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Leptinotarsa texana Schaeffer and *Gargaphia arizonica* Drake & Carvalho as potential biocontrol agents for the noxious weed *Solanum elaeagnifolium* Cav

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ABSTRACT

Silverleaf nightshade (*Solanum elaeagnifolium* Cav.; SLN) is a perennial forb native to the southern United States, Mexico and South America that has become a serious agricultural weed across the world. Biological control has emerged as a significant alternative for the management of (SLN) due to the challenges and high costs associated with chemical and mechanical controls. In this study, we used a combination of field and laboratory studies to (1) explore the fundamental and realized host ranges of two North American insects, *Leptinotarsa texana* Schaeffer and *Gargaphia arizonica* Drake & Carvalho and (2) assess their suitability as potential biological control agents for SLN. Field trials over 2 years with SLN planted alongside close relatives, eggplant (*Solanum melongena* L.) and potato (*Solanum tuberosum* L.), showed that *L. texana* fed on both eggplant and SLN causing similar leaf damage to both, but no feeding damage was observed on potato. Over the same period, *G. arizonica* was found feeding only on SLN. In no-choice laboratory experiments, *G. arizonica* fed on both eggplant and potato, but nymph survival and feeding damage were much lower on these nontarget plants, when compared to SLN. Collectively, these results suggest that biosecurity risks of *G. arizonica* are relatively low, and its biological control potential should be explored further, especially when compared to other candidate agents, such as *L. texana*.

1 | Introduction

Silverleaf nightshade (SLN, *Solanum elaeagnifolium* Cav.) is an herbaceous perennial native to the southern United States, Mexico, Central and South America, with the northernmost range extending to southern Texas and Arizona (Brunel 2011; Kariyat and Chavana 2018; Petanidou et al. 2018). SLN has been introduced through anthropogenic activities in at least 40 countries such as Australia, Greece, and South Africa

(Knapp et al. 2017; Gharde et al. 2018; Krigas et al. 2021; Roberts and Florentine 2022; Krigas et al. 2023). SLN negatively impacts agricultural and pastureland productivity as it competes aggressively for nutrient uptake with crops, exudes allelopathic compounds that hinder crop growth, has excellent fitness traits, a persistent seed bank, a suite of physical and chemical defenses against herbivores, and hosts harmful phytophagous pathogens and pests (Chavana et al. 2021; Kasper et al. 2021; Vasquez et al. 2024).

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Persistent economic and environmental effects of SLN warrants the continued search for more appropriate management strategies for extensive infestations (Kwong et al. 2006; Stanton et al. 2011; Lefoe et al. 2020). Since chemical and mechanical control of SLN have proven costly and largely ineffective, the search for more economically efficient management has turned to classical biological control methods (DeLoach 1997; Cullen et al. 2012). SLN has been a target of biocontrol efforts for decades, with candidate agents including *Leptinotarsa texana* (Coleoptera: Chrysomelidae), *Leptinotarsa defecta* (Coleoptera: Chrysomelidae), *Ditylenchus phyllobius*, *Orrina phyllobia*, *Symmetrischema ardeola* (Lepidoptera: Gelechiidae), and *Gargaphia arizonica* (Hemiptera: Tingidae) (Siebert 1975; Wapshere 1988; Wapshere et al. 1989; Olckers and Zimmermann 1991; Karowe 1990; Roberts and Florentine 2022) to name a few.

L. texana and *L. defecta* were released in South Africa in 1992 and both beetles have become established there and achieved substantial reductions in SLN vigor (Olckers and Hulley 1994; Olckers et al. 1995; Hoffmann et al. 1998; Olckers 2004). The two *Leptinotarsa* beetles have been considered strong candidates for SLN biocontrol, particularly *L. texana* due to its tendency to congregate in high densities and strip SLN plants of all edible parts, severely stunting growth and seed set (Hoffmann et al. 1998; Olckers et al. 1999). In host specificity testing, *L. texana* were able to survive and develop on solanaceous crops like eggplant (*S. melongena* Linnaeus) and some potato varieties (*S. tuberosum* Linnaeus) as well as other wild plants in the *Solanum* genus, especially during no-choice starvation tests (Olckers et al. 1995; Lefoe et al. 2020). The findings from Lefoe et al. (2023) indicate that adult females of *L. texana* preferred SLN over *S. melongena* for settling and laying eggs. However, it was also found that *L. texana* larvae can cause damage to *S. melongena* when co-occurring SLN is defoliated in field experiments (Lefoe et al. 2023).

Host specificity is often considered a prerequisite for the importation of invertebrate biological control agents, but South African risk-assessments in the 1990s considered the risk to nontarget native and agricultural *Solanum* species acceptably low compared to the potential of SLN control (Olckers et al. 1999; Hunt et al. 2008). Although Kwong (2008) suggested that *G. arizonica* could be a promising agent, they lacked data on its host specificity. Historical records indicate that *G. arizonica* was observed on populations of SLN in the Rio Grande Valley during the late 1960s and was mentioned as likely host specific in biocontrol surveys conducted in the 1980s (Goeden 1971; Kwong et al. 2008; Wapshere 1988). Despite these observations, there has been limited research conducted to further elucidate the fundamental and realized host range of *G. arizonica*, which creates a significant gap in our knowledge regarding its potential as a biocontrol agent. In classical biological control programs, initial host-specificity trials are often conducted as pilot studies to provide proof-of-concept before committing to extensive multi-site, multi-year assessments (Briese et al. 2002; Schaffner et al. 2018; Hinz et al. 2020). Such early-stage evaluations are critical for identifying promising agents, refining experimental designs, and prioritizing candidates for more exhaustive testing. This study was designed within that framework, providing the first targeted comparison of the realized and fundamental host ranges of *G. arizonica* and *L. texana* in the native range of SLN in south Texas.

