Captive Rearing Success and Critical Thermal Maxima of *Bombus griseocollis* (Hymenoptera: Apidae): A Candidate for Commercialization?

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Abstract

Commercialized bumble bees (*Bombus*) are primary pollinators of several crops within open field and greenhouse settings. However, the common eastern bumble bee (*Bombus impatiens* Cresson, 1863) is the only species widely available for purchase in North America. As an eastern species, concerns have been expressed over their transportation outside of their native range. Therefore, there is a need to identify regionally appropriate candidates for commercial crop pollination services, especially in the western U.S.A. In this study, we evaluated the commercialization potential of brown-belted bumble bees (*Bombus griseocollis* De Geer, 1773), a broadly distributed species throughout the U.S.A., by assessing nest initiation and establishment rates of colonies produced from wild-caught gynes, creating a timeline of colony development, and identifying lab-reared workers’ critical thermal maxima (CT Max) and lethal temperature (ecological death). From 2019 to 2021, 70.6% of the wild-caught *B. griseocollis* gynes produced brood in a laboratory setting. Of these successfully initiated nests, 74.8% successfully established a nest (produced a worker), providing guidance for future rearing efforts. Additionally, lab-reared workers produced from wild-caught *B. griseocollis* gynes had an average CT Max of 43.5°C and an average lethal temperature of 46.4°C, suggesting *B. griseocollis* can withstand temperatures well above those commonly found in open field and greenhouse settings. Overall, *B. griseocollis* should continue to be evaluated for commercial purposes throughout the U.S.A.

**Key words:** bumble bee, nest initiation, nesting success, CT MAX, commercialization

Bumble bees (Hymenoptera: Apidae: Bombus) are effective pollinators of cultivated and wild plant communities (Velthuis and van Doorn 2006). The use of *Bombus* species in open field and greenhouse production is increasing around the world because commercialized pollinators reduce the need for labor-intensive hand pollination practices and chemical hormones (Velthuis and van Doorn 2006; Williams et al. 2014), and due to the growing industry of crop production in protected environments (Despommier 2011, Lawrence et al. 2016, McCartney and Lefsrud 2018). However, of the 265 described *Bombus* species worldwide, only a few species have been commercialized to provide pollination services. As a result, the few commercialized species are purchased and intentionally released often well outside of their native ranges (Goulson 2010, Strange 2015). This human-mediated movement has led to the unrestricted release of non-native *Bombus* species into novel ecosystems (Ruz 2002, Morales 2007, Yokoyama and Inoue 2010, Looney et al. 2019), subsequently causing negative impacts on the local environment (Tsuchida et al. 2010).

The western bumble bee (*Bombus occidentalis* Greene, 1858) was the primary commercialized bumble bee species in western North America until the late 1990s. While once common throughout the western U.S.A. (Koch and Strange 2009, Cameron et al. 2011, Sheffield et al. 2016), *B. occidentalis* has been assessed as vulnerable by the International Union for the Conservation of Nature (IUCN) and is currently being considered for listing by the U.S. Fish and Wildlife Service (USFWS) under the Endangered Species Act (ESA) (Hatfield et al. 2015, Graves et al. 2020). Additionally, this species is more susceptible to infection by *Vairimorpha bombi* (previously *Nosema bombi*) (Cameron et al. 2011) than many other *Bombus* species (Fries et al. 2001, Whittington and Winston 2004, Velthuis 2006).
and van Doorn 2006, Koch and Strange 2012). These high infection rates harm colony development and increase the potential of pathogen introductions to wild Bombus species, which led to the abandonment of B. occidentalis as a commercially viable species in the late 1990s (Flanders et al. 2003, Whittington and Winston 2004, Velthuis and van Doorn 2006). As a result, production shifted to B. impatiens (Whittington and Winston 2004, Velthuis and van Doorn 2006).

