (mm) | - | - | 2.226 | 1 | 0.136 |
| No. seeds | 0.056 | 0.007 | 62.558*** | 1 | <0.001 |
| Humidity (%) | - | - | 2.053 | 1 | 0.152 |
| Temperature (°C) | - | - | 0.010 | 1 | 0.921 |
| **Adults in 2025 pods** | | | | | |
| Month | - | - | 1.199 | 18 | 0.273 |
| Pod width (mm) | 0.212 | 0.104 | 4.194* | 1 | 0.041 |
| Pod length (mm) | -0.123 | 0.060 | 4.258* | 1 | 0.039 |
| No. seeds | 0.209 | 0.052 | 16.156*** | 1 | <0.001 |
| Humidity (%) | - | - | 0.004 | 1 | 0.952 |
| Temperature (°C) | - | - | 0.200 | 1 | 0.655 |
| **Nymphs in 2025 pods** | | | | | |
| Month | - | - | 7.291** | 18 | 0.007 |
| Pod width (mm) | - | - | 0.120 | 1 | 0.729 |
| Pod length (mm) | - | - | 0.626 | 1 | 0.429 |
| No. seeds | 0.171 | 0.057 | 8.895** | 1 | 0.003 |
| Humidity (%) | - | - | 0.407 | 1 | 0.523 |
| Temperature (°C) | - | - | 0.036 | 1 | 0.850 |

[1]β is the log-scale coefficient, only shown for significant covariates, Standard Error (SE), chi-square *P* reported from Type II Wald Test. *$p$<0.05, **$p$<.01, ***p<.001.

## Chapter 3:

## Surveys in California for natural enemies of cotton seed bug, *Oxycarenus hyalinipennis* (Costa) (Hemiptera: Oxycarenidae)

## Introduction

Before developing a biological control program for an invasive insect pest, the first step is to determine whether resident natural enemies provide some level of population suppression in the introduced range. If existing natural enemies provide adequate control, sometimes referred to as "natural control" (DeBach 1964), then strategies to conserve or augment natural enemy populations may be implemented (Hogg et al. 2022). This is similar to the concept of "biotic resistance," where existing species in a community limit the spread of an invasive species, through different mechanisms such as competitive exclusion (Byun and Lee 2017).

Sometimes, invasive species spread rapidly because they are no longer exposed to natural enemy species that regulated their population densities in their native range and existing natural enemies do not provide adequate levels of pest control, sometimes referred to as the "enemy escape hypothesis" (Heger et al. 2024). In this case, exotic agents may be imported from the pest's native range and used in a classical biological control program targeting the pest of concern (Hogg et al. 2022). While host-specific parasitoids are often preferred for use in classical biological control programs, naturally-occurring generalist predators have, in the absence of specialist natural enemies, been shown to significantly reduce pest populations in some instances, such as when predator killing efficiency or when predator numbers early in the pest season are high (Chang and Kareiva 1999, Symondson et al. 2002). However, these generalist predators may be limited in the prey they consume based on their own body size. The handling-size hypothesis suggests that larger predators have a larger diet breadth because big predators

can feed on both small and large prey relative their body size, whereas smaller predators can only feed on small prey relative to their body size (Michalko and Pekár 2025).

Cotton seed bug (CSB), *Oxycarenus hyalinipennis* (Costa) (Hemiptera: Oxycarenidae), native to Africa and a specialist herbivore on seeds of plants in the Malvales order (USDA 2010; CAPS 2023); it has successfully invaded Asia, the Middle East, Europe, South America, and the Caribbean (Dueñas-López 2022). CSB was first documented in the US in 2010 in the Florida Keys (Halbert and Dobbs 2010), but was successfully eradicated in 2014 (USDA-APHIS 2024). In 2019, CSB was detected in Los Angeles, California (CDFA 2019). By 2021, the California Department of Food and Agriculture (CDFA) confirmed that CSB spread to Orange, San Diego, and Riverside Counties in California (Hoddle and Hoddle 2023, Hoddle 2024). Since January 2026, iNaturalist posts have documented CSB in San Bernardino, Santa Barbara, Santa Clara, and Ventura counties (Hoddle et al 2026). In Riverside County, CSB has been detected on the introduced ornamental cow-itch tree, *Lagunaria patersonia* (Andrews) G. Don (Malvales: Malvaceae), and the California natives' Indian mallows, *Abutilon* sp. (Mill) (Malvales: Malvaceae), desert mallows, *Sphaeralcea ambigua* (A. Gray) (Malvales: Malvaceae), and island mallows, *Malva rosa* (Kellogg) M.F. Ray (Malvales: Malvaceae) (Hoddle et al 2026).

CSB is an economic pest due to the damage it can inflict on cotton, *Gossypium hirsutum* (L.) (Malvales: Malvaceae). CSB feeding can damage cotton seeds by reducing cotton seed weight by up to 15 percent (USDA 2010), and germination rates by as much as 88 percent (Kirkpatrick 1923). Moreover, cotton lint may be stained if any CSB stage

are crushed during the ginning process (Henry 1983). At time of writing, CSB has not been detected in commercial cotton production areas in California or other major cotton producing states in the US.

In California, upland and pima cotton are primarily grown in San Joaquin Valley, including the counties of Fresno, Kern, Kings, Madera, Merced, and Tulare and is also grown in Riverside and Imperial counties (CDFA 2022). Using online production statistics and estimated bale values, the cotton crop grown in California in 2024 was worth approximately $135.2 million (Hoddle et al. 2026). If the current CSB populations infesting wilderness and urban areas are not adequately controlled, it is likely that CSB will invade commercial cotton fields in eastern Riverside County and elsewhere in California (CDFA 2022). Therefore, the detection and identification of naturally occurring predator and/or parasitoid species of CSB may provide opportunities for biological control of this pest. However, there are no documented parasitoids of CSB and only three documented predators attacking this pest: green lacewing larvae, *Chrysoperla* sp. (Neuroptera: Chrysopidae), the garden cross spider, *Argiope pulchella* (Thorell) (Araneae: Araneidae), and the tropical tent web spider, *Cyrtophora cicatrosa* (Stoliczka) (Araneae: Araneidae) (Sabarinathan et al. 2007). Two of these putative natural enemies, the garden cross spider and the tropical tent web spider, are only found in Southern and Southeast Asia (Dyal 1935, Sen et al 2015), in the CSB's introduced range, while species of *Chrysoperla* are known from California and other parts of the US (Henry et al. 2002). Thus, as part of developing emerging proactive management programs for CSB before detection in commercial cotton production areas, it would be beneficial to survey for and

identify natural enemies, especially egg parasitoids and predators, associated with CSB in California.

There are multiple ways to survey parasitoids and predators of pests. Sentinel egg cards are a method used to identify the presence of predators and/or parasitoids that attack eggs of a pest of interest (Lövei and Ferrante 2017). Sentinel egg cards are deployed in areas infested with the pest of concern, then collected from the field after a set period of time; in the laboratory, emergence of egg parasitoids, numbers of missing and predated eggs, and hatched hosts are recorded (Lövei and Ferrante 2017). Time of field deployment is optimized so it is long enough to maximize opportunities to be discovered by naturally occurring parasitoids and predators, but short enough that deployed eggs do not complete their development and hatch before retrieval (Hougardy et al. 2024). Another method to identify natural enemies is the enclosure method. In this instance, predators found in association with the pest of interest are captured, identified, and contained inside study arenas with different life stages (e.g., eggs, nymphs, or adults) of the pest. Visual observations are made to determine predation rates and life stage preferences for consumption by predators (Birkhofer et al. 2017). In addition, we can also determine predation potential, for example, are no-choice tests, where a predator is placed in a study arena with a potential prey item and no other food choices, and predation events are recorded (Withers and Mansfield 2005). Thus, no-choice tests can be used to determine the propensity of generalist predators to feed on CSB eggs, small and large nymphs, and adults when there are no competing food sources.

The abundance and species composition of natural enemies from these surveys may be affected by seasonality. While specialists, such as parasitoids, are commonly synchronized with their hosts (Saska and Honek 2008), generalist natural enemies, like predators, are less tied to a specific prey species, and thus possibly, exhibit seasonal patterns that differ from prey population phenology (Southwood and Comins 1976, Hassell and May 1986). For example, generalist predator populations usually build slowly and may increase as pest prey decline towards the end of the season or, instead, predators may feed on a preferred prey species before switching to the pest species of interest (Settle et al. 1996). Furthermore, temperature variations can alter the intensity of predator-prey interactions which can further decouple generalist predator populations from prey (Laws and Joern 2013).

Ultimately, the purpose of this study is to survey for and identify natural enemies, especially egg parasitoids and predators, associated with CSB in California in search for potential biological control agents. To determine the species composition and abundance of natural enemies associated with CSB, twice weekly surveys were undertaken on *L. patersonia* trees heavily infested with CSB on the University of California Riverside Campus (UCR, Riverside, CA, USA). Surveys used sentinel egg cards deployed in CSB-infested *L. patersonia* trees and *S. ambigua* shrubs to monitor for egg parasitoids, and predator species found inside *L. patersonia* seed pods infested with CSB were captured, identified, and assessed for their ability to feed on different life stages of CSB.

## Materials and Methods

### CSB Egg, Nymph, and Adult Collection

CSB eggs (≤ 24 hours old) were used as sentinel eggs for field surveys. Approximately five mating pairs of CSB were maintained in colony boxes in climate-controlled cabinets (Darwin Chambers, St. Louis, MO, USA) with a 24 h fluctuating temperature cycle that changed hourly and averaged 27°C ± 1°C, a 60-70% relative humidity, and photoperiod of at 12:12 h (L:D). CSB mating pairs were reared predominantly on organic upland cotton seeds (*Gossypium hirsutum*), but seeds from native Californian Malvaceae plants (e.g., *Abutilon* sp. and *Sphaeralcea ambigua*), were occasionally used as a supplemental food source.

Sheets of KimTech wipes (11.2 cm x 21.3 cm, 100% virgin wood fiber, Kimberly-Clark Professional, Irving, TX, USA) were used as an oviposition substrate and to absorb excess moisture to reduce unwanted mold growth on seeds and water sources. Seeds, water, and KimTech wipes were replaced as needed, usually once a week. CSB eggs were collected daily and five to fifteen eggs were placed on each egg card, depending on the availability of CSB eggs. Nymphs and adults from colony boxes were used in predator feeding bioassays.

### Egg Card Set-up

Egg cards, 7.6 cm long by 2.5 cm wide, were cut from 7.6 cm x 12.7 cm blank index cards (Office Depot, Boca Raton, FL, USA). To each card, double-sided tape (Scotch™ 3M, Cynthiana, KY, USA), approximately 1.2 cm x 0.4 cm was attached to the center of the egg card. A water moistened fine paintbrush was used to transfer fresh CSB

eggs, ≤ 24 hours old, to double-sided tape. Fine quartz sand (National Geographic™, Phoenix, AZ, USA) was added to the double-sided tape to cover exposed adhesive thereby allowing natural enemies to access CSB eggs without getting trapped (Lara et al. 2019, Ganjisaffar et al. 2020). Cards were then labeled with dates of deployment, retrieval, and numbers of CSB eggs added to cards, and an identification code unique to each card.

Sentinel egg cards were transported to field sites in 10-dram vials (Thorton Plastics, Salt Lake City, UT, USA). Egg cards were deployed on heavily infested *L. patersonia* trees UCR from May 1st, 2024, to January 8th, 2026. Starting on September 6th, 2024, egg cards were placed on CSB-infested *S. ambigua* growing at the UCR Entomology Research Museum. Egg cards were attached to the undersides of leaves and stems of CSB host plants, *L. patersonia* or *S. ambigua*, respectively, with two paper clips (No.1 paper clips, Office Depot, Boca Raton, FL, USA). Starting on September 7th, 2025, smaller egg cards (2.5 cm by .95 cm) were placed inside CSB-infested *L. patersonia* pods. Egg cards were deployed inside pods as this placement aligned more closely with the habitat in which CSB egg parasitoids would be expected to forage.

Egg cards were checked daily to monitor egg development. When eggs turned red, the egg cards and paper clips were retrieved and transported to the laboratory in plastic 10-dram vials. In the laboratory, egg cards were checked daily post-retrieval. The numbers of missing and predated eggs, CSB nymphs that hatched, and emerged parasitoids were recorded. Egg cards were discarded when all eggs hatched or if there was no emergence from intact eggs after eight weeks in the laboratory. CSB egg retrieval

rate (%) was determined by dividing the total number of eggs that returned to the laboratory by the total number of eggs originally deployed on labeled egg cards. CSB egg hatch rate (%) was determined by dividing the number of eggs from which CSB nymphs emerged by the total number of CSB eggs that were retrieved on egg cards to the laboratory for rearing.

The climate in Riverside California is classified as Mediterranean. Therefore, seasons were broken down in the following officially recognized ranges: winter (November 24-March 20), spring (March 21-June 12), summer (June 13-September 7), and fall (September 8–November 23) (Kotsias et al. 2021b).

**Feeding Bioassays with Predators Collected from *Lagunaria patersonia* Seed Pods**

Generalist predators collected opportunistically from *L. patersonia* tree pods (see Chapter 2) were used in prey exposure bioassays. Collected predators consisted of seven species: long-legged sac-spiders, *Cheiracanthium* sp. (C. L. Koch) (Araneae: Cheiricanthiidae), buttonhook leafbeetle jumping spiders, *Sassacus vitis* (Cockerell), (Araneae: Salticidae), green lacewing larvae, *Chrysoperla Comanche* (Banks) (Neuroptera: Chrysopidae), pirate bugs, *Buchananiella continua* (White), (Hemiptera: Anthocoridae), leafhopper assassin bug, *Zelus renardii* (Kolenati), (Hemiptera: Reduviidae), ground sac spider, *Trachelas* sp. (L. Koch) (Araneae: Trachelidae), and plant bug nymphs, *Phytocoris* sp. (Fallén) (Hemiptera: Miridae) (Table 3.6).