To assess biological control candidates, the fundamental host range and realized host range throughout their life cycle must be well understood (Briese et al. 2002; Park et al. 2018; Lefoe et al. 2022). No-choice cage testing provides information on fundamental host range by pairing a single biocontrol agent to a single experimental plant species under controlled conditions where the insect must either feed on the plant, die or attempt to disperse (Withers and Mansfield 2005; Sutton et al. 2017). However, insects that feed on a plant in a no-choice assay may not feed when they encounter that same plant in open-field conditions or may not even encounter that plant in the field (Moffat et al. 2013; Hinz et al. 2014; Park et al. 2018). Well-designed open-field testing in the insect's natural habitat can help circumscribe the realized host range of proposed biocontrol agents (Briese et al. 2002; Clement and Cristofaro 1995; Park et al. 2018).

Combining these, in our study, we used open-field, reverse interspersed common garden experiments to investigate the realized host range of both *G. arizonica* and *L. texana*. Additionally, we conducted no-choice cage experiments to examine the fundamental host range of *G. arizonica*. Open-field experiments were chosen to simulate natural conditions and assess the actual host plants that *G. arizonica* and *L. texana* interact with in the field. While we recognize that open-field experiments do not account for certain variable environmental factors, their use provides valuable insights into potential host interactions under natural conditions. The reverse interspersed common garden design allowed us to compare the feeding preferences and behaviors of these insects among different plant species, including their target host, SLN, and closely related crops such as eggplant and potato (see Schaffner et al. 2018 for a detailed description of reverse interspersed experiments).

We also conducted no-choice cage experiments to evaluate the fundamental host range of *G. arizonica*. By isolating the insects in controlled environments, we observed their feeding preferences without the influence of alternative host plants to better understand the inherent capabilities and limitations of *G. arizonica* while feeding on various plant species. The combination of open-field experiments and no-choice cage experiments provides an understanding of the realized and fundamental host ranges of *G. arizonica* and the realized host range of *L. texana*. These approaches collectively allow us to assess the potential suitability of them as biological control agents for SLN and provide insights into their feeding preferences and potential impacts on nontarget plant species.

2 | Materials and Methods

All research presented here was conducted in Edinburg, Texas, in the native range of both SLN and the candidate biocontrol agents (Kariyat and Chavana 2018; Chavana et al. 2021; Kasper et al. 2021; Vasquez et al. 2024). Edinburg lies in the semi-arid region along the US-Mexico border, with an annual average temperature of 24°C (16.3–30.2°C) and annual rainfall of 572 mm (Texas Water Development Board 2012).

2.1 | Plant Propagation

2.1.1 | Silverleaf Nightshade (*S. elaeagnifolium* Cav.)

SLN used in all experiments was collected from a naturally growing population in Edinburg, Texas (26.3089718, -98.202935) (Chavana et al. 2021; Kasper et al. 2021). Seedlings of SLN around 2.5 cm tall were then dug from the ground and transplanted to 10 cm pots. These plants were used for the reverse interspersed choice field trials. Also, 15 SLN plants for no-choice tests were planted in pots from field-collected seeds (Chavana et al. 2021) and grown in a growth chamber (Percival Scientific; Perry, IA) at 27°C, 70% relative humidity, and a 16:8 day-night period.

2.1.2 | Eggplant (*S. melongena* L.)

Two varieties of eggplant, Black beauty and Nadia, were grown under greenhouse conditions from USDA-certified organic seed (Johnny's Selected Seed; Winslow, Maine) and planted in 4-inch pots with potting medium (Sunshine Mix Sun Gro Horticulture, Bellevue, WA, United States). Only Black beauty variety was used in experiment 1 while Nadia was introduced in experiment 2 to explore varietal preferences of *L. texana* and *G. arizonica*. When seedlings reached 15–20 cm in height, they were transplanted for the reverse interspersed open-field trials and for use in the no-choice tests described below.

2.1.3 | Potato (*S. tuberosum* L.)

The potato cultivar Nadine was grown from seed potatoes (Glacial Ridge Potato Seed Trail, Minnesota USA). In experiment 1, plants were initially grown in a greenhouse using 6-inch pots filled with potting mix (Sunshine Mix Sun Gro Horticulture, Bellevue, WA, United States) and then transplanted for reverse interspersed field trials. In experiment 2, Nadine potato (Real Potatoes Ltd.; Charlottetown, Prince Edward Island, Canada supplied from Washington USA) was directly planted into the field trials in early February. Additionally, potato cultivar Atlantic was obtained locally (Jack Wallace Farms, Edinburg, TX, USA) and directly planted in the field for reverse interspersed trials in Experiment 2. The Atlantic variety was introduced in Experiment 2 to evaluate varietal preferences of *L. texana* and *G. arizonica*. The Nadine cultivar was also used for no-choice trials with *G. arizonica*, as previous research indicated that *L. texana* feeds on this variety (Lefoe et al. 2020).