Bombus impatiens has been the only species widely available for purchase in the U.S.A. and Canada since the early 2000s (Whittington and Winston 2004, Velthuis and van Doorn 2006, Strange 2010). However, as an eastern species, concerns have been expressed about the potential of this species to expand its range, compete with native species, disrupt plant-pollinator interactions, cause genetic deterioration due to interspecific mating, and introduce and increase pathogenic loads in habitats surrounding release sites, specifically west of the Rocky Mountains (Whittington and Winston 2003, 2004; Colla et al. 2006; Velthuis and van Doorn 2006; Otterstatter and Thompson 2008; Vilsac et al. 2012; Looney et al. 2019). These concerns were underscored when B. impatiens was imported to British Columbia, Canada for greenhouse pollination in the early 2000s, subsequently became established in the wild (Ratti and Colla 2010), and expanded throughout the Pacific Northwest (Looney et al. 2019). As a result, several states have restrictions on importing non-native Bombus species for pollination. For example, B. impatiens is restricted to greenhouse use in California (open field release is prohibited) to reduce pathogen introductions and a queen-excluder must be used to prevent accidental bumble bee introductions (California Food and Agriculture 1973; Velthuis and van Doorn 2006; Strange 2010, 2015). Further, it is illegal to import B. impatiens into Oregon for open field or greenhouse pollination (Strange 2010, Oregon Department of Agriculture 2017). In response to these concerns, the yellow-faced bumble bee (Bombus vosnesenskii Radoszkowski, 1862) and Hunt’s bumble bee (Bombus huntii Greene, 1860) became available for commercial purposes in North America. Bombus vosnesenskii is available for purchase throughout its native range in California, Oregon, and Washington, U.S.A. (Koppert 2022a), and Bombus huntii is being produced and distributed in western Canada (Biobest 2022). Therefore, there is a need to identify regionally appropriate candidates for commercial crop pollination, especially throughout the non-coastal states in the western U.S.A.

Several facets must be considered when developing a pollinator species for commercialization, including captive rearing success, mating success, diapause conditions in a controlled laboratory setting, effective pollination of target crop(s) within open field and greenhouse settings, life history traits, and pathogen and pest resistance (Macfarlane et al. 1994, Strange 2010). Despite previous work on captive rearing, nesting initiation and establishment rates can be low when rearing colonies from wild-caught gynes (Kwon et al. 2006, Strange 2010). Additionally, given biological differences among species, rearing methods should be tested on individual species to maximize rearing success (Kwon et al. 2006, Yoneda 2008, Strange 2010). Maximizing rearing success and establishing year-round production of offspring is necessary to provide pollination services and to create sources of reproductive drones and gynes (Velthuis and van Doorn 2006). Further, identifying additional species outside of the subgenera commonly used for commercialization (Pyrobombus) can increase the sustainable production of colonies in the face of pathogen outbreaks.

One potential candidate species for commercialization is Bombus (Callihamantobombus) griseocollis. Bombus griseocollis is a broadly distributed species, occurring throughout much of U.S.A. and southern Canada (Fig. 1; Koch et al. 2012, GBIF 2022). This makes them a good candidate to be released in eastern and western environments for crop pollination, with the exception of some of the southwestern U.S.A. (i.e., Arizona, Nevada, and Southern California) where they are not native to the region, thus increasing the potential to make one species
available as a commercial source in a much wider native range than is currently possible. Bombus griseocollis survives well throughout a range of habitat types including open farmland and fields, urban parks and gardens, and wetlands (Koch et al. 2012, Williams et al. 2014). Additionally, given their wide spatial distribution, they are exposed to increased climate variability, which may allow them to be less sensitive to climatic disturbances (Kingsolver et al. 2013). In the face of ongoing and projected climate change, species with a wider thermal range may have a competitive advantage over other species, which is particularly important for open field released pollinators (IPCC 2014, Verble-Pearson et al. 2015, Soroye et al. 2020).

In this study, we evaluated the commercialization potential of B. griseocollis by assessing nest initiation and establishment rates of colonies produced from wild-caught gynes, creating a timeline of colony development in laboratory settings, and identifying lab-reared workers’ critical thermal maxima (CTmax) and lethal temperature (ecological death). Results from this study establish systematic nesting biology knowledge on B. griseocollis should the species continue to be evaluated for commercial purposes throughout the U.S.A.