On October 2nd, 2025, extra *Z. renardii* (two fifth instars and one adult) were collected on horseshoe geranium, *Pelargonium zonale* (L.) L'Hér. ex Aiton, (Geraniales: Geraniaceae) to assess propensity for feeding on small nymphs, first-third instars. *Zelus*

*renardii* collected on *L. patersonia* on June 23rd, 2025, died before CSB eggs, first and second instars nymphs could be offered as prey in feeding bioassays.

All collected predator species were kept individually in ventilated clear plastic 10 dram vials and labeled with collection date. A green bean, *Phaseolus vulgaris* (L.), (Fabales: Fabaceae), or a wet cotton wick was added to the vial to provide a source of moisture and CSB of various stages (i.e., eggs, small nymphs [first-third instars], large nymphs [fourth-fifth instars], or adults) were added as potential food sources.

Predators were offered one individual CSB at a time. The first CSB stage offered was of comparable size to the predator to provide an estimate of the maximum size threshold of prey a predator may feed on. If that CSB stage was eaten, the next larger CSB stage was offered. If that CSB stage was not eaten within three days, the CSB life stage under evaluation was removed and the next smaller stage was offered. CSB stages offered were not duplicated until every stage was offered. For example, *Cheiracanthium* sp. (5-7 mm) were first offered fifth instar CSB (3.7 mm). If *Cheiracanthium* sp. fed on the fifth instar, an adult CSB was offered. If the adult CSB was not eaten within three days, a fourth instar was offered. When the fourth instar was eaten, a third instar was offered. When the third instar was eaten, a second instar was offered. This process was repeated until *Cheiracanthium* sp. fed on CSB eggs up until the fifth instar. Afterwards, *Cheiracanthium* sp. were offered adult CSB. Predators were offered every stage of CSB, pending on predator lifespan within the laboratory.

Feeding on CSB (i.e., life stage and numbers consumed) by predators, CSB molting, and predator molting was recorded. Upon death, predators were preserved in 95% ethanol in labeled vials.

**Feeding Bioassays with First and Second Instar *Zelus renardii***

The adult leafhopper assassin bug, *Z. renardii*, collected on June 23rd, 2025, on *L. patersonia* laid 25 and 7 eggs on June 25th, 2025, and June 28th, 2025, respectively. The first instars that emerged were transferred to 20 ml polyethylene terephthalate (PET) vials (SKS science products, Saratoga Springs, NY, USA) with 22/400 white polypropylene (PP) lids (SKS science products, Saratoga Springs, NY, USA), with a fine mesh lid to allow for ventilation (radius 0.9 cm). A cotton wick was placed on the lid and wet daily to provide moisture. One stage of CSB (i.e. CSB eggs only) was offered to *Z. renardii* nymphs and consumption of CSB life stages, molting of CSB, and molting of first instar *Z. renardii* were recorded. CSB life stages were replaced with different CSB life stages when they were not fed upon for three days. Trials ended when *Z. renardii* nymphs died and dead nymphs were preserved in 95% ethanol in labeled vials.

**Phenology CSB and Associated Predators Infesting *Lagunaria patersonia* Seed Pods**

From July 4th, 2024, to January 29th, 2026, 64-80 *L. patersonia* pods were collected, biweekly (Mondays and Thursdays), to determine the population dynamics of CSB (Chapter 2). Other arthropods (e.g. psocids, *C. comanche, S. vitis, B. continua,* etc.) found inside sampled pods were stored in labeled vials with 95% ethanol. These records of CSB populations, predator numbers, and the dates predators used in prey exposure bioassays were collected from the pods, were used to generate phenology graphs of taxa

abundance. These phenology graphs were used to assess if the predator to prey ratio was at or greater than 1 predator per 450 prey, the maximum ratio required for successful biological control (Zhang et al. 2022). *Zelus renardii* collected on October 2nd, 2025, from *P. zonale* were excluded from the phenology data set as they did not originate from *L. patersonia* growing on the UC Campus.

**Statistical Analyses**

All statistical analyses were done in IBM SPSS Version (31.0.0.0). If Levene's test for equality of variances was found to be significant, independent sampled Welch's t-tests were used. Otherwise, independent sampled t-tests were used to compare the retrieval rates and egg hatch rates between egg cards placed on *L. patersonia* vs *S. ambigua*, and to compare the mean retrieval rates and hatch rates between large egg cards attached to leaves and small egg cards inserted into *L. patersonia* seed pods. One-way analysis of variance (ANOVA) was used to assess the effect of season on CSB egg retrieval rates and hatch rates of field deployed egg cards. If results were statistically significant, post hoc Tukey tests of significance were used for pairwise comparisons at the 0.05 significance level across seasons.

## Results:

**Egg Hatch and Retrieval Rates**

The retrieval rate for CSB eggs deployed on *S. ambigua* stems and *L. patersonia* leaves and pods was 53.92% (Table 3.1). Of those eggs that were returned to the laboratory, 72.27% hatched (Table 3.1). For egg cards placed on *S. ambigua*, 28.50%

returned to the laboratory and 31.92% hatched (Table 3.2). For egg cards placed on *L. patersonia* leaves and inside pods 65.69% returned and 80.37% hatched, respectively (Table 3.2). The larger retrieval rates and hatch rates on *L. patersonia* were significant (Table 3.3). For eggs deployed on large egg cards (i.e., 7.6 cm x 2.5 cm) attached to *L. patersonia* leaves, 66.80% returned and 80.25% hatched (Table 3.2). For eggs deployed on small egg cards (i.e., 0.95 cm x 2.5 cm) inside *L. patersonia* seed pods, 58.95% returned and 81.25% hatched, respectively (Table 3.2). The differences in retrieval rate and hatch rate were not statistically significant (Table 3.3).

**Effects of Season on Retrieval Rate and Hatch Rate**

For CSB eggs placed on *L. patersonia* leaves and inside pods, a significant difference existed for the retrieval rate of eggs across seasons ($F = 5.419$; $df = 7,212$; $P < 0.001$) (Table 3.4). The highest retrieval rate was in spring 2024 (93.17% ± 2.46%) and the lowest retrieval rate was in spring 2025 (41.85% ± 7.04%) (Table 3.4). The retrieval rate was consistently higher for all seasons in 2024 vs spring 2025 and the results from statistical comparisons are presented in Table 3.4. The retrieval rate for spring 2024 was also significant compared to summer 2025 (Table 3.4). All other comparisons for retrieval rate vs. season were not significant for sentinel eggs deployed on *L. patersonia* leaves and in pods (Table 3.4)**.**

For eggs placed on *S. ambigua,* the effect of season on retrieval rate was significant ($F = 6.563$; $df = 5,85$; $P < 0.001$) (Table 3.4). The highest retrieval rate was in fall 2024 (93.74% ± 6.25%) and the lowest retrieval rate was in spring 2025 (11.87% ± 4.33%) (Table 3.4). The retrieval rate was consistently higher for fall 2024 vs. the

following seasons except winter 2024/2025 (Table 3.4). All other comparisons of retrieval rate and season were not significant for sentinel eggs deployed on *S. ambigua* (Table 3.4)**.**

The collective hatch rate (67.91%) of CSB eggs deployed on egg cards across both host plant species, *L. patersonia* and *S. ambigua*, was not statistically significant (Table 3.5). The same can be seen for hatch rate comparisons within the plant species, with a mean hatch rate of 78.85% and 25.28%, on *L. patersonia* and *S. ambigua*, respectively (Table 3.5).

**Predator Activity on Field Deployed Egg Cards**

Predators were found feeding on CSB eggs while attached to sentinel egg cards. One field observation was of a whirligig mite, *Anystis* sp. von Heyden (Trombidiformes: Anystidae), which fed on CSB eggs and newly emerged first instars (Fig. 3.1). Argentine ants, *Linepithema humile* Mayr, (Hymenoptera: Formicidae), were observed removing CSB eggs from egg cards. Predation behavior by *L. humile* was more commonly seen on *S. ambigua* rather than *L. patersonia* (Fig. 3.1).

**Egg Parasitoid Activity on Field Deployed Egg Cards**

No egg parasitoids were observed to attack, lay eggs, or emerge from CSB eggs.

**Predator Assays**

Seven species of generalist predators were found associated with CSB infesting *L. patersonia* egg pods. Predator species inhabiting seed pods included: long-legged sac-spiders, *Cheiracanthium* sp. (Araneae: Cheiricanthiidae), jumping spiders, *S. vitis*, (Araneae: Cheiricanthiidae), leafhopper assassin bugs, *Zelus renardii*, (Hemiptera:

Reduviidae), green lacewing larvae, *C. comanche*, (Neuroptera: Chrysopidae), pirate bugs, *B. continua*, (Hemiptera: Anthocoridae), a ground sac spider, *Trachelas* sp., (Araneae: Trachelidae), and plant bugs, *Phytocoris* sp. (Hemiptera: Miridae) (Table 3.6). Laboratory-reared first and second instar nymphs of the leafhopper assassin bug, *Zelus renardii*, (Hemiptera: Reduviidae), originally collected from *L. patersonia*, also attacked CSB in feeding bioassays.

In laboratory bioassays, all seven predator species fed on at least one stage of CSB. Two species, *S. vitis* adult and *C. comanche* larvae, fed on all stages of CSB (i.e., eggs, small and large nymphs, and adults), (Table 3.6). The *S. vitis* juvenile only fed on first, second, and third instar CSB (Table 3.6). No *Z. renardii* stages fed on CSB eggs and all *Z. renardii* stages fed on CSB instars one through five (Table 3.6). However, *Z. renardii* adults, and second and fifth instar nymphs also fed on adult CSB (Table 3.6).

*Buchananiella continua* fed on eggs and first instar nymphs only. *Phytocoris* sp. only fed on CSB eggs (Table 3.6). First instar CSB nymphs were consumed most frequently and 75% of predators fed on this stage. While adult CSB were least vulnerable to predation, with 50% of predators feeding on this stage (Fig. 3.2).

**Population dynamics of CSB and associated predators infesting *Lagunaria patersonia* seed pods**

Overall CSB population densities were generally low during the period July 2024 to September 2024 (Fig. 3.3). Population densities quickly increased in October 2024 and peaked in December 2024, coinciding with a peak in nymphs in October 2024 and adults in December 2024 (Fig. 3.3). However, CSB densities steadily declined from January

2025 to January of 2026, with a small peak being observed in June 2025, due to an increase in the population of nymphs (Fig. 3.3).

The densities of *S. vitis* spiders was steady, at around seven to nine specimens being collected from *L. patersonia* pods every three months over the period July 2024 to September 2025 (Fig. 3.4a). The *S. vitis* abundances in pods peaked in November 2025 at eight, before decreasing to three in January 2026 (Fig. 3.4a). *Cheiracanthium* sp. were rarely observed, with a maximum on one specimen observed per month and long intervals, up to two months with no observations being recorded (Fig. 3.4b). *Buchananiella continua*, followed a consistent pattern with a sharp peak in abundance observed in July 2024 and 2025, after which numbers decline in August through June (Fig. 3.5a). *Zelus renardi*, were rarely observed, with only three observations being made over July 2024 to January 2026 (Fig. 3.5b). *Chrysoperla comanche* numbers in pods peaked in June 2025, with most other months having four of fewer observations (Fig. 3.5c). However, exceptions to this generalized statement occurred over the period May to July 2025, when more than eight *C. comanche* larvae were observed per month (Fig. 3.5c). *Phytocoris* sp. abundance in pods peaked in May of 2025 (10 plant bugs) (Fig. 3.5c). There was only one observation of *Trachelas* sp. in August 2025, and this solitary specimen was collected and used for feeding bioassays (Fig. 3.4c).

**Discussion**

The goal of this study was to determine the presence of egg parasitoids and the propensity of generalist predators to feed on specific CSB stages. Generalist predators

associated with CSB inside *L. patersonia* pods will feed on this pest. CSB life stages consumed by immature and adult predators appear to be mediated by predator-prey body size relationships, as seen with jumping spiders, leafhopper assassin bugs, and pirate bugs. The predictions from the handling-size hypothesis suggests were confirmed by jumping spider feeding preferences for CSB life stages with small instars being attacked. Additionally, juvenile jumping spiders were similar to the size of a third instar CSB (approximately 2.25 mm in length), and fourth instars (approximately 2.86 mm in length) are likely too large to attack (Kirkpatrick 1923). Therefore, attacks on these larger CSB life stages were not observed. This same trend was observed for leafhopper assassin bugs where first instar nymphs (2.4 mm in length) were able to feed on fifth instars (3.7 mm in length), but were unable to feed on adult CSB (approximately 4-5 mm in length) (El-Tom 1965, Kirkpatrick 1923). Second instar assassin bugs (3.7 mm in length) were able to feed on fifth instar nymphs and adults (El-Tom 1965). Thus, for first instar assassin bugs there appears to be a maximum size threshold for prey. Furthermore, there was no minimum size threshold of CSB prey for adult leafhopper assassin bugs, as the 14 mm female fed on the 1.2 mm first instars and adults (El-Tom 1965, Kirkpatrick 1923). It is also assumed that that non-tested third and fourth instar *Z. renardii* would feed on every instar and adult CSB based on the lack of size thresholds for second instar and adult *Z. renardii* (Michalko and Pekár 2025). Adult pirate bugs exhibited a small maximum size prey threshold as these 2 mm insects did not feed on second instar CSB nymphs which were 1.58 mm in length (Kirkpatrick 1923, Schneider 2018). Therefore, the size of

immature and adult natural enemies strongly affects the CSB life stages that are attacked and consumed.

