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Original Communication

Differential acceptability of conifer hosts to feeding by western conifer seed bug, *Leptoglossus occidentalis* Heidemann (Heteroptera: Coreidae)

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ABSTRACT

The western conifer seed bug, *Leptoglossus occidentalis* Heidemann (Heteroptera: Coreidae) is an important pest of conifer seed orchards in western North America. In laboratory studies, we tested the feeding and oviposition activity of adult *L. occidentalis* when presented with four different hosts from the inter-mountain western United States and also tested the survival and development of nymphs when confined to these individual hosts. Adult *L. occidentalis* preferentially fed on pine hosts over non-pine hosts, and females oviposited on ponderosa pine more frequently than on any other surface. *L. occidentalis* nymphs survived the longest and were most likely to complete development on second-year cones of lodgepole pine. Survival of nymphs was shortest in non-fed control, water-only and first-year pine cone treatments, none of which resulted in maturation to adulthood. Pine foliage was preferred as an oviposition site over the non-pine material and was more suitable for maturation compared with non-pine hosts. The differences between our results and those of prior reports are discussed in terms of phylogenetic distance between Douglas-fir in inland versus coastal areas, potential for seasonal shifts in host preference over the course of a summer and the potential adaptations by the insect in various geographic areas.

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KEYWORDS: conifer seed orchards, host suitability, host selection

INTRODUCTION

Conifer seed orchards are used to produce high quality seeds from selected genotypes of important tree species. Orchards are important for the improvement of genetic stock in timber applications and for reforestation efforts following disturbance events such as wildfire and harvesting. These orchards can be expensive to establish and may require intensive management. Further, in western North America, these orchards often produce multiple species of native conifers.

The western conifer seed bug *Leptoglossus occidentalis* Heidemann (Heteroptera: Coreidae) is a major pest of seed orchards throughout western North America [1]. Seed bugs overwinter as adults and initiate flight and feeding activity with warm weather in late spring or early summer. Females oviposit on host tree needles during the course of a season, averaging just under a total of 80 eggs per female [2]. Nymphs emerge after approximately ten days and complete five instars before maturation. Both nymphs and adults feed on maturing cones and seeds of a number of conifer species belonging to multiple genera (including *Abies* (Plin. ex Tourn.) Miller, *Pinus* Linnaeus and *Pseudotsuga* (Carriére) until the onset of cold weather [1, 3]. The cones of these major host tree genera develop over different time periods, *Pinus* cones require two summers to mature [4, 5] while cones of *Pseudotsuga* and *Larix* mature during a single summer [6, 7].

Leptoglossus occidentalis feeding on Douglas-fir, *Pseudotsuga menziesii* (Mirb.) Franco, cones can result in reductions of 78% of seed lipids and 97% of insoluble proteins [8]. Additionally, feeding on mature seeds reduces seedling emergence by up to 80%. Seed bugs have been estimated to be responsible for the loss of 5% to > 50% of mature seeds in Douglas-fir in western British Columbia [9, 10], and early season feeding by the insect may account for an additional 50% increase in aborted seeds of Douglas-fir in western Oregon [11]. Feeding by *L. occidentalis* nymphs on first-year cones of lodgepole pine, *Pinus contorta* var. *latifolia* Engelmann, reduced seed production by 75% [12]. Similarly, seed bug feeding on western white pine, *P. monticola* Dougl. ex D. Don, resulted in abortion of 75% of the first-year cones and a reduction in seed production of 47% in the surviving cones [12]. Life tables for *L. occidentalis* on lodgepole pine indicate that the cumulative damage from a single adult seed bug and its offspring could result in an average loss of approximately 310 seeds per season [2].

Prior studies that have examined host location and selection by *L. occidentalis* have focused on the insect's choice among trees within a species and cones within a tree [13, 14]. In the wild, adult *L. occidentalis* use infrared-sensing organs on their abdomen to find cones on conifers at dusk [14] but they also respond to some physical characteristics of both Douglas-fir and lodgepole pine in selecting host trees [13]. In seed orchards, *L. occidentalis* preferred trees of moderate height, with moderate numbers of large, well-laden cones for feeding and oviposition. No controlled, direct examination of host selection by *L. occidentalis* adults when simultaneously presented with multiple host species has been reported. Therefore, the specific objectives of this study were to: 1) examine feeding and oviposition preference of adult *L. occidentalis* when presented with multiple potential conifer hosts in a laboratory setting and 2) compare the suitability of potential conifer hosts for survival and development of *L. occidentalis* nymphs.