**Materials and Methods**

**Bombus griseocollis Rearing**

Bombus griseocollis gynes were net collected as they emerged from winter dormancy in northern Utah from May to June 2019–2021. A total of 214 gynes were captured across the three years: 80 in 2019, 82 in 2020, and 52 in 2021. The captured gynes were removed from the net, transferred into individual 7 × 3 cm plastic collection vials (W. W. Grainger Inc., Lake Forest, IL) with ventilation holes, and stored in an insulated container with ice packs until they could be transported to the United States Department of Agriculture, Agricultural Research Service, Pollinating Insect – Biology, Management, and Systematics Research Unit in Logan, UT. Once at the laboratory, the captured gynes were placed in individual plastic rearing chambers (15 × 15 × 10 cm; Biobest Canada, Leamington, Ontario) in a rearing room that was maintained between 26 and 30°C and 60% relative humidity in complete darkness. Each gyne was initially provided a 2-g provision of commercial pollen (Fresh Bee Gathered Pollen: Wildflower Varietal, Moon Shine Trading Company, Z Specialty Food, Woodland, CA) mixed with artificial nectar, and a wicking feeder filled with artificial nectar. As offspring were produced, each colony was fed pollen provisions and artificial nectar ad libitum (Strange 2010). Once the queen produced five workers, the colony was transferred to a larger plastic colony box (29 × 22 × 13 cm; Biobest Canada, Leamington, Ontario). General set up and preparation of pollen provisions and artificial nectar (equal parts cane sugar and invert syrup) followed Smith et al. (2020).

Colonies were assessed every other day over the course of their development in the rearing room under red light to avoid disturbing and stressing the colonies (Fig. 2). Days to first brood, days to first worker, days to five workers, and total emerged offspring (workers, drones, and gynes) were documented for each colony to provide information on nest initiation and establishment, and to create a timeline of colony development in a controlled laboratory setting. Nest initiation was defined as the ability of a queen to produce brood. Nest establishment was defined as the ability of a queen to rear one adult female offspring (worker) from brood (Strange 2010). An analysis of variance (ANOVA) was used to

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**Table 1.** Rearing success of B. griseocollis as defined by the production of brood (nest initiation) and emergence of one worker (nest establishment) from 2019 to 2021. Colony development of B. griseocollis within captivity as defined by days to nest initiation ± SD, days to nest establishment ± SD, and days to five workers from 2019 to 2021

| Year | Successful Nest Initiation | Successful Nest Establishment | Days to First Brood | Days to First Worker | Days to Five Workers |
|------|-----------------------------|------------------------------|---------------------|----------------------|---------------------|
| 2019 | 64/80                       | 53/64                        | 5 ± 5.6             | 30 ± 10.6            | 42.9 ± 13.8         |
| 2020 | 50/82                       | 34/50                        | 10 ± 8.9            | 40 ± 14.6            | 53 ± 22.7           |
| 2021 | 37/52                       | 26/37                        | 9 ± 4               | 38.2 ± 9.9           | 46.9 ± 11.3         |
| Combined | 151/214                   | 113/151                     | 7.6 ± 7             | 34.8 ± 12.6          | 46.5 ± 16.6         |

**Table 2.** Average and maximum number of emerged offspring ± SD within B. griseocollis colonies from 2019 to 2021

| Year | Average Offspring Emerged | Maximum Offspring Emerged |
|------|---------------------------|---------------------------|
| 2019 | 4.7 ± 2.1                 | 9                         |
| 2020 | 8.4 ± 9.9                 | 46                        |
| 2021 | 9 ± 7.5                   | 25                        |
| Combined | 6.8 ± 6.9                | 46                        |
determine the differences in nest establishment among years ($p < 0.05$).

**Bombus griseocollis Thermal Tolerance**

In 2020, workers from lab-reared colonies were used in thermal tolerance trials to measure the critical thermal maximum ($CT_{max}$) of *B. griseocollis*, which is the temperature at which organisms experience loss of coordinated muscle function (Terblanche et al. 2007). Using a modified approach from Barnes et al. (2019) and Verble-Pearson et al. (2015), forty *B. griseocollis* workers from fifteen colonies were placed within individual stoppered glass vials (9.5 x 2.5 cm; Berlin Packaging, Chicago, IL), and submerged approximately nine cm into a water bath with an initial temperature of 25.44 ± 3.65°C for five minutes to allow the worker to acclimate to the chamber. Air holes were provided at the top of the glass vials to allow for respiratory gas exchange. The water bath was established using a hot plate stirrer (Fisher Scientific 1152049SH) and a beaker filled with 800 mL of water. A small stir bar was included at the bottom of the beaker and the hot plate was set to the lowest stir setting to promote even, consistent heating of the water. One empty vial was submerged with each trial to verify that the internal vial temperature was consistent with the water temperature. Temperatures were determined using a Twindc K-type thermocouple and a HOBO 4-channel thermocouple data logger (#UX120-014M, Onset Computer Corporation, Bourne, MA). The temperature of the water bath was increased at a constant rate until the critical thermal maximum ($CT_{max}$) and lethal temperature (ecological death) were determined for all workers. The rate of heating for the empty vial (0.45 ± 0.04°C per minute) was not significantly different from the rate at which the water was heated (0.45 ± 0.05°C per minute) ($t = −0.35$, df = 34, $p = 0.72$), indicating that the internal vial temperature was consistent with the water temperature.