2.2 | Reverse Interspersed Field Trials

Field trials were conducted in a certified organic research plot (26.3078, -98.1708) in Edinburg, Texas, in both 2020 (experiment 1) and 2021 (experiment 2). Using a reverse interspersed design, two central 8 m rows of single plants of SLN were surrounded on both sides by blocks of eggplant and potato in both field experiments (Supporting Figures A and B).

In the field trial experiment 1, a total of 24 plants each of SLN, eggplant Black beauty, and potato Nadine were included within

six rows, with each row having 12 plants. In field trial experiment 2, a total of 20 plants were used per species, including 10 plants each from the two varieties of eggplant, Black beauty and Nadia, and 10 plants each from the two potato varieties, Nadine and Atlantic.

Both field experiments were conducted in the same field from March to June in two consecutive years 2020 and 2021. Both experiments were set up as a choice experiment that mimics agricultural field conditions. Plants were irrigated through drip irrigation daily for 30 min to facilitate establishment and recovery from transplanting shock.

2.2.1 | Monitoring and Assessment

For each experiment, each plant was assessed for observations of *G. arizonica* and *L. texana*. Assessments were made every other day in mid-late afternoon throughout the growing period of both crops. Plants were assessed by examining the adaxial leaf surface for 1 min and then the abaxial surface by slightly lifting each leaf while observing for any adults that fell to the ground. In field trials (experiments 1 and 2), we assessed the relative feeding preferences of *G. arizonica* and *L. texana* by recording the number of adults observed on each plant (Supporting Figure C). In experiment 2, we expanded our observations to include *L. texana* feeding preferences by counting the total number of larvae and associated feeding damage. We also attempted to count *G. arizonica* nymphs; however, their small size and clustered distribution made accurate field counts challenging. *G. arizonica* feeding damage was estimated based on distinct feeding damage including evidence of black spots and desiccated leaves. *L. texana* feeding damage was estimated by observing characteristic chewing patterns on the leaves.

We assessed the feeding preferences by recording the total number of adults and quantifying feeding damage using a relative scale ranging from 0 to 4, where 0 indicates no feeding, 1 represents 1–25% feeding damage, 2 represents 25–50%, 3 represents 50–75%, and 4 represents 75–100% (Kariyat and Chavana 2018; Kasper et al. 2021).

Due to a severe, prolonged winter storm in February 2021, populations of *G. arizonica* were difficult to find within the experimental sites. However, *L. texana* was found in experimental sites. To facilitate timely observations, 80 adults of *G. arizonica* were collected from other locations where populations were emerging and introduced into the experiment.

2.3 | No-Choice Tests

No-choice cage experiments of *L. texana* were previously published by Lefoe et al. (2020), showing that more than 50% of the leaf area was consumed by *L. texana* on plants of 12 Australian *Solanum* species and two crop species (potato and eggplant). Consequently, no-choice experiments in our study focused on *G. arizonica* during Spring 2021 (SLN and eggplant) and 2022 (potato), as *G. arizonica* is a candidate biological control agent, and such cage experiments had not been conducted for this species before. Fifteen individual plants of potato, eggplant, and

SLN were used in this study in cohorts of five (5 plants in each of three cohorts). Each plant was individually placed in a mesh-sided bug dorm (Bioquip, Rancho Dominguez Hills, CA) (Supporting Figure D) and watered daily using automatic sprinkler irrigation in a temperature-controlled greenhouse. In each cage, 10 late-stage nymphs of *G. arizonica* were placed on the leaves of the target plant (150 late-stage nymphs total). This process was repeated three times in April 2022.

2.3.1 | Rearing of *G. arizonica*

All *G. arizonica* used in the no-choice trials were reared under laboratory conditions. Field collected *G. arizonica* were placed on potted SLN on April 28, 2021. Initially, two mesh-bug dorms (Bioquip, Rancho Dominguez Hills, CA) were set up on the lab countertop, with two pots of SLN plants in each dorm. Lab conditions were set at 22°C, with natural lighting. 28 adult *G. arizonica* were placed randomly on the leaves of SLN in each bug dorm. Observations of each bug dorm were made daily. Nymphs appeared in both bug dorms after 3 days. An additional bug dorm was added to keep up with the colony for no-choice cage experiments. Several generations of reared insects were used for additional no-choice testing.

2.3.2 | Monitoring and Assessment

50 min of observation were conducted sequentially in each of the bug dorms. Data on the total number of alive nymphs, number of adults, and number of dead insects were taken at 48, 96, and 144 h. At each of these observations, leaf damage from *G. arizonica* was estimated using a scale of 0–4, as described above (Kariyat and Chavana 2018; Kasper et al. 2021).

2.4 | Statistical Analysis

For leaf damage response variables, which were measured on an ordinal scale (0–4), we used ordinal logistic regression to analyze the data, with Wald tests conducted to assess the significance of predictor variables. This method is appropriate for ordinal data, ensuring proper interpretation of the relationships between treatments and leaf damage severity. For count data, including nymph count and adult count, we conducted a generalized regression analysis using a Poisson distribution to account for the non-normality and discrete nature of the response variables. Tukey's post-hoc tests were performed for pairwise comparisons when significant differences were detected. All statistical analyses were performed using JMP Pro. Statistical significance was determined at $p < 0.05$.