In addition to size, other natural history attributes of CSB affect predator feeding. For instance, the leaf hopper assassin bug did not feed on the egg stage of CSB, possibly because assassin bugs prefer to attack mobile prey (Lahbib et al. 2022). However, immobile prey may be preferred by some predators. *Phytocoris* sp. are zoophagous and various species have been documented to feed on insect eggs, mites, aphids, and scale insects (Hradil et al. 2013, Stonedahl 2013). *Phytocoris* sp. did not recognize first instar CSB as potential prey (Heger et al. 2024), but fed on CSB eggs, possibly because they are immobile and easy to procure (Morrison et al. 2016, Parker et al. 2023). Finally, specific attributes that differ between adults and nymphs may affect predator feeding. In addition to being larger in comparison to nymphs, adult CSB are more heavily sclerotized than nymphs, which may act as a potential deterrent to some predators, such as long-legged sac spiders (USDA 2021).

Phenological and density data presented here strongly suggests that low densities of generalist predators likely provided little to no natural control (DeBach 1964) of CSB inhabiting *L. patersonia* pods on the UCR campus. Life stage data collected for CSB during phenology studies indicated that for most of the year, a significant proportion of the CSB population is greater than the maximum consumable prey size, a body-size refuge for prey, for most predator species (Michalko and Pekár 2025). Additionally, the predator-prey ratio during the peak CSB population densities (November and December

2024) was around 1 predator per 500 prey, beyond the regulatory capacity for low density generalist predators.

Salticid spiders, *B. continua, Phytocoris* sp., and *Chrysoperla* sp. have been documented feeding on psocids (i.e., barklice), which are also found inside of *L. patersonia* pods (Smith 1922, Hradil et al. 2013, Stonedahl 2013, Nentwig et al. 2024). Thus, it is likely that these generalist predator species may have feed primarily on psocids, and not CSB, especially if psocids are preferred over CSB. However, further research needs to be done to determine if predators may prefer psocids over CSB.

With respect to CSB egg cards deployed at field sites, there were no collections of egg parasitoids indicating that it is very unlikely that species of egg parasitoids (either self-introduced with CSB or present prior to CSB establishing in California) attacking CSB exist at the UCR Campus study site. This is common when invasive species first establishes in a new area because there is usually a lag period when a natural enemy is able to follow or be introduced to a new area with the invasive species (Heger et al. 2024).

There were differences in egg retrieval rates for CSB eggs between the *L. patersonia* and *S. ambigua* locations. This could be due to the diversity of arthropod communities on the plants, where native California plants, like *S. ambigua*, are known to support a higher species richness in comparison to non-native plants like *L. patersonia* (Padovani et al. 2020). For example, aphids infest *S. ambigua* (Neveln 2026) and produce honey dew, a carbohydrate-rich waste product, that attracts ants (Banks 1962). Ants, such as the invasive Argentine ant, *L. humile* (Hymenoptera: Formicidae), are known to be

opportunistic predators of insect eggs, tend honeydew producing aphids infesting *S. ambigua* (Cerdá and Dejean 2011, Pickett et al. 2022, Neveln 2026).

Season did not have a distinct effect on the retrieval rate of sentinel eggs on egg cards (i.e. there was no consistent differences for egg return rates across consecutive seasons). However, there was a significant annual effect, where the retrieval rate for eggs in 2025 was consistently lower than the retrieval rate in 2024. This may be due to generalist predators adapting to novel food sources, like CSB eggs, for example (Parker et al. 2023).

The significant difference in egg hatch rates between egg cards placed on *L. patersonia* and *S. ambigua* may be due to sunlight exposure (Campbell et al. 2016). Egg cards on *S. ambigua* stems were oriented towards the sky, inadvertently exposing eggs to direct sunlight, and heat and UV radiation may have damaged exposed eggs (Beard 1972, Zhang et al. 2014). Additionally, in comparison to *L. patersonia*, *S. ambigua* bushes provide relatively little shade and protection for eggs on egg cards against sun, wind, and rain. Therefore, egg cards attached to *L. patersonia* leaves and inside seed pods experienced less direct exposure to sunlight and other potentially adverse abiotic events. The insect community inhabiting *L. patersonia* pods did not affect egg retrieval rates and egg hatch rates as these were similar to retrieval and hatch rates of eggs on egg cards attached to leaves. This suggests that overall, natural enemy activity on CSB was similarly equal in these two habitats and was generally very low.

In conclusion, this is the first study to provide insights into the lack egg parasitism and predation of CSB by generalist predators. Bioassays indicated that generalist

predators associated with CSB have prey size thresholds related to their body size with small predators being restricted to smaller-sized CSB (e.g., pirate bugs) while larger predators (e.g., assassin bug adults) were able to consume a range of CSB life stages, from small nymphs to adults. Sampling of predators in pods strongly indicated that generalist predator species provided negligible suppression of CSB populations in the field. Season had negligible effects on hatch rates of CSB eggs on egg cards, but significant yearly effects on egg retrieval rates were observed. At this time, it would appear that natural enemy species associated with CSB infesting *L. patersonia* are unlikely to provide effective population-level control of CSB. Consequently, CSB populations are very high inside *L. patersonia* pods. This finding may support the "enemy escape hypothesis" (Heger et al. 2024) where CSB, an invasive pest in California, has escaped control of natural enemy species that may have regulated population densities in the native range. Future studies, especially in the home range of CSB, could identify parasitoids or specialist predators that may have potential for use in a classical biological control program.

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**Fig. 3.1** Images of predator activity seen on CSB egg cards. (A High resolution image of the whirligig mite (*Anystis* sp.). (B) Whirligig mite feeding on freshly emerged first instars. (C Argentine ant walking on egg card placed on *S. ambigua*. (D) Argentine ants removing CSB eggs from egg card placed on *L. patersonia*.

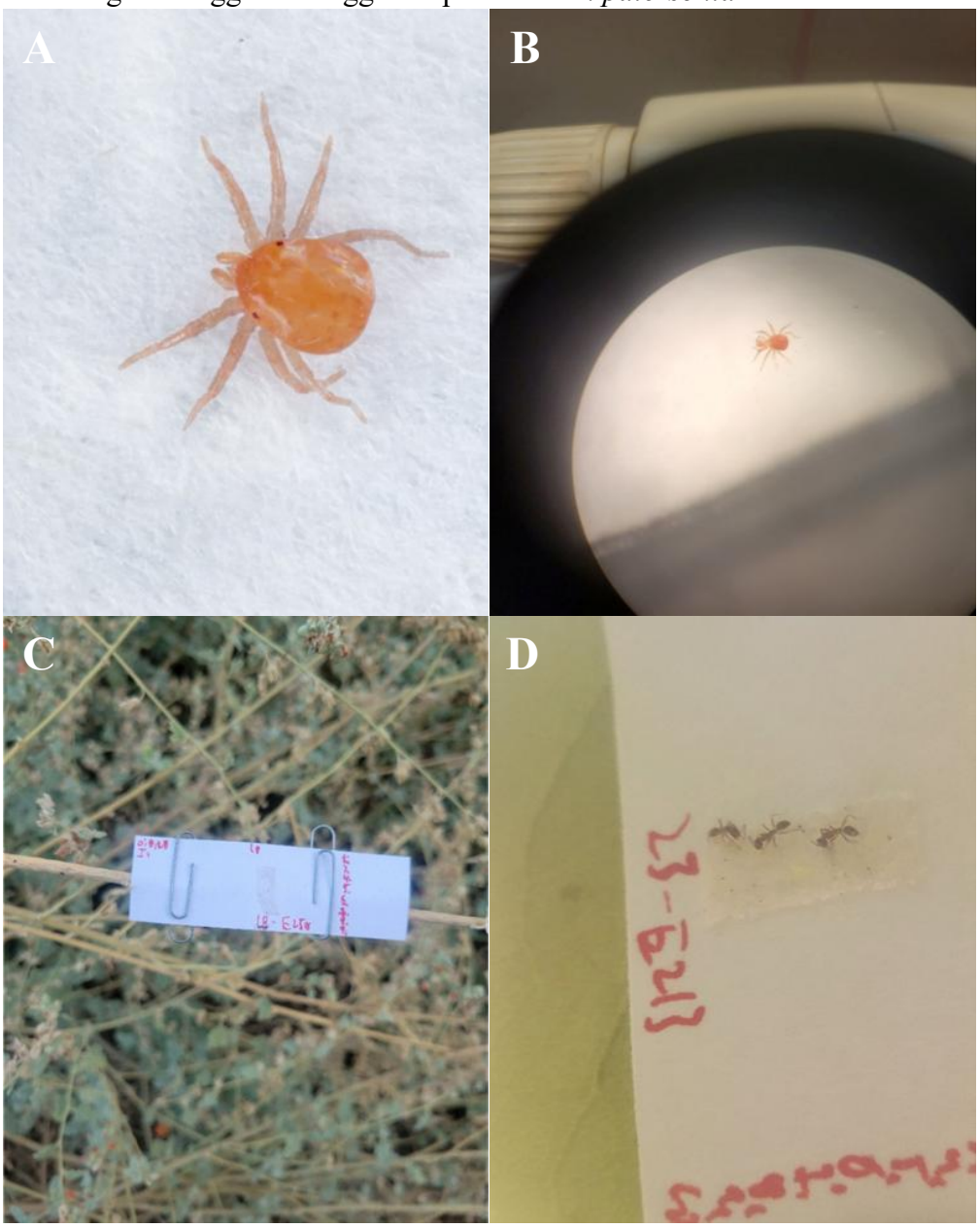
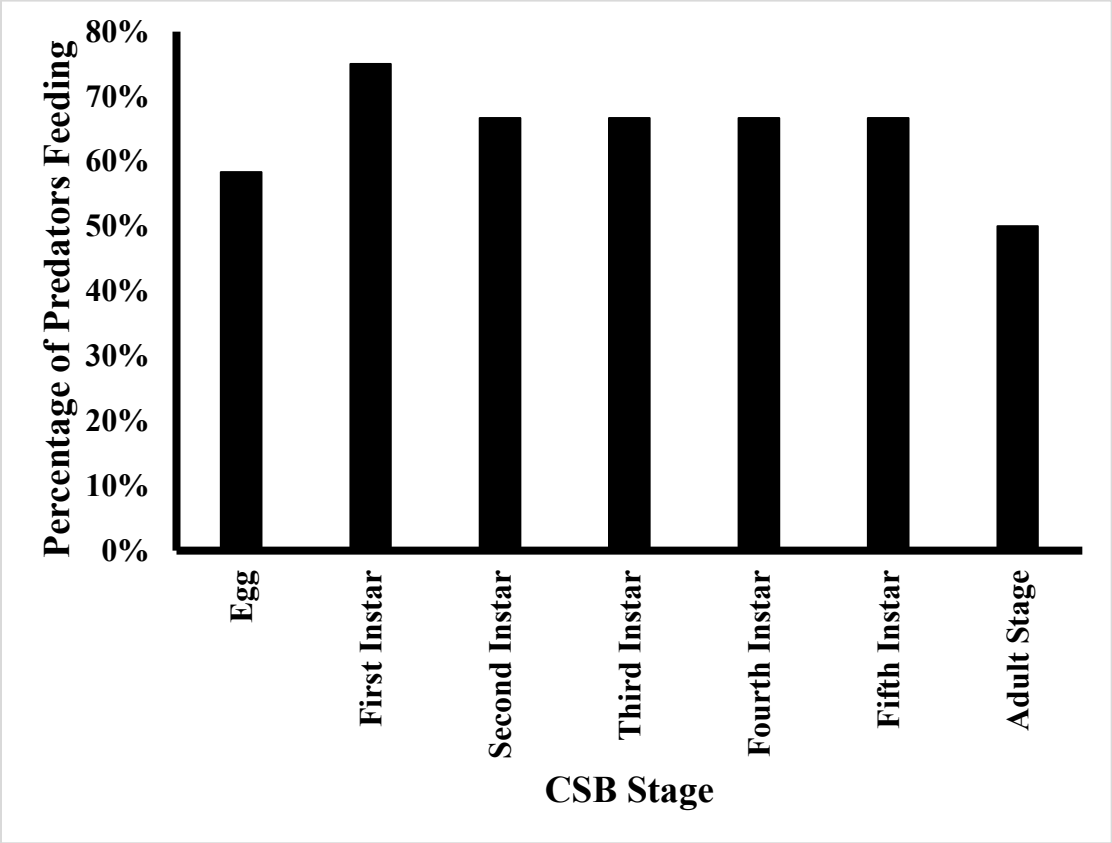


**Fig. 3.2** The percentage of predators (out of the 12 categories) that fed on each CSB stage determined by predator bioassays in the laboratory.




The twelve categories are the predator groups listed in Table 3.6.

