Received: 28 January 2025 | Accepted: 6 August 2025

DOI: 10.1111/eea.70008

Entomologia Experimentalis et Applicata

ORIGINAL ARTICLE

Oviposition behavior and development of the invasive lily leaf beetle on native host plants from the Pacific Northwest United States

Maggie Freeman[1,2,3] | **Chris Looney**[2] | **Liesl Oeller**[3] | **David W. Crowder**[3]

[1]Oregon State University Mid-Columbia Research and Extension Center, Hood River, Oregon, USA

[2]Washington State Department of Agriculture, Olympia, WA, USA

[3]Department of Entomology, Washington State University, Pullman, WA, USA

Correspondence
David W. Crowder, Washington State University Department of Entomology, 166 FSHN Bldg, PO Box 646382, Pullman, WA, 99164-6382, USA.
Email: dcrowder@wsu.edu

Funding information
Washington State Department of Agriculture, Grant/Award Number: SCBG K-1985

Abstract

Invasive insects often feed on novel plant species related to hosts in their native range, including species of conservation concern. The lily leaf beetle, *Lilioceris lilii* Scopoli (Coleoptera: Chrysomelidae), a Eurasian pest of cultivated lilies, is one such invader first detected in Canada in 1943 that has spread throughout the Northeastern United States and Canada, reaching the Pacific coast by 2011. Although *L. lilii* is known to feed on nearly 100 plant species across several genera, it is unknown what hosts it will feed on in its new range. We addressed this knowledge gap with experiments of *L. lilii* on native host plants found in the Pacific Northwest, United States, where it is now well-established and has the potential for population expansion. We conducted oviposition and larval feeding trials with *Calochortus tolmiei* Hook. & Arn. (Liliaceae), *Fritillaria affinis* (Schult. & Schult.f.) Sealy (Liliaceae), *Lilium columbianum* Leichtlin (Liliaceae), and *Prosartes hookeri* Torr. (Liliaceae). Trials showed that female *L. lilii* oviposited on all hosts tested, and larvae fed on all hosts except *P. hookeri*. Larvae reared on *L. columbianum* performed similarly well to larvae that fed on the control lilies. Individuals were only able to be reared to the adult stage on *L. columbianum* and *C. tolmiei*, although survival rates were low for *C. tolmiei*. With numerous susceptible native Liliaceae species of conservation concern in the Pacific Northwest, the establishment of the lily leaf beetle may constitute a new threat to these already vulnerable species across broad regions.

KEYWORDS
Coleoptera, egg laying, exotic insects, feeding behavior, host preference, host range, host shifts, Liliales, *Lilioceris lilii*, species conservation

INTRODUCTION

Invasive insect herbivores can widely threaten crops and native species of conservation concern (Mack et al., 2000; Vilà et al., 2011). The hosts threatened by an invasive herbivore depend on their host breadth and host availability. Host shifts between native and invasive ranges can also occur when species invade new regions, such as *Leptinotarsa decemlineata* Say and *Rhinocyllus conicus* Frölich (Hare, 1990; Louda et al., 2011). In North America, *L. decemlineata* has expanded its host range from native *Solanum rostratum* Dunal to cultivated potato *Solanum tuberosum* L. and has since become one of the worst US potato pests (Alyokhin et al., 2015; Hare, 1990; Hsiao & Pasteels, 1999). Host plant shifts by invasive species threaten native flora and crops (Hare, 1990; Louda et al., 2011). However, it is often unknown when species invade a new area, which host plants may be most threatened.

One invasive pest that threatens native and ornamental plants is the lily leaf beetle, *Lilioceris lilii* Scopoli (Coleoptera: Chrysomelidae), a Eurasian pest that feeds on lilies (*Lilium* spp.) and fritillaries (*Fritillaria* spp.). *Lilioceris lilii* has a broad

natural range spanning from southern Greece to Siberia and the United Kingdom to China (Bouchard et al., 2008; Orlova-Bienkowskaja, 2013). It was first detected in North America near Montreal, Canada, in 1943, likely via introduced Asiatic lilies (Bouchard et al., 2008; Lesage, 1983). Populations are now known to occur in 10 Canadian provinces (Cappuccino, 2017; Cappuccino et al., 2013; Hicks & Sellars, 2014; LeSage, 1992; LeSage & Elliott, 2003). In 1992, *L. lilii* was detected in Massachusetts (Bouchard et al., 2008) and now is found in 17 northeastern and central states as far west as Minnesota; recently, it was detected on the Pacific coast in Washington state (Cappuccino, 2017; Hicks & Sellars, 2014; Maier, 2012; Majka & Kirby, 2011; Murray et al., 2016). Across this range, there is a wide diversity of potential hosts for *L. lilii* and various genetic backgrounds of invasive populations that may vary in host preferences, but it remains unclear which host species may be threatened in any given region.

Across the lily leaf beetle's invaded range, it typically feeds and reproduces on cultivated lilies (*Lilium* spp.) (Ernst et al., 2007). However, adults have been observed to feed on at least 20 additional genera (Salisbury et al., 2010). Studies with eastern North American plants found that *L. lilii* larvae feed on at least five genera, three in the Liliaceae and two in the Asparagaceae (Asparagales) (Ernst et al., 2007). This work also showed there was a strong positive correlation between the estimated age of divergence of genera in the Liliaceae family and suitability for *L. lilii* larval feeding, with genera more closely related to *Lilium* being more suitable (Ernst et al., 2007). Larvae completed development on species such as *Medeola virginiana* L., *Streptopus aplexifolius* L., and *Lilium philidelphicum* L., with adults developing from all species except *M. virginiana* (Ernst et al., 2007). Additionally, *L. lilii* eggs and larvae have been observed on native plants throughout Canada. However, in newer regions, the threatened species are often unknown.

The first detection of lily leaf beetle in the western United States was in Bellevue, Washington, in 2011 (Murray et al., 2016). At least 10 native Liliaceae genera occur in this region (*Calochortus, Clintonia, Erythronium, Fritillaria, Lilium, Lloydia, Medeola, Prosartes, Scoliopus, Streptopus*) (USDA NRCS, 2019). Several species of *Lilium*, *Fritillaria*, and *Calochortus* are of conservation concern, including some federally listed species (USDA NRCS, 2019), and may be especially vulnerable to impacts from *L. lilii* as it expands its range in the western US. To address variation in the ability of *L. lilii* to feed and develop on host plants from the western US, we conducted studies of the lily leaf beetle's oviposition preference and development on native Liliaceae. We conducted oviposition and survival trials on a variety of host species as well as preference tests. Our results revealed which hosts might be suitable for lily *L. lilii* across the Liliaceae. These results can help guide monitoring and mitigation strategies for this damaging invasive insect.