3 | Results

3.1 | Field Experiment 1

3.1.1 | *L. texana* Adult Count

Pairwise comparison revealed significantly more *L. texana* adults on eggplant Black beauty and SLN than potato Nadine (Tukey test; $p \leq 0.0003$) but the difference was nonsignificant

between SLN and eggplant Black beauty (Tukey test; $p = 1.000$) (Figure 1).

3.1.2 | *G. arizonica* Adult Count

Pairwise comparison revealed on average significantly more *G. arizonica* adults on SLN than on eggplant Black beauty and potato Nadine ($p \leq 0.0001$). No adults were observed potato Nadine and eggplant Black beauty (Figure 2).

3.2 | Field Experiment 2

3.2.1 | *L. texana* Adult Count

Pairwise comparison revealed significantly more *L. texana* adults on SLN than on eggplant, Black beauty and Nadia, and potato

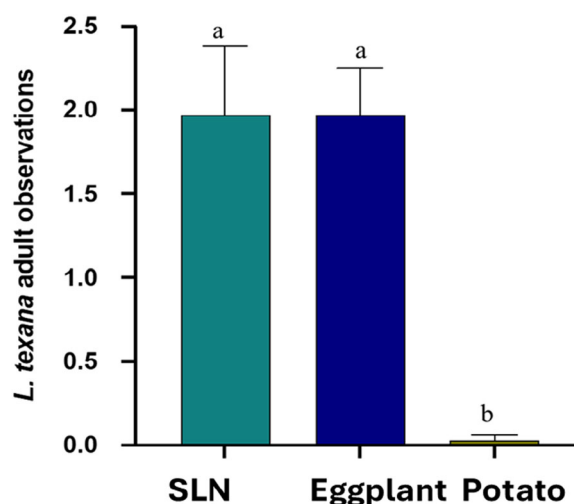


FIGURE 1 | *L. texana* observed on silverleaf nightshade (SLN), eggplant Black beauty, and potato Nadine in field experiment 1. Different letters above bars denote statistically significant differences between plant species. Error bars represent standard errors.

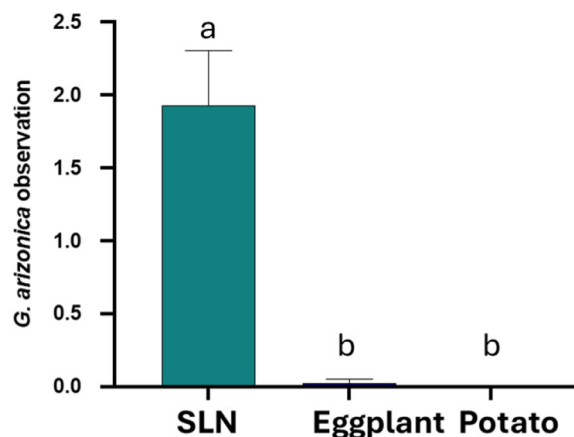


FIGURE 2 | *G. arizonica* observed on silverleaf nightshade (SLN), eggplant Black beauty, and potato Nadine in field experiment 1. No adults were observed on eggplant and potato. Different letters above bars denote statistically significant differences between plant species. Error bars represent standard errors.

Nadine ($p \leq 0.0001$) for each pair. Eggplant Nadia had significantly more adults than eggplant Black beauty, and potato Nadine ($p = 0.0016$). Eggplant (Black beauty) had significantly more adults than potato (Nadine) ($p = 0.0245$) and there was no difference between potato Nadine and potato Atlantic ($p = 0.9998$). We did not find any insects in the potato Atlantic. Therefore, we did a pairwise comparison only with SLN and eggplant (Black beauty and Nadia), and potato Nadine (Figure 3A).

3.2.2 | *L. texana* Larvae Count

Pairwise comparison revealed significantly more *L. texana* larvae on SLN than on eggplant (Black beauty and Nadia), and potato Nadine ($p \leq 0.0001$). Eggplant Nadia had significantly

more larvae than eggplant Black beauty ($p = 0.0001$). We did not find any larvae in potato (Nadine and Atlantic). Therefore, we did a pairwise comparison only with SLN and eggplant (Black beauty and Nadia). There was no difference in larvae number between potato Nadine and potato Atlantic ($p = 1.000$) (Figure 3B).

3.2.3 | Leaf Damage (*L. texana*)

Higher leaf damage was found on SLN and eggplant Nadia than on eggplant Black beauty and potato Nadine and Atlantic. Damage done by *L. texana* was significantly higher on eggplant Black beauty than potato Nadine and Atlantic which is also confirmed by Wald Chi-square value of 42.53 ($p < 0.0001$).