**Fig. 3.3** Number of nymph and adult CSB found inside *Lagunaria patersonia* pods on UC Riverside Campus.

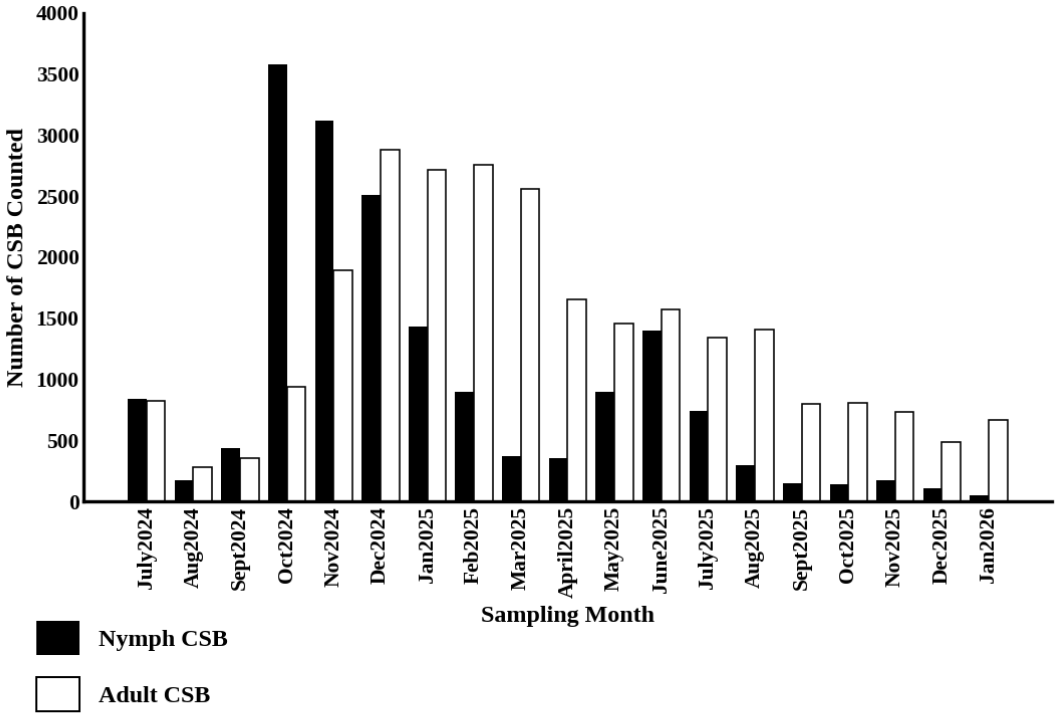


**Fig. 3.4** Number of spiders, order Aranea, found inside *Lagunaria patersonia* pods on UC Riverside Campus, including dates listed in Table 3.6. Number of (A) *Sassacus vitis* (B) *Cheiracanthium* sp., (C) *Trachelas* sp. Note the scale of the y-axis differs among panels.

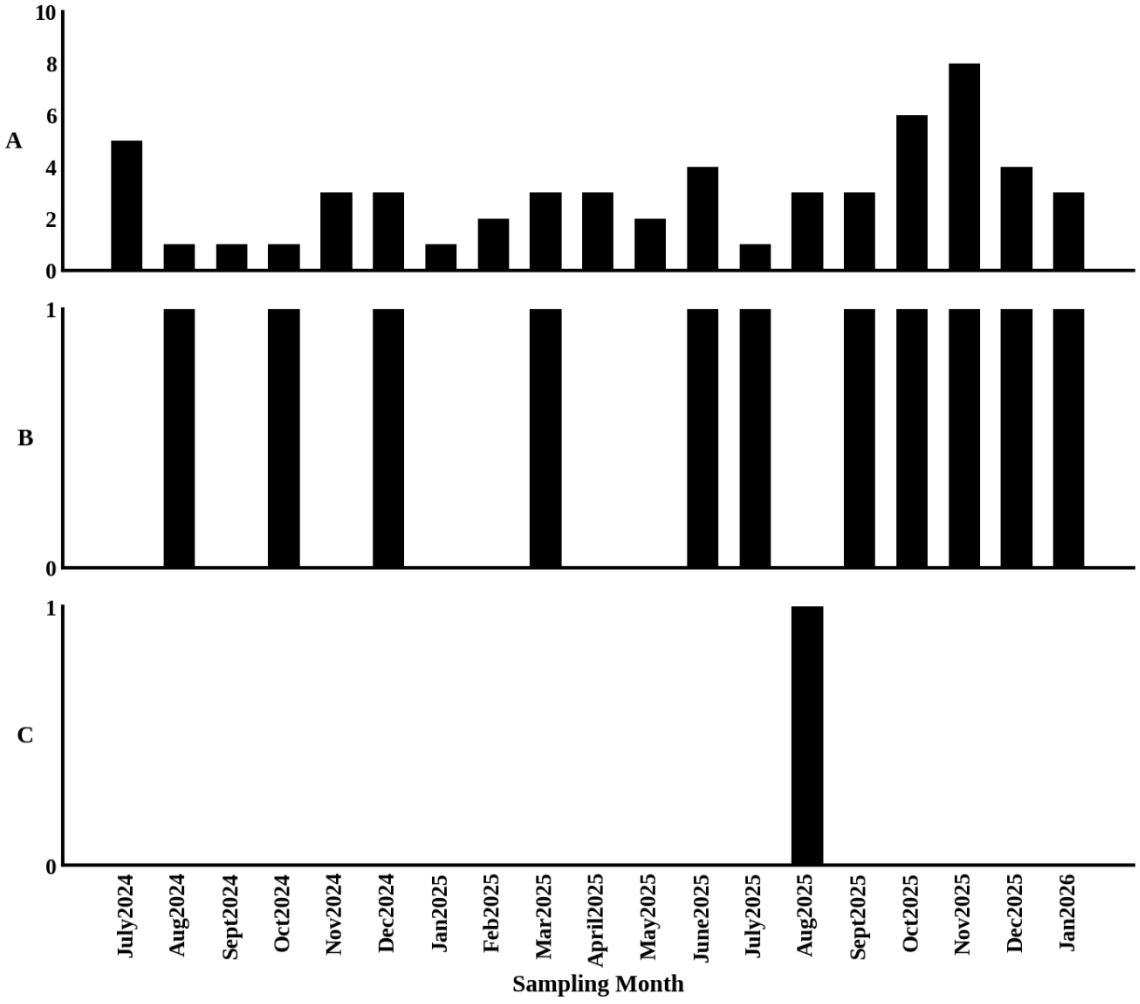


**Fig. 3.5** Number of insects, class Insecta, found inside *Lagunaria patersonia* pods on UC Riverside Campus, including dates listed in Table 3.6. Number of (A) *Buchananiella continua*, (B) *Zelus renardii*, (C) *Chrysoperla comanche,* (D) *Phytocoris* sp. Note the scale of the y-axis differs among panels.

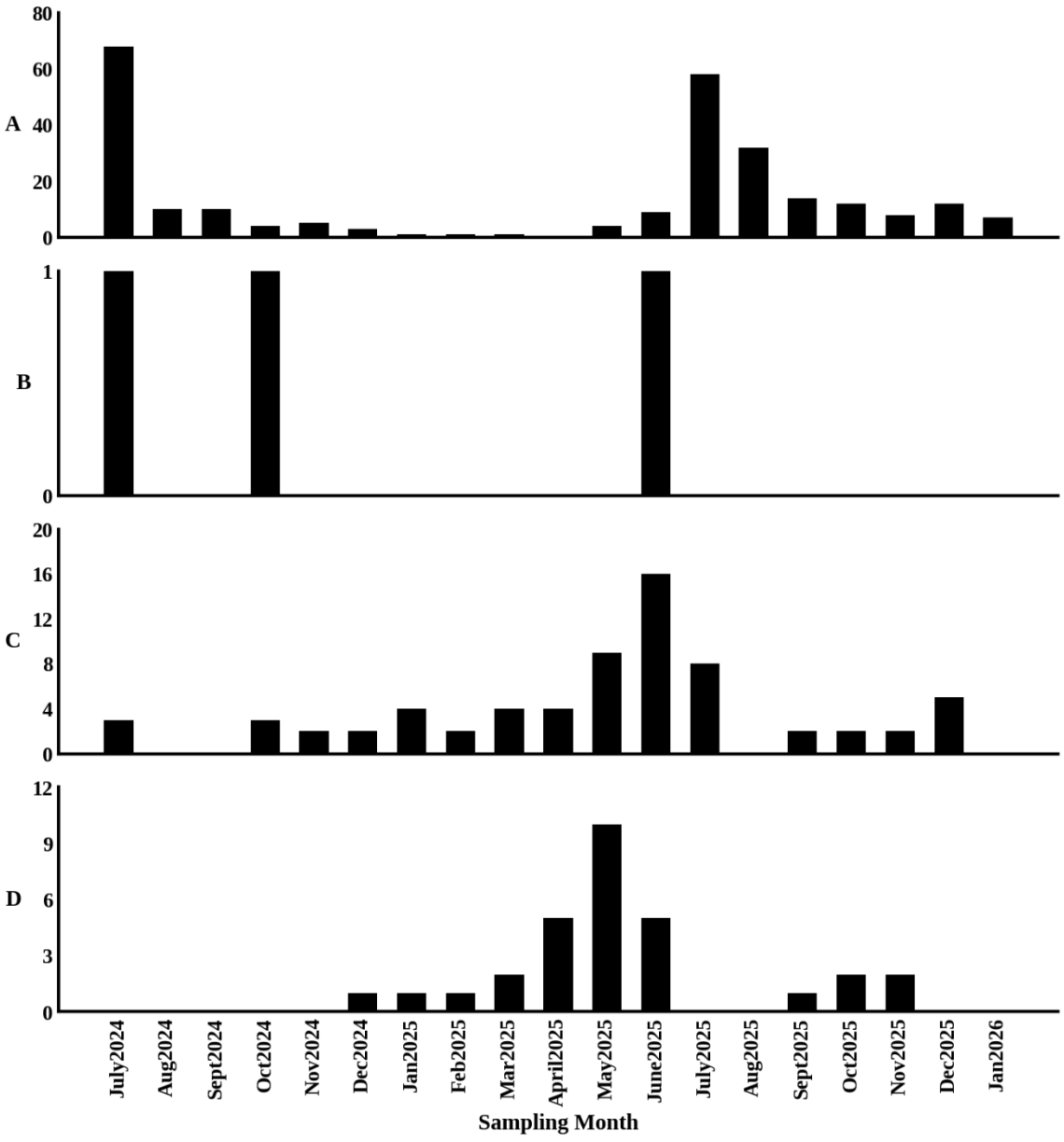


**Table 3.1** Summary table of the totals for CSB egg cards used for duration of the experiment

| Number of eggs deployed | Number of eggs not retrieved | Number of eggs retrieved | Number of eggs that hatched | Number of eggs that never hatched |
|---|---|---|---|---|
| 3926 | 1809 | 2117 | 1530 | 587 |

**Table 3.2** Summary table of CSB egg cards placed on *L. patersonia* and *S. ambigua*. Rows with dimensions only include egg cards placed on *L. patersonia*.

| | **Eggs deployed** | **Eggs retrieved (%)** | **Eggs hatched (%)** |
|---|---|---|---|
| *Sphaeralcea ambigua* | 1242 | 354 (28.50%) | 113 (31.92%) |
| *Lagunaria patersonia* | 2684 | 1763 (65.69%) | 1417 (80.37%) |
| Small cards | 380 | 224 (58.95%) | 182 (81.25%) |
| Large cards | 2304 | 1539 (66.80%) | 1235 (80.25%) |

**Table 3.3** Mean retrieval rate and hatch rate (mean percentage ± SE) for CSB eggs placed on *Lagunaria patersonia*, *Sphaerlacea ambigua*, and for large and small egg cards placed on *Lagunaria patersonia*.

| | Host plant | | | | |
|---|---|---|---|---|---|
| | *Lagunaria patersonia* | *Sphaeralcea ambigua* | *t* | df | *P* |
| Retrieval rate | 68.87% ± 3.17%[a] | 30.41% ± 4.32%[b] | 6.829*** | 309 | <0.001 |
| Hatch rate | 79.03% ± 2.00%[a] | 23.74%± 5.60%[b] | 9.297*** | 54.431 | <0.001 |
| | Egg card dimensions | | | | |
| | 7.6 cm x 2.5 cm | 0.95 cm x 2.5 cm | | | |
| Retrieval rate | 69.79% ± 3.19%[a] | 65.16% ± 10.69%[a] | 0.415 | 40.282 | 0.680 |
| Hatch rate | 79.57% ± 2.04%[a] | 76.26% ± 7.33%[a] | 0.435 | 24.361 | 0.667 |

Means within rows followed by the different letters are significantly different as determined by t-tests. *$p$<0.05, **$p$<.01, ***p<.001.

**Table 3.4** Mean retrieval rates (± SE) for CSB eggs by season and host plant.

| | *Lagunaria patersonia* | *Sphaerlacea ambigua* |
|---|---|---|
| Season | Retrieval rate (mean ± SE) | Retrieval rate (mean ± SE) |
| Spring 2024 | 93.17% ± 2.46%[a] | N/A |
| Summer 2024 | 88.97% ± 8.74%[ac] | N/A |
| Fall 2024 | 88.54% ± 7.86%[ac] | 93.75% ± 6.25%[a] |
| Winter 2024/2025 | 76.52% ± 7.21%[ac] | 30.00% ± 10.01%[bc] |
| Spring 2025 | 41.85% ± 7.04%[bc] | 11.87% ± 4.33%[bc] |
| Summer 2025 | 56.17% ± 6.82%[c] | 22.10% ± 8.58%[bc] |
| Fall 2025 | 59.53% ± 11.59%[ac] | 31.03% ± 11.19%[bc] |
| Winter 2025/2026 | 72.67% ± 19.95%[ac] | 46.94% ± 19.15%[ac] |

Means followed by the same letter are not significantly different ($p > 0.05$) as determined by Tukey tests following a significant One-Way ANOVA.