MATERIALS AND METHODS

Study system

Lilioceris lilii is a univoltine insect that overwinters as an adult and emerges from March to June (Cappuccino et al., 2013; Ernst, 2005). Adults feed for a few weeks prior to mating, and females can lay 200–300 eggs in a season (Cappuccino et al., 2013; Ernst, 2005). Larvae emerge after 7–10 days and feed communally on leaf undersides. Larvae cover their dorsal surface with a layer of excrement, termed a "fecal shield," which protects against predators and prevents desiccation, although it may also attract parasitoids (Bouchard et al., 2008; Schaffner & Müller, 2001). Larval development takes 3–4 week, after which they dislodge their fecal shield and move into soil to pupate (Cappuccino et al., 2013; Ernst et al., 2007). Adult beetles emerge after 3–4 week, feed until fall, and then move to soil or leaf litter to overwinter (Cappuccino et al., 2013). However, all of these data are from studies in the eastern United States and Canada, and the hosts used by *L. lilii* in the Pacific Northwest United States (and effects on development parameters) are unknown.

Oviposition preference

To assess oviposition preferences of *L. lilii*, choice and no-choice experiments were conducted with five Liliaceae species in the laboratory and field. Field experiments were conducted near Bellevue, Washington State, USA, where beetles already occur to reduce the risk of spreading beetles. Experiments were conducted in May of 2017 to 2019, a period when daily temperatures varied from nighttime minimums of 5–11°C to daytime highs of 13–21°C, with relative humidity between 70% and 76% and approximately 15 h of daylength. Four species of Liliaceae were tested: (i) *Lilium columbianum* Leichtlin (Liliaceae) (Columbia lily), (ii) *Prosartes hookeri* Torr. (Liliaceae) (Hooker's fairy bells), (iii) *Calochortis tolmiei* Hook. & Arn. (Liliaceae) (Tolmie's star tulip), and (iv) *Fritillaria affinis* (*Schult. & Schult.f.*) *Sealy* (Liliaceae) (checker lily or chocolate lily). These native plants occur across British Columbia, Washington, Oregon, California, Idaho, and Montana (Utech, 2003) and are common where *L. lilii* has invaded in Washington. Plants were purchased from a nursery specializing in native species (SevenOaks Native Nursery, Albany, Oregon). Stock of *C. tolmiei* and *F. affinis* plants was limited and partially determined the number of replicates. Previous studies show *L. lilii* does not develop on *Clintonia*, *Erythornium*, and *Tulipa* (Ernst et al., 2007), so they were excluded. *Lloydia* and *Scoliopus* are potential hosts also present but were not available for purchase. All lilies used as controls in experiments were grown from a bag of mixed Asiatic and Oriental lily bulbs purchased at a local hardware store. As Asiatic and Oriental lily hybrids are highly suitable host plants

(Salisbury et al., 2010), they were used interchangeably as the control.

No-choice field experiments were conducted to assess the suitability of hosts for oviposition of *L. lilii*. In May 2017, two 1.2 × 1.5 m raised beds were established in Bellevue, WA. In each bed, nine plants were grown in three rows of three plants each; one *L. columbianum*, one *P. hookeri*, and one *C. tolmiei* were ordered randomly in each row. A 61 × 20 cm cage of chicken wire and fine mesh fabric was placed over each plant. One mated pair of *L. lilii* adults, collected from gardens in the area, was placed into each cage and allowed to feed and oviposit for 72 h, after which they were removed. All plants were in their vegetative growth stage when the experiment was conducted. Each plant and cage was then inspected and all eggs counted and locations recorded. After 1 week, eggs were evaluated every 24 h until all larvae eclosed. If no larva emerged after 2 week, the egg was designated unviable. Subsequently, the larvae on plants were recorded every 48 h until all larvae died or pupated, and the number of days to reach the pupal stage was recorded. Cages were checked for adult emergence starting 20 days after larvae began to pupate. As adults emerged, the emergence date was recorded and each adult's length and weight were measured.

Laboratory no-choice trials (at room temperature and humidity, 20°C and 15% relative humidity, with an L14:D10 photoperiod) were conducted using *C. tolmiei* and *F. affinis*, as both emerge and flower earlier than the *Lilium* species and are challenging to maintain in the field. In 2018, eight *C. tolmiei* plants were individually placed into cages with one mating pair of *L. lilii* and left for 72 h. After the beetles were removed, plants were checked for eggs. Any larvae that eclosed were monitored for development using the same procedures as the field experiment. This experiment was repeated in 2019 with eight *F. affinis* plants; although larval development could not be monitored in this experiment due to plants rapidly senescing after beetles were removed.

Finally, choice trials in the field were used to assess oviposition preference by *L. lilii* on other Liliaceae species when their preferred *Lilium* host was available. Plants used were *L. columbianum*, *C. tolmiei*, *F. affinis*, and a control lily. In the spring of 2018 and 2019, two 1.2 × 1.5 m raised beds in Bellevue, WA, were each planted with four rows (8 total) of one randomly arranged control lily, one *L. columbianum*, one *F. affinis*, and one *C. tolmiei*. Each row of plants was caged using a PVC frame (1.2 × 0.3 × 0.9 m) and covered with mesh fabric. Fabric ends were secured to the soil using fabric staples to prevent insects from leaving or entering the cage. Adult lily leaf beetles were collected from residential and public gardens in the Bellevue area. Four mating pairs of *L. lilii* were placed in each cage for 72 h and were then removed. Each plant was carefully inspected, and the number of eggs laid on each plant was recorded.