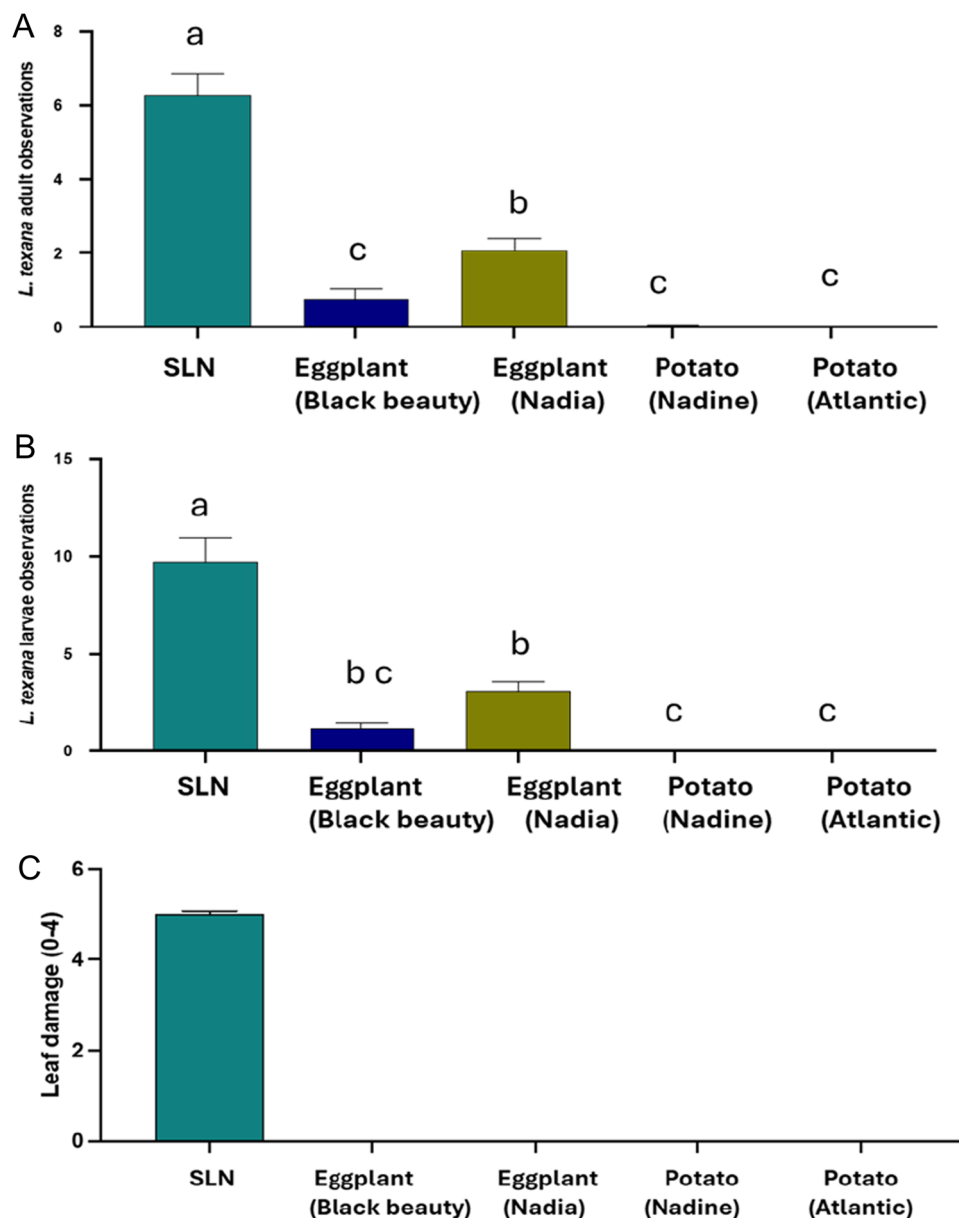


FIGURE 3 | Field experiment 2 observations of *L. texana* on silverleaf nightshade (SLN), eggplant varieties (Black beauty and Nadia), and potato varieties (Nadine and Atlantic). (A) Adult counts. (B) Larval counts. (C). Leaf damage ratings (0–4 scale). Different letters above bars denote statistically significant differences between plant species. Error bars represent standard errors.

We did not find any leaf damage on potato Nadine and Atlantic (Figure 3C).

3.2.4 | *G. arizonica* Adult Count

G. arizonica adults were more abundant on SLN than on eggplant, Black beauty and Nadia, and potato, Nadine and Atlantic, ($p < 0.0001$). Pairwise comparisons showed nonsignificant difference in abundance between eggplant Black beauty, eggplant Nadia, potato Nadine, and potato Atlantic ($p = 1.000$). *G. arizonica* adults were never observed on eggplant Black beauty and Nadia, potato Nadine and Atlantic (Figure 4A).

3.2.5 | Leaf Damage (*G. arizonica*)

Higher leaf damage was found on SLN than on eggplant (Nadia and Black beauty) and potato (Nadine and Atlantic) as confirmed by Wald Chi-square value of 81.07 (Ordinal logistic regression; $p < 0.0001$). We did not find any leaf damage of *G. arizonica* in eggplant (Black beauty and Nadia), potato (Nadine and Atlantic) (Figure 4B).

3.3 | No-Choice Cage Experiment (*G. arizonica*)

3.3.1 | Total Adults

Significantly more adults were found on SLN than on eggplant Black beauty and potato Nadine at 48 h, 96 h and 144 h (Poisson distribution fit model regression: $p < 0.0001$) but the difference was nonsignificant between eggplants Black beauty and potato Nadine at 48 h ($p = 0.239$), 96 h ($p = 0.629$) and 144 h ($p = 0.560$) (Figure 5).

3.3.2 | Leaf Damage

Ordinal logistic regression showed significantly higher feeding damage of *G. arizonica* on SLN than on eggplant Black beauty and potato Nadine which was also confirmed with Wald χ^2 tests. χ^2 values of 10.95 ($p = 0.0042$) at 48 h, 18.56 ($p \leq 0.0001$) at 96 h and 17.53 ($p = 0.0002$) at 144 h confirmed that feeding damage on SLN remained consistently higher throughout the experiment (Figure 6).