MATERIALS AND METHODS

Locations and experimental organisms

All experiments were conducted during the summer of 2010 in Moscow, ID, USA. Adult *L. occidentalis* were collected from the University of Idaho campus in Moscow from ornamental pines (a combination of mugo pine *P. mugo* Turra, European black pine *P. nigra* J. F. Arnold, limber pine *P. flexilis* E. James, and Scots pine *P. sylvestris* Linnaeus). Non-native hosts were used as collection substrate to limit the possibility of host acclimation by the insects to the tested host species prior to their use in the experiments. Insects were maintained at 4°-6 °C for less than 48 hours before trials were initiated. Immature seed bugs were reared in the laboratory from eggs laid by the collected adults. Laboratory insects were maintained at ambient temperature (approximately 23°-28 °C) and lighting (approximately 12:12 light:dark hour cycle).

Cones to be used in the experiments were obtained from 3 locations, all in Latah County, ID. Douglas-fir, lodgepole pine and western larch cones were obtained from the University of Idaho campus. Douglas-fir, lodgepole pine and ponderosa pine cones were obtained from the University of Idaho Experimental Forest, located 6.4 km south of Harvard, ID. Western white pine cones were obtained from the East Fork Western White Pine Seed Orchard maintained by the Idaho Department of Lands, located 5.4 km east of Boville, ID.

Choice trials, adult selection and oviposition

Host selection trials occurred from late June through mid-July in 30 x 30 x 30 cm fabric mesh enclosures (BugDorm-1 Insect Rearing Cages, Mega View Science Education services Co., Ltd.). Sprigs of foliage, with a single cone per sprig, of Douglas-fir, ponderosa pine, lodgepole pine and western larch were randomly assigned to locations and placed in the enclosures approximately equidistant from one another, the cage wall, and the release point for the insects. There were not enough western white pine cones available to use in both adult selection trials and nymph survival trials (see below). Sprigs were supplied with water in glass tubes and stabilized in glass jars.

Trials were maintained at ambient temperature, with a 16:8 hour (light:dark) cycle. Individual *L. occidentalis* adults were introduced to the test enclosures and monitored for three successive days for their location within the enclosure and apparent activity. In the first trial, insects were monitored daily (N = 6 male, 6 female; total N = 12 insects) while in the second and third trials, insects were monitored every two hours (N = 6 male, 6 female per trial; total N = 12 insects per trial). At the end of each three-day trial, the number of eggs per clutch, the number of clutches, and location of each clutch (on which host and/or enclosure structure) were recorded. For the two trials in which insects were monitored every two hours, the host it was located on during each observation period was recorded. All of the tested females oviposited at least one clutch of eggs during the test periods. The insertion of the mouthparts into host material was recorded during each period and used as an indication of feeding by the individual insects. If the insects were on a host but did not have their mouthparts inserted into plant tissue, they were not recorded as feeding.

No-choice trials of adult oviposition

To test the acceptability of the two hosts that were not selected for oviposition sites in the tests involving multiple hosts, no-choice tests were conducted using the 30 x 30 x 30 cm enclosures. Within an enclosure, only Douglas-fir or western larch was presented as a potential oviposition host to determine if, in the absence of pine, the insects would utilize them as an oviposition substrate. Individual *L. occidentalis* females (N = 7 females per host) were added to each enclosure. Trials were maintained at ambient temperature, with a 16:8 hour (light:dark) cycle for three days, at which point the number of eggs per clutch, and the number and location of clutches were recorded. All of the tested females oviposited at least one clutch of eggs during the test periods.

No-choice trials of nymph survival

Five, newly-hatched (within 48 hours) *L. occidentalis* with no previous exposure to any food source were placed into 1.0 liter mesh-covered glass containers with one of the following potential nutrient sources: first-year cones of Douglas-fir, lodgepole pine and ponderosa pine; second-year cones of western white pine, lodgepole pine and ponderosa pine; water only, or no-water control. Western white pine first-year cones were not available from the areas where cones were being obtained for this study. There were also not enough western larch cones available for the full trial so this host was dropped from the trial. The water-only and no-water control treatments were replicated five times (N = 25 nymphs per treatment). All host trials were replicated ten times (N = 50 nymphs per treatment). Mortality and approximate instars were determined daily, and insects reaching adulthood were weighed and sexed.