As temperatures increased, bees became more agitated before losing motor function, causing them to fall onto their backs and experience leg spasms (Oyen et al. 2016). The temperature at the initial onset of spasms for each *B. griseocollis* was recorded as the $CT_{max}$ (Lutterschmidt and Hutchison 1997, Hanna and Cobb 2007, Oyen et al. 2016, Barnes et al. 2019, Burdine and McCluney 2019). Temperatures continued to increase at a constant rate until complete leg curling occurred, indicating the lethal temperature (ecological death) of the individual (Burdine and McCluney 2019). To reduce inconsistencies, the same observer was used to determine the $CT_{max}$ and lethal temperature of each *B. griseocollis* worker in real time. After death, the distance between wing-attachment points on the thorax (inter tegular distance, ITD) of each bee was measured using a Keyence digital microscope VHX-500F (Keyence Corp. Itasca, IL) to determine the body size of each worker. Additionally, each bee was dried at 60°C for 24 hours and weighed to the nearest microgram to determine the dry mass of each worker.

A Pearson’s correlation test was used to determine if ITD and dry body mass, proxies for body size, were correlated. ITD and dry body mass were correlated ($r > 0.79$, $n = 40$); therefore, only ITD was used in the following analyses to reduce redundancy. A Pearson’s correlation test was also used to determine if correlations existed between the $CT_{max}$ and lethal temperature. Further, linear regressions were used to determine the effects of ITD on $CT_{max}$ and lethal temperature for *B. griseocollis* workers. All conditions for the linear regression were met.

![Fig. 3. Range of $CT_{max}$ and lethal temperatures (°C) for *B. griseocollis* workers from 2020.](image)

![Fig. 4. Linear regression describing the relationships between intertegular distance (mm) and (A) $CT_{max}$ and (B) lethal temperatures (°C) for *B. griseocollis* workers from 2020. Shaded regions represent the 95% confidence interval.](image)
(linearity, normality, independence, and homoscedasticity). Statistics were performed using base functions in R version 4.0.3 (R Core Team 2020).

**Results**

*Bombus griseocollis* Rearing

From 2019 to 2021, 70.6% of the wild-caught *Bombus griseocollis* gynes produced brood (the criterion for nest initiation) in a laboratory setting 7.6 ± 7 days after the gynae collection date. Of the 151 successfully initiated nests, 74.8% produced at least one worker (the minimum criterion of successful nest establishment) 34.8 ± 12.6 days after nest initiation. There was no significant difference in nest establishment among years ($F_{1,151} = 18.07, p = 0.147$). Further, 70 of the nests had five workers emerge 46.5 ± 16.6 days after nest initiation (Table 1). On average, colonies produced 6.8 ± 6.9 offspring (workers, drones, and gynes) over the course of colony development, with a single queen producing a maximum of 46 offspring (Table 2).

*Bombus griseocollis* Thermal Tolerance

We found a positive correlation between the CT$_{Max}$ and lethal temperature for *B. griseocollis* ($r > 0.5, n = 40$). The average CT$_{Max}$ was 43.5 ± 0.49°C, while the average lethal temperature was 46.4 ± 0.27°C for *B. griseocollis* workers (Fig. 3). The mean difference of 2.89 ± 0.4°C between CT$_{Max}$ and lethal temperatures suggests a short time period (approximately 6 minutes at a heating rate of 0.45°C) between the loss of critical motor function and death. Further, we found that CT$_{Max}$ and lethal temperatures increased with lower ITD (Fig. 4). For every 1 mm gained in ITD, the CT$_{Max}$ decreased by 0.85°C ($F_{1,38} = 8.55, p = 0.005$) and the lethal temperature decreased by 0.61°C ($F_{1,38} = 8.55, p = 0.005$).