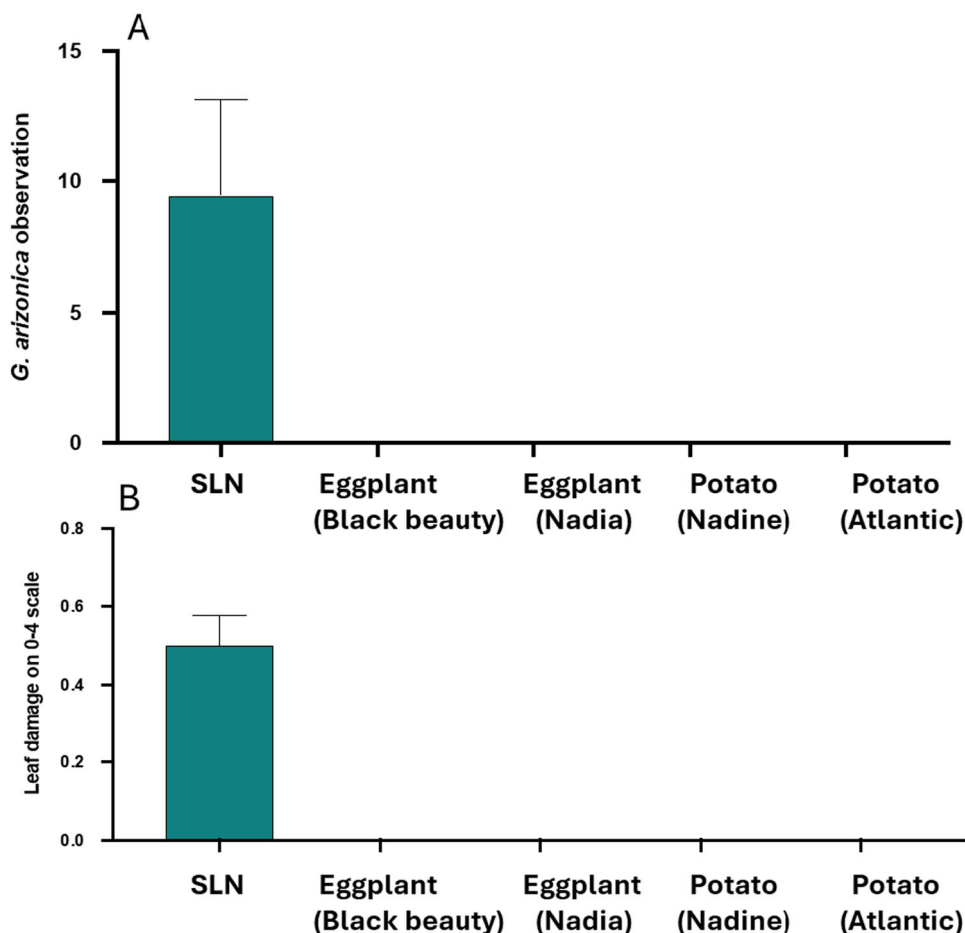


FIGURE 4 | Field experiment 2 observations of *G. arizonica* on silverleaf nightshade (SLN), eggplant varieties (Black beauty and Nadia), and potato varieties (Nadine and Atlantic). (A) Adult counts. (B) Leaf damage ratings (0–4 scale). Different letters above bars denote statistically significant differences between plant species. Error bars represent standard errors.

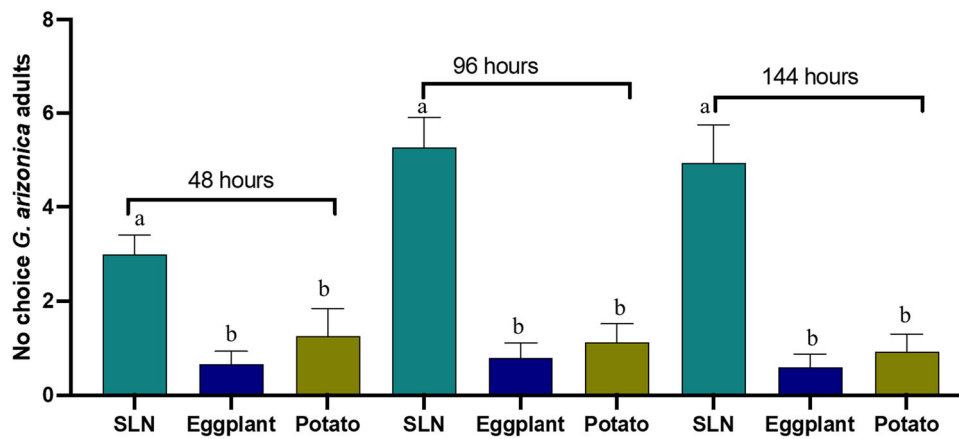


FIGURE 5 | *G. arizonica* adults observed on SLN, eggplant, and potato at 48 h, 96 h, and 144 h in no-choice cage experiments. Different letters above the bars denote statistically significant differences between plant species. Error bars represent standard errors.