Statistical analysis

Oviposition data from selection trials were compared using an analysis of variance with a protected Fisher's least significant difference (LSD) test on all-pair wise comparisons. For each replicate of the suitability trials that were monitored every two hours, observations of feeding behavior on all hosts were totaled, and the number of observations on a given host were divided by the total to calculate the proportion of feeding on each host. Proportions were log transformed, and differences among transformed values were analyzed using a generalized linear model (PROC GENMOD in SAS 9.2) assuming a binomial distribution [15]. Pair-wise contrasts were analyzed by comparing the differences among least squares means. No-choice oviposition data were analyzed using Student's t-tests [16]. Survival curves of host suitability were analyzed using Kaplan-Meier product limit survivorship analysis [16]. Kaplan-Meier survivorship analysis is a non-parametric procedure that estimates the survival function of a population from lifetime data as a series of horizontal steps of declining magnitude [17]. Survival time and proportion of insects to reach adulthood were compared using analysis of variance tests with a protected Fisher's LSD test [16]. Adult weight and time taken to reach adulthood were analyzed using analysis of variance (by gender) with all of the data from conifer species for which means and standard errors could be calculated [16].

RESULTS

Choice trials, adult selection and oviposition

When placed in enclosures with four hosts to choose from, female *L. occidentalis* deposited significantly more eggs on ponderosa pine than on other surfaces ($F_{4,75}$ = 17.44, $P < 0.0001$, Figure 1). The surface of the enclosure had the second highest number of eggs deposited on it, followed by lodgepole pine. No eggs were laid on either Douglas-fir or western larch.

Feeding behavior was not recorded during the trial that was monitored once per day. In the trials that were monitored every two hours, there were significant differences among species in the proportion of *L. occidentalis* feeding on a specific host (June trial, $\chi^2 = 109.17$, $P < 0.0001$; July trial, $\chi^2 = 57.04$, $P < 0.0001$, Figure 2). The proportion of *L. occidentalis* feeding on ponderosa pine during June was significantly higher than the feeding on western larch, lodgepole pine or Douglas-fir. During July, proportions of *L. occidentalis* feeding on ponderosa pine did not differ from feeding on lodgepole pine and both were significantly higher than the proportion of feeding that occurred on Douglas-fir. There was no observed feeding on western larch during the July trial.

When comparing the proportional feeding of *L. occidentalis* by host species, there was a gender-by-host species interaction between the June and July trials ($\chi^2 = 66.63$, $P < 0.0001$, Figure 3). Males consistently preferred to feed on ponderosa pine during both June and July, while females fed predominantly on ponderosa pine during June but fed equally on ponderosa pine and lodgepole pine during July. Further, western larch was not recorded being fed upon by either males or females during the July trial period.

No-choice trials of adult oviposition

No-choice tests were used to determine if female *L. occidentalis* would oviposit on Douglas-fir or western larch foliage if there was no pine host available. Females did oviposit on both Douglas-fir and western larch foliage but there was no significant difference in the number of eggs laid on the foliage versus on the surface of the enclosure (Figure 4).

No-choice trials of nymph survival

L. occidentalis nymphs survived significantly longer on the second-year cones of lodgepole pine than on any other host, followed by survival on second-year cones of western white pine and