**Table 3.5** Mean hatch rates (± SE) for CSB eggs by season and host plant.

| | Overall | *Lagunaria patersonia* | *Sphaerlacea ambigua* |
|---|---|---|---|
| Season | Hatch rate (mean ± SE) | Hatch rate (mean ± SE) | Hatch rate (mean ± SE) |
| Spring 2024 | 80.84% ± 4.49% | 80.84% ± 4.49% | N/A |
| Summer 2024 | 68.91% ± 4.63% | 68.91% ± 4.63% | N/A |
| Fall 2024 | 66.29% ± 7.47% | 83.64% ± 4.63% | 33.75% ± 13.87% |
| Winter 2024/2025 | 67.55% ± 6.66% | 81.61% ± 5.36% | 17.47% ± 13.92% |
| Spring 2025 | 67.16% ± 7.45% | 89.65% ± 3.51% | 2.50% ± 2.50% |
| Summer 2025 | 62.42% ± 5.86% | 75.16% ± 4.62% | 4.20% ± 4.20% |
| Fall 2025 | 67.78% ± 8.72% | 83.79% ± 7.07% | 43.76% ± 45.70% |
| Winter 2025/2026 | 62.31% ± 12.31% | 67.23% ± 13.63% | 50.00% ± 28.87% |

Means within columns were not determined to be significantly different ($p > 0.05$) by One-Way ANOVA.

**Table 3.6** Feeding matrix (number of predators feeding on the stage / total number of predators offered the stage) of generalist predators found in *Lagunaria patersonia* pods determined by predator assys. CSB stages that were not eaten were offered to the predators. The dates listed are days the predators were sampled from the field.

| | CSB stage | | | | | | |
|---|---|---|---|---|---|---|---|
| | Egg | First | Second | Third | Fourth | Fifth | Adults |
| Long-legged sac-spider, *Cheiracanthium* sp. (Araneae: Cheiricanthiidae), 3/31/2025, 6/30/2025, 7/31/2025, 9/29/2025 | 3/4 | 1/1 | 2/3 | 1/2 | 1/4 | 1/3 | 0/3 |
| Jumping spider juvenile, *Sassacus vitis*, (Araneae: Salticidae), 8/4/2025 | 0/1 | 1/1 | 1/1 | 1/1 | 0/1 | 0/1 | 0/1 |
| Jumping spider adult, *Sassacus vitis*, (Araneae: Salticidae), 4/7/2025, 6/5/2025, 6/12/2025, 9/1/2025, 9/11/2025, 10/13/2025 | 1/5 | 4/5 | 4/6 | 2/5 | 5/6 | 1/5 | 1/6 |
| Leafhopper assassin bug, *Zelus renardii,* (Hemiptera: Reduviidae) first instar, 7/3/2025 (x17), 7/4/2025 (x3), 7/6/2025 (x6) | 0/3 | 4/8 | 9/13 | 7/11 | 10/19 | 13/21 | 0/2 |
| Leafhopper assassin bug, *Zelus renardii,* (Hemiptera: Reduviidae) second instar, 7/9/2025, 7/10/2025, 7/13/2025, 7/18/2025 | 0/4 | 1/2 | 1/2 | 1/2 | 3/4 | 3/4 | 1/2 |
| Leafhopper assassin bug, *Zelus renardii*, (Hemiptera: Reduviidae) fifth instar, 10/2/2025 (x2) | 0/2 | 1/2 | 1/2 | 1/2 | 1/2 | 1/2 | 1/2 |
| Leafhopper assassin bug, *Zelus renardii*, (Hemiptera: Reduviidae) Adult, 6/23/2025, 10/2/2025 | 0/2 | 2/2 | 2/2 | 1/2 | 2/2 | 2/2 | 2/2 |
| Green lacewing larva, *Chrysoperla comanche,* (Neuroptera: Chrysopidae), 6/23/2025, 7/3/2025, 7/21/2025, 7/24/2025, 11/16/2025 | 5/5 | 2/2 | 1/1 | 1/1 | 2/2 | 4/4 | 4/5 |
| Pirate bug, *Buchananiella continua,* (Hemiptera: Anthocoridae), 7/3/2025, 7/28/2025, 7/31/2025, 9/4/2025 | 1/4 | 1/4 | 0/4 | 0/2 | 0/2 | 0/2 | 0/2 |
| Ground sac spider, *Trachelas* sp. (Araneae: Trachelidae), 8/21/2025 | 1/1 | 0/1 | 0/1 | 0/1 | 1/1 | 1/1 | 1/1 |
| Plant bug nymph, *Phytocoris* sp. (Hemiptera: Miridae), 10/31/2025 | 1/1 | 0/1 | 0/1 | 0/1 | 0/1 | 0/1 | 0/1 |
| Plant bug adult, *Phytocoris* sp. (Hemiptera: Miridae), 11/3/2025 | 1/1 | 0/1 | 0/1 | 0/1 | 0/1 | 0/1 | 0/1 |

# Chapter 4:

## Developmental biology of cotton seed bug, *Oxycarenus hyalinipennis* (Costa) (Hemiptera: Oxycarenidae), on cow-itch trees, *Lagunaria patersonia* (Andrews) G. Don (Malvales: Malvaceae) and upland cotton, *Gossypium hirsutum* (L.) (Malvales: Malvaceae)

## Introduction

Understanding the life cycle of an insect is a foundational component of successful Integrated Pest Management (IPM) programs (Alston 2011). IPM strategies are often developed to disrupt insect life cycles, thus reducing pest densities and their subsequent negative impacts on crops (Balraj and Singh 2025). One key component affecting an insect's life cycle is diet (Kang et al. 2022). Diet nutritional quality can significantly influence fitness which can be measured in terms of survival rates, speed of development, and reproductive output (i.e., fecundity), which collectively affect rates of population growth and spread (Wang et al. 2018). Importantly, diet quality can vary greatly between different host plant species which may have direct effects on insect fitness (Behmer 2009, Behmer and Nes 2003).

Cotton seed bug (CSB), *Oxycarenus hyalinipennis* (Costa) (Hemiptera: Oxycarenidae), has a hemimetabolous life cycle, which includes the egg, five nymphal instars, and adult stage. Each motile stage is polyphagous but primarily feeds on seeds from plants in the Malvales order (CAPS 2023). One host species of economic concern is upland cotton, *Gossypium hirsutum* (L.) (Malvales: Malvaceae). CSB feeding can reduce cotton seed weight by up to 15 percent (USDA 2010), and germination rates by as much as 88 percent (Kirkpatrick 1923). Moreover, cotton lint may be stained, reducing the sale price of cotton, if any CSB stage is crushed during the ginning process (Henry 1983).

CSB, native to Africa, is considered an invasive pest in Asia, the Middle East, Europe, South America, and the Caribbean (Dueñas-López 2022). In 2010, this insect was first documented in the U.S. in the Florida Keys (Halbert and Dobbs 2010) but was

declared successfully eradicated (by detecting CSB populations early, quarantining potential Malvales host plants, and destroying infested host plants) in 2014, (USDA-APHIS 2024). In 2019, CSB was detected on Indian mallows, *Abutilon palmeri* (Mill) (Malvales: Malvaceae) in urban areas of Los Angeles, California (CDFA 2019). The California Department of Food and Agriculture (CDFA) confirmed populations of CSB in Orange, San Diego, and Riverside counties in 2021 (Hoddle and Hoddle 2023; Hoddle 2024). As of January 2026, iNaturalist records have documented CSB in additional California counties, including, San Bernardino, Santa Barbara, Santa Clara, and Ventura (Hoddle et al 2026). In Riverside County, CSB has been detected on cow-itch trees, *Lagunaria patersonia* (Andrews) G. Don (Malvales: Malvaceae), an ornamental landscape tree (Hoddle et al 2026). Thus far, CSB has not been detected in commercial cotton production areas in California or other major cotton producing states in the US but may cause significant economic damage if CSB infect cotton production areas.

In California, upland and pima cotton are primarily grown in San Joaquin Valley, including the counties of Fresno, Kern, Kings, Madera, Merced, and Tulare and is also grown in Riverside and Imperial counties (CDFA 2022). Using online production statistics and estimated bale values, the cotton crop grown in California in 2024 was worth approximately $135.2 million (Hoddle et al. 2026). If the current CSB populations infesting urban and wilderness areas are not controlled, and programs to limit spread via the movement of live plants are not implemented, especially through the nursery trade (Hoddle et al. 2026), it is likely that CSB will invade commercial cotton production areas (CDFA 2022).

In California, CSB can be found infesting *L. patersonia* seed pods growing in urban areas. *Lagunaria patersonia* is native to Australia (i.e., Queensland and New South Wales) and some Pacific Islands (i.e., Lord Howe Island, Norfolk Island) (Iamonico and Ridha El Mokni 2020). These trees are evergreen with showy pink flowers and are commonly planted as ornamental trees in urban landscapes (Esmaiel et al. 2014, Roy et al. 2025). *Lagunaria patersonia* seed pods that develop from flowers are approximately 2-4 cm in diameter (Iamonico and Ridha El Mokni 2020). Seed pods contain red seeds (Iamonico and Ridha El Mokni 2020) that CSB nymphs and adults feed on. The nutritional quality of *L. patersonia* seeds for CSB development and reproduction is not known, and these life history outcomes may differ markedly when compared to CSB reared on organically grown upland cotton seeds.

Life tables can be used to summarize survivorship rates across different life stages and the reproductive output of female insects (Harcourt 1969). Life table statistics for CSB reared on *L. patersonia* and upland cotton may provide important analytical insights in the quality of these two different seed types for CSB development and population growth. For example, life tables can used to generate metrics to estimate rates of population growth (e.g., net reproductive rates $R_o$) (Morris and Miller 1954; Carey 1982; Gabre et al. 2005; Satpute et al. 2005; Hoddle 2006; Pilkington and Hoddle 2007; Polanco et al. 2011). To the best of our knowledge, no life tables have been constructed for CSB, even though studies have described various characteristics of CSB development in the laboratory, including egg incubation periods, egg hatch rates, nymphal duration, and female fecundity (El-Taher et al. 2022, Saveer et al. 2024). Furthermore, these

studies were done on cotton seeds, and similar studies have not been conducted with seeds of *L. patersonia* or other malvaceous hosts (El-Taher et al. 2022, Saveer et al. 2024). Therefore, the purpose of work conducted here was to generate vertical life tables (Polanco et al. 2011) for CSB to enable comparison of the effects of two different seed diets, *L. patersonia* and *G. hirsutum*, on the development, reproduction, survivorship, and potential population growth of CSB.

## Materials and Methods

### Source of Experimental CSB

CSB used for experiments were collected from various Malvaceae plants in Riverside County, such as *L. patersonia*, *A. palmeri*, and *Sphaeralcea ambigua* (A. Gray) (Malvales: Malvaceae). Field-collected insects were used to create colonies where adult males and females (20-30 males and females) were confined in modified ventilated plastic containers (2.2 L Rectangle, Sistema® Plastics, Auckland, NZ) with aeration holes covered with fine mesh, to allow for air circulation and to prevent escape.

CSB colonies were reared predominantly on organic upland cotton seeds. However, seeds from native Californian Malvaceae plants (e.g., *A. palmeri*, and *S. ambigua*), were occasionally used as supplemental food sources. Small petri dishes (35 mm, polystyrene, Fisher Scientific, St. Louis, MO, USA) with water-saturated cotton wicks were provided as a moisture source. Sheets of KimTech wipes (11.2 cm x 21.3 cm, 100% virgin wood fiber, Kimberly-Clark Professional, Irving, TX, USA) were used as oviposition substrates, shelter for nymphs and adults, and to absorb excess moisture to

reduce unwanted mold growth on seeds and water sources. Seeds, water, and KimTech wipes were replaced as needed (usually once a week). The progeny of field-collected adult CSB was used to initiate the experiments.

Colony boxes were maintained in climate-controlled cabinets (Darwin Chambers, St. Louis, MO, USA) with a 24 h fluctuating temperature cycle that changed hourly and averaged 27°C (Table 4.1). Humidity in cabinets was maintained at 60-70% and photoperiod was set at 12:12 h (L:D) with a light intensity of 100 μE $m^{-2}$ $s^{-1}$ for every experimental temperature profile. To ensure the desired experimental temperature profiles and relative humidity were achieved, HOBO Pro V2 Temperature/RH loggers (Onset Computer, Pocasset, Massachusetts, USA) were programmed to record temperature and humidity at 60-min intervals inside the climate-controlled cabinets throughout the duration of the experiment. These data were used to confirm temperature cabinets were performing the programmed temperature cycles.