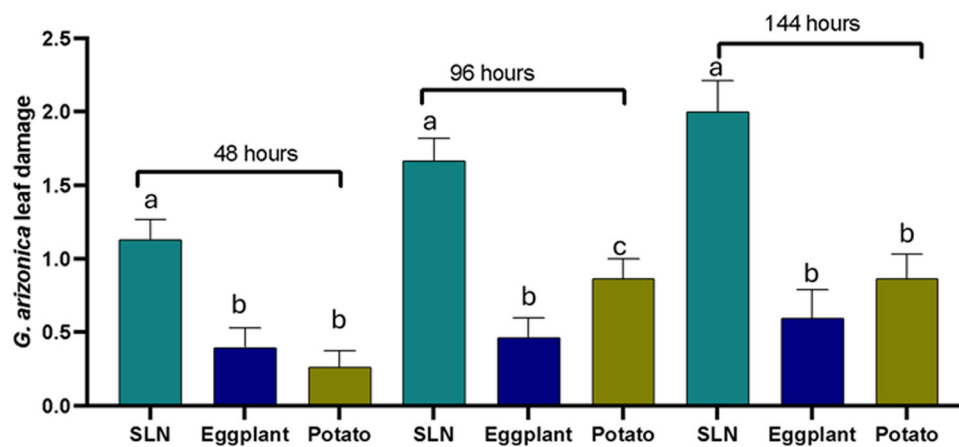


FIGURE 6 | Feeding damage by *G. arizonica* on SLN, eggplant, and potato at 48 h, 96 h, and 144 h in no-choice cage experiments. Different letters above the bars denote statistically significant differences between plant species. Error bars represent standard errors.

3.4 | Total Nymph Survival

Significantly more nymphs were found on SLN than on eggplant Black beauty and potato Nadine at 48 h, 96 h and 144 h (Poisson distribution fit model regression: $p < 0.0001$). Pairwise comparison revealed significantly more nymphs on SLN than on eggplant Black beauty and potato Nadine at 48 h, 96 h, 144 h (Tukey's test; $p \leq 0.0001$). More nymphs were found on potato Nadine than on eggplant Black beauty at 48 h (Tukey's test; $p = 0.004$) but the difference was nonsignificant between eggplant Black beauty and potato Nadine at 96 h ($p = 0.097$) and 144 h ($p = 0.173$) (Figure 7).

3.4.1 | Total Mortality

Significantly more *G. arizonica* deaths were found on eggplant Black beauty and potato Nadine than on SLN at 48 h, 96 h and 144 h (Poisson distribution fit model regression: $p < 0.0001$). Pairwise comparisons revealed significantly more deaths on eggplant Black beauty and potato Nadine than on SLN at 48 h, 96 h, 144 h, (Tukey's test; $p \leq 0.0001$). Significantly more deaths

were found on eggplant Black beauty than potato Nadine at 48 h (Tukey's test; $p = 0.039$) but the difference was nonsignificant between eggplant Black beauty and potato Nadine at 96 h (Tukey's test; $p = 0.706$) and 144 h (Tukey's test; $p = 0.866$) (Figure 8).

4 | Discussion

Using field and lab-based experiments over 2 years, in this study we explored the potential of two insects as biocontrol agents for the noxious and invasive weed, SLN. No-choice cage experiments showed that *G. arizonica* will feed on eggplant and potato, suggesting that these two solanaceous crops lie within its fundamental host range. However, our results also indicate that they develop, survive, and feed on SLN significantly more than on the other test plants. More live nymphs and adults were found on SLN than on eggplants and potato for nearly all measurements except live nymphs at 48 h and mortality rates were higher on eggplant and potato at all time periods. These observations of insect feeding, maturity, and mortality indicate that while eggplant and potato may be within the fundamental

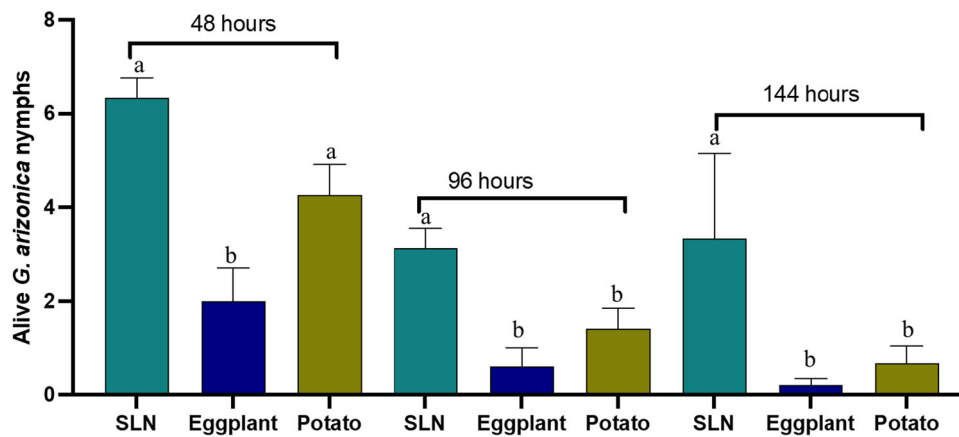


FIGURE 7 | Nymph survival of *G. arizonica* on SLN, eggplant, and potato at 48 h, 96 h, and 144 h in no-choice cage experiments. Different letters above the bars denote statistically significant differences between plant species. Error bars represent standard errors.