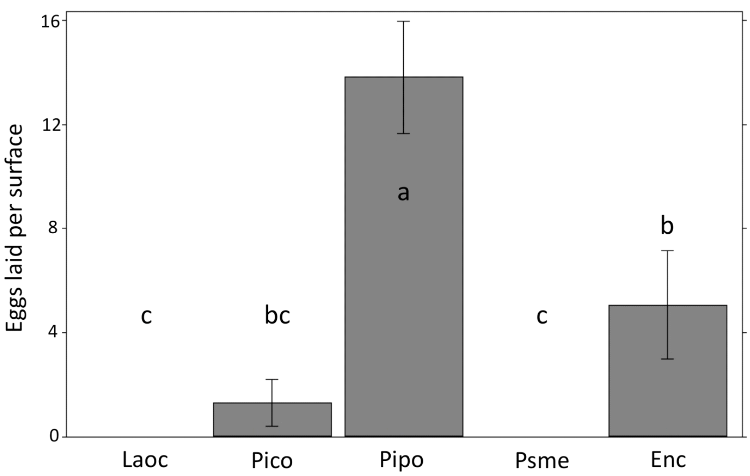

Figure 1. Mean number of eggs (± SEM) on surfaces oviposited by adult *Leptoglossus occidentalis* when simultaneously presented with four hosts in preference trials. Bars with different letters ('a', 'b' or 'c') are significantly different based upon analysis of variance with protected Fisher LSD tests. Oviposition surface abbreviations are: western larch, *Larix occidentalis* = Laoc; lodgepole pine, *Pinus contorta* = Pico; ponderosa pine, *Pinus ponderosa* = Pipo; Douglas-fir, *Pseudotsuga menziesii* = Psme; experimental enclosure = Enc.

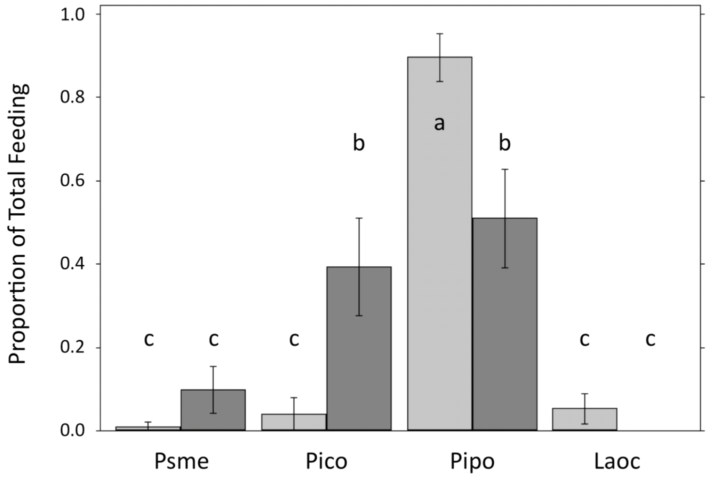

Figure 2. Mean proportion of feeding (± SEM) by adult *Leptoglossus occidentalis* by conifer species when simultaneously presented with four hosts in preference trials. Bars with different letters ('a', 'b' or 'c') are significantly different (Logit-transformed least-squares means). Lighter bars indicate June test period and darker bars indicate July test period. Tree species abbreviations are: western larch, *Larix occidentalis* = Laoc; lodgepole pine, *Pinus contorta* = Pico; ponderosa pine, *Pinus ponderosa* = Pipo; Douglas-fir, *Pseudotsuga menziesii* = Psme.