Laboratory larval feeding and development trials

We next assessed larval development on native plants. In 2017, we tested *L. columbianum*, *P. hookeri, C. tolmiei*, and a control lily. Lily leaf beetle eggs were collected from residential areas by removing lily leaves with eggs. Leaf material to feed captive larvae was collected from plants at the Bellevue field. Four leaves were collected from control lilies, four from *L. columbianum*, two from *C. tolmiei*, and eight from *P. hookeri*. In the laboratory, each leaf was placed in a plastic petri dish with a moist paper towel. Two mature eggs were placed on opposite ends of each leaf using a soft paint brush. Petri dishes were kept in an incubator (L16:D8 photoperiod at 22°C:15°C). Eggs and hatched larvae were checked every 24 h, and leaves were replaced ad libitum. This was repeated daily until each larva had died or developed to pre-pupa. Larvae that became pre-pupae were moved to a 118-mL plastic container with moist soil. The number of days it took to develop into adults was recorded, and each adult was measured for length and weighed.

This experiment was repeated in 2019, substituting *F. affinis* for *P. hookeri*. Plant material used was collected from (i) eight *C. tolmiei* plants in 11-cm-round plastic pots, (ii) eight *F. affinis* plants with one to two leaves in 9-cm plastic pots, (iii) eight leaves from potted *L. columbianum*, and (iv) eight control lily leaves collected from a single plant for the control. For the individual leaf treatments, each leaf was placed into a plastic petri dish with a moistened paper towel. For all treatments, one mature egg was placed onto each leaf using the tip of a soft paint brush.

All treatments were placed in an incubator (L16:D8 photoperiod at 22°C:15°C). Eggs were checked every 24 h for hatching, and larvae were removed every 48 h to be weighed. Each larva's fecal shield was removed with a soft paint brush before it was weighed. This was repeated until each larva died or pupated, and the time to reach the pre-pupal stage was noted. Leaves were replaced ad libitum for controls and *L. columbianum*; no plants were available to replace *C. tolmiei* or *F. affinis*. Larvae that reached the pre-pupal stage were transferred to 118 mL plastic containers with moistened soil. The time it took larvae to develop into adults was recorded. After emergence, each adult beetle was measured for length and was weighed.

Data analysis

A generalized linear model (GLM) with a negative binomial distribution was used to assess the effects of various host plant treatments on the number of eggs laid in the no-choice and choice oviposition trials, and the number of eggs laid on the cage in the no-choice trial. This statistical distribution was used based on the variation in the count data. Post hoc Tukey tests were used to perform multiple comparisons between each treatment. Logistic regression was used to evaluate the

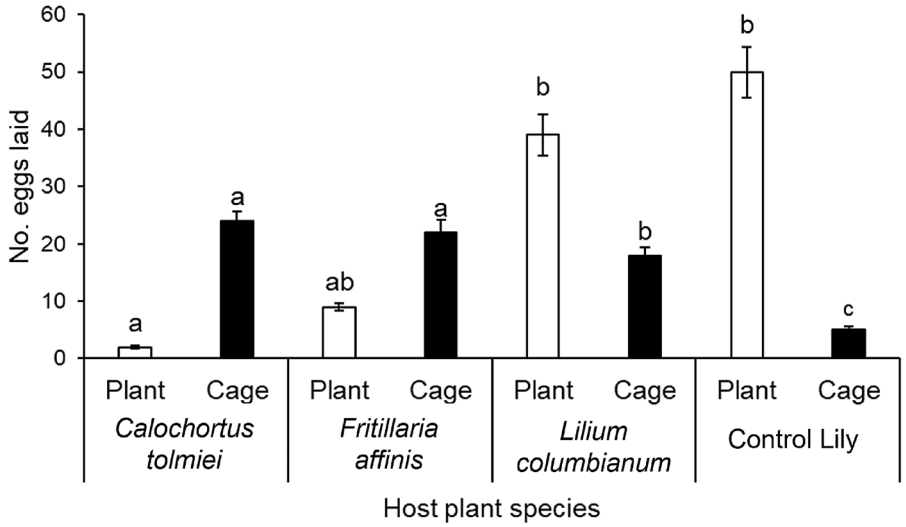

FIGURE 1 Total number of eggs (mean ± standard error) laid on host plants or cages in the no-choice oviposition trial. Different letters indicate significant differences in the number of eggs laid among plant treatments and among cage treatments according to the generalized linear model results ($p = 0.05$).

proportion of eggs laid on the plant versus on the cage in the no-choice oviposition trial. A Tukey post hoc test was used to perform multiple comparisons between each treatment.

To assess whether hosts affected the number of days larvae survived in the feeding and development trial, a Cox proportional hazards model was used. For the 2017 and 2019 feeding and development trials, as controls were directly compared to specific hosts, we used two-sample *t*-tests to assess whether the weight and length of adult beetles that completed their lifecycles, the number of days it took larvae to reach the pre-pupal stage, and the number of days it took pupated larvae to emerge as adults differed between controls and each host plant. For these multiple *t*-tests, we used a Bonferroni correction to control for multiple comparisons. As each experiment involved four hosts, we adjusted the α required for significance to 0.05/4 = 0.0125.

In the 2018 laboratory feeding and development trial, as all hosts were compared with the control at the same time, a one-way ANOVA was used to assess if host plant affected the weight and length of adult beetles, the time it took larvae to reach the pre-pupal stage, and the time it took pupated larvae to emerge as adults. This test was also used to assess whether the host treatments affected larval weight gain in the 2019 laboratory feeding and development trial. When a significant effect was found in the one-way ANOVA, a Tukey HSD post hoc test was used to perform multiple comparisons between each host plant treatment. All statistical analyses were conducted in R Studio version 3.4.1 (R Core Team, 2017).

RESULTS

No-choice oviposition trials

The number of eggs laid by *L. lilii* differed across hosts (GLM: $\chi^2 = 12.0$, df = 3, $p = 0.007$) (Figure 1). Fewer eggs were laid on *C. tolmiei* (0.33 ± 0.21) than *L. columbianum* (6.5 ± 3.51) and control lilies (8.33 ± 4.39), with *P. hookeri* intermediate between these groups (1.5 ± 0.72). The number of eggs laid on cages did not vary strongly across treatments (GLM: $\chi^2 = 3.19$, df = 3, $p = 0.36$) (Figure 1). However, the ratio of eggs laid on plants versus cages varied significantly between hosts (logistic regression: $\chi^2 = 75.5$, df = 3, $p < 0.001$). The ratio of eggs on plants was significantly higher for control lilies (95% CI: 0.79–0.96) than for the other hosts, followed by *L. columbianum* (95% CI: 0.55–0.79), which was higher than *P. hookeri* (95% CI: 0.16–0.47), and *C. tolmiei* (95% CI: 0.02–0.26).