**Experimental Set-Up for Nymph and Adult Survival and Development**

CSB colony boxes were checked daily to ensure that eggs used in experiments were recently laid. Egg color is a useful indicator of egg age. Eggs ≤ 24 h of age tend to be white, and older eggs have a reddish-orange coloration. Eggs collected for use in experiments were recorded as being laid on the date they were harvested. A damp fine paintbrush was used to collect and transfer CSB eggs into 20 ml polyethylene terephthalate (PET) vials (SKS science products, Saratoga Springs, NY, USA; i.e. ~5-10 similar aged eggs were placed in a vial) labeled with the date of collection and set up. Eggs were checked daily for emergence. When eggs hatched, newly emerged first instars

were collected with a moistened paint brush and moved into separate 20 ml PET vials with 22/400 white polypropylene (PP) lids (SKS science products, Saratoga Springs, NY, USA). Vials contained an abundant amount of food, either *L. patersonia* or upland cotton seeds. A cotton wick that absorbed excess moisture and provided a surface for the CSB to rest on was provided. Vial lids were modified and had a 0.9 cm radius opening that was covered with a fine mesh lid to prevent CSB from escaping while allowing air circulation. A water-moistened cotton wick was placed on top of the ventilation mesh on the outside of the vial lid as a source of moisture. Placement of the water source on top of the ventilated lid reduced mold growth on seeds. Cotton wicks were moistened daily. Vials with nymphs or adults were checked daily, and nymphal instar or adult survival was recorded. If the vial and/or seed had mold, the vial was cleaned and cotton wicks and seeds were replaced. The dates of vial refreshments were recorded. A trial ended when CSB went unobserved for three or more consecutive days, suggesting that nymphs had escaped, or when CSB died of natural causes. A total of 130 individual first instar nymphs, 50 on *L. patersonia* seeds and 80 on upland cotton seeds, were set up for daily observations. Daily mortality data were recorded for CSB nymphs and adults that died of natural causes. Dead CSB adults were preserved in 95% ethanol in vials labeled by diet type, unique identification number, and date of death.

**Fecundity of Adult CSB**

A sample of seven males and seven females on *L. patersonia* seeds and ten males and ten females on upland cotton from the prior experiment were set up as mating pairs in Munger cells (Munger 1942). These mating pairs were supplied with a wet cotton wick as

a moisture source, a dry cotton wick as a substrate for oviposition, and *L. patersonia* or upland cottons seeds were provided as food. Mating pairs were checked daily, mating events, if observed, were recorded, the number of eggs laid each day was recorded, and seeds were replaced if mold was developing on them. If eggs were laid, seeds and the cotton wick with eggs were transferred to a new labeled vial and observed for emergence. A new cotton wick and seeds were added to replace the removed substrates in the mating pair's Munger cell. Vials containing the eggs were labeled with the date eggs were laid and the identification number of mating pair that laid the eggs. Eggs were checked daily to determine time to eclosion in days. The number of eggs laid, the number that hatched, and days to hatch were recorded. Vials were provided with excess seeds to ensure nymphs had sufficient food to reach adulthood. Vials were checked daily, and the date of adult appearance was recorded and used to determine how many females emerged from laid eggs. Upon death, adult CSB were preserved in 95% ethanol in labeled vials.

**Life Table Calculations**

Daily development, survivorship data, and daily progeny production data were used to create life tables. The number of nymphs that progressed through each developmental stage were used to calculate the "number entering the stage," (i.e., the $l_x$ column) (Deevey 1947). Survivorship of eggs was determined by the hatch rate of the eggs produced by mating pairs (i.e. total number of first instars that hatched/total number of eggs laid). The $m_x$ column, a measure of age specific fecundity (i.e., the average number of female offspring produced per female at age $x$) was calculated by dividing the number of females produced by a batch of eggs by the number of females alive in the

mating pair set ups (Deevey 1947). To calculate $m_x$, for example, the number of females that emerged from eggs produced on the fifth day were divided by the number of females alive in the mating pairs on the fifth day after each mating pair was set up.

The following demographic parameters were calculated from $l_xm_x$ life Tables:

1. Net reproductive rates, $R_o = \sum l_xm_x$ (Deevey 1947).

2. Mean generation time, $T_c = \sum xl_xm_x/R_o$ (Deevey 1947).

3. The intrinsic rate of natural increase, $r_m$ (Birch 1948). The value of $r_m$ is iterated until the equation $1 = \sum l_xm_x\exp(=r_mx)$ was solved.

4. Finite rate of increase, $k = \exp(r_m)$ (Birch 1948).

5. Population doubling time, $T_d = \ln(2)/r_m$ (Carey 1989).

**Stage-Specific Survivorship**

Survivorship of eggs was determined by egg hatch rate (i.e. number of first instars/number of eggs laid). Survivorship rates of the five nymphal stages as they progressed through to adults were calculated by dividing the number of CSB entering a stage by the initial number of first instar nymphs used to generate that cohort. These stage-specific survivorship were analyzed using Kaplan-Meier curves by stage.

**Statistical Analyses**

All statistical analyses were done in IBM SPSS Version (31.0.0.0). If Levene's test for equality of variances was found to be significant, independent sampled Welch's t-tests were used. Otherwise, independent sampled t-tests were used to compare the means of development time of the CSB stages (egg, first instar, second instar, third instar, fourth instar, fifth instar, adult, egg to adult, and egg to adult to egg) between CSB fed on *L.*

*patersonia* or upland cotton seeds, and the mean number of eggs laid and hatched from mating pairs fed on *L. patersonia* or upland cotton seeds. Two-way ANOVAs with diet and mating status as factors were used to compare the longevity of male and female CSB. Kaplan-Meier curves by stage were generated from stage-specific survivorship of CSB nymphs fed on *L. patersonia* or upland cotton seeds.

## Results

### Development Time

At 27 °C, CSB reared on *L. patersonia* seeds developed significantly faster across all stages (around 1.5 days faster as an egg, around 3.5 days faster as a first instar, around 3 days faster as a second instar, around five days faster as a third instar, around 4 days faster as a fourth instar, and around 5.5 days faster as a fifth instar) than CSB reared on upland cotton seeds (Table 4.2). The same result was observed for mean development time from egg to adult, with CSB developing >40% quicker on *L. patersonia* seeds when compared to individuals reared on upland cotton seeds (Table 4.2). Furthermore, adult CSB maintained on *L. patersonia* seeds lived about 2.4 times longer than adults that were provided upland cotton seeds (Table 4.2).

### Fecundity

The mean number of eggs produced by mated females on *L. patersonia* and upland cotton seeds was not significantly different (Table 4.3). Egg hatch rate was similar for females reared on *L. patersonia* and upland cotton seeds (Table 4.3).

**Mated vs Unmated Adult Longevity**

On *L. patersonia* seeds, unmated males and females lived about 2.07 times and 4.36 times longer than their mated counter parts of the same sex, respectively (Fig. 4.4, Fig. 4.5, Table 4.5, Table 4.6). On average, unmated females lived the longest and they lived about 1.51 times longer than unmated males (Table 4.5, Table 4.6). Mated females and mated males had similar life spans (Table 4.5, Table 4.6).

For CSB on upland cotton seeds, mated males and females lived about 1.87 times and 2.42 times longer than their unmated counterparts, respectively (Fig. 4.4, Fig. 4.5, Table 4.5, Table 4.6). On average, mated females and males, lived approximately the same length of time (Table 4.5, Table 4.6). Unmated females had the shortest lifespan, but duration was not significantly less than unmated males (Table 4.5, Table 4.6).

Unmated males fed on *L. patersonia* seeds lived about 3.32 times longer than unmated males reared on upland cotton seeds (Table 4.5). This trend is similar to what was observed between unmated females on *L. patersonia* seeds vs unmated females on upland cotton seeds (Table 4.6). There was no statistically significant difference in longevity between mated males and females regarding seed type they were reared on (Table 4.4, Table 4.5).

**Fecundity and Female Longevity**

CSB females on *L. patersonia* seeds exhibited two peaks for egg production. The first larger peak was in week four and the second smaller peak was in week eight (Fig. 4.2). Females did not produce eggs after week eight. CSB females experienced the

highest mortality between weeks 8 (i.e., 83% survival) and 9 (i.e., 33% survival) (Fig. 4.2).

CSB on upland cotton also had two peaks for egg production. The first larger peak was in week 13 and the second peak was in week 17 (Fig. 4.3). There were no eggs produced after week seventeen. CSB females experienced the highest mortality between weeks 13 (i.e., 83% survival) and 14 (i.e., 50% survival), and weeks 17 (i.e., 33% survival) and 18 (i.e., 0% survival) (Fig. 4.3).

**Stage-Specific Survivorship**

CSB nymphs reared on *L. patersonia* seeds had a significantly higher probability of surviving to adulthood (66.67%) compared to nymphs on upland cotton seeds (34.69%) ($\chi^2$=10.363, *df* = 1; Fig. 4.1). CSB on *L. patersonia* seeds had a 100% survival rate after reaching third instar (i.e., 40/40 third instars made it to adulthood) (Fig. 4.1). On upland cotton seeds, 55.74% of CSB made it to adulthood after reaching third instar (i.e., 34/61 third instars made it to adulthood) (Fig. 4.1). Mating pairs fed on *L. patersonia* seeds had a hatch rate of 83.03% (137 first instar nymphs/165 eggs) whereas upland cotton had a hatch rate of 79.92% (191 first instar nymphs/239 eggs) (Table 4.3).

**Life Tables**

Based on the life tables, CSB reared on *L. patersonia* (Table 4.8) and upland cotton (Table 4.9) both had $R_0$>1 and λ >1 (i.e., $R_0$=8.16 and λ=1.05, and $R_0$=10.17 and λ=1.03, *L. patersonia* and upland cotton, respectively), suggesting that the population of CSB resulting from this cohort would increase (Table 4.9). On *L. patersonia, g*eneration times were estimated to be around 43 days ($T_c$=43.08), larger than the observed

experiment generation time of 36.5 ± 5.18 days (Table 4.9). On upland cotton, generations were estimated to be around 89-90 days ($T_c$=89.48), larger than the observed experiment generation time of 58.5 ± 3.28 days (Table 4.9). This is because $T_c$ is the mean age of mothers at the birth of their offspring, weighted by reproductive output and survivorship. Expected population doubling times were between 14-15 days ($T_d$=14.22) and between 26-27 days ($T_d$=26.74) on *L. patersonia* and upland cotton, respectively (Table 4.9).

**Discussion**

The purpose of this study was to compare the effects of two different seed diets, *L. patersonia* and *G. hirsutum*, on the development, survivorship, reproduction, and potential population growth of CSB. Findings from the study indicate that CSB develops significantly faster, has greater survivorship, and lives longer as adults on *L. patersonia* seeds when compared to upland cotton seeds. Egg hatch time of 6.09 ± 0.27 days on upland cotton at a fluctuating 27°C is similar to values found in the literature, 6.92 days at 26 ± 1°C (Saveer et al. 2024) and 6.66 days at 25 ± 1°C (El-Taher et al. 2022). This suggests that the average egg hatch time for CSB of 6-7 days at temperatures ranging 25-27°C on cotton seeds is significantly slower than average egg hatch times for CSB on *L. patersonia* seeds (4-5 days). However, other studies using cotton seeds reported that CSB develop faster from first instar to adult, including 28.96 days at 26 ± 1°C (Saveer et al. 2024) and 25.2 days at 25 ± 1°C (El-Taher et al. 2022). These nymph to adult development times are more similar to the average development time on *L. patersonia* seeds which was 28.5 ± 1.21 days vs the 48.62 ± 2.06 days found on upland cotton in this

study. One explanation for this outcome could be the rearing conditions that CSB were exposed to, notably a 75% relative humidity (RH) and 16 h light/8 h dark cycle (Saveer et al. 2024) and the 70-75± 5% RH (El-Taher et al. 2022), compared to this study at 60-70% RH. Differences in RH and light and dark cycles have been demonstrated to affect insect development and egg production (Farnesi et al. 2018, Mądra-Bielewicz and Matuszewski 2025). Alternatively, the cotton cultivars used across these studies differed (El-Taher et al. [2022] used dry cotton bolls from different localities in Egypt); Saveer et al. [2024] used cotton seeds from Cotton Fiber Bioscience Research Unit, USDA-ARS, New Orleans, LA, USA) and this study used upland cotton, suggesting, that even within commercial cotton, the nutritional quality of seeds from different cultivars may differ and affect CSB fitness.

This study also looked at the effects that mating had on the longevity of adult CSB reared on *L. patersonia* and upland cotton seeds. Mated males and females on upland cotton survived longer than their unmated counterparts. This finding is consistent with other published studies. For example, on a diet of cotton seeds, mated females survived significantly longer than unmated females, (median survival of 25 days vs 9.5 days) and mated males survived significantly longer than unmated males (median survival 28 days vs 9.5) (Saveer et al. 2024). This may be due to upland cotton having a low amount of protein, delaying reproduction and diverting resources towards body maintenance (Collins et al. 2023). However, further research needs to be done to determine the protein content of this studies' upland cotton. Reduced longevity of mated males and females when fed on *L. patersonia* seeds suggests mating may reduce the

lifespan of males and females, this phenomenon has been described as the "cost of reproduction" (Williams 1966). Reduced lifespan following mating has been observed in other hemipterans, such as the green belly stink bug, *Dichelops furcatus*, (Hemiptera: Pentatomidae) (Cingolani et al. 2020), and the seed bug *Togo hemipterus* (Hemiptera: Lygaeidae) (Himuro and Fujisaki 2010).

Although fecundity and egg hatch rates were similar between *L. patersonia* and upland cotton seeds, these values were much lower than those in the literature. El-Taher et al. (2022) found that each female produced an average of 227.7 eggs and a 95% hatch rate (2022), whereas Saveer et al. (2024) reported that females laid an average of 151 eggs and had a 90% hatch rate. Again, these differences may be attributed to the conditions (relative humidity and light/dark cycle) that the insects were exposed to (Farnesi et al. 2018, Mądra-Bielewicz and Matuszewski 2025), variation in nutritional quality of seeds from different cotton cultivars (Huang et al. 2025), and genetic constitution of experimental CSB populations (Lainhart et al. 2015, Ross et al. 2019). Furthermore, these previous studies provide evidence that the CSB may be able to lay more eggs and have a higher percentage of eggs reach first instar than this study suggests (El-Taher et al. 2022, Saveer et al. 2024).