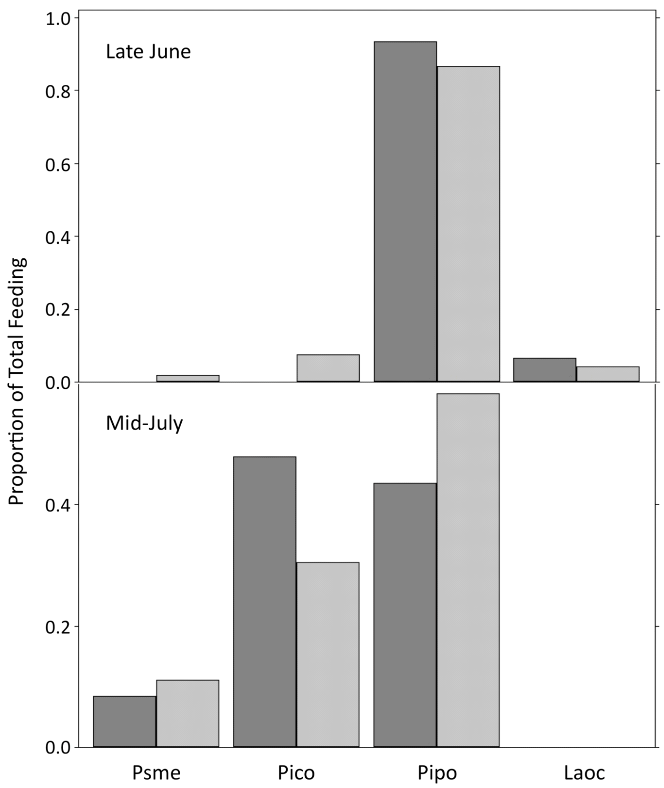

Figure 3. Mean proportion of feeding (± SEM) by female and male *Leptoglossus occidentalis* adults when simultaneously presented with four host species during two test periods, June and July. Lighter bars indicate female feeding and darker bars indicate male feeding. Tree species abbreviations are: western larch, *Larix occidentalis* = Laoc; lodgepole pine, *Pinus contorta* = Pico; ponderosa pine, *Pinus ponderosa* = Pipo; Douglas-fir, *Pseudotsuga menziesii* = Psme.

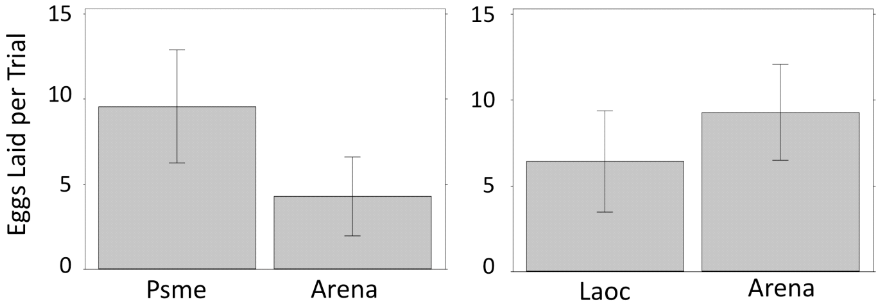

Figure 4. Mean number of eggs (± SEM) oviposited by female *Leptoglossus occidentalis* on surfaces in 30 x 30 x 30 cm fabric mesh arenas containing either western larch, *Larix occidentalis* = Laoc or Douglas-fir, *Pseudotsuga menziesii* = Psme.

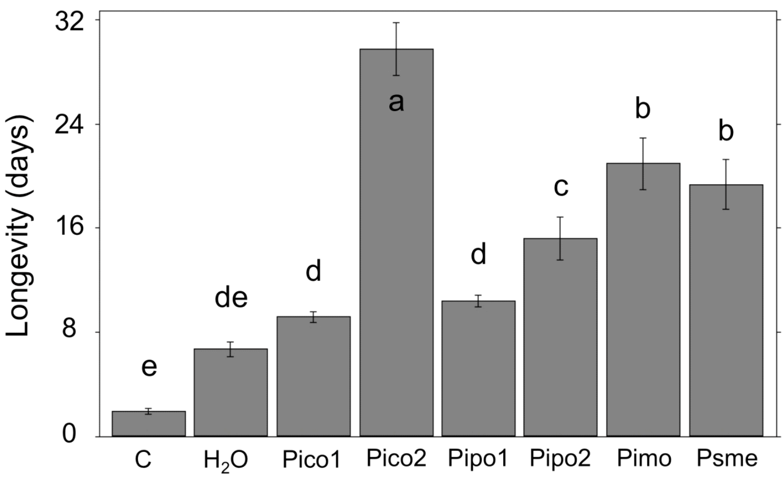

Figure 5. Mean longevity (± SEM), in days, of *Leptoglossus occidentalis* nymphs feeding on material of varying nutrient status. Bars with different letters ('a', 'b', 'c', 'd' or 'e') indicate significant differences among nutrient sources based upon analysis of variance with protected Fisher LSD test. Abbreviation for the nutrient sources are: control, no nutrient source = C; water only = H_2O; first-year cones of lodgepole pine, *Pinus contorta* = Pico1; second-year cones of lodgepole pine = Pico2; first-year cones of ponderosa pine, *Pinus ponderosa* = Pipo1; second-year cones of ponderosa pine = Pipo2; second-year cones of western white pine, *Pinus monticola* = Pimo; cones from Douglas-fir, *Pseudotsuga menziesii* = Psme.

Douglas-fir cones (Figure 5). Survival of nymphs was significantly shorter when they were provided second-year cones of ponderosa pine. Survival of nymphs on first-year cones of either lodgepole pine or ponderosa pine did not significantly differ from survival on the water-only treatment.