Host plants had a strong effect on larval survival time (Cox proportional hazards, $Z = 4.09$, df = 3, $p < 0.001$). Larvae on control lilies (12.3 ± 0.83 days) and *L. columbianum* (12.7 ± 0.44 days) lived longest. For all hosts, survivorship dropped from day 0 to 4 (Figure 2). All larvae that hatched on *P. hookeri* ($N = 9$) and *C. tolmiei* ($N = 1$) died within 48 h. Survival to pre-pupae was 32% for controls, 33% for *L. columbianum*, and 0% for *C. tolmiei* and *P. hookeri* (Figure 2). Time to reach the prepupal stage was similar on control lilies (13.4 ± 0.35 days) and *L. columbianum* (12.7 ± 0.44) (two-sample *t*-test: $t_{27} = 1.03$, $p = 0.31$). The number of days it took larvae to become adults was marginally longer on control lilies (24.3 ± 0.3) than on *L. columbianum* (23.0 ± 0.71) (two-sample *t*-test: $t_{12} = 1.03$, $p = 0.065$). There was no significant difference in the length (mm) of adults reared on control lilies (7.4 ± 0.16) and *L. columbianum* (6.8 ± 0.25) (two-sample *t*-test: $t_{12} = 1.03$, $p = 0.081$). However, adults reared on control lilies weighed notably more (mg) (28.5 ± 0.91) than those on *L. columbianum* (23.75 ± 1.38) (two-sample *t*-test: $t_{12} = 2.82$, $p = 0.015$).

For the 2018 *C. tolmiei* oviposition trial, a total of 15 eggs were laid on the eight *C. tolmiei* plants (1.88 ± 1.23). Of the 15 eggs, 11 hatched, five larvae pupated, and three adults emerged. For the 2019 *F. affinis* trial, a total of 55 eggs were laid on eight *F. affinis* plants (6.88 ± 0.88).

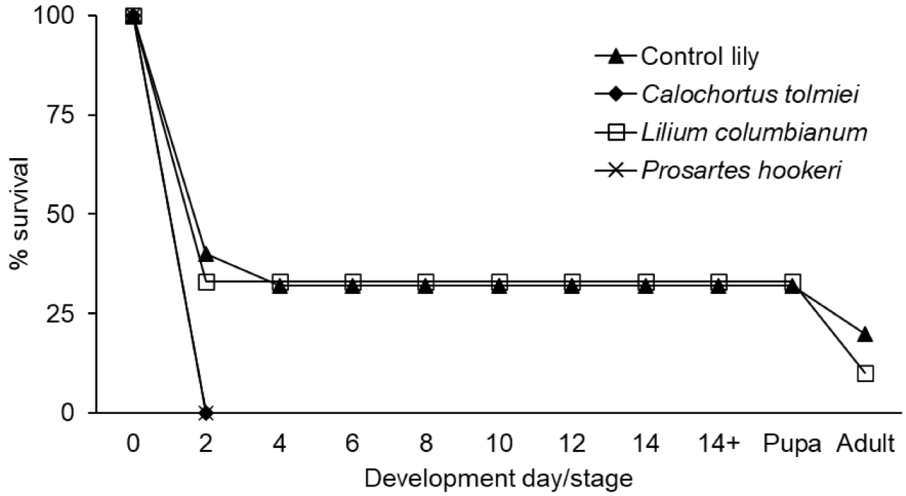

FIGURE 2 Survival of *Lilioceris lilii* larvae on four species of Liliaceae in no-choice oviposition trials. Survival is the proportion of the initial cohort of larvae for each host plant species alive at the start of each 2-day interval during the larval period, the pupal stage, or adult life stage.

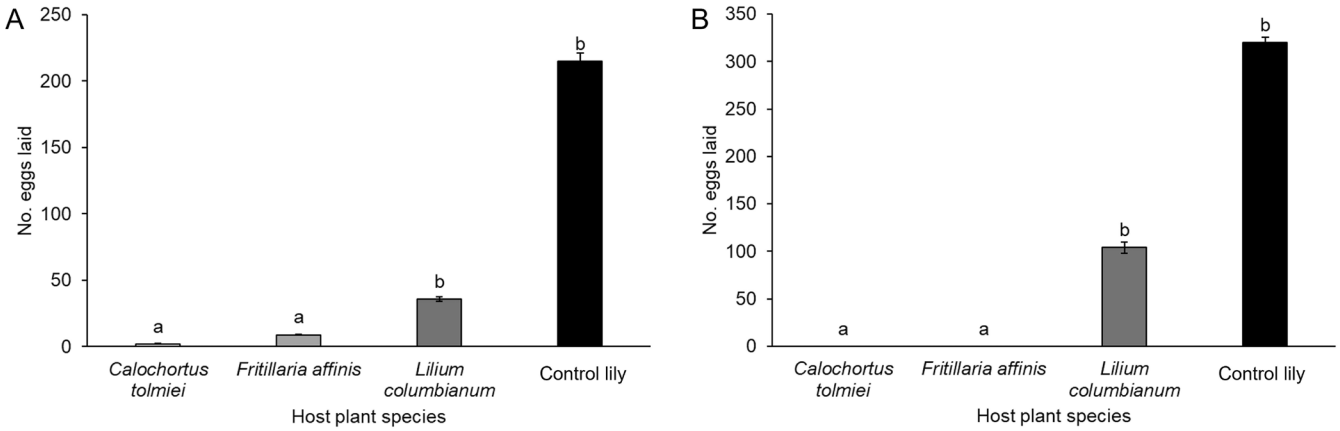

FIGURE 3 Total number of eggs (mean ± standard error) laid by *Lilioceris lilii* in the (A) 2018 and (B) 2019 choice oviposition trials. Different letters above the bars indicate a significantly different number of eggs laid on plants of various host species according to the generalized linear model results ($p = 0.05$).