**Discussion**

*Bombus griseocollis* are broadly distributed throughout the U.S.A. and southern Canada (Koch et al. 2012). If commercially produced, this broad distribution reduces the risk of *B. griseocollis* escaping from greenhouse settings and accidentally establishing outside their native range and may decrease the likelihood of adverse effects on local ecosystems. Additionally, their ubiquity exposes them to a range of habitat types and climates (Koch et al. 2012, Williams et al. 2014). This may allow them to be less sensitive to habitat and climatic disturbances, and may therefore, be able to tolerate higher temperatures when placed in open field settings.

We found that lab-reared workers produced from wild-caught *B. griseocollis* gynes in Utah had an average CT$_{Max}$ of 43.5 ± 0.49°C and an average lethal temperature of 46.4 ± 0.27°C. Previous studies have identified that there is little variation in CT$_{Max}$ across geographic thermal gradients (Sunday et al. 2012, Pimsler et al. 2020), which is supported by the similar CT$_{Max}$ (45.31°C) documented in wild-caught *B. griseocollis* in North Carolina (Hamblin et al. 2017). Additionally, CT$_{Max}$ and lethal temperatures increased with lower ITD, suggesting smaller individuals had a higher CT$_{Max}$ and lethal temperature than their larger counterparts. Smaller organisms may dissipate heat better through more rapid thermoregulation strategies, such as thoracic or evaporative cooling and wing fanning, but may be more prone to desiccation (Heinrich 1976, Willmer and Stone 1997, Gardner et al. 2011, Burdine and McClane 2019). However, it is important to note that ambient temperatures rarely reach 40°C throughout much of the contiguous U.S.A., reducing the risk of *B. griseocollis* exposure to CT$_{Max}$ and lethal temperatures within well-shaded and well-ventilated colonies in open field settings. Further, greenhouses are typically held between 23.8 and 29.4°C, which is well below the CT$_{Max}$ and lethal temperature of *B. griseocollis*. Additionally, the CT$_{Max}$ of *B. impatiens*, the widely available commercial *Bombus* species in the U.S.A., was also tested following the same methodology outlined in this study. *Bombus impatiens* workers from commercially produced colonies had an average CT$_{Max}$ of 44.2 ± 0.46°C and a lethal temperature of 45.4 ± 0.36°C (Supp. Fig. 1 [online only]). Given that the CT$_{Max}$ and lethal temperatures for *B. griseocollis* and *B. impatiens* are within 1°C of each other, this suggests that the upper thermal tolerance of *B. griseocollis* may be conducive to commercialization. Next steps should involve evaluating the critical thermal minima (CT$_{min}$) of *B. griseocollis*, which is influenced by regional and local temperatures (Pimsler et al. 2020). Determining the CT$_{Min}$ would also allow the thermal tolerance range (CT$_{Max}$ to CT$_{Min}$) to be calculated, which is expected to be broad given the wide thermal tolerance of *B. griseocollis*.

Evaluating the commercialization potential of *B. griseocollis* identified that they were successfully reared within a laboratory setting from wild-caught gynes in Logan, UT, with high nest initiation and establishment rates (70.6 and 74.8%, respectively). A timeline of colony development identified an average of 7.6 ± 7 days to nest initiation and 34.8 ± 12.6 days to nest establishment. This research provides a set of techniques and expectations on rearing success that can be considered by other researchers rearing bumble bees. Additionally, management practices can be optimized using this information to enhance the production of workers and future reproductive drones and gynes, which are needed to establish year-round production of colonies in laboratory settings (Velthuis and Van Doorn 2006, Strange 2015). Next steps should evaluate colony biology (i.e., rearing success, colony development, CT$_{Min}$) throughout the U.S.A., as local adaptations may differ spatially. Additional research is also needed on other aspects of *B. griseocollis* production in captivity. For example, previous research has identified low prevalence of pathogen infections (e.g., Varimorpha, Crithidia, and Apicystis) in wild *B. griseocollis* populations, rarely above 13% (Kissinger et al. 2011, Cordes et al. 2012, Malfi and Roulston 2014, Averill et al. 2021, L. B. Whiteman, personal communication), which may support the sustainable production of colonies in the face of pathogen outbreaks. Therefore, *B. griseocollis* susceptibility to pathogen infections (e.g., Varimorpha bombi, Crithidia bombi, and Apicystis bombi) in controlled environments should be evaluated, as high infection rates reduce colony development and increase transmission to wild *Bombus* species, causing adverse effects on the local environment.