Despite this lower comparative fecundity, life table analyses suggest that CSB populations have the potential to increase quickly at 27ºC, the experimental temperature used in these studies. The doubling time of CSB was 14.22 days and 26.74 days on *L. patersonia* seeds and upland cotton, respectively. This shorter development time on *L. patersonia* is primarily due to when CSB begin laying eggs (week 4 vs week 7 on *L.*

*patersonia* and upland cotton, respectively), allowing for more generations per year that can more quickly amplify pest populations (Bale et al. 2002). Thus, CSB populations are expected to increase at a quicker rate and reach higher populations when fed on *L. patersonia* seeds. Additionally, the net reproductive rate ($R_0 = 8.16$ and $R_0 = 10.17$, on *L. patersonia* and upland cotton, respectively) and finite rate of increase ($\lambda = 1.05$ and $\lambda = 1.03$, on *L. patersonia* and upland cotton, respectively) were both greater than one regardless of diet, indicating that CSB population increase is probable under favorable laboratory conditions.

Mean development time from egg to adult to the first batch of eggs that hatched is considerably shorter than the calculated generation time ($T_c$) for both seeds (i.e., $36.5 \pm 1.58$ days vs $T_c = 43.08$ days and $58.5 \pm 3.28$ days vs $T_c = 89.48$ days, on *L. patersonia* and upland cotton, respectively). This is because the calculated generation time ($T_c$) is the mean age of mothers at the birth of their offspring, weighted by reproductive output and survivorship. Thus, fecundity and survivorship later in life of CSB causes the calculated generation time ($T_c$) to be greater than the observed development time from egg to adult to egg. The later fecundity can be seen in CSB from the presence of a dual-peak fertility pattern. The occurrence of a second fertility peak approximately one-month post-adulthood suggests that CSB females can maintain their reproductive output as they age. This could cause populations to rebound unexpectedly after an initial population surge, necessitating the need for regular population monitoring to inform control decision-making. However, on *L. patersonia*, as females enter their second month, fecundity significantly decreases and, in many instances, egg laying ceases entirely. On upland

cotton, females continue to produce eggs during the first two months of adulthood, reaching peak fecundities at the beginning of their third and fourth months. Multiple fecundity peaks has been observed in another hemipteran, *Pseudacysta perseae* (Hemiptera: Tingidae) (Dadlani et al. 2025).

Data from the observed stage-specific survivorship rates suggest that *L. patersonia* seeds provide better nutrients for survival than upland cotton. Before adulthood, CSB on *L. patersonia* seeds exhibit survivorship that is more representative of a Type III pattern, where there is relatively high mortality in the early stages (egg, first instar, and second instar), but limited mortality in later instars (fourth and fifth instar) (Clark et al. 2018). Conversely, CSB fed upland cotton seeds exhibited patterns of survivorship more consistent with a Type II pattern, with relatively constant mortality for eggs and the five nymphal stages (Clark et al. 2018). This has been observed in another hemipteran, *Halyomorpha halys* Stål (Hemiptera: Pentatomidae), where different diets affected development duration and survival of later instars more so than earlier instars (Acebes-Doria et al. 2016). Furthermore, El-Taher et al. (2022) observed a much higher survivorship rate, with 95% of nymphs surviving to adults at 25 ± 1°C. Thus, depending on laboratory conditions, CSB has potential for high survivorship rates from nymph to adulthood, which would predict rapid population growth is possible under highly favorable conditions.

In conclusion, this study provides the first insight into the performance of CSB on *L. patersonia* seeds. Overall, CSB developed faster, had higher survivorship, and could potentially reach population densities faster on *L. patersonia* seeds when compared to

CSB reared on upland cotton seeds. Because *L. patersonia* seeds are a very good host for CSB, surveys monitoring the distribution, spread, and phenology of CSB should sample *L. patersonia* seed pods if this host plant is available in areas of interest.

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**Fig. 4.1** Kaplan-Meier survival probability curves displaying estimated survival probability for CSB on a diet *Lagunaria patersonia* or upland cotton, *Gossypium hirsutum*, seeds. Eggs symbolized by 0.00, First instars symbolized by 1.00, Second instars symbolized by 2.00, Third instars symbolized by 3.00, Fourth instars symbolized by 4.00, Fifth instars symbolized by 5.00, Adults symbolized by 6.00, Adults that died symbolized by 7.00.

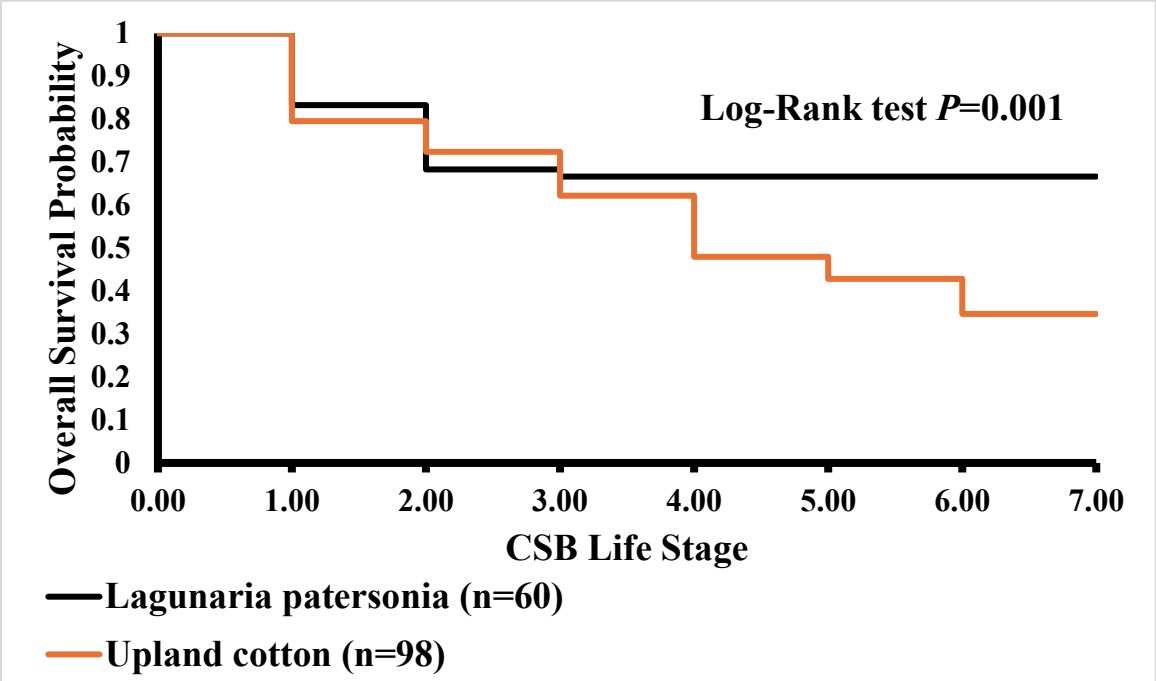

**Fig. 4.2** Number of daughter eggs produced weekly by each female ($m_x$) by survivorship curve ($l_x$) of the CSB fed on *Lagunaria patersonia* seeds.

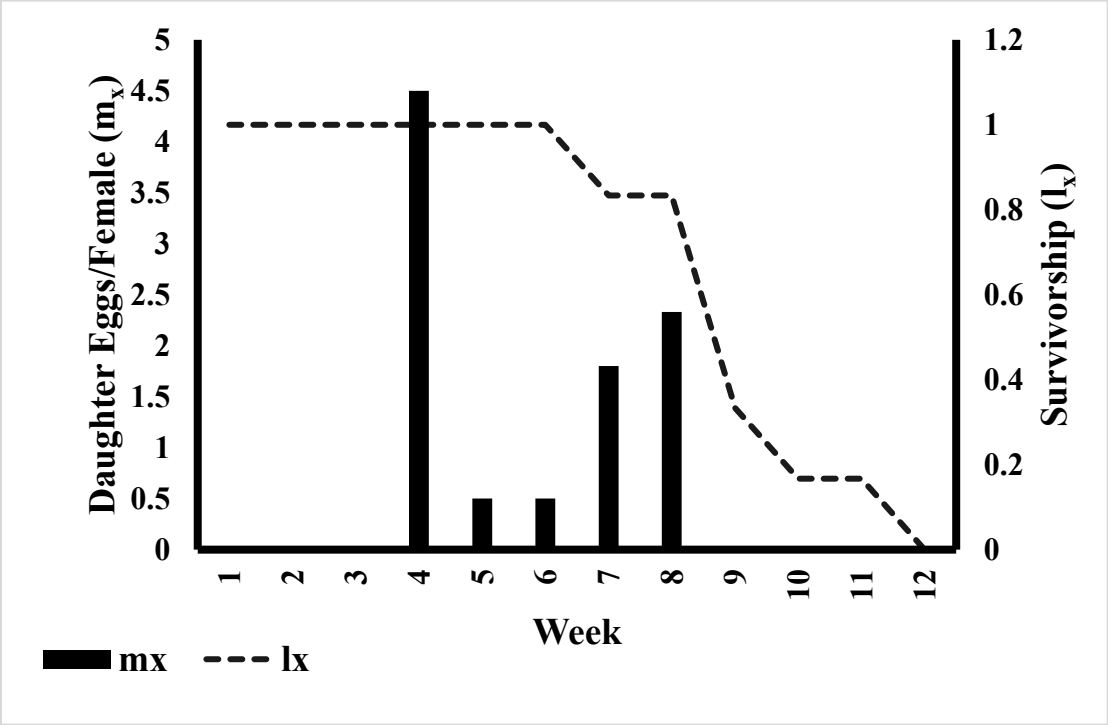

**Fig. 4.3** Number of daughter eggs produced weekly by each female ($m_x$) by survivorship curve ($l_x$) of the CSB fed on upland cotton, *Gossypium hirsutum*, seeds.