Oviposition choice trial

In 2018, the number of eggs laid differed across hosts (GLM: $\chi^2 = 74.7$, df = 3, $p < 0.001$). More eggs were laid on control lilies (26.9 ± 8.27) than on other hosts. *Lilium columbianum* (4.5 ± 1.55) had more eggs than *C. tolmiei* (0.25 ± 0.19), with *F. affinis* intermediate between them (1.12 ± 0.51) (Figure 3A). The number of eggs laid by *L. lilii* in 2019 also differed significantly across hosts (GLM: $\chi^2 = 76.4$, df = 3, $p < 0.001$). Significantly more eggs were laid on control lilies (40 ± 5.5) and *L. columbianum* (4.5 ± 1.55) than on *C. tolmiei* (0 ± 0) and *F. affinis* (0 ± 0) (Figure 3B).

Laboratory larval feeding and development trial

Larvae feeding on *P. hookeri* all died between Day 0 and Day 2 (Figure 4A). Half of the larvae feeding on *C. tolmiei* died between Day 0 and Day 2, with one more dying on Day 5 when it drowned in condensation that formed on the petri dish. A single larva on *L. columbianum* died on Day 16. The overall larval survivorship to the pre-pupal stage between plant species was 100% for the control lily, 87.5% for *L. columbianum*, 25% for *C. tolmiei*, and 0% for *P. hookeri* (Figure 4A).

The number of days it took larvae to reach the pre-pupal stage varied significantly between the control lily (14.5 ± 0.19), *L. columbianum* (15.4 ± 0.57) and *C. tolmiei* (19.0 ± 0) treatments (one-way ANOVA: $F_{2,13} = 7.75$, $p = 0.006$); larvae on both control lilies and *L. columbianum* had significantly shorter development times than larvae fed on *C. tolmiei* (Tukey HSD). The number of days it took pupated larvae to emerge as adults did not significantly vary between the control lily (27.5 ± 0.19), *L. columbianum* (28.3 ± 0.33) or *C. tolmiei* (28.0 ± 0) (one-way ANOVA: $F_{2,13} = 2.64$, $p = 0.11$).

The length (mm) of emerged adults from control lilies (7.25 ± 0.16), *L. columbianum* (6.75 ± 0.31), and *C. tolmiei*

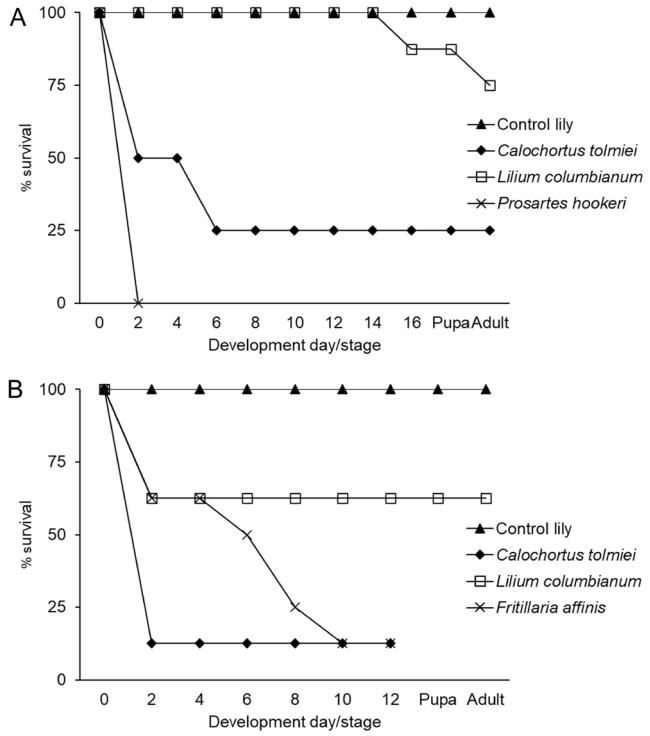

FIGURE 4 Survival of *Lilioceris lilii* larvae reared on four species of Liliaceae in the (A) 2017 and (B) laboratory larval feeding and development trial. Survival is the proportion of the initial cohort of larvae for each host plant species alive at the start of each 2-day interval during the larval period, the pupal stage, or adult life stage.

(5.5 ± 0) varied significantly across hosts (one-way ANOVA: $F_{2,12} = 1.53$, $p = 0.04$), with individuals on control lilies significantly larger than those on the C. *tolmiei* treatment (Tukey HSD). No other significant difference was detected for other pairwise comparisons between any hosts (Tukey HSD). The weight (mg) of the emerged adults reared on the control lilies (18.8 ± 0.98), *L. columbianum* (17.3 ± 0.84), and *C. tolmiei* (14.0 ± 0) did not vary significantly across hosts (one-way ANOVA: $F_{2,12} = 1.836$, $p = 0.20$).

For the second feeding trial in 2019, seven larvae in the *C. tolmiei* treatment, two larvae in the *F. affinis* treatment, and three larvae in the *L. columbianum* treatment died within 2 days. Larval attrition continued for *F. affinis* until Day 10; after which, a single larva survived (Figure 4B). All available host plant material was consumed by the remaining larva *on C. tolmiei* on Day 12 and the larva on *F. affinis* on Day 14. Thus, larval survivorship was only compared between the plant hosts until Day 12. On Day 12, overall larval survivorship between plant species was 100% for the control lily, 63% for *L. columbianum*, and 13% for both *C. tolmiei* and *F. affinis*.

Both the control lily and *L. columbianum* hosts supported larval development to the pre-pupal stage, and all pupated larvae successfully emerged as adults. Larval weight gain remained fairly similar across host plant treatments, with a significant difference in mass only detected on Day 2 (one-way ANOVA: $F_{3,15} = 10.3$, $p = 0.0006$) and Day 12 (one-way ANOVA: $F_{3,15} = 13.8$, $p = 0.0005$) (Figure 5). On

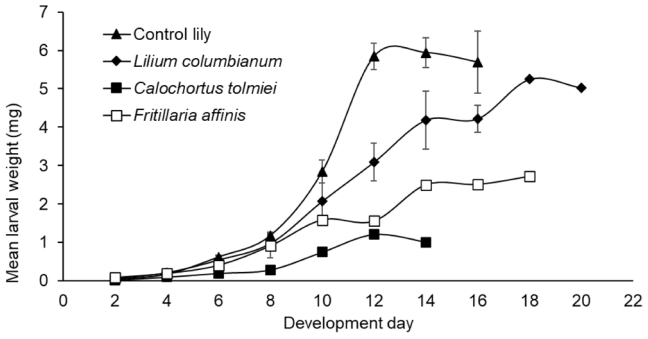

FIGURE 5 Weight (mean ± standard error; in mg) of *Lilioceris lilii* larvae reared on four species of Liliaceae for the 2019 laboratory larval development trial, at 2-day intervals.