The length of time that nymphs survived on each type of host material is less important than the proportion of the population that can reach maturity on the host. Nymphs matured to adulthood only when feeding on second-year cones of the pine species or on Douglas-fir cones (Figure 6). A significantly higher proportion of nymphs ($F_{3,39}$ = 15.79, P < 0.0001) did mature on second-year lodgepole pine than in any other treatment. Only one nymph reached adult maturity when feeding exclusively on Douglas-fir cones. There was no significant difference among treatments in adult weight ($F_{1,36}$ = 0.39, P = 0.5365) or time taken to reach adulthood ($F_{1,36}$ = 0.63, P = 0.4334).

DISCUSSION

Under laboratory conditions using host material from the inter-mountain western United States,

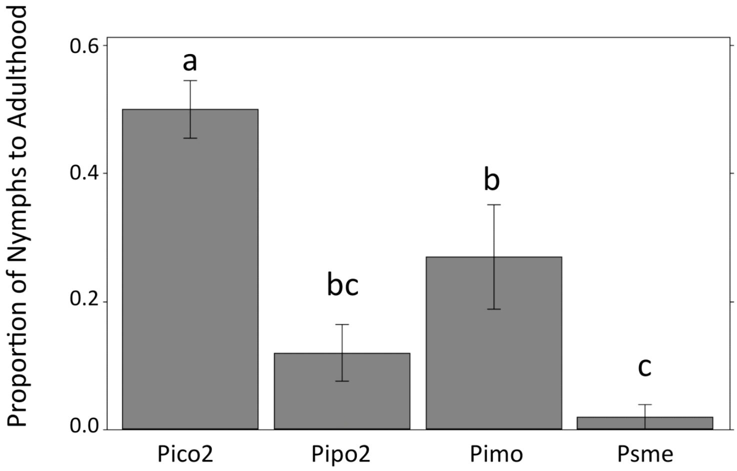

Figure 6. Mean proportion (± SEM) of *Leptoglossus occidentalis* nymphs to reach maturity while feeding on individual species of host conifer cones. Bars with different letters ('a', 'b' or 'c') indicate significant differences among nutrient sources based upon analysis of variance with protected Fisher LSD test. Only the nutrient sources on which some nymphs reached adulthood are presented. Abbreviations for the nutrient sources are: second-year cones of lodgepole pine, *Pinus contorta* = Pico2; second-year cones of ponderosa pine, *Pinus ponderosa* = Pipo2; second-year cones of western white pine, *Pinus monticola* = Pimo; cones of Douglas-fir, *Pseudotsuga menziesii* = Psme.

pine hosts were more likely to be fed and oviposited upon by adult *L. occidentalis* than any of the non-pine species that were tested. Specific cues responsible for the preferential use of pine were not elucidated. Under field conditions, the number of eggs on individual lodgepole pine and Douglas-fir appear to be related to host shape and cone density [13]. Tree shape and cone density are not cues that would have been available in our laboratory tests. Also, with multiple hosts in close proximity, females would have probably received a mix of host-specific chemical cues. While oviposition preference may be expected to correlate with host suitability, in the tests in which only the less preferred Douglas-fir or western larch were provided, females appeared to have difficulty stabilizing themselves on the foliage while ovipositing. No such difficulty was observed on the thicker, stiffer pine needles or on the surface of the enclosure. Therefore, the observed preference in oviposition substrate may be related to the ease with which the female can adhere to the surface.

There was an apparent shift in feeding preference of adults from June to July. During June, adult *L. occidentalis* preferentially fed upon ponderosa pine, while during July they fed with similar frequency on ponderosa pine and lodgepole pine. While only ten days apart, there was fresh plant material used at the start of each trial and the two tests occurred during a time of rapid cone expansion. Therefore, physical and chemical changes in cone and seed composition were possibly occurring during the period and these may have resulted in the observed changes in host preference.

