Materials and methods

## 2.1 Plant characteristics and living conditions

Potato clones were provided by the USDA-ARS, Small Grains and Potato Germplasm Research Unit Aberdeen, ID, USA. We used three sibling clones derived from *Solanum chacoense* L. with putative resistance to Lso: A07781-3LB (‘3LB’), A07781-4LB, (‘4LB’), and A07781-10LB (‘10LB’) (Rashidi et al. 2017). Russet Burbank was used as a susceptible control due to its susceptibility to Lso (Munyaneza et al. 2011) and its large role in Idaho agriculture. Russet Burbank comprised 48.3% of Idaho’s total potato acreage planted in 2017 (USDA 2017). Potatoes were grown in a greenhouse maintained between 25-32°C, 32% RH, with a photoperiod of 16:8 (L:D). Plants were grown in pots of approximately 8.5 cm length x 8.5 cm width x 9.5 cm height, with a soil mixture of 4:4:4:1 peat moss: compost: coconut coir: perlite. Fertilizer was not used on experimental plants to avoid nitrogen increases which may affect insect feeding behaviors (Pfeiffer and Burts 1983, 1984). We used plants in their vegetative growth stage (growth stage II) (Dwelle et al. 2003).

## 2.2 Insect characteristics and living conditions

A Lso-positive potato psyllid colony was reared in the same greenhouse conditions as described above to avoid phenological asynchrony (Hodkinson et al. 2015). The ‘Central’ haplotype was confirmed via the methods described in Swisher and Crosslin (2014) and analyzed with ethidium bromide-stained agarose gels. Psyllids were allowed free access to both Russet Burbank potatoes and ‘Yellow Pear’ tomatoes (*Solanum lycopersicum* L.). Colony plants were fertilized once weekly with approximately 17 g of 24:8:16 NPK fertilizer per gallon of water (MiracleGro® All Purpose Plant Food, Scotts Company, Marysville, OH). Plants were replaced as needed.

## 2.3 Lso detection

Forty psyllids were transferred to individual microcentrifuge tubes filled with 70% ethanol. Lso incidence was tested at the Aberdeen Research and Extension Center (Aberdeen, ID, USA). DNA extraction was based on the methods described by Marzachi et al. (1998). Individual psyllids were ground by a homogenizer (Omni International Inc., Kennesaw, GA), macerating each psyllid for 1 minute at high speed and an additional minute at medium speed in 500 µl of CTAB 2% % (Alpha Teknova, Inc., Hollister, CA, Cat. No. C2190) (Composition: 2% CTAB, 100mM Tris-HCl, pH 8.0, 20mM EDTA, pH 8.0, 1.4M Sodium Chloride (NaCl). Microcentrifuge tubes were then incubated at 60°C for 30 minutes and gently mixed by inversion every 10 minutes while incubating. Tubes were then spun in a centrifuge at 14,000 rpm for 5 minutes and then the supernatant was transferred to clean 2 ml tubes. The supernatant was vortexed for approximately 20 seconds with 500 ml of chloroform:isoamyl alcohol (24:1 v:v) (Sigma-Aldrich, Inc., Atlanta, GA; Catalogue number C0549), then centrifuged at 14,000 rpm for 5-10 minutes at 4℃. The clean supernatant was transferred to a new tube, then refrigerated isopropanol (Sigma-Aldrich, Inc., Atlanta, GA; Catalogue number I9516) was added at a rate of 2/3 of the volume of the supernatant. The mixture was then refrigerated at ˗20°C for 20-30 minutes. DNA was precipitated by centrifuging the mixture for 20 minutes at 14,000 rpm at 4℃, gently pouring off the supernatant and keeping the precipitated DNA pellet. The pellet was washed in 300 µl of 70% ethanol and centrifuged for 5 mins at 10,000 rpm. The pellet was then dried overnight in a fume hood. Once dry, 30 µl of nuclease-free water was added. DNA was stored at ˗20°C.

Extracted DNA samples were then processed using a Sybgreen method. SsoAdvanced™ Universal SYBR® Green Supermix (Biorad, Hercules, CA) was mixed in a CFX Connect Real-Time PCR Detection System (Biorad, Hercules, CA). HLBr (5’-GCG TTA TCC CGT AGA AAA AGG TAG-3’) and LsoF (5’-GTC GAG CGC TTA TTT TTA ATA GGA-3’) were used as primers and 10 µl of Sybgreen supermix was added to 150 nM of each primer with 1 µl of DNA template. The program cycle was as follows: one cycle at 98°C for 2 mins followed by 40 cycles of 95°C for 10 sec and 62°C for 20 sec. The melt curve was 65°C to 95°C, with increments of 0.5°C sec-1. DNA of a healthy tuber was used as a negative control. DNA of a Lso-infected tuber was used as a positive control and water was used as a no-template control in all tests. pIDTSmart Kan (Synthetic Genomics, SGI-DNA, CA) with a 250 bp region was amplified with the primer HLBr. The plasmid was diluted 10-fold and used with the following dilutions: 1 x 10-2, 1 x 10-3, 1 x 10-4, 1 x 10-6, 1 x 10-7, and 1 x 10-8 ng. Pathogen quantity was reported as copy number of Lso; copy numbers were determined using the methods of Levy et al. (2011).