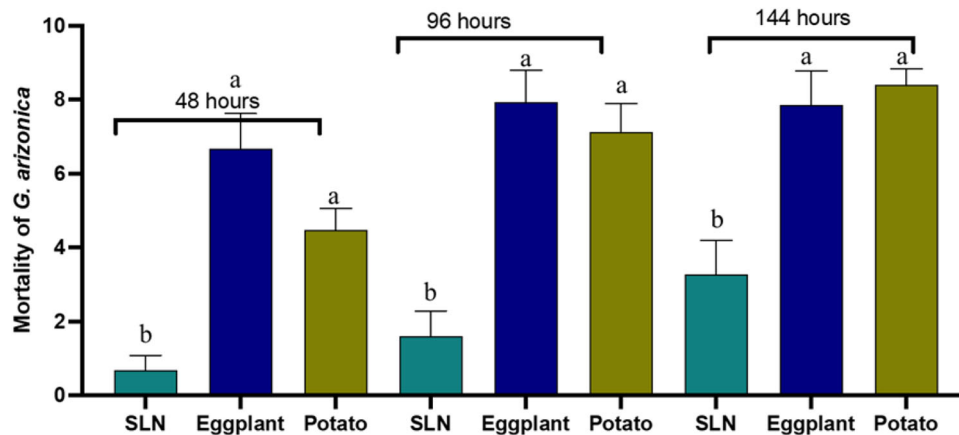


FIGURE 8 | *G. arizonica* mortality rate observed on eggplant, potato and SLN at 48 h, 96 h and 144 h in no-choice cage experiments. Different letters above bars denote statistically significant differences between plant species. Error bars represent standard errors.

host range of *G. arizonica*, they are able to better survive and reproduce on SLN than on alternative agricultural hosts. However, our study was limited to late-stage nymphs and did not assess the completion of full life cycle. Thus, further studies are required to determine whether *G. arizonica* can complete at least one full generation on these host plants under controlled conditions. Results from open-field trials of *G. arizonica* does make a stronger case for host specificity to SLN. During the study, *G. arizonica* was only rarely observed on any other test plants besides SLN and no evidence of feeding on non-SLN plants was ever observed. Both eggplant and potato appear to be outside of the realized host range when observed under field conditions.

Two years of reverse interspersed field observations confirmed that *L. texana* does feed on eggplant under field conditions. The presence of *L. texana* was similar on SLN and eggplant (avg. 1.97 insects per observation) in the first experiment, while Experiment 2 recorded over 50% fewer *L. texana* adults and larvae on eggplant. Despite this, leaf damage levels on SLN and eggplant Nadia were comparable. Further research is needed to determine if this damage affects eggplant yield. While no-choice cage trials showed *L. texana* feeding on potato (Lefoe et al. 2020), field trials found no

evidence of its presence or feeding damage, suggesting potato is at the fringe of its host range. These findings collectively indicate that *L. texana* poses a greater biosecurity risk to eggplant than potato if used as a biocontrol agent for SLN. Further studies should assess the impact of *L. texana* on eggplant yield and explore factors influencing its host preference, behavior, and potential risks to other solanaceous crops.

Both no-choice and open-field observations suggest that *G. arizonica* could serve as a more promising classical biological control candidate for SLN than *L. texana*, due to its relatively narrow realized host range. However, further efforts are needed to fully understand the biotic and abiotic factors that influence population dynamics and the efficacy of *G. arizonica* as a biological control agent. Studies suggest that there are abiotic and anthropogenic predictors of the population and distribution of these SLN herbivores, which may prove critical in a classical biological control program (Chavana et al. 2021; Kasper et al. 2021). The potential of *G. arizonica* (a sap sucking insect) to transmit or vector other pathogens should also be examined. However, these results suggest that biosecurity risks of *G. arizonica* are relatively low, especially when compared to other agents, like *L. texana*.

This study represents an initial proof-of-concept in the classical biological control pathway, aligning with the first phase of host-specificity testing (Briese et al. 2002; Schaffner et al. 2018; Hinz et al. 2020). While limited in scope and life stage, the findings provide clear evidence to prioritize *G. arizonica* for further evaluation and to re-examine the risk-benefit profile of *L. texana*. Future research should refine knowledge of *G. arizonica*'s host range, population dynamics, efficacy, and potential risks through comparative studies evaluating its performance and effectiveness against other candidate biocontrol agents. Multi-site, multi-year trials will be essential to capture environmental variability, alongside mechanistic studies on the physiological and behavioral drivers of host preference. Long-term assessments should examine its impact on SLN populations and the overall ecological dynamics of the system, including interactions with natural enemies and effects on beneficial organisms and nontarget species. Considering abiotic factors such as climate and habitat characteristics will be valuable for developing region-specific management approaches, while targeted risk assessments will be critical to ensuring its safe, effective deployment and contributing to sustainable strategies for managing SLN and reducing its impacts across agricultural and natural systems.

Author Contributions

Samikshya Subedi, Greg Lefoe, Alexis Racelis, and Rupesh Kariyat designed the experiments. Samikshya Subedi, Stephanie Kasper, Alexis Racelis, and Rupesh Kariyat collected the data. Samikshya Subedi, Greg Lefoe, Stephanie Kasper, and Rupesh Kariyat analyzed the data. Samikshya Subedi wrote the first draft, and all authors contributed to the revisions and approved.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

Data that support the findings of this study are available from the corresponding author, [Kariyat, R], upon reasonable request.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.
SupMat.