Female *L. occidentalis* laid more eggs on ponderosa pine than any of the other hosts while nymphs survived significantly longer on lodgepole pine, western white pine and Douglas-fir. In addition, nymphs reached adulthood with greater frequency on pine versus non-pine hosts. Our laboratory results confirmed a previous report that first-year lodgepole pine cones are an inadequate food source for maturation of *L. occidentalis* nymphs [12]. Nymphs feeding on first-year ponderosa pine cones also did not reach maturity. There was an initial molt from first to second instars when nymphs were provided with water but no source of nutrients, confirming that host material is not necessary for first instar nymphs to molt [18]. Under field conditions, utilization of ponderosa pine as a host may be advantageous to *L. occidentalis* if there is increased survival due to decreased predation or parasitism as has been described for other forest insects that oviposit on less suitable hosts [19].

The selection trials demonstrated a low preference by *L. occidentalis* for Douglas-fir relative to pine hosts. In contrast, the presence of large numbers of *L. occidentalis* on Douglas-fir in seed orchards in western British Columbia has been reported [9] and there is evidence for substantial loss of Douglas-fir seeds to the insect in the interior region of British Columbia [10]. In addition, development to the adult stage occurred with less frequency when the nymphs were feeding on Douglas-fir compared with second-year pine cones. As with our adult tests, the low suitability of Douglas-fir differs from previous studies [10, 13, 20]. The differences between our results and prior studies is possibly related to spatial, temporal and/or phylogeographic discrepancies among the studies. Spatially, our experiments restricted adult *L. occidentalis* to small enclosures containing multiple hosts from which to select for oviposition and feeding. Physical characteristics such as tree stature and density of cones are important in host selection by *L. occidentalis* in the field [13], and these factors were not present in the laboratory conditions we tested. Further, discrete chemical cues from individual host species would not have been present in the laboratory setting. Temporally, our experiments provide evidence of variability through time of *L. occidentalis* feeding preference among conifer species. Feeding damage to Douglas-fir also varies through the period of cone development [10, 11]. Our experiments were confined to June and July and any preferential feeding by *L. occidentalis* on Douglas-fir either earlier or later in the season would not have been observed. Douglas-fir in northern Idaho have been genetically separated from its conspecifics on the Pacific coast for an estimated 2.11 million years [21]. This separation may have resulted in physical and/or chemical differences or differences in nutritional quality between the phylogeographic areas that resulted in inter-mountain Douglas-fir being less attractive to *L. occidentalis*.

Similar to the potential variation in host trees among different geographic locations, there is the potential that populations of *L. occidentalis* may also differ among areas. The insect has proven to be a successful invader in many regions of the world such as the southeastern United States [22], Mexico [23], Italy [24], other parts of Europe [25] and Russia/Ukraine [26]. Within these diverse geographic locations, the insect has adapted to novel hosts and demonstrated the ability to modify life history characteristics such as voltinism [23, 24]. Such adaptability in host usage or life history parameters may be responsible for the observed differences between our results and those reported from western Oregon and Canada.

The adult insects used for this study were all collected from ornamental species of pine. The pine hosts may have resulted in a bias in these insects during our laboratory trials; we rarely encountered *L. occidentalis* on non-pine species during our field collections and the pine species used in the experiments were different than the species from which adults were collected. Also, *L. occidentalis* adults are very mobile, often flying to other trees (including non-pine conifers) when disturbed. Further, the adult feeding preference for pine accompanied by the greater degree of maturation of the nymphs on pine is unlikely to be influenced by parental preferences in this generalist species.

CONCLUSION

In our laboratory studies, adult *L. occidentalis* preferentially fed upon pine hosts over non-pine hosts, and females more frequently oviposited on ponderosa pine compared with all other surfaces. In general, pine foliage was preferred as an oviposition site over the non-pine material and was more suitable for maturation compared with non-pine hosts. Immature *L. occidentalis* survived longest and were most likely to complete development when provided second-year cones of lodgepole pine. In contrast, survival of immature *L. occidentalis* was shortest in non-fed control treatments, water-only treatments and when provided first-year cones from pine. The differences between the current results and those of prior reports are possible due to phylogenetic distance between Douglas-fir in the inter-mountain west versus coastal populations of Douglas-fir, the potential for seasonal shifts in host preference as cones mature over the course of a summer and potential adaptations to available host material by the insect in different geographic areas.

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CONFLICT OF INTEREST STATEMENT

The work reports the results of research and does not represent an endorsement of any of the products used. None of the authors have personal or financial associations with companies or their products.

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