## 2.4 No-choice behavior assays

No-choice assays were conducted in a climate-controlled room maintained at 26°C. Assays were conducted on a wire shelving unit, which allowed the testing arena to be lit both from above and below. Three Smith-Victor Digilight fixtures (Smith-Victor Corporation, Bartlett, IL) were used with three Azlo (Akces Media LLC dba ALZO Digital, Bethel, CT) full-spectrum CFL bulbs per light fixture (100-240 volts, 60 Hz, color temp 5500K CRI 91, 750 lumens, 15 watts). Two lights were placed with their light sources 35 cm above the testing arena and the light was softened with a diffusion cloth. The remaining light fixture was placed so that its light source was 45 cm below the testing arena and was softened with diffusion material as well. Illuminance was 3600 lx at the surface of the arena (Sekonic L-308DC-U Light Meter, Sekonic Corporation, Tokyo, Japan).

The observation arena (Fig. 1) was modelled after the design described by Liu and Trumble (2004) and modified to observe psyllids on leaflets of intact, living plants. A recording arena was formed by sandwiching a panel of glass, a wetted filter paper, a leaf, and a piece of Plastazote® polyethylene foam (Zotefoams Inc., Croydon, UK), with a circular opening in the center (28 mm diameter). The arena was held together with two clips. This arena was then suspended by a suction cup held by an adjustable burette clamp. This allowed each psyllid to move freely on the abaxial surface of a leaf attached to a living plant. We used leaves from the upper canopy of the plants. The filter paper was discarded between observations. The glass pane and foam were replaced with each new plant and washed and dried at 90°C before reuse to remove potential volatile accumulation. Recordings were done with a L3CMOS C-mount USB camera and ToupView recording software (L3CMOS14000KPA, Hangzhou ToupTek Photonics Co., Ltd, Hangzhou, Zhejiang, China).

We collected psyllids from the colony by aspiration and transferred them to 8 x 35 mm glass shell vials. All psyllids were used within 90 minutes from the time of collection.Psyllids were introduced to the arena and recorded for five minutes, after which we preserved them in 95% ethanol for later PCR testing. We recorded the same categories as Butler et al. (2011): probing/feeding, walking, cleaning, and whether the psyllid was on or off the leaf. Probing and feeding behaviors were a combined category due to putative significance with disease transmission (Prager et al. 2014). Behavior was scored using CowLog3 (Hänninen and Pastell 2009), which recorded incidence and timestamps for the behaviors observed.

## 2.5 Fecundity assays

Fecundity assays were conducted with greenhouse conditions, plants, and insects as previously described (see *‘Plant characteristics and living conditions’* and *‘Insect characteristics and living conditions’*, above). A female/male pair of teneral psyllids (identified by their green body color) was introduced to plants covered with an insect rearing sleeve (MegaView Science Co., Ltd., Taiwan). Rearing sleeves were supported over the plant using two lengths of galvanized steel wire with a diameter of 1.63 mm. Each wire was curved into a parabolic shape and each end of the wire was inserted into the soil on opposite corners of the plant pot (Fig. 2). Plants were blocked by variety in rows of four and placed inside 60 cm length x 60 cm width x 60 cm height mesh-covered PVC-framed cages. Plants were watered on alternating days by soaking pots in 56 cm length x 28 cm width x 6 cm height plastic trays until the soil became saturated (approximately 45 mins). After six days the male was removed from plants using an aspirator and the female was transferred to a new plant of the same variety. Females were moved to new plants every four days until 18 days had elapsed. Eggs were counted on each plant after the female was removed. Nymphs were counted three times over the four days following removal of the female. Each nymph was removed as it was counted. The percentage of nymphs which hatched was considered an indicator of egg fertility and was calculated as the ratio of nymphs divided by initial egg counts for each sample.

## 2.6 Statistical analysis

Generalized linear mixed modeling with a Poisson link was used to analyze no-choice experiments, which had a non-normal distribution (Stroup 2015). Data were analyzed in R Version 3.4.4 (R Core Team 2013), using the glmer() function from the package lme4 (Bates 2015). No-choice assays were modelled with fixed factors of sex, germplasm and the interaction between them. Psyllid replicate was treated as a random factor. Egg fertility assays were

Wald's *χ2* tests were conducted to test for significant effects. Estimated marginal means were used to discriminate significant differences from *χ2* tests, using pairwise comparisons among means. Off-leaf data was not analyzed due to low occurrences (20 out of 182 observations), which did not allow an interaction to be estimated. P values were multiplicity adjusted for the model.

For the fecundity studies, both the number of eggs that females laid and the percent egg fertility were compared among germplasms using repeated-measures ANOVA. Fisher’s least significant difference tests were used to determine differences between treatments.

Statistical significance was considered at *α* = 0.05.