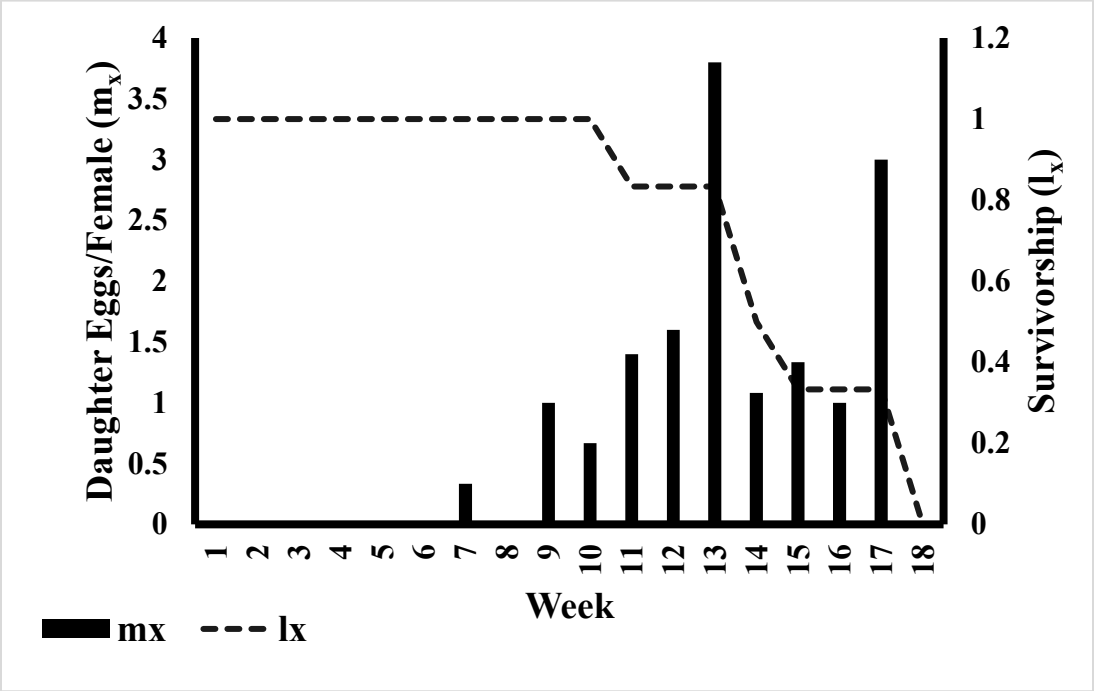

**Fig. 4.4** Interaction of mating status and diet among adult male CSB longevity.

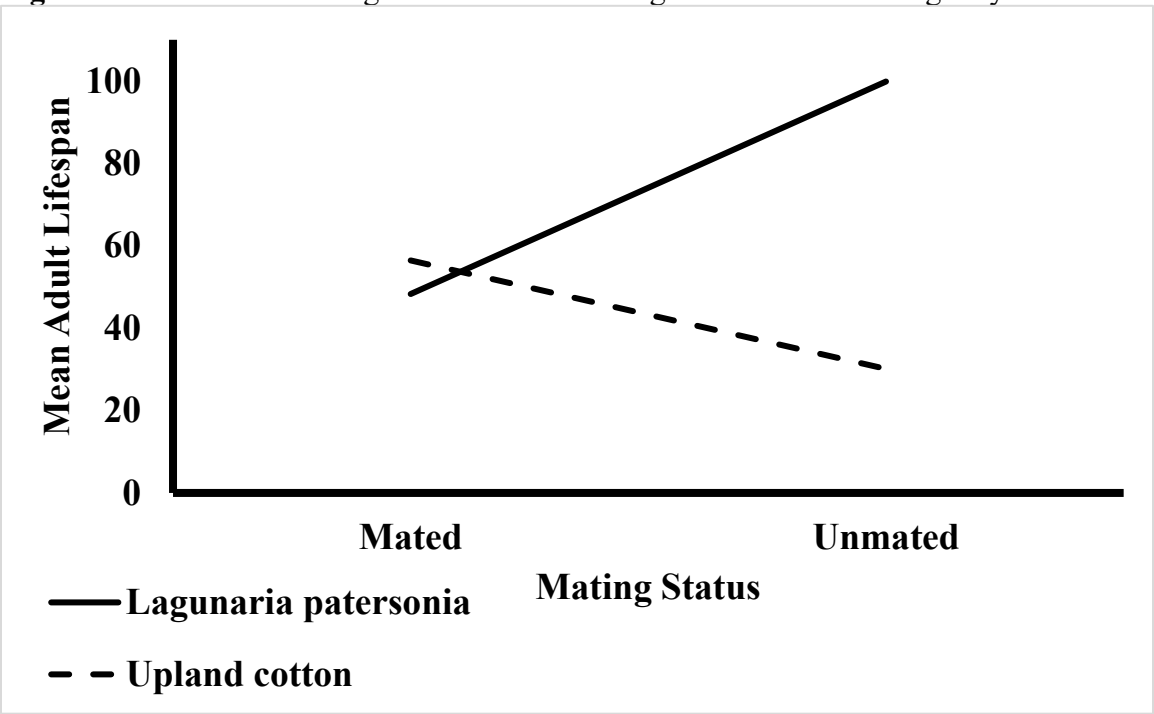

**Fig. 4.5** Interaction of mating status and diet among adult female CSB longevity.

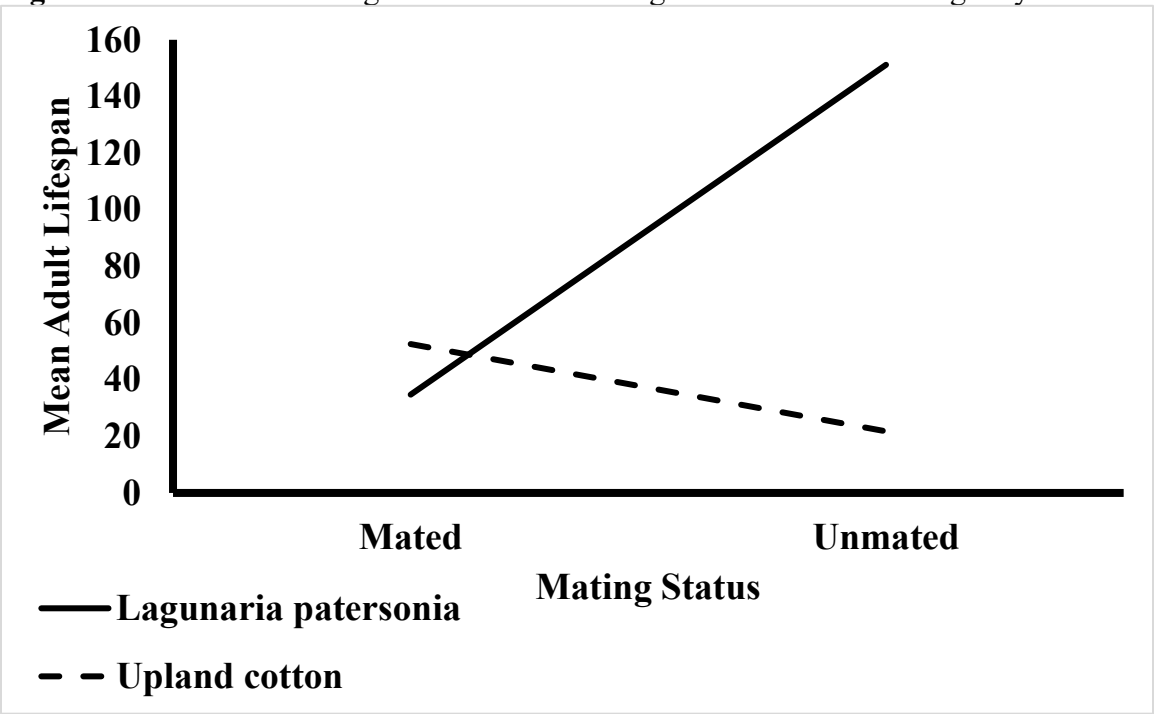

**Table 4.1** Stepwise hourly temperature ramping in temperature cabinets used for fluctuating temperature regimens for assessing the development and reproductive biology of CSB on *L. patersonia* or upland cotton.

| Hour | Mean Temp (°C) | Photoperiod |
|---|---|---|
| 100 | 21 | |
| 200 | 21 | |
| 300 | 20 | Dark |
| 400 | 19 | |
| 500 | 19 | |
| 600 | 19 | |
| 700 | 21 | |
| 800 | 25 | |
| 900 | 27 | |
| 1000 | 29 | |
| 1100 | 30 | |
| 1200 | 32 | Light |
| 1300 | 33 | |
| 1400 | 34 | |
| 1500 | 34 | |
| 1600 | 35 | |
| 1700 | 35 | |
| 1800 | 33 | |
| 1900 | 31 | |
| 2000 | 28 | |
| 2100 | 26 | Dark |
| 2200 | 25 | |
| 2300 | 24 | |
| 2400 | 23 | |
| Total Steps | 24 | |

**Table 4.2** Mean (± SE) development time for CSB eggs, first, second, third, fourth, and fifth instar nymphs, adult longevity (males and females combined), combined egg-to-adult development, and egg-to-adult-to-egg (i.e., [E-A-E] generation time) observed on a diet of *Lagunaria patersonia* or upland cotton, *Gossypium hirsutum*, seeds.

| | Average duration times (mean days ± SE) | | | | |
|---|---|---|---|---|---|
| CSB stage | *Lagunaria* | Upland cotton | *t* | *df* | *P* |
| Egg | 4.68 ± 0.08[a] | 6.09 ± 0.27[b] | -8.879*** | 113.403 | <0.001 |
| First instar | 5.78 ± 0.37[a] | 9.37 ± 0.97[b] | -5.774*** | 109.971 | <0.001 |
| Second | 5± 0.54[a] | 7.98 ± 1.06[b] | -3.676*** | 98 | <0.001 |
| Third instar | 3.7 ± 0.31[a] | 8.51 ± 1.26[b] | -6.551*** | 65.127 | <0.001 |
| Fourth | 3.7 ± 0.27[a] | 7.79 ± 1.21[b] | -5.991*** | 57.292 | <0.001 |
| Fifth instar | 5.78 ± 0.73[a] | 11.41 ± 1.36[b] | -5.466*** | 72 | <0.001 |
| Adult | 96.25± 10.24[a] | 40.09 ± 4.10[b] | 5.015*** | 51.340 | <0.001 |
| Egg-adult | 28.5 ± 1.21[a] | 48.62 ± 2.06[b] | -8.192*** | 53.687 | <0.001 |
| E-A-E | 36.5 ± 5.18[a] | 58.5 ± 3.28[b] | -3.487** | 11 | <0.01 |

Means within columns followed by the same letter are not significantly different ($p > 0.05$) as determined by t-tests. *$p$<0.05, **$p$<.01, ***p<.001.

**Table 4.3** Mean fecundity rates (i.e., egg hatch), represented by the mean number CSB eggs laid (± SE) and mean number of CSB eggs that hatched (± SE) a diet of *Lagunaria patersonia* or upland cotton, *Gossypium hirsutum*, seeds.

| Food Source | Mean Number of Eggs Laid by Mating Pairs | Mean Number of Eggs Hatched from Mating Pairs |
|---|---|---|
| *Lagunaria patersonia* | $27.50 \pm 12.68^{a}$ [6] (165) | $22.83 \pm 10.86^{a}$ [6]{137} |
| Upland cotton | $29.88 \pm 9.74^{a}$ [8] (239) | $22.88 \pm 7.72^{a}$ [8]{191} |
| *t* | -0.151 | -0.081 |
| *df* | 12 | 12 |
| *P* | 0.882 | 0.937 |

Means within columns followed by the same letter are not significantly different ($p > 0.05$) as determined by t-tests. The number in [square brackets] denotes the quantity of evaluated female CSB, the number in (round brackets) indicates the total count of eggs laid, and the number in {parentheses} represents the total number of resulting CSB eggs that hatched at each temperature profile.

**Table 4.4** Two-way ANOVA of adult male and female CSB longevity to mating status and diet.

| | | | *df* | | |
|---|---|---|---|---|---|
| | | *F* | Numerator | Denominator | *P* |
| Male | Mating status | 1.392 | 1 | 36 | 0.246 |
| | Seed type | 8.287 | 1 | 36 | 0.007 |
| | Interaction | 13.259 | 1 | 36 | <0.001 |
| Female | Mating status | 4.792 | 1 | 26 | 0.038 |
| | Seed type | 8.134 | 1 | 26 | 0.008 |
| | Interaction | 14.176 | 1 | 26 | <0.001 |

**Table 4.5** Mean (± SE) adult survival times in days for mated or unmated male CSB on a diet of *Lagunaria patersonia* or upland cotton, *Gossypium hirsutum*, seeds.

| | Average duration times (mean days ± SE) | |
|---|---|---|
| | Mated | Unmated |
| *L. patersonia* | 48.29 ± 9.52 | 99.87 ± 10.99 |
| Upland cotton | 56.44 ± 7.63 | 30.11 ± 4.81 |

**Table 4.6** Mean (± SE) adult survival times in days for mated or unmated female CSB on a diet of *Lagunaria patersonia* or upland cotton, *Gossypium hirsutum*, seeds.

| | Average duration times (mean days ± SE) | |
|---|---|---|
| | Mated | Unmated |
| *L. patersonia* | 34.71 ± 5.13 | 151.17 ± 20.20 |
| Upland cotton | 52.57 ± 4.69 | 21.75 ± 14.02 |

**Table 4.7** Abridged survivorship and fecundity life table for CSB mating pairs on a diet of *Lagunaria patersonia* seeds.

| Age, x (days) | Life stage | $l_x$ | $m_x$ | $l_xm_x$ | $xl_xm_x$ |
|---|---|---|---|---|---|
| 0 | Eggs | 1 | 0 | 0 | 0 |
| 5 | First Instar | 1 | 0 | 0 | 0 |
| 11 | Second Instar | 1 | 0 | 0 | 0 |
| 16 | Third Instar | 1 | 0 | 0 | 0 |
| 21 | Fourth Instar | 1 | 0 | 0 | 0 |
| 26 | Fifth Instar | 1 | 0 | 0 | 0 |
| 32 | Adult | 1 | 0 | 0 | 0 |
| 35 | Adult | 1 | 5 | 5 | 175 |
| 48 | Adult | 1 | 0.5 | 0.5 | 24 |
| 56 | Adult | 0.833 | 1.8 | 1.500 | 83.997 |
| 59 | Adult | 0.5 | 2.33 | 1.165 | 68.735 |
| 68 | Adult | 0.167 | 0 | 0 | 0 |
| 81 | Adult | 0 | 0 | 0 | 0 |

$l_x$ is age-specific survivorship and $m_x$ is the mean number of daughter eggs per female alive at age x.

**Table 4.8** Abridged survivorship and fecundity life table for CSB mating pairs on a diet of upland cotton, *Gossypium hirsutum*, seeds.

| Age, x (days) | Life stage | $l_x$ | $m_x$ | $l_xm_x$ | $xl_xm_x$ |
|---|---|---|---|---|---|
| 0 | Eggs | 1 | 0 | 0 | 0 |
| 6 | First Instar | 1 | 0 | 0 | 0 |
| 16 | Second Instar | 1 | 0 | 0 | 0 |
| 24 | Third Instar | 1 | 0 | 0 | 0 |
| 32 | Fourth Instar | 1 | 0 | 0 | 0 |
| 40 | Fifth Instar | 1 | 0 | 0 | 0 |
| 52 | Adult | 1 | 0 | 0 | 0 |
| 57 | Adult | 1 | 0.5 | 0.5 | 28.5 |
| 69 | Adult | 1 | 1.5 | 1.5 | 103.5 |
| 75 | Adult | 0.833 | 1.4 | 1.167 | 87.450 |
| 93 | Adult | 0.833 | 5.4 | 4.450 | 418.483 |
| 96 | Adult | 0.667 | 0.75 | 0.500 | 48.002 |
| 101 | Adult | 0.5 | 0.667 | 0.333 | 33.668 |
| 114 | Adult | 0.333 | 5 | 1.667 | 189.981 |
| 125 | Adult | 0 | 0 | 0 | 0 |

$l_x$ is age-specific survivorship and $m_x$ is the mean number of daughter eggs per female alive at age x.

**Table 4.9** Demographic statistics derived from $l_x m_x$ life tables for CSB reared on *Laguaria patersonia* or upland cotton, *Gossypium hirsutum*, seeds.

| Food source | Net reproductive rate ($R_0$) | Generation time ($T_c$) | Intrinsic rate of natural increase ($r_m$) | Finite rate of increase ($\lambda$) | Doubling time ($T_d$) |
|---|---|---|---|---|---|
| *Lagunaria patersonia* | 8.16 | 43.08 | 0.05 | 1.05 | 14.22 |
| Upland cotton | 10.17 | 89.48 | 0.03 | 1.03 | 26.74 |