Day 2, the larvae feeding on the control lilies were significantly heavier than the larvae feeding on *F. affinis* and on *L. columbianum* (Tukey HSD). On Day 12, the larvae feeding on the control lilies were significantly heavier than the larva on *C. tolmiei*, *L. columbianum*, or on *F. affinis* (Tukey HSD).

Larvae that fed on control lilies (13.4 ± 0.18) reached prepupae in marginally fewer days than larvae on *L. columbianum* (16.8 ± 1.2) (two-sample *t*-test: $t_{11} = 2.82$, $p = 0.05$). The number of days it took pupae to emerge as adults did not vary between control lilies (31.9 ± 0.85) and *L. columbianum* (29.6 ± 0.81) (two-sample *t*-test: $t_{27} = 1.93$, $p = 0.080$). There was no difference in the length (mm) of adult beetles reared on the control lilies (7.63 ± 0.16) and *L. columbianum* (7.2 ± 0.12) (two-sample *t*-test: $t_{11} = 2.14$, $p = 0.060$). There was also no significant difference in weight (mg) of the adult beetles reared on control lilies (23.9 ± 0.14) and *L. columbianum* (21.0 ± 0.63) (two-sample *t*-test: $t_{11} = 1.84$, $p = 0.09$).

DISCUSSION

Female *L. lilii* oviposited on all hosts tested, and larvae survived for over a week on all hosts except *P. hookeri*. However, the only hosts on which larvae developed to adulthood were *L. columbianum* and *C. tolmiei*. This suggests *L. lilii* could impact native hosts across the Pacific Northwest US, although there may be large variability in risk to various hosts. Records for native species of *Calochortus*, *Fritillaria*, and *Lilium* show species from all three genera occur within 20 km of the current infestation site of *L. lilii* in Washington State. Lily leaf beetle adults are strong fliers (Ernst, 2005), such that these native plants could easily be reached as the beetle disperses. With nearly 70 Pacific Northwest species of *Calochortus*, *Fritillaria*, and *Lilium* that are endangered, threatened, or sensitive, the spread of *L. lilii* may present a threat to natural areas (California Department of Fish and Wildlife, 2018; Idaho Bureau of Land Management, 2016; Oregon Biodiversity Information Center, 2016; Washington Natural Heritage Program, Washington Department of Natural Resources, 2017).

Our assays show there was widespread variation in oviposition behavior and development across hosts tested. When given a choice between a control lily and native species of Pacific Northwest Liliaceae, mated female *L. lilii* preferred the control. Moreover, larvae that fed on the control lilies had the quickest development time, the highest survival, and the largest adults. The native *Lilium columbianum* was also readily used as an oviposition site even when control lilies were available. Larval survival was high on *L. columbianum*, with many larvae completing their lifecycles. Larvae that fed on *L. columbianum* displayed similar development time to the larvae that fed on control lilies. The adults that emerged from the *L. columbianum* treatments, though sometimes having a lower weight, were very similar in size to the adults that fed on the control lilies. These results, along with observations from Ernst et al. (2007) and Blackman et al. (2016) indicate that native *Lilium* across North America are suitable hosts for *L. lilii*.

We also observed that female *L. lilii* will oviposit *on C. tolmiei* (divergence time 38 mya from *Lilium*). Although larval attrition was high for *C. tolmiei*, surviving larvae readily fed on the host plant and pupated. Larvae that successfully fed on *C. tolmiei* had similar development time, although adult and larval weight gain were lower than that of larvae fed *Lilium* species. This indicates *C. tolmiei* can be a viable host plant for *L. lilii*, particularly when species of Lilium are not available. Although *L. lilii* oviposit on *P. hookeri* (divergence time 36 mya from *Lilium*) when no lily is available, the death of each larva within 48 h indicates that this species is not a suitable *L. lilii* host plant. Even though none of the larvae in our experiments were able to fully complete their development on *F. affinis* (divergence time 4 mya from *Lilium*), many eggs were laid on *F. affinis*, and larvae steadily gained weight feeding on this species for up to 14 days.

Ernst et al. (2007) found lily leaf beetle larvae could feed and reach the pre-pupal stage on multiple native eastern North American Liliaceae, and that genera with a closer divergence time to *Lilium* were more likely to be acceptable hosts (*Lilium philidelphicum* L., *Medeola virginiana* L. [divergence time 30 mya from *Lilium*], and *Streptopus amplexifolius* (L.) DC.[divergence time 37 mya from *Lilium*]). However, we show the genus *Prosartes* (divergence time 36 mya from *Lilium*) is an unsuitable host, whereas *Calochortus* (divergence time 38 mya from *Lilium*) is suitable, such that divergence time is not the only predictive factor of host suitability. More broadly, our study adds to the literature showing invasive insects particularly threaten hosts similar to hosts in their native region (Uden et al., 2023). Assessing phylogenic relationships among host species can generate risk profiles, as was done with 47 conifer species in North America and 62 potentially invasive Eurasian insects (Uden et al., 2023). Deployment of sentinel plants, along with field and laboratory experiments like those conducted here, can be a useful tool to assess the potential risk of invasive species to native hosts, as has been done with invasive aphids (Redlich et al., 2019).

The risk posed by *L. lilii* to native host plants occurring in the Pacific Northwest United States will depend not only on host suitability but also on the spatial and temporal overlap between the hosts and the insect (Dang et al., 2021; Koch, 2021; Rand & Louda, 2006). Habitat suitability models indicate considerable areas of western Oregon and Washington States, northern Idaho, and Montana are susceptible to the future spread of *L. lilii* (Freeman et al., 2020). Such models could predict risk to native species by considering how *L. lilii* spread may overlap with native host distributions (Dang et al., 2021; Koch, 2021). Host plants that have suitable tissue during periods when *L. lilii* are active will likely be at greater risk than host plants that occur during periods of low beetle activity. Future management and risk assessment activities should consider both the spatial and temporal overlap between *L. lilii* and native host plants as the invasion spreads.