Mating success and diapause conditions in a controlled laboratory setting should also be considered when evaluating *B. griseocollis* for commercialization (Macfarlane et al. 1994, Strange 2010). Mating of bumble bees in controlled laboratory settings is both challenging and necessary to establish year-round production of reproductive drones and gynes. Therefore, management strategies for captive rearing conditions need to be obtained, including information on optimal age for mating, mating behavior, number of preferred mates, mating duration, inbreeding, and environmental conditions (Tasei et al. 1997, Sauter and Brown 2001, Brown and Baer 2005, Trenore et al. 2021). The duration of gynae diapause also needs to be determined along with establishing optimal diapause conditions for captive rearing. Additional research is required to determine the success rates of overwintering *B. griseocollis* gynes in cold storage or subjecting gynes to CO$_2$ narcosis, causing them to bypass diapause and begin nest initiation (Roseler 1985, Beckman and Van Stratum 2001, Gosterit and Gurel 2009, Amsale and Grozinger 2017).
Both methodologies can impact colony life cycle and development, such as the number and timing of producing reproductive drones or gynes, so determining the best method for establishing year-round B. griseocollis production is essential (Treonore et al. 2021). Although we established high rearing success of B. griseocollis in the lab, previous research has identified challenges with nest initiation following diapause or CO₂ narcosis. For example, some colonies require a social stimulus, pleometrosis, to initiate brood production (Treonore et al. 2021). Therefore, B. griseocollis should be evaluated to determine if pleometrosis increases nest initiation and establishment rates, which may help enhance the production of workers and reproductive drones and gynes.

Future research should also determine the efficacy of B. griseocollis pollination efforts within diverse open field and greenhouse settings (Strange 2015). Our lab-reared B. griseocollis colonies were small, with colony sizes never exceeding 50 workers. This differs from B. impatiens, which are sold with 100–125 workers per colony and can contain between 300–400 workers at maturity (Cnaani et al. 2002, Koppert 2022b). Commercial B. impatiens colonies are used for pollinating a wide range of crops grown on surfaces larger than 2 km² that produce 25–35 flowers per m² every week (Koppert 2022c). Given their small colony size, B. griseocollis may not be effective at pollinating crops in greenhouse settings unless several colonies are used or overall colony sizes can be increased in controlled/commercial settings. However, low B. griseocollis densities could be beneficial in small greenhouses to avoid flower damage from excessive pollination and over-visitation (Strange 2015). Research into the stocking densities of B. griseocollis is needed to determine the optimal number of bees for pollinating specific crops in different sized greenhouses (Strange 2015). Crops should also be selected based on the phenological overlap between crop pollination and worker emergence periods.

In summary, B. griseocollis exhibit traits that are conducive to commercialization. As a broadly distributed species, they present lower risk of causing adverse effects to the ecosystems in which they are placed. Further, we demonstrated high success rates when rearing B. griseocollis from wild caught gynes in Utah and identified a time-line for colony development within a laboratory setting. Overall, B. griseocollis should continue to be evaluated for commercial purposes throughout the U.S.A.

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Data Availability

The data and code supporting the findings of this study are openly available on Zenodo at https://doi.org/10.5281/zenodo.6364010.

Conflict of Interest

The authors declare that they have no known competing financial interests or personal connections that could have raised the question of bias in the work reported or in the conclusions, implications, or opinions stated.

Author Contributions

MEC: Conceptualization; Methodology; Data curation; Formal analysis; Investigation; Software; Validation; Visualization; Writing – original draft. LRS: Funding acquisition; Project administration; Resources; Supervision; Writing – review & editing. JBUK: Resources; Supervision; Writing – review & editing. JPS: Resources; Supervision; Writing – review & editing. CLB: Conceptualization, Methodology, Validation, Writing – Review & Editing. RAR: Funding acquisition; Project administration; Resources; Supervision; Writing – review & editing.

Supplementary Data

Supplementary data are available at Journal of Insect Science online.

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