Our larval performance trials on *F. affinis* and *C. tolmiei* should be interpreted cautiously given that *Fritillaria* are known hosts for *L. lilii* in other regions (Bouchard et al., 2008; Ernst et al., 2007) and because some plants senesced while trials were conducted. Moreover, we ran out of leaves in laboratory trials; that may have contributed to low survival. In western Washington, *C. tolmiei* and *F. affinis* develop earlier than most *Lilium* species (Turner & Gustafson, 2006). Our experiments were conducted in late May, when most *Fritillaria* and *Calochortus* were senescing, and both genera may be more attractive when their leaves are younger. Our results may indicate a phenological mismatch between *L. lilii* and some native species, making them less suitable for oviposition and development even if they are otherwise viable host plants. Even so, the beetle is active as early as March in western Washington, suggesting that in some climates early season plants could be at risk as the only available food source.

Findings from these experiments help provide important data to assess the lily leaf beetle's potential to invade and impact native Liliaceae species in the Pacific Northwest, United States. However, there are several avenues of future research that require exploration. First, an in-depth analysis of the various Liliaceae species' chemical composition, leaf structures, and secondary metabolites would help explain why *L. lilii* oviposit and feed on some but not all genera in the Liliaceae family. Second, an analysis of chemical and defensive traits of various lilies could be compared to historical divergence times. Finally, future studies should examine how climate change impacts the lily leaf beetle's emergence time in the spring to see whether the beetle's phenology overlaps with Liliaceae genera such as *Calochortus*. Despite these caveats, to help mitigate the impacts of this invasive beetle, the Washington State Department of Agriculture initiated a classical biological control program with releases of two parasitoid wasp species, *Tetrastichus setifer* Thomson and *Diaparsis jucunda* Holmgren. These specialist wasps have previously been released in Connecticut and Rhode Island, where reduced

beetle populations and parasitism rates of over 90% were observed (Tewksbury et al., 2017). Although this may be promising, these tiny wasps only spread up to 5 km a year (Tewksbury et al., 2017) and will take considerable time to spread throughout the Pacific Northwest. In the interim, threatened native species should be closely monitored as the beetle expands its range. Further host plant testing should also be conducted in field sites with overlap to beetles to help define management priorities.

AUTHOR CONTRIBUTIONS

Maggie Freeman: Writing – original draft; writing – review and editing; conceptualization; methodology; investigation; data curation; formal analysis. **Chris Looney:** Conceptualization; supervision; writing – original draft; writing – review and editing. **Liesl Oeller:** Resources; writing – review and editing; project administration. **David W. Crowder:** Conceptualization; supervision; writing – original draft; writing – review and editing.

ACKNOWLEDGEMENTS

We thank Bellevue College for space and Seven Oak Native Nursery for plants. We thank the Bellevue Demonstration Garden and residents that allowed us access to collect beetles. We thank H. Amms for aid with statistical analysis.

FUNDING INFORMATION

Support was provided by the Washington State Department of Agriculture (SCBG K-1985).

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

Data and code used for analysis are available upon request from Figshare (https://doi.org/10.6084/m9.figshare.28057418.v1) and will be made publicly available in this repository pending acceptance of this manuscript.

ORCID

Liesl Oeller https://orcid.org/0000-0003-3894-2880
David W. Crowder https://orcid.org/0000-0002-3720-1581

REFERENCES

Alyokhin, A., Mota-Sanchez, D., Baker, M., Snyder, W.E., Menasha, S., Whalon, M. et al. (2015) The red queen in a potato field: integrated pest management versus chemical dependency in Colorado potato beetle control. *Pest Management Science*, *71*, 343–356.

Blackman, C.K., Cappuccino, N. & Mason, P.G. (2016) First record of *Lilioceris lilii* (Scopoli) (Coleoptera: Chrysomelidae) on *Lilium michiganense* Farwell and confirmation of its association with *Streptopus lanceolatus* (Aiton) reveal (Liliaceae). *The Coleopterists Bulletin*, *70*, 482–484.

Bouchard, A., McNeil, J.N. & Brodeur, J. (2008) Invasion of American native lily populations by an alien beetle. *Biological Invasions*, *10*, 1365–1372.

California Department of Fish and Wildlife. (2018) California natural diversity database. State and federally listed endangered, threatened, and rare plants of California. https://nrm.dfg.ca.gov/FileHandler.ashx?DocumentID=109390&inline.

Cappuccino, N. (2017) Lily leaf beetle tracker. http://lilybeetletracker.weebly.com.

Cappuccino, N., Haye, T., Tewksbury, L. & Casgrande, R. (2013) *Lilioceris lilii (Scopoli), LLB (Coleoptera: Chrysomelidae). Biological Control Programmes in Canada 2001–2010*. Wallingford, UK: CAB International, pp. 208–213.

Dang, Y.Q., Zhang, Y.L., Wang, X.Y., Xin, B., Quinn, N.F. & Duan, J.J. (2021) Retrospective analysis of factors affecting the distribution of an invasive wood-boring insect using native range data: the importance of host plants. *Journal of Pest Science*, *94*, 981–990.

Ernst, C. (2005) The lily leaf beetle (*Lilioceris lilii*): an unwelcome invader. *Lily Yearbook of the North American Lily Society*, *58*, 29.

Ernst, C., Cappuccino, N. & Thor, A. (2007) Potential novel hosts for the lily leaf beetle *Lilioceris lilii* Scopoli (Coleoptera: Chrysomelidae) in eastern North America. *Ecological Entomology*, *32*, 45–52.

Freeman, M., Looney, C., Orlova-Bienkowskaja, M.J. & Crowder, D.W. (2020) Predicting the invasion potential of the lily leaf beetle, *Lilioceris lilii* Scopoli (Coleoptera: Chrysomelidae), in North America. *Insects*, *11*, 560.

Hare, J.D. (1990) Ecology and management of the Colorado potato beetle. *Annual Review of Entomology*, *35*, 81–1000.

Hicks, B. & Sellars, R. (2014) Record of the lily leaf beetle *Lilioceris lilii* (Coleoptera: Chrysomelidae) in Newfoundland. *Journal of the Acadian Entomological Society*, *10*, 10–11.

Hsiao, T.H. & Pasteels, J.M. (1999) Evolution of host-plant affiliation and chemical defense in Chrysolina-Oreina leaf beetles as revealed by mtDNA phylogenies. In: Cox, M.L. (Ed.) *Advances in Chrysomelidae Biology 1*. Leiden, the Netherlands: Backhuys, pp. 321–342.

Idaho Bureau of Land Management. (2016) Bureau of Land Management Idaho Special Status Plant List. https://www.blm.gov/sites/blm.gov/files/programs_natural-resources_native-plant-communities_about-native-plants_Idaho_special-status-species.pdf.

Koch, F.H. (2021) Considerations regarding species distribution models for forest insects. *Agricultural and Forest Entomology*, *23*, 393–399.

Lesage, L. (1983) Note sur la distribution présente et future du Criocére du lys, *Lilioceris lilii* (Scopoli) (Coleoptera: Chrysomelidae) dans l'est du Canada. *Le Naturaliste Canadien*, *110*, 95–97.

LeSage, L. (1992) The lily beetle, *Lioceris lilii* (Scopoli), in Canada (Coleoptera: Chrysomelidae). *La Revue Canadienne Des Insectes Nuisibles Aux Cultures*, *70*, 88–96.

LeSage, L. & Elliott, B. (2003) Major range extension of the lily leaf beetle (Coleoptera: Chrysomelidae), a pest of wild and cultivated Liliaceae. *The Canadian Entomologist*, *135*, 587–588.

Louda, S.M., Rand, T.A., Kula, A.A.-R., Arnett, A.E., West, N.M. & Tenhumberg, B. (2011) Priority resource access mediates competitive intensity between an invasive weevil and native floral herbivores. *Biological Invasions*, *13*, 2233–2248.

Mack, R.N., Simberloff, D., Lonsdale, W.M., Evans, H., Clout, M. & Bazzac, F.A. (2000) Biotic invasions: causes, epidemiology, global consequences, and control. *Ecological Applications*, *3*, 689–710.

Maier, C.T. (2012) New distributional records of invasive Coleoptera found in Connecticut and other eastern states: *Agrilus cyanescens* (Ratzeburg) (Buprestidae), *Lilioceris lilii* (Scopoli), and *Pyrrhalta viburni* (Paykull) (Chrysomelidae). *Proceedings of the Entomological Society of Washington*, *114*, 501–512.

Majka, C.G. & Kirby, C. (2011) Lily leaf beetle, *Lilioceris lilii* (Coleoptera: Chrysomelidae), in Maine and the maritime provinces: the continuing dispersal of an invasive species. *Journal of the Acadian Entomological Society*, *7*, 70–74.

Murray, T., Looney, C., LaGasa, E. & Collman, S. (2016) Distribution of two invasive leaf beetles, *Pyrrhalta viburni* (Paykull) and *Lilioceris lilii* (Coleoptera Chrysomelidae), in Washington State. *The Coleopterists Bulletin*, *70*, 368–371.

Oregon Biodiversity Information Center. (2016) Rare, threatened and endangered vascular plant species of Oregon. http://inr.oregonstate.edu/sites/inr.oregonstate.edu/files/2016-rte-vascs.pdf.

Orlova-Bienkowskaja, M.J. (2013) Dynamics of the range of lily leaf beetle (*Lilioceria lilii*, Chrysomelidae, Coleoptera) indicates its invasion from Asia to Europe in the 16th-17th century. *Russian Journal of Biological Invasions*, *4*, 93–104.

R Core Team. (2017) *R: A language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing. https://www.R-project.org/.

Rand, T.A. & Louda, S.M. (2006) Invasive insect abundance varies across the biogeographic distribution of a native host plant. *Ecological Applications*, *16*, 877–890.

Redlich, S., Clemens, J., Bader, M.K.-F., Pendrigh, D., Perret-Gentil, A., Godsoe, W. et al. (2019) Identifying new associations between invasive aphids and Pinaceae trees using plant sentinels in botanic gardens. *Biological Invasions*, *21*, 217–228.

Salisbury, A., Clark, S.J., Powell, W. & Hardie, J. (2010) Susceptibility of six *Lilium* to damage by the lily beetle, *Lilioceris lilii* (Coleoptera: Chrysomelidae). *Annals of Applied Biology*, *156*, 103–110.

Schaffner, U. & Müller, C. (2001) Exploitation of the fecal shield of the lily leaf beetle, *Lilioceris lilii* (Coleoptera: Chrysomelidae), by the specialist parasitoid *Lemophagus pulcher* (hymenoptera: Ichneumonidae). *Journal of Insect Behavior*, *14*, 739–757.

Tewksbury, L., Casagrande, R.A., Cappuccino, N. & Kenis, M. (2017) Establishment of parasitoids of the lily leaf beetle (Coleoptera: Chrysomelidae) in North America. *Environmental Entomology*, *46*, 226–236.

Turner, M. & Gustafson, P. (2006) *Wildflowers of the Pacific northwest*. Portland, OR, USA: Timber Press Field Guide.

Uden, D.R., Mech, A.M., Havill, N.P., Schulz, A.N., Ayres, P., Herms, D.A. et al. (2023) Phylogenetic risk assessment is robust for forecasting the impact of European insects on north American conifers. *Ecological Applications*, *33*, e2761.

USDA NRCS. (2019) The plants database. http://www.plants.usda.gov/.

Utech, F.H. (2003) Liliaceae. In: *Flora of North America Editorial Committee, eds*. New York and Oxford: Flora of North America North of Mexico, pp. 124–166.

Vilà, M., Espinar, J.L., Hejda, M., Hulme, P.E., Jarošík, V., Maron, J.L. et al. (2011) Ecological impacts of invasive alien plants: a meta-analysis of their effects on species, communities and ecosystems. *Ecology Letters*, *14*, 702–708.

Washington Natural Heritage Program, Washington Department of Natural Resources. (2017) Endangered, threatened, and sensitive vascular plant list. https://www.dnr.wa.gov/publications/amp_nh_vascular_ets.pdf?mvznr.

How to cite this article: Freeman M, Looney C, Oeller L & Crowder DW (2025) Oviposition behavior and development of the invasive lily leaf beetle on native host plants from the Pacific Northwest United States. *Entomologia Experimentalis et Applicata 173*: 1196–1204. https://doi.org/10.1111/